MULTIPLE SCLEROSIS IN ASIAN POPULATIONS: THE GENETIC AND ENVIRONMENTAL DETERMINANTS OF VARIABLE SUSCEPTIBILITY AND CLINICAL PROFILE

by

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Abstract

Multiple sclerosis is a chronic, inflammatory disorder of the central nervous system, thought to primarily affect persons of Caucasian ancestry. Despite growing recognition of multiple sclerosis and clinical variants such as neuromyelitis optica in persons of other ethnicities, relevant research in emerging populations is comparatively scant. Consequently, current understanding with respect to clinical outcomes and risk factors in this ethnic group is remarkably under-developed. The overarching objective of this dissertation was to address these knowledge gaps using a multi-disciplinary approach focusing on Asian-ethnic populations in Canada and China.

The prevalence of multiple sclerosis in an Asian-ethnic population of Canada was found to be intermediate to that typically reported in Asia and in the general Canadian population. Longitudinal analysis also revealed an increase in incidence among females of Asian ancestry in Canada. In comparative analysis in a Canadian clinic population, long-term clinical outcomes of multiple sclerosis in patients of Asian ancestry were remarkably similar to those in non-Asian patients. Male sex and later age at onset were associated with less favourable clinical outcomes, irrespective of ethnicity or region. In immigrant and Canadian-born patients, duration of exposure to the Canadian environment prior to onset was associated with multiple sclerosis, whereas exposure to the regional environment of Asia was associated with neuromyelitis optica. Case-control analysis revealed a robust association of smoking with multiple sclerosis risk and clinical outcomes in Canadians of Asian ancestry. Genetic studies confirmed the overall low rate of familial recurrence in this ethnic group, but also identified novel variants associated with risk and clinical phenotypes.
The findings underscore the importance of ethnicity-related genetic and environmental factors in modifying susceptibility to and clinical presentation of multiple sclerosis and related disorders in persons of Asian ancestry. Nevertheless, these results also suggest that the clinical trajectory in patients of Asian ancestry may be more comparable to classic clinical descriptions than previously believed. Taken together, the findings presented in this dissertation contribute new perspectives to the epidemiology and etiology of multiple sclerosis and related disorders, and advance knowledge in an emerging patient population in Canada and globally.
Preface

The work presented in this dissertation was completed under the auspices of a research program established by Drs. Anthony Traboulsee and A. Dessa Sadovnick. An operating grant from the Canadian Institutes of Health Research (China-Canada Joint Health Research Initiative, CCI-102935) funding this research was obtained by Dr. Anthony Traboulsee (Principal Investigator). This research was approved by the University of British Columbia Clinical Research Ethics Board (Certificate No.: H11-01360) and the Vancouver Coastal Health Research Institute (Certificate No.: V11-01360).

With assistance from Irene Yee, I prepared and submitted the initial ethics application and annual renewal applications to the UBC Clinical Research Ethics Board. With contributions from Talitha Greenwood, Madonna de Lemos, Dr. Gui-Xian Zhao, and Dr. Zhi-Ying Wu, I jointly acquired clinical data, patient-reported data, and biological specimens used in studies presented in Chapters 4-8. Under the guidance of Dr. Anthony Traboulsee, I performed all clinical chart reviews for studies presented in Chapters 3-5. I jointly acquired and analyzed data presented in Chapter 8 with Dr. Carles Vilariño-Güell. With guidance from Drs. A. Dessa Sadovnick and Anthony Traboulsee, I conceived the studies, designed the experiments, oversaw data collection, performed data analysis, and interpreted the findings for all research presented in this dissertation. I drafted all manuscripts to be submitted for publication based on work presented in this dissertation with contributions from co-authors with respect to the interpretation of findings and substantive revisions to the manuscript. I composed this dissertation under the guidance of Dr. A. Dessa Sadovnick, and with additional input from Drs. Anthony Traboulsee, Jan Friedman, and Matthew Farrer.
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<th>Description</th>
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<tr>
<td>ADEM</td>
<td>acute disseminated encephalomyelitis</td>
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<tr>
<td>BC</td>
<td>British Columbia</td>
</tr>
<tr>
<td>CCHS</td>
<td>Canadian Community Health Survey</td>
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<tr>
<td>CCPGSMS</td>
<td>Canadian Collaborative Project on Genetic Susceptibility to Multiple Sclerosis</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CIS</td>
<td>clinically isolated syndrome</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>DMT</td>
<td>disease-modifying therapy</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
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<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</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>IFNβ</td>
<td>Interferon-beta</td>
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<tr>
<td>IIDD</td>
<td>idiopathic inflammatory demyelinating disorder</td>
</tr>
<tr>
<td>IQR</td>
<td>inter-quartile range</td>
</tr>
<tr>
<td>LESCL</td>
<td>longitudinally extensive spinal cord lesion</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>MS</td>
<td>multiple sclerosis</td>
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<tr>
<td>MSSS</td>
<td>Multiple Sclerosis Severity Score</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NMO</td>
<td>neuromyelitis optica</td>
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<tr>
<td>NMO-IgG</td>
<td>neuromyelitis optica immunoglobulin-G</td>
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<tr>
<td>NMOSD</td>
<td>neuromyelitis optica spectrum disorders</td>
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<tr>
<td>OCB</td>
<td>oligoclonal banding</td>
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<tr>
<td>ON</td>
<td>optic neuritis</td>
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<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>OSMS</td>
<td>opticospinal multiple sclerosis</td>
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<tr>
<td>PPMS</td>
<td>primary progressive multiple sclerosis</td>
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<tr>
<td>PRMS</td>
<td>progressive-relapsing multiple sclerosis</td>
</tr>
<tr>
<td>RRMS</td>
<td>relapsing-remitting multiple sclerosis</td>
</tr>
<tr>
<td>rs</td>
<td>reference SNP</td>
</tr>
<tr>
<td>SKAT</td>
<td>sequence kernel association test</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>SPMS</td>
<td>secondary progressive multiple sclerosis</td>
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<tr>
<td>TM</td>
<td>transverse myelitis</td>
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<tr>
<td>UBC</td>
<td>University of British Columbia</td>
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<tr>
<td>$\beta$</td>
<td>linear regression coefficient</td>
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To those whose lives have been touched by multiple sclerosis and related disorders
Chapter 1: Introduction

1.1 Multiple sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory disorder of the central nervous system (CNS), primarily affecting white matter in the brain and spinal cord\textsuperscript{1,2}. The principal pathological features of MS are autoimmune destruction of myelin and subsequent axon degeneration, both of which give rise to neurological injury and consequent clinical disability. MS develops in genetically susceptible individuals exposed to one or more environmental triggers. Its precise etiology, however, is unclear.

The symptoms of MS vary widely, in large part due to the multitude of functions mediated by affected areas of the CNS. Commonly impacted faculties include mobility, balance, bowel and bladder function, vision, hearing, speech, and cognition\textsuperscript{2}. The clinical onset of symptoms typically occurs in early adulthood, usually between ages 20 and 40 years. In addition, MS in pediatric or older adult populations is now increasingly recognized.

Excluding trauma, MS is the most common cause of neurological disability among young adults in regions of the world predominantly inhabited by populations of European ancestry\textsuperscript{3}. It is estimated that over 2.3 million persons worldwide are currently living with MS\textsuperscript{3}. In Canada, which has one of the highest rates of MS in the world, there are approximately 100,000 persons living with MS\textsuperscript{4}. The global economic burden of MS is staggering. In Canada, the estimated cost of illness is between $30,000 and $70,000 per patient per year\textsuperscript{5}; this figure amounts to an economic burden on the order of several billion dollars annually. The non-financial costs of the
illness, including psychosocial well-being and quality of life, are also prodigious, if not innumerable.

1.2 MS variants and related disorders

MS is the prototypic disease among the CNS idiopathic inflammatory demyelinating disorders (IIDD). The IIDDs encompass several heterogeneous disorders that overlap considerably with MS and with one another in terms of clinical and pathological features. These disorders are routinely considered among the possible diagnoses when a patient initially presents with a clinical picture suspicious for MS. Conversely, misdiagnosis of atypically presenting MS with another IIDD is not uncommon, given the extensive overlap in clinical features. These disorders, collectively termed MS and related disorders in this dissertation, include clinically isolated syndromes suggestive of MS, acute disseminated encephalomyelitis, and neuromyelitis optica and its spectrum disorders. For clarity, conventional MS variants are simply referred to as MS throughout this dissertation.

1.2.1 Clinically isolated syndrome

In approximately 85% of MS cases, the disease begins with an episode of acute or subacute neurological dysfunction. This initial clinical episode characterized by signs and symptoms suggestive of MS is termed a clinically isolated syndrome (CIS). As such, CIS is often the first clinical manifestation of MS. In a study with a mean follow-up period of 20 years, 63% of CIS patients experienced a relapse and therefore progressed to clinically definite MS status. Abnormal brain magnetic resonance imaging (MRI) at the time of the initial CIS episode is the most reliable predictor of an eventual MS diagnosis. CIS cases with an abnormal baseline brain
MRI have an 82% risk of conversion, whereas those with normal imaging (disregarding the symptomatic lesion associated with the CIS episode) have a 21% risk of developing MS\(^8\).

In many patients ultimately diagnosed with MS, an episode of optic neuritis (ON) or transverse myelitis (TM) is the first clinical manifestation. When occurring in the context of brain lesions suggestive of demyelination on MRI, monofocal ON or TM is highly predictive of eventual development of clinically definite MS. In one study, 88% of such cases with a mean follow-up of 14 years experienced a second attack fulfilling clinical criteria for MS, whereas those with normal brain MRI at first clinical event had a 19% risk of developing clinically definite MS\(^9\).

ON is caused by inflammatory demyelination of the optic nerve and is characterized by acute visual deficits, such as partial or complete blindness, blurriness, colour desaturation, or scotoma. In ON, these signs and symptoms frequently manifest with pain elicited by eye movement. ON may be recurrent\(^10\); in cases in which both optic nerves are affected, a diagnosis of MS may be warranted on grounds that diagnostic criteria of “dissemination in time and space”\(^11\) have been fulfilled. Additionally, patients with recurrent ON are at considerable risk to develop neuromyelitis optica (NMO) (discussed in Section 1.2.3), particularly if episodes are frequent or characterized by severe visual deficits\(^12\).

TM is caused by focal inflammation of the spinal cord. Symptoms include sensory disturbances, bladder dysfunction, motor weakness, and paralysis. Recovery from TM is variable, as approximately one-third of patients are left with minimal, moderate, or severe residual disability following recovery\(^13\). In some individuals, TM is a monophasic disease, whereas in others, it may be the onset symptom of MS or NMO. Similar to ON, transition to MS occurs more frequently in patients with focal demyelination appearing on brain MRI at onset of TM\(^14\).
Recurrent TM may occur in the context of MS, NMO, or other inflammatory syndromes affecting the spinal cord, such as systemic lupus erythematosus\(^\text{15}\). Recurrent myelopathies in the context of normal brain MRI is strongly suggestive of NMO, and in an appropriate setting, may be classified as a limited form of NMO\(^\text{16}\).

### 1.2.2 Acute disseminated encephalomyelitis

Acute disseminated encephalomyelitis (ADEM) is a severe but self-limited, inflammatory CNS demyelinating disorder with rapid onset\(^\text{17}\). ADEM is generally considered to be nosologically distinct from MS, on account of its typically monophasic course and probable immunogenic trigger (infectious illness or vaccination)\(^\text{17}\), although ADEM is often included in the broader spectrum of MS and related disorders\(^\text{18}\). ADEM is more common in children than in adults, and most patients recover completely. However, apparent onset of ADEM should raise clinical suspicion of possible MS, as 27% of patients initially diagnosed with ADEM were subsequently diagnosed with MS following one or more relapses in a study with a mean follow-up of approximately six years\(^\text{18}\). In such cases, an ADEM-like syndrome was the initial manifestation of an underlying pathological process culminating in MS.

### 1.2.3 Neuromyelitis optica

NMO, also known as Devic’s disease, is characterized by selective and classically severe attacks of inflammatory demyelination of the optic nerve(s) and spinal cord\(^\text{16}\). In NMO, episodes of ON and TM need not occur simultaneously; demyelinating events may in fact be separated by an interval of several weeks, and in some patients, several years. This restricted topographical distribution of lesions is the \textit{sine qua non} of NMO, as the brain is spared of clinical involvement.
(at least in the disease’s early stages), unlike in MS. NMO is of particular relevance, as it is exceedingly rare in Caucasian populations, but accounts for approximately one-third of MS and related disorders in Asian-ethnic populations.

Similar to MS, NMO follows a relapsing course in most patients, and its clinical symptoms and signs overlap considerably with MS. For this reason, NMO was long considered an aggressive variant of MS. The clinical prognosis of NMO is considerably less favourable than MS due to the increased severity and frequency of relapses\textsuperscript{16}. ON in NMO is more frequently associated with severe or bilateral vision loss and incomplete recovery compared to MS\textsuperscript{16}. Episodes of myelitis in NMO, which tend to be more devastating than in MS, often manifest as severe motor weakness or symmetric paraplegia. Again, residual deficits typically persist following a period of convalescence\textsuperscript{16}.

The nosological position of NMO in relation to the MS spectrum has long been a matter of debate. Only recently, with the discovery of a highly specific serological biomarker to facilitate diagnosis\textsuperscript{19}, has there been general acceptance that NMO should be considered a distinct disease rather than a variant of MS. Subsequent studies have reaffirmed the concept of NMO as a disease related to but distinct from MS, owing to recognized clinical, epidemiological, pathological, and immunological differences\textsuperscript{16}. However, more recent observations establishing a wider range of clinical heterogeneity than previously recognized suggests that NMO and its spectrum disorders do not represent a pathologically uniform disease entity\textsuperscript{20}. Further, it is now recognized that opticospinal MS (OSMS), a presumptive variant of MS characterized by clinical involvement largely restricted to the optic nerves and spinal cord (and frequently reported in the Asian MS literature), likely represents a \textit{forme fruste} of NMO\textsuperscript{21}.
The historical definition of NMO—that is, a monophasic illness characterized by bilateral ON and TM occurring simultaneously or within weeks of each other—has recently been supplanted by increasingly broader definitions with successive revisions to the diagnostic criteria\textsuperscript{22,23}. This inclusive approach is manifest in the concept of the “NMO spectrum disorder” (NMOSD), which continues to garner acceptance in the field. NMOSD comprises diverse syndromes suggestive of incipient NMO or with features associated with a high-risk to evolve into definite NMO. These include limited forms of NMO (e.g., isolated or recurrent longitudinally extensive TM; severe, recurrent ON; severe, recurrent TM; simultaneous, bilateral ON), as well as ON or TM comorbid with a systemic (e.g., Sjögren syndrome, systemic lupus erythematosus) or organ-specific (e.g., myasthenia gravis, thyroid disease, type 1 diabetes) autoimmune disorder or NMO-characteristic brain lesions (e.g., in the hypothalamus, corpus callosum, fourth ventricle, or long internal capsule)\textsuperscript{16}. High frequency of NMO-IgG seropositivity (discussed below) is a unifying feature of NMOSD, thus suggesting common pathobiology\textsuperscript{24}.

1.3 History of MS research in Asian populations

The earliest detailed clinical description of MS is credited to Jean-Martin Charcot in 1868\textsuperscript{25}, but the first peer-reviewed publication on MS in an East or Southeast Asian (hereafter, collectively termed “Asian”) population\textsuperscript{26} did not occur until some 90 years later in a Japanese study. Peer-reviewed reports of MS in Asian populations remained scarce prior to 1970. The first published study on MS in an Asian population outside of Asia was a report in 1969 that focused on patients in the state of Hawaii\textsuperscript{27}. Subsequent studies in Hawaii\textsuperscript{28–31} were some of the earliest published works to directly examine the putative differences in the clinical presentation of MS between Asian-ethnic and Caucasian populations.
Despite several decades of research on MS in Asian populations, the epidemiology, clinical features, and etiology of MS in these populations remain poorly understood. This deficit in knowledge is a consequence of a relative dearth of MS research in these populations, which is itself largely due to the rarity of MS and related disorders in these populations. Perhaps unsurprisingly, the proportion of peer-reviewed publications on MS and related disorders in Asian-ethnic populations reflects the comparatively low burden of disease in these populations. To illustrate, of the more than 42,000 English-language publications indexed with the medical subject heading term “Multiple Sclerosis” in the US National Library of Medicine’s MEDLINE database (on December 31, 2013), fewer than 400 (<1%) were indexed with the term “Asian Continental Ancestry Group” or a medical subject heading term for any East or Southeast Asian country. Notably, the number of such publications has increased steadily in the last decade. Remarkably, more than half of these works were published in the last 10 years, conceivably an indication of growing awareness of MS and related disorders in Asian-ethnic populations.

1.4 Epidemiology of MS and related disorders

Although MS occurs in every region of the world, its global distribution is not homogeneous\(^3\). Geographic differences in the burden of MS, including regional clusters and latitudinal clines, have long been recognized (Figure 1.1). Similar to other disorders considered to be of autoimmune origin, MS affects females more frequently than males. However, regional variation in sex ratio is widely recognized\(^3\).
Figure 1.1 Worldwide prevalence of MS by country in 2013.

Prevalence estimates were derived from the Multiple Sclerosis International Federation Atlas of MS 2013.
1.4.1 Frequency of MS

A remarkable epidemiological feature of MS is its irregular global distribution. A latitudinal gradient in MS prevalence, characterized by an upward cline in MS frequency with increasing distance from the equator, has long been recognized. Numerous studies since Davenport’s seminal work\textsuperscript{32} have corroborated the latitudinal effect, including regional studies\textsuperscript{33–37} and large meta-analyses\textsuperscript{38,39}. However, the existence of a genuine latitudinal effect has recently been challenged by some\textsuperscript{40–42}, who suggest that the phenomenon may be an artifact. They reason that the more robust geographic trend in MS prevalence is the differential frequency of MS between continental regions, which in large measure reflects insularity of populations sharing common ethnic origins\textsuperscript{42}.

The global median prevalence of MS is approximately 33 cases per 100,000\textsuperscript{3}. MS is more common in Caucasians, particularly those with Northern or Central European ethnic origins\textsuperscript{40,43}. Accordingly, the frequency of MS is greatest in Europe and North America, where estimated prevalence is 108 and 140 cases per 100,000, respectively\textsuperscript{3}. Canada has one of the highest reported MS prevalence rates in the world, recently estimated at 240 to 285 cases per 100,000\textsuperscript{44}. The burden of MS in Canada likely exceeds that in regions of Northern Europe with historically high rates of MS. Studies conducted within the last 10 years have yielded estimates of prevalence ranging from 118 to 231 per 100,000 in the British Isles, and 93 to 219 per 100,000 in Scandinavian countries\textsuperscript{45}.

Estimates of the incidence of MS also vary considerably, even within the same geographic area. In Canada, recent estimates of the annual incidence of MS have ranged from 4.2 to 8.1 per 100,000\textsuperscript{46}. High MS incidence rates have also been documented in many regions of Europe,
including the British Isles (4.0-12.2 per 100,000), Sweden (5.2-6.4 per 100,000), Norway (3.0-8.7 per 100,000), Finland (5.1-11.6 per 100,000), France (4.3-7.5 per 100,000), and Germany (6.0-8.0 per 100,000)\textsuperscript{45}.

On the other hand, MS is exceedingly rare in most regions predominantly inhabited by non-Caucasian populations, including East and Southeast Asia. Reports from China\textsuperscript{47}, Japan\textsuperscript{36,48}, South Korea\textsuperscript{49,50}, Hong Kong\textsuperscript{51,52}, Taiwan\textsuperscript{53,54}, Malaysia\textsuperscript{55}, and Thailand\textsuperscript{56} have collectively shown prevalence rates ranging from 0.70 to 2.00 per 100,000. However some studies have reported higher prevalence rates in Japan (7.7 to 16.2 per 100,000)\textsuperscript{57-61}, Hong Kong (4.8 per 100,000)\textsuperscript{62}, Taiwan (2.96 per 100,000)\textsuperscript{63}, and South Korea (3.5-3.6 per 100,000)\textsuperscript{64} compared to previous studies in the respective regions.

An increase in the incidence of MS in Japan was recently reported, with the mean annual incidence increasing from 0.17 to 0.77 per 100,000 between 1980 and 2009\textsuperscript{61}, and a similar trend was reported in Taiwan\textsuperscript{63}. Importantly, most studies of MS frequency in East and Southeast Asia (particularly those conducted prior to widespread adoption of the most recent revisions to the diagnostic criteria for NMO\textsuperscript{23}) included clinical variants such as opticospinal MS, which is now usually recognized as NMO or a variant thereof. Therefore, it is perhaps more accurate to consider most estimates of prevalence and incidence previously reported in these regions to reflect cumulative rates for MS and related disorders, especially NMO and NMOSD.

The apparent temporal increase in the frequency of MS in some parts of East Asia reflects a broader trend that has also been observed elsewhere in populations considered “high-risk” for MS\textsuperscript{42}. Although these trends may be indicative of genuine increases in MS risk in these populations, other possible explanations warrant consideration. For instance, temporal
improvements in case ascertainment (for example, due to more sensitive diagnostic criteria and tools facilitating diagnosis, as well as improvements to access thereof) may be in part driving this upward trend in MS frequency. The potential for under-recognition of MS is of particular concern in low-prevalence regions such as East Asia where awareness of rare diseases like MS has historically been limited among health care providers65.

In addition, other factors, such as changes in population structure due to aging, migration, and ethnic admixture, as well as increasing survival in those diagnosed with MS and related disorders, could also influence prevalence estimates over time independently of any changes in MS risk66. These possibilities notwithstanding, others have suggested that the increasing incidence of MS in these populations reflects a true increase in MS risk, a claim that is corroborated by concomitant changes in the clinical phenotype and sex ratio among incident cases60.

1.4.2 Demographics of MS

In most patients, the onset of MS symptoms occurs in the third or fourth decade of life. A recent global survey of MS revealed regional variations in the age at clinical onset67. In general, MS onset was earlier in high-prevalence regions such as Canada and northern parts of Europe (29.2-29.4 years) compared to low-prevalence regions such as East Asia (33.3 years)67. However, regional variation in the age at onset was modest overall.

Although it is now universally accepted that MS affects females more frequently than males, reports from the early part of the 20th century demonstrated generally equal rates68, and some even reported excess of MS in males69. However, early estimations of the sex ratio were almost
certainly inaccurate due to incomplete and biased case ascertainment, owing to pervasive socially prescribed gender roles at the time: Males, who almost exclusively assumed the role of primary income-earner in households during this epoch, were more likely to receive medical care for neurological symptoms. The notion of female preponderance in MS did not gain wide acceptance until the latter half of the 20th century.

It is now widely acknowledged that MS affects females more often than males at a ratio of at least 2:1, although regional and population variations to this ratio have been noted. It has long been assumed, for example, that female preponderance of MS is greater in Asian-ethnic populations on the basis of several case series with female-to-male ratios greater than 3:1, and in some cases equaling or exceeding 5:1. However, an important consideration when appraising these data is the reasonable likelihood that patients with NMO, a diagnosis typified by greater female predilection, were included in these case series due to nosological uncertainty at the time. More recent work from this region paying heed to diagnostic distinction of MS and NMO appears to suggest that the sex ratio may not actually differ substantially from that in Caucasian populations.

Several recent studies have reported an increase in the female-to-male ratio in MS, suggestive of increasing incidence among females when juxtaposed with relative stability of incidence in males. This trend has also been observed in lower-prevalence regions, such as Japan and Iran. It may be argued that the apparent increase in female preponderance may simply reflect the gradual diminution of gender biases in ascertainment, which have historically favoured greater medical scrutiny in males. Others have speculated that the trend may be an artifact of evolving diagnostic practices and tools that have enabled better identification of benign MS.
cases assumed to be more common among females\textsuperscript{79}. However, this explanation is countered by studies demonstrating equivalent trends in diagnostic delay between males and females\textsuperscript{77,78}.

1.4.3 Epidemiology of NMO

Relatively little is known about the epidemiology of NMO, in part because of its uncertain nosological position with respect to MS for most of the last several decades. The current body of knowledge in this regard is informed almost entirely by a limited number of case reports and case series. There is no consensus on the global prevalence of NMO. Estimates of prevalence range from 0.52 to 4.4 per 100,000\textsuperscript{24}, which suggests that NMO is perhaps 100 times less prevalent than MS overall. However, even studies from the same geographic region have reported divergent estimates of prevalence\textsuperscript{82,83}.

It has long been assumed that NMO is more common in East and Southeast Asia compared to regions predominantly inhabited by Caucasians. This notion was predicated on the established observation that the ratio of NMO to MS in Asian-ethnic populations is categorically higher than in Caucasian populations. Whereas in Europe and North America NMO accounts for 1-2% of diagnoses of MS and related disorders\textsuperscript{84,85}, NMO and its spectrum disorders collectively comprise approximately one-third of cases in Asian populations\textsuperscript{86}. The proportion of such cases has been as high as 48-65\% in some Asian case series\textsuperscript{26,55,74,87}. However, recent studies in Caucasian populations have demonstrated higher NMO prevalence rates than previously reported\textsuperscript{88,89}, and there is now considerable debate as to whether regional differences truly exist with respect to the absolute prevalence of NMO.
The demographic distribution of NMO differs from that of MS, in that the female preponderance and mean age at onset are greater in the former. NMO is several times more prevalent among females than males, but the sex ratio in several large case series has ranged considerably, from 2.8:1 to 10.6:1.\textsuperscript{85,88,90,91} Clinical onset of NMO typically occurs in the fourth or fifth decade of life. In recent large studies in the US, Denmark, and Japan, mean age at onset was 41.1, 35.6, and 42.9 years, respectively.\textsuperscript{85,88,91} Recognition of these demographic differences has strengthened the case for considering NMO as a distinct disease from MS.

1.5 Clinical characteristics of MS and related disorders

In terms of clinical features, MS and related disorders are extraordinarily heterogeneous.\textsuperscript{6} These disorders span a broad continuum of clinical presentation, and collectively, are characterized by notoriously unpredictable clinical course and diverse symptomatology. Consequently, the diagnosis and clinical management of MS and related disorders are continually evolving, and in recent decades have undergone significant changes in several domains, including neuroimaging, diagnostic biomarkers, and therapeutics.

1.5.1 Signs and symptoms of MS and related disorders

The classic signs and symptoms of MS arise when focal inflammation or axon degeneration localize to functionally relevant areas of the CNS. The possible anatomical sites of injury in MS encompass several CNS structures, including the optic nerves, spinal cord, brainstem, cerebellum, and cerebrum.\textsuperscript{2} The disseminated topography of inflammatory lesions and axon degeneration accounts for the diversity of signs and symptoms in MS and related disorders. Neurological deficits can manifest as disruptions to the visual, sensory, motor, cognitive and
autonomic systems. The timing and location of new MS lesions, as well as the pace and extent of their repair, are extraordinarily variable. The clinical presentation of MS is, therefore, remarkably heterogeneous.

Involvement of structures in the brain is an expected feature of conventional MS. Injury localizing to the cerebellum and its associated neural pathways may give rise to problems with balance, limb coordination, tremor, and gait ataxia. Lesions in the cerebrum can manifest as cognitive impairment, such as deficits in attention, executive function, and memory. In addition, mood disorders such as depression, which affects up to 50% of MS patients, putatively localize to disruptions in cerebral neural networks.

Inflammatory injury to the optic nerve gives rise to symptoms of ON, which include scotoma (typically occupying the central visual field), colour desaturation, and pain accompanying voluntary eye movement. The signs of ON include reduced visual acuity and relative afferent pupillary defect. Optic nerve involvement is common in MS, being the initial symptom in approximately 19% of cases. ON in MS is typically unilateral, although it is relatively common for both eyes to eventually be affected. Severe, recurrent, or simultaneous bilateral ON are more common in NMO.

MS lesions in the brainstem can give rise to a multitude of clinical signs and symptoms, owing to its numerous critical autonomic functions and its role as a conduit for tracts ascending to and descending from the brain. Symptomatic manifestation of brainstem injury associated with MS include visual dysfunction such as diplopia and oscillopsia; difficulties with swallowing; speech problems such as dysarthria; paroxysmal sensory or motor disturbances, including trigeminal neuralgia and painful, tonic spasms in the limbs or face; and emotional lability, such as
pseudobulbar affect (pathological laughing and crying); and vertigo\textsuperscript{2}. Clinical signs of brainstem dysfunction include nystagmus, internuclear ophthalmoplegia, and pupillary defects\textsuperscript{2}.

Demyelination and subsequent atrophy of the spinal cord give rise to the prototypic impairments in mobility that are characteristic of progressive MS. Initial neurological injury to the spinal cord from episodic relapses can result in sensory disturbances known as paresthesias and dyesthesias, which include tingling, numbness, itchiness, burning, and pain\textsuperscript{2}. More pernicious presentations of spinal cord relapse include limb paresis or paralysis, which is rare in MS, but relatively common in NMO\textsuperscript{16}.

Chronic spinal cord dysfunction in MS, which is characteristic of patients in the progressive phase of disease, may undermine a variety of functional systems. Common symptoms in this regard include stiffness or spasticity in the limbs, bladder or bowel problems, and sexual dysfunction\textsuperscript{2}.

Some symptoms of MS are more global in distribution and are therefore not readily attributed to a specific anatomical target in the CNS. Pathological fatigue, which is perhaps the most common symptom of MS, affecting up to 80\% of all patients, is one such symptom\textsuperscript{95}. Although not as readily apparent as other functional deficits in MS, fatigue is regarded as the most debilitating symptom by a large proportion of patients\textsuperscript{96}. Other global symptoms include generalized neuropathic pain and Uhtoff’s phenomenon, which is an exacerbation of MS symptoms triggered by a rise in core body temperature\textsuperscript{2}. Diffuse symptoms such as these may involve multiple CNS sites.
Patients of Asian ancestry exhibit the same repertoire of signs and symptoms, although the respective frequencies may differ, largely on account of the greater relative frequency of clinical phenotypes consistent with NMO. Acute and fulminant ON and TM cause the classical symptoms of NMO and NMOSD, including severe vision loss and limb paralysis. Spinal cord lesions encroaching on the brainstem may also give rise to peculiar symptoms of intractable hiccups or vomiting. Early studies in Japanese patients demonstrated a greater propensity toward ON at onset, bilateral involvement of the optic nerves, and spinal cord symptoms during the course of the disease relative to Caucasian patients. A subsequent neuroimaging study in Japanese patients reported a significantly lower frequency of cerebellar symptoms compared to patients of European ancestry. Similar findings of an enrichment of optic nerve and spinal cord involvement have been reported in studies in other East Asian populations.

### 1.5.2 Clinical course of MS and related disorders

Although the clinical trajectory of MS varies considerably from one patient to the next, clinical activity at any point in time can be characterized as a relapse, progression, or stability. Relapses (also commonly and interchangeably referred to as “exacerbations”, “attacks”, or “flare-ups”) are new episodes of acute or sub-acute worsening of neurological function attributable to CNS inflammatory demyelination. Such attacks must persist for at least 24 hours, occur in the absence of fever or infection, and be followed by variable degrees of recovery to be considered a relapse. Progression refers to a continuous or nearly continuous pattern of worsening neurological function that occurs in the absence of relapses. Periods of stability in MS refer to intervals of clinical quiescence that are free of relapses and are not accompanied by appreciable worsening in neurological function. There are four recognized disease course categories of
MS, each characterized by one or more of the above clinical descriptions: relapsing-remitting, primary-progressive, secondary-progressive, and progressive-relapsing (Figure 1.2).

Relapsing-remitting MS (RRMS) is the most common clinical course in a cross-section of patients. At least 80% of patients have RRMS at the time of diagnosis\(^2\). RRMS is characterized by clear-cut, intermittent relapses separated by variable periods of clinical stability (Figure 1.2A), which can range from months to years\(^{103}\). Recovery from relapses in RRMS is generally complete or nearly complete such that there is minimal or no residual deficit in functioning. Benign MS is a specific subtype of RRMS in which full functionality is retained in all neurological systems after 15 or more years of disease\(^{103}\).

Primary-progressive MS (PPMS), which accounts for only 10% of patients at diagnosis, is characterized by gradual clinical progression from the onset of disease that occurs in the absence of relapses (Figure 1.2B). There are allowances for brief plateaus and temporary minor improvements in clinical disability in the consensus definition of PPMS\(^{103}\). Clinical progression is generally ascribed to a neurodegenerative process, as opposed to the focal inflammation underlying relapses\(^{104}\). By extension, it has been argued by some that relapsing variants of MS and PPMS may represent distinct disease entities\(^{105}\). Lending support to this hypothesis are consistent observations that PPMS is the only clinical subtype for which a conspicuous female preponderance is not observed, and onset of PPMS occurs at a markedly later age than RRMS\(^{106}\).

Secondary-progressive MS (SPMS) is characterized by an initial RRMS course of variable duration (several months to decades) followed by a progressive phase during which relapses diminish in frequency (or disappear completely), but neurological functioning declines steadily, even between any relapses\(^{103}\) (Figure 1.2C). The point in time at which the functional baseline
between exacerbations continuously declines is when the transition from RRMS to SPMS is
demed to have taken place\textsuperscript{103}. The median time interval from onset of symptoms to the start of
the secondary-progressive phase is approximately 15-20 years\textsuperscript{107,108}.

Progressive-relapsing MS (PRMS) is the least common clinical course of MS, typically
accounting for less than 5\% of cases at diagnosis\textsuperscript{4,109}. PRMS is characterized by progressive
course from onset with superimposed, clearly-defined relapses associated with variable
recovery\textsuperscript{103} (Figure 1.2D).
Figure 1.2. Range of clinical course in MS.

(A) Relapsing-remitting MS (RRMS) is characterized by distinct, intermittent relapses interspersed between variable periods of clinical stability. (B) Primary-progressive MS (PPMS) is characterized by gradual clinical progression from onset occurring in the absence of relapses. (C) Secondary-progressive MS (SPMS) is marked by an initial relapsing course followed by a progressive phase during which neurological functioning declines steadily in the absence of relapses. (D) Progressive-relapsing MS (PRMS) is typified by continuous progressive course from onset with interspersed, distinct relapses superimposed.
The most reliable predictor of disability progression is the disease course at onset\textsuperscript{110}. On this basis, some investigators have advocated for a simplified rubric for classifying disease course in MS in which the four recognized disease courses are collapsed to two categories: relapsing-onset and progressive-onset MS. Several recent studies applying this dichotomous classification scheme for disease course have demonstrated its validity insofar as it was reliably predictive of clinical outcomes\textsuperscript{104,111,112}.

There is some debate as to whether the relative frequencies of each clinical course differ between Asian-ethnic patients relative to those of European ancestry\textsuperscript{86}. Several studies in Asia, which collectively reported PPMS at a frequency ranging from 0 to 5.3\%, suggest that primary-progressive course may be less common in Asian-ethnic populations\textsuperscript{62,75,100,113–115}. As before, one should entertain the possibility that these case series may have been “contaminated” to varying degrees with NMO cases, particularly given that progressive course is rare in NMO\textsuperscript{16}.

1.5.3 Natural history of MS and related disorders

The clinical outcomes of MS are extraordinarily variable, such that it is nigh impossible to accurately predict the prognosis of a given patient at disease onset. On one end of the spectrum are patients with so-called benign course, characterized by minimal or non-existent neurological dysfunction 15 or more years after onset\textsuperscript{103}. At the other extreme are those burdened with a malignant course, which is typified by severe disability or death due to MS within five years of disease onset\textsuperscript{103}. It is worth noting that MS is generally not considered a fatal disorder, although life expectancy in persons with MS is reduced by 6-14 years on average\textsuperscript{116,117}. In reality, most patients occupy an intermediate position along the spectrum of clinical outcomes, and consistent with heterogeneity in presentation, there is also considerable variability in disability progression.
It is a useful exercise, nonetheless, to determine a “typical” disease course—that is, one defined by a meaningful average measure of disability progression—in order to guide prognostication and counseling of the newly diagnosed patient. Longitudinal natural history studies in large cohorts have contributed to our understanding of the expected disease course in typical MS patients.

A litany of instruments to grade disability in MS exists, including those that assess global functioning as well as those that assess specific functional domains, such as cognition or walking\textsuperscript{118}. The most enduring and widely used measure of disability in MS is the Expanded Disability Status Scale (EDSS), which is a standardized index of global neurological dysfunction\textsuperscript{119}. The EDSS is an ordinal scale with values increasing in increments of 0.5 from zero (normal neurological examination) to 10 (death due to MS). The EDSS score is derived from component functional system scores assigned through a neurological examination assessing pyramidal, brainstem, cerebellar, sensory, bowel/bladder, visual, and cerebral system function, as well as ambulation\textsuperscript{119}. The EDSS is not without limitations; it is inherently more sensitive to detecting changes in mobility and ambulation, while capturing changes in cognitive and visual function to a lesser degree. However, it remains the most widely used instrument for measuring global disability in MS in both clinical and research settings.

The most commonly selected EDSS milestone in studies of long-term disability progression in MS is EDSS 6.0, which denotes disability to an extent that a walking aid (such as a cane, crutches, or brace) is required to walk 100 metres, with or without rest\textsuperscript{119}. The time from onset of symptoms to EDSS 6.0 disability, most commonly determined from survival analysis using the Kaplan-Meier estimator\textsuperscript{120}, is therefore a meaningful index of disability progression. In
particular, the median time to event, which is understood as the time at which half of the patients have reached the disability endpoint, is a useful outcome measure with which to compare cohorts.

Recently published natural history studies on longitudinal patient cohorts in the US, Canada, and France reported median survival times from onset to EDSS 6.0 ranging from 24 to 28 years\textsuperscript{121–123}. These findings support the belief that disability progression in MS appears to be slowing when compared to earlier studies in a Canadian patient cohort\textsuperscript{124,125} reporting a median time to EDSS 6 of only 15 years. The reasons for the longer times to reach critical disability endpoints are unclear, but it is likely that several factors are involved. For instance, earlier diagnosis, improvements to clinical management of patients, routine use of disease-modifying therapies in newly diagnosed patients, and a temporal shift toward a greater proportion of relapsing-onset cases\textsuperscript{38} may be driving the trend of slowing disability progression.

The long-held and pervasive notion that the clinical course of MS is more aggressive in patients of Asian ancestry compared to Caucasians derives primarily from early studies in Asia that depicted a rapidly progressive disease\textsuperscript{53,126}. In one illustrative study, the time from symptom onset to death was 7.6 years\textsuperscript{55}. More recently, a study in a Taiwanese cohort reported a median time of 4.7 years from onset to reach EDSS 7.0 disability level, which corresponds to dependency on a wheelchair for mobility\textsuperscript{115}. However, several contemporary publications demonstrated overall similarity in the clinical outcomes of MS between these ethnic groups\textsuperscript{31,51,97,98}. The data in this regard are therefore inconsistent.

The apparent enrichment of cases with opticospinal presentation in many of these studies arouses suspicion that NMO cases were likely included in some of these studies\textsuperscript{55,97,98,126,127}. The clinical
course of NMO is unquestionably more aggressive than that of MS. Left untreated, approximately 50% NMO patients experience permanent vision loss in at least one eye or inability to walk without assistance within five years of disease onset. It therefore stands to reason that ethnic differences in the relative proportions of MS and NMO could in part explain the ostensible differences in clinical outcomes between Asian and Caucasian patients. In recent studies in Asian-ethnic patients, for instance, an enrichment of confirmed NMO cases as well as less favourable clinical outcomes overall was observed. Few studies have directly compared Asian and non-Asian patient cohorts ascertained from the same clinic population.

More recent investigations undertaken concurrently with widespread adoption of revised diagnostic criteria for NMO have nevertheless been conflicting. In a retrospective cohort study involving 95 RRMS cases in Hong Kong, 40% of patients reached EDSS 6.0 level disability after a median interval of 6.5 years from onset. In striking contrast, a subsequent study in Hong Kong reported a median survival time of 22 years to reach the same disability endpoint. Similar results showing modest disease progression were reported in a recent retrospective cohort study in South Korea, in which the investigators obviated the misclassification biases of earlier studies by taking into account patients with putative NMO. After rigorously screening cases to exclude NMO and NMOSD, median time from onset to EDSS 6.0 disability among MS cases was 20 years. In contrast to early reports, these studies in East Asian populations demonstrate that disability progression largely conforms to a pace similar to that recently reported in natural history studies in predominantly Caucasian populations. Thus, the evidence to date concerning the pace of disease progression in Asian-ethnic patients has been
inconsistent. On the balance of the conflicting findings, it remains unclear if the prognosis of MS in Asian-ethnic patients is any less favourable than in Caucasians.

1.5.4 Paraclinical features of MS and related disorders

Several laboratory studies are important in the diagnosis and clinical management of MS and related disorders. Most prominent among the paraclinical tools is MRI, enabling a non-invasive means of visualizing macroscopic injury to CNS tissue in vivo. MRI is a critical tool to aid rapid and accurate diagnosis, as well as ongoing monitoring of disease activity in MS\(^{131}\). In addition, cerebrospinal fluid (CSF) studies can support the diagnosis of MS, and serologic testing for an antibody biomarker for NMO can help to differentiate NMO and its spectrum disorders from MS.

1.5.4.1 MRI in MS and related disorders

Since the first application of MRI to image MS lesions some 30 years ago, its role and importance in the diagnosis and monitoring of disease progression in MS has grown substantially. MRI enables more accurate detection of CNS abnormalities relative to a clinical history or neurological exam. The high sensitivity of MRI is critical to clinical management, as 30% and 70% of radiologically apparent spinal cord and brain lesions, respectively, are not accompanied by a clinical relapse\(^{132}\). These “clinically silent” lesions are indicative of ongoing biological activity that fails to break the clinical threshold of detection, but may nonetheless be relevant to disease evolution.

Conventional MRI also enables temporal characterization of MS lesions by monitoring lesion growth over sequential scans or highlighting compromised integrity of the blood-brain barrier. In
the latter, imaging is performed following administration of a contrast agent, gadolinium. Areas of active inflammation are differentiated from existing MS plaques as enhancement, which localizes to CNS regions where gadolinium enters the intrathecal space through gaps in the blood-brain barrier tight junctions caused by inflammation. Serial scanning enables monitoring of disease evolution by tracking changes in the lesion burden and brain volume, which are indices of focal inflammation and atrophy, respectively. The MS lesion burden increases 5-10% annually in a typical patient, while brain volume decreases 0.7-1.0% per year, some 2-3 times more rapidly than in the healthy population. Regions of severe and chronic damage associated with axon degeneration and subsequent atrophy appear as hypo-intense foci ("black holes") on T1-weighted scans.

Brain lesions on MRI are almost always seen in MS. The characteristic radiological appearance of MS is multiple focal white matter lesions in the brain, with the greatest burden in the periventricular regions. In addition, MS lesions are commonly observed in the corpus callosum, temporal lobes, cerebellum, brainstem, and juxtacortical regions (at the interface of white and gray matter). Large lesions with ill-defined boundaries and signal intensity intermediate to that of focal lesions and normal-appearing white matter (termed "dirty-appearing" white matter) are less frequently observed, but indicative of diffuse pathological changes that are distinct from acute, focal lesions.

Focal spinal cord lesions, while less common than brain lesions, are observed in up to 92% of MS cases at some point during the disease. The simultaneous presence of brain and spinal cord lesions frequently observed in MS is seldom seen in any other disease. In MS, spinal cord
lesions are most common in the cervical cord, generally occupy the posterior or lateral cord, and seldom exceed two vertebral segments in length or half of the cross-sectional area of the cord\textsuperscript{139}.

The characteristic MRI appearance of NMO early in the disease course is that of extensive spinal cord and optic nerve abnormalities in combination with a normal brain scan or one that features non-specific lesions that do not fulfill diagnostic criteria for MS\textsuperscript{16,24}. Spinal cord lesions in NMO and NMOSD are usually longitudinally extensive, occupying a length of the cord and spanning more than three contiguous vertebral segments\textsuperscript{16}. The longitudinally extensive spinal cord lesion (LESCL) typical of NMO are centrally located in the cord and occupy the majority of the cord’s cross-sectional area along the affected plane. In addition, swelling and atrophy of the spinal cord are more commonly seen in NMO compared to MS\textsuperscript{24}. The resulting clinical manifestation, longitudinally extensive TM, is a hallmark of NMO and accounts for much of the mobility impairment in the disease.

Lesions that localize to the brainstem are observed on MRI in up to 60\% of NMO patients later in the disease course. Brain lesions are often clinically silent in NMO\textsuperscript{140}. Other brain lesions with a distinctive pattern of involvement localizing to aquaporin-4-rich CNS regions are relatively uncommon, being observed in approximately 10\% of cases, but appear to be specific to NMO. These NMO-characteristic lesions include lesions in the hypothalamus, area postrema, periaqueductal area, and proximal to the lateral ventricles; tumefactive lesions in the corpus callosum, brainstem, and cerebellum; optic nerve lesions extending into the optic chiasm; and linear brainstem lesions extending into the cervical cord\textsuperscript{24,141}. NMO-characteristic lesions have also been reported in Asian-ethnic patients diagnosed with NMO or NMOSD\textsuperscript{142–146}. 

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MRI features in Asian patients with classical MS appear to be similar to those in Caucasian patient populations\textsuperscript{86,147–149}, although the frequency of asymptomatic lesions may be lower in patients of Asian ancestry\textsuperscript{150}. A study in Japanese patients assessing the diagnostic utility of early MRI diagnostic criteria for MS that were originally developed in predominantly Caucasian populations\textsuperscript{151} reported sensitivity and specificity of 86\% and 93\%, respectively\textsuperscript{152}. Subsequent studies applying more recent diagnostic criteria corroborated these findings, but also indicated limited sensitivity of current diagnostic criteria in patients with opticospinal or NMO-like presentation\textsuperscript{153,154}.

In other studies in Japan in which cases were stratified into conventional and opticospinal clinical phenotypes (prior to widespread recognition that the latter diagnosis typically embodies NMO), OSMS cases had a significantly lower lesion burden on brain MRI than conventional MS cases\textsuperscript{155–158}. Moreover, the frequency of optic nerve enhancement, LESCL, cord swelling, and atrophy were also significantly greater in OSMS\textsuperscript{149,155,156}. In general, MRI findings in OSMS cases in Asian populations were consistent with those reported in Caucasian NMO patients\textsuperscript{128,129,159}. One notable study\textsuperscript{99} demonstrating a markedly lower frequency of cerebellar hemispheric lesions in Japanese MS patients (6.4\%) compared to rates typically reported in Caucasian patients (49–88\%)\textsuperscript{160,161} had subsumed OSMS cases with MS.

\subsection{1.5.4.2 CSF studies in MS and related disorders}

Other than MRI, CSF analysis has been the most important paraclinical tool supporting the diagnosis of MS in the last two decades. The presence of CSF-specific oligoclonal IgG banding (OCB) in samples subjected to isoelectric focusing and immunoblotting is robustly associated with MS, being consistently observed at a frequency greater than 90\% in most clinically definite
MS case series\textsuperscript{162–164}. Some 60-70\% of CIS patients also exhibit CSF OCB\textsuperscript{165,166}, suggesting that intrathecal IgG production begins early in the disease process. Although CSF OCB is not normally seen in healthy individuals\textsuperscript{167}, it is observed at varying frequency in other CNS inflammatory disorders, including systemic lupus erythematosus (30-50\%), Behçet disease (20-50\%), neurosyphilis (90-95\%), neurosarcoïdosis (40-70\%), and Sjögren syndrome with neurological involvement (75-90\%)\textsuperscript{132}. CSF OCB status may be helpful in discriminating MS from NMO, as the frequency of OCB in the latter is considerably lower (20\%) and less stable over serial assays\textsuperscript{168}. Limited specificity of the assay underscores the importance of diagnosing MS based on the totality of the available clinical and paraclinical evidence.

The frequency of OCB positivity in MS patients of Asian ancestry is thought to be markedly lower than in Caucasians. Several studies published prior to widespread diagnostic distinction of NMO and MS clinical phenotypes in Asia indicated OCB frequencies between 19 and 63\%\textsuperscript{51,52,74,169–174}. In studies that distinguished conventional MS from OSMS\textsuperscript{115,159,175}, the frequency of OCB in conventional MS (57-77\%) was higher than in OSMS (0-31\%), but notably lower than that typically reported in Caucasian MS patients (>90\%).

\textbf{1.5.4.3 \textit{NMO-IgG in MS and NMO}}

A biomarker for NMO, an autoantibody subsequently termed NMO immunoglobulin G (NMO-IgG), was discovered in 2004 and reportedly discriminated NMO from prototypical MS with high specificity\textsuperscript{19,176}. Prior to its discovery, NMO was widely considered an aggressive variant of MS, owing to the substantial overlap in the clinical signs and symptoms. The definition of NMO was to that point limited in scope, inflexibly requiring a triad of ON, acute myelitis, and absence of brain involvement to warrant a diagnosis\textsuperscript{22}. NMO-IgG was subsequently found to exist across
a broader range of clinical presentation, encompassing diverse syndromes falling outside the boundaries of the classical description, but showing features consistent with NMO or limited presentations thereof. Increasing recognition of heterogeneity in clinical presentations of NMO prompted revisions to the diagnostic criteria\textsuperscript{23} and broader acceptance of NMOSD being subsumed under the umbrella of NMO\textsuperscript{16}.

Subsequent studies determined that the target antigen of NMO-IgG was aquaporin-4, a cell membrane water channel that is highly expressed in CNS tissues on the foot processes of astrocytes\textsuperscript{177}. Aquaporin-4 is especially enriched near endothelial cells of the blood-brain barrier, including those in the region of the spinal cord, optic nerves, hypothalamus and lateral ventricles, thereby accounting for the distinct CNS topography of NMO lesions\textsuperscript{140}. NMO-IgG deposition in functionally relevant CNS regions initiates a pathogenic sequence of complement and macrophage activation leading to astrocytopathy, which gives rise to the typically acute and severe symptoms of NMO\textsuperscript{178}. Thus, unlike in MS where demyelination is a consequence of targeted destruction by autoreactive T-cells recognizing myelin antigens, demyelination in NMO is likely a secondary process triggered by antibody-mediated damage to non-myelin structures, namely astrocytes\textsuperscript{178}.

NMO-IgG seropositivity is associated with a range of clinical syndromes beyond prototypical NMO\textsuperscript{23}. As detailed in Section 1.2.3, NMOSD includes limited forms of the disease, as well as ON or TM concurrent with an autoimmune disorder or NMO-typical brain lesion\textsuperscript{16}. Patients with a clinical presentation satisfying one of these definitions of NMOSD are likely to eventually fulfill diagnostic criteria for NMO, given adequate follow-up\textsuperscript{90}.
The high specificity of NMO-IgG for NMO and its spectrum disorders, and by extension, clinical utility of the autoantibody test in distinguishing NMO from conventional MS, are the primary basis for its inclusion in the most recent revisions to the diagnostic criteria for NMO\textsuperscript{23}. A recent review\textsuperscript{179} of 59 studies encompassing over 15,000 assay results comparing NMO and NMOSD patients to control subjects (drawn from the healthy population, MS patients, or other neurological disease cohorts) reported a median specificity of 98.2\% across all studies. In the comparison of NMO versus MS, median specificity was 99.1\%\textsuperscript{179}. However, notable ethnic differences in assay accuracy were also evident. Among a subset of 27 studies in Asian-ethnic populations, median specificity in comparisons of MS versus NMO was 95.4\%. However, 12 of these studies reported specificities ≤90\%, including one in which specificity was a meagre 62.5\%\textsuperscript{46}. It has been suggested that the lower reported specificity of NMO-IgG in Asian populations may, in part, be due to historical classification of presumptive NMO or NMOSD cases as OSMS or misclassification as other variants of MS\textsuperscript{179}. It is now known that approximately 60\% of Asians diagnosed with OSMS are seropositive for the autoantibody\textsuperscript{19,128}.

Diagnostic sensitivity of the NMO-IgG assay is modest by comparison. A review of assay results in a combined sample of 2,384 patients with a clinical diagnosis of NMO revealed a median assay sensitivity of 64\%\textsuperscript{179}. In general, higher sensitivity was noted for relapsing NMO (74-81\%) compared to monophasic NMO (0-53\%)\textsuperscript{180,181}, suggesting the possibility of pathogenic distinction between the variants. Among 29 studies in Asian-ethnic patients, median diagnostic sensitivity was similar to that in the overall NMO patient population (61.9\%)\textsuperscript{179}, reaffirming the assay’s limitation in reliably detecting NMO.
1.5.5 Diagnosis of MS and related disorders

The clinical work-up leading to a diagnosis of MS integrates multiple lines of evidence from the patient’s clinical history, physical examination, and paraclinical studies that now invariably include MRI\textsuperscript{132}. MS is classically considered a diagnosis of exclusion, owing to an extensive list of other diagnoses that may mimic MS. For example, many autoimmune disorders (e.g., systemic lupus erythematosus, Sjögren syndrome), infectious myelopathies (e.g., Lyme disease, neurosyphilis, HTLV-1 associated myelopathy), metabolic conditions (e.g., vitamin B12 deficiency, folate deficiency), and heritable diseases (Leber’s hereditary optic neuropathy, mitochondrial encephalomyopathy, spinocerebellar ataxia), may feature signs and symptoms that overlap with those of MS to varying degrees\textsuperscript{182,183}. The requirement that there be no better explanation for the patient’s signs and symptoms has, in fact, been a tenet of MS diagnosis that has remained unchanged from the earliest established diagnostic criteria. Demonstrating separation in time and space of CNS demyelination is a guiding principle of MS diagnosis that has also remained unchanged over the years. In the several decades following establishment of the first consensus set of criteria for the diagnosis of MS\textsuperscript{102}, several successive iterations of MS diagnostic criteria, each incorporating insights gleaned from clinical practice and the literature, have resulted in earlier and more accurate diagnosis.

1.5.5.1 Diagnosis of MS

The first widely accepted diagnostic criteria for MS\textsuperscript{102} confirmed the diagnosis based on clinical features alone. A diagnosis of MS required clinical evidence of inflammatory demyelination affecting at least two CNS locations during two or more episodes of exacerbation lasting at least 24 hours and separated by at least 30 days (or in the case of progressive MS, gradual progression
of signs and symptoms over a period of at least six months). The Poser Criteria, published some two decades later, retained the requirement for at least two clinical attacks separated in time and space, but augmented the strictly clinical diagnosis of “definite MS” with “probable MS” diagnosis based on a single clinical attack supported by paraclinical evidence from CSF or evoked potential laboratory studies. Consequently, the dissemination of MS lesions in time and space could thereafter be demonstrated with laboratory (in addition to clinical) evidence.

Since publication of the Poser Criteria, widespread adoption of MRI in clinical practice prompted the development of new criteria integrating this emerging neuroimaging tool. The 2001 McDonald Criteria formalized the use of MRI observations (along with previous laboratory studies) to supplement clinical evidence as a means of fulfilling criteria for dissemination in time and space in patients with a single clinical episode suggestive of MS (i.e., CIS). Subsequent prospective studies confirmed the superior sensitivity of the McDonald Criteria vis-à-vis the Poser Criteria in diagnosing MS at 12-months following the initial clinical attack, thereby enabling earlier diagnosis of patients with CIS. Revisions to the McDonald Criteria in 2005 further enhanced sensitivity while preserving specificity by reducing the minimum interval required between baseline and follow-up MRI scans to fulfill dissemination-in-time criteria, and expanding the role of spinal cord lesions to fulfill dissemination-in-space criteria. The most recent revisions to the McDonald Criteria in 2010 again improved sensitivity by further simplifying requirements for demonstrating dissemination in time and space by MRI. These latest revisions enable immediate diagnosis of definite MS in CIS patients with supportive paraclinical investigations, which may be as early as the time of baseline MRI scan in approximately 45% of patients.
1.5.5.2 Diagnosis of NMO

Clinical guidelines for the diagnosis of NMO have also undergone substantial changes in the last few decades. The classical description of NMO was that of a monophasic illness characterized by acute, bilateral ON and TM occurring simultaneously or in close succession (not more than several weeks apart)\textsuperscript{16,86}. This restricted definition of NMO remained unchanged for many decades, and due to a lack of consensus regarding the definition of NMO, numerous studies included patients with relapsing disease or only partially fulfilling the classical clinical profile\textsuperscript{191–193}. In East and Southeast Asia, however, the monophasic definition of NMO was generally favoured until recently. Therefore, relapsing syndromes consistent with NMO were historically classified as a variant of MS, namely OSMS\textsuperscript{86}.

In 1999, new diagnostic criteria unifying monophasic and relapsing variants of NMO were proposed\textsuperscript{22}. In addition to satisfying at least one major or two minor supportive criteria based on clinical or paraclinical evidence, diagnosis of NMO required fulfilment of three absolute criteria: ON, acute myelitis, and no evidence of clinical activity in CNS locations outside of the optic nerves or spinal cord. Although these criteria were more inclusive than previous definitions of NMO, they precluded limited forms of NMO and NMO with brain involvement, the latter of which is now recognized as a common feature of NMO. Further revisions to the diagnostic criteria in 2006 addressed some of these shortcomings (for example, clinical involvement in the brain was removed as an exclusionary criterion), simplified the diagnostic algorithm, and emphasized the utility of NMO-IgG serological status\textsuperscript{23}. Recent advances in understanding the clinical diversity of NMO and related disorders have broadened the nosological scope of NMO to include NMOSD\textsuperscript{16,24}. 

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1.5.5.3  Diagnosis of MS and related disorders in Asian patients

In Asian-ethnic patients with IIDDs, the diagnosis of MS is confounded by a much higher relative frequency of NMO compared to Caucasian populations in which conventional MS dominates (discussed in Section 1.4.3). The dichotomy of clinical phenotypes in Asian patients—that is, conventional MS versus NMO—as well as the considerable degree of overlap between clinical features thereof, has resulted in lingering uncertainty with respect to the nosology of clinical variants consistent with NMO, such as OSMS\textsuperscript{21,194}. It has been recognized that most OSMS patients would be classified as NMO or NMOSD by current diagnostic criteria, and only a small proportion would be diagnosed with conventional MS\textsuperscript{195}. On the other hand, some observers have proposed modifications to MS diagnostic criteria for application in Asian populations that would result in some patients with NMO-like paraclinical features being classified as MS\textsuperscript{196}.

These concerns were addressed by the International Panel on Diagnosis of MS in the most recent revisions to the McDonald Criteria\textsuperscript{189}. The consensus of the panel was that in Asian patients with equivocal clinical presentation, application of the 2006 NMO diagnostic criteria in tandem with the 2010 McDonald Criteria should be sufficient to accurately distinguish MS from definite NMO, insofar as the respective criteria concerning brain MRI findings are mutually exclusive in the vast majority of cases. For suspected cases of NMOSD not otherwise fulfilling criteria for “definite NMO”, NMO-IgG testing was recommended by the panel. In such cases, a seronegative result would not necessarily rule out NMO or NMOSD, particularly in light of the limited sensitivity of the assay, but a seropositive result would effectively “rule in” a diagnosis of NMO or NMOSD on account of the high specificity of NMO-IgG\textsuperscript{189,197}. The panel concluded
that, once NMO and NMOSD have been systematically excluded, the McDonald Criteria should be adequate to accurately diagnose MS in Asian populations\textsuperscript{189}.

1.5.6 Clinical management of MS and related disorders

The clinical management of MS can be broadly simplified into four domains: treatment of acute relapses, prevention of future disease activity, treatment of symptoms, and recovery of function. The diverse symptomatology and unpredictable course of MS dictate a care plan that is guided by weighing the circumstances specific to a particular individual in combination with empirical evidence. The overarching goal is to rapidly suppress active disease, prevent future relapses, and enhance quality of life by alleviating or masking symptoms and mitigating disability.

1.5.6.1 Acute relapse therapies

Approximately 85\% of MS patients experience a disease course with interspersed acute relapses associated with temporary functional impairment and potential residual deficits. Prompt treatment of moderate to severe relapses is therefore strongly advocated in the interest of minimizing disability and improving overall quality of life. Clinical practice guidelines with respect to treatment of acute MS relapses are generally consistent across populations, as there is no convincing evidence to date of population-specific response to relapse therapies.

The consensus first-line therapy for MS relapses is systemic corticosteroids, which work by stifling inflammation\textsuperscript{198}. Standard treatment is high-dose (20-30 mg/kg per day) intravenous methylprednisolone for 3-5 days, whereas a less commonly used alternative is high-dose oral prednisone (1000-1250 mg/day for three days)\textsuperscript{199}. In patients with incomplete resolution of symptoms following this course, oral prednisone taper is considered on a case-by-case basis\textsuperscript{200}.  

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Short-term corticosteroid treatment of MS relapses is generally considered to be very safe. More commonly reported adverse events include edema, gastrointestinal irritation, weight gain, acne, insomnia, and increased susceptibility to infection\textsuperscript{198}. Less frequent adverse events include hyperglycemia, hypertension, Cushingoid facies, and aseptic necrosis of a major joint such as the hip\textsuperscript{198}.

Second-line relapse therapies may be considered for patients with an unsatisfactory response to high-dose steroids. Plasmapheresis is occasionally used, although the evidence basis for it is somewhat limited\textsuperscript{201–203}. The standard course of plasmapheresis consists of 5-10 exchanges performed on alternating days\textsuperscript{204}. Immunosuppressants (discussed below) may also be considered as an adjunct or alternative to plasmapheresis in patients with recalcitrant or exceptionally disabling relapses\textsuperscript{198}.

\subsection*{1.5.6.2 Disease-modifying therapies}

The first MS therapy proven in randomized, placebo-controlled clinical trials to reduce the frequency of relapses was interferon beta (IFNβ)-1b in 1993\textsuperscript{205}. Prior to its approval by the US Food and Drug Administration in 1993 and by Health Canada in 1995, there were no effective therapies available to alter the natural history of MS. There are now 10 disease-modifying therapies (DMT) approved by Health Canada for use in patients with relapsing MS. All of the approved therapies have been shown to reduce the frequency and severity of relapses, which are thought to contribute to the accrual of disability in MS\textsuperscript{206–208}. In addition, several clinical trials have shown the first-line DMTs to be effective in delaying progression of CIS to clinically definite MS\textsuperscript{209–212}. Therefore, early initiation of therapy is strongly advocated, a position that is
further supported by studies demonstrating an association between early relapses and poor long-term prognosis\textsuperscript{213,214}.

The most commonly prescribed first-line therapies in MS are IFNβ-1a, IFNβ-1b, and glatiramer acetate, which are administered subcutaneously or intramuscularly. All three first-line DMTs are equally efficacious\textsuperscript{215,216}, reducing relapse rates by approximately 30\% in RRMS, and reducing risk of a second clinical attack in CIS by 45\% through a 3-year follow-up period\textsuperscript{209,212}. As all three of the injectable first-line DMTs have comparably favourable safety profiles and efficacy, preference is often dictated by non-clinical factors such as frequency of administration\textsuperscript{200}.

Second-line therapies are more efficacious than first-line DMTs, but generally have higher risk of serious adverse effects. In addition to patients who are unable to tolerate first-line DMT injections, those with aggressive disease (e.g., multiple severe relapses with poor recovery within a two-year interval) from the outset or breakthrough disease while on a first-line therapy may be suitable candidates for second-line therapies. Treatment non-response or suboptimal response may be due to several factors, including disease stage, ethnicity\textsuperscript{217,218}, and the development of neutralizing antibodies to the drug\textsuperscript{219,220}. The threshold for therapy escalation is assessed on an individual basis and may depend on several factors determined from clinical and imaging studies. Observations that would weigh in favour of initiating second-line therapy include frequent or severe relapses\textsuperscript{213,214,221}; poor recovery from relapses\textsuperscript{222}; continued accumulation of disability, especially when caused by changes in multiple functional systems\textsuperscript{200}; and evidence of new T2 or gadolinium-enhancing lesions on follow-up MRI\textsuperscript{223–226}.

Commonly prescribed second-line therapies for MS in Canada are natalizumab, which is administered by intravenous infusion, and the oral medication, fingolimod\textsuperscript{200}. Potent
immunosuppressants, such as cyclophosphamide and mitoxantrone, also qualify as second-line treatments, and may be used as induction or maintenance therapies in patients with particularly aggressive disease.

Second-line therapies are associated with greater overall risk due to rare, but serious adverse events. There is a 0.2% empirical risk of progressive multifocal leukoencephalopathy\textsuperscript{227}, a potentially fatal brain infection caused by an opportunistic viral infection, in patients receiving natalizumab treatment. Mitoxantrone therapy is associated with cumulative cardiotoxicity, limiting its long-term use in MS patients, as well as an approximately 0.2% risk of acute leukemia\textsuperscript{228}. Additionally, many of the second-line therapies are associated with an increased risk of infections, owing to their immunosuppressive effects. In light of the additional safety concerns, second-line therapies are typically indicated in patients with otherwise severe or intractable disease. Emerging therapies approved by Health Canada within the last year (\textit{i.e.}, teriflunomide, alemtuzumab, dimethyl fumarate)\textsuperscript{229} will continue to the shape the rapidly evolving landscape of MS therapies.

The greater potential for severe and permanent disability arising from NMO relapses generally warrants a more vigilant and aggressive therapeutic approach. Similar to standard practice for MS relapses, acute NMO relapses are initially treated with intravenous corticosteroids\textsuperscript{24}. In NMO, however, induction is typically followed by a longer maintenance phase of oral prednisone for one month, then a 6-12 month taper\textsuperscript{24}. Plasmapheresis is also indicated for steroid-non-responsive exacerbations of NMO\textsuperscript{24}. It is now generally acknowledged that conventional MS DMTs, including the interferons\textsuperscript{230–234} and fingolimod\textsuperscript{235}, are not only inefficacious for NMO, but may actually trigger acute exacerbations and are therefore
contraindicated. Long-term stability in NMO is typically achieved with ongoing immunosuppression through low-dose steroids or one of a number of potent immunosuppressants, including azathioprine and mycophenolate mofetil. While these therapies have been mainstays in preventing relapses in NMO, rituximab, a B-cell-depleting monoclonal antibody against the cell surface marker CD20, is increasingly prescribed, although cost remains an impediment to its widespread use.

In Asian-ethnic patients with MS and related disorders, the greater relative proportion of NMO compared to Caucasians necessitates a greater degree of vigilance during diagnosis and therapeutic management, given the distinct treatment approaches for MS and NMO. Recent studies from Japan and Thailand have also corroborated earlier findings in Caucasian populations that IFNβ therapy may trigger severe relapses in patients with NMO. However, a subsequent study demonstrated efficacy of IFNβ-1b therapy in patients with “genuine” OSMS—that is, patients with optico-spinal clinical presentation but with MS-characteristic paraclinical features. In general, use of first- and second-line DMTs to treat conventional MS in Asian-ethnic patients is supported by evidence from clinical studies in these populations. IFNβ and fingolimod were recently shown to be efficacious for RRMS in Japanese and Thai patients. For disease-modifying treatment of NMO and NMOSD, efficacy of rituximab in Chinese patients was shown in two small cases series.

1.6 Environmental factors associated with MS and related disorders

Incomplete concordance in monozygotic twins and geographic clines in prevalence leave little doubt that environmental factors play an important role in modifying susceptibility to MS and related disorders. Migration studies have shown that the long-known uneven global
distribution of MS\textsuperscript{38,39} is not entirely accounted for by the geographic distribution of genetic factors alone\textsuperscript{250}. Within the last few decades, significant strides have been made in understanding the nature and impact of environmental risk factors for MS, but many aspects remain poorly understood. Seminal population-based studies of adopted and half-siblings of MS probands\textsuperscript{251,252} have all but ruled out the familial microenvironment in the search for environmental risk factors, and have drawn attention to environmental factors at the level of the population or geographic region. Many candidates have subsequently been proposed and studied, but few have stood up to scrutiny. Moreover, the period during which environmental factors are operative in modifying risk remains unclear, with evidence to date supporting a broad at-risk interval spanning from the prenatal (or even ancestral) period to adulthood\textsuperscript{253}.

\textbf{1.6.1 Geography and migration in MS and related disorders}

The most enduring environmental factor associated with MS risk is geographic latitude, first noted early in the 20\textsuperscript{th} century and reliably observed in numerous populations since\textsuperscript{254}. Myriad studies demonstrating north-south as well as east-west\textsuperscript{255} gradients in the prevalence of MS suggest spatial clines in the distribution of environmental risk. Established geographic patterns of MS frequency undoubtedly reflect the influence of both genetic and environmental factors (as well as interactions thereof), as anthropological patterns of migration and assortative mating have strong genetic underpinnings\textsuperscript{255,256}. Nevertheless, studies in ethnically homogeneous populations have been particularly informative in parsing the effect of geography from genetic influences. A study in French farmers, a relatively ethnically uniform and non-migratory population, revealed striking spatial patterns of MS prevalence that were best explained by geographic differences in solar UV penetration\textsuperscript{37}. Similar findings of a marked latitudinal
gradient have been reported in other regional studies in North America\(^{33,257,258}\), Europe\(^{259–263}\), Asia\(^{57}\), South America\(^{264,265}\), and Australasia\(^{35,266,267}\), suggesting that the latitudinal effect operates at a global level and extends to both hemispheres. However, some reports (mostly studies with smaller geographic scope) failed to corroborate the latitudinal effect in MS\(^{268–273}\).

Studies in migrant populations have contributed tremendously to current understanding of the role and timing of the geographic environment on MS risk. Migration studies draw insight from a natural experiment of sorts, whereby the environment to which an at-risk population is exposed is altered while its genetic composition remains unchanged\(^{274}\). In general, migration tends to conform to one of two patterns of movement based on the directionality of risk alteration: migration from a region of higher MS risk to one of lower risk; and migration from a low-risk region to one of higher risk, a pattern that is applicable, for example, to Asian-ethnic individuals migrating from their country of origin to Canada.

The majority of migration studies in MS describe populations moving from a region of high prevalence to one of low prevalence. Studies to this effect include populations migrating from the UK or other European countries to South Africa\(^{275,276}\), Europe to Israel\(^{277–279}\); UK to Australia or New Zealand\(^{34,280–284}\); and France to the West Indies\(^{285,286}\). Studies of internal migration to regions of lower risk within the same country include migrant populations within Norway\(^{287}\), as well as those moving from the northern states to the southern states in the contiguous US\(^{288–291}\) or Hawaii\(^{29}\). Despite great heterogeneity in the populations and time periods encompassed by these studies, their findings have been remarkably consistent. The overarching conclusion is that populations migrating from regions of high prevalence to low prevalence
exhibit a reduction in overall MS risk to a level that is typically intermediate to that of the respective regions.

Studies involving migration proceeding in the opposite direction, from a region of low risk to one of higher risk, have also contributed significantly to the body of knowledge on MS risk acquisition. Insights have been gleaned by observing MS risk in populations migrating from India, Africa, and the Caribbean to the UK\textsuperscript{282,292,293}; Asia and Africa to Israel\textsuperscript{277,279,294}; Vietnam to France\textsuperscript{295}; and East Asia to the US\textsuperscript{289}. Internal migration from low-prevalence to high-prevalence regions has also been studied with respect to MS risk in Norwegian\textsuperscript{287} and US\textsuperscript{289–291} populations.

The findings of these studies are somewhat less consistent compared to studies in which the migration proceeds from high-risk region to low-risk region. Whereas some studies report retention of low risk in migrants relocating to high-prevalence regions\textsuperscript{277,279,289,292–294}, others suggest that there is an appreciable acquisition of risk, as indicated by subsequent prevalence rates intermediate to that of the regions of origin and destination\textsuperscript{287,291,296,297}. Some studies have demonstrated a marked increase in risk among migrants relocating from a low-prevalence region to one of higher prevalence, including Vietnamese\textsuperscript{295} and northern African\textsuperscript{298} immigrants to France, as well as Iranian immigrants to Canada\textsuperscript{299}. The prevalence of MS in these immigrant populations, in fact, exceeded that in the general population of their adoptive, high-prevalence countries, suggestive of a profound increase in overall risk. The inconsistent conclusions of studies examining migration in the direction of increasing risk may be attributable to a number of factors. For instance, regional differences in the recognition and diagnosis of MS in atypical ethnic populations may give rise to spuriously low estimates of prevalence, particularly in
regions with limited access to MS care. Moreover, because the majority of these studies focus on
ethnic groups with intrinsically low risk of MS, the corresponding prevalence estimates are
generally characterized by wide confidence intervals. Consequently, meaningful interpretation of
apparent differences in these dubious prevalence rates becomes challenging.

Studies of MS risk in migrant populations of Asian ancestry are scarce. Research in the 1970s in
Americans of Japanese and Chinese ancestry in California and Washington appeared to confirm
retention of low MS prevalence and mortality in these populations, some 4-12 times less frequent
than in resident Caucasians. In contrast to these findings, however, studies in
populations of East Asian and mixed Vietnamese/French ancestry conducted in Hawaii and
France, respectively, reported strikingly high MS prevalence rates that were in fact similar to
or greater than rates in their respective general population. Offering still another perspective, a
small Canadian study reported MS prevalence in the Asian-ethnic population that was
intermediate to that in the region of origin and in the general Canadian population. Notably,
there have not been any recent studies revisiting the theme of MS risk in Asian-ethnic immigrant
countries.

While migration studies shed light on the impact of wholesale changes in environment on overall
MS risk, it is important to pay heed to their limitations. Foremost, it should be recognized that
migrants are seldom representative of those remaining in their region of origin. Migrants tend to
be younger, healthier, better educated, and wealthier than their non-migrant compatriots. The
highly selective nature of migration therefore obscures the contribution of environmental factors
to changes in risk, particularly because assumptions of genetic uniformity between migrant and
non-migrant populations may be invalid, even in ethnically matched populations. Disparities
between migrant and non-migrant ethnic populations in terms of access to health services, care-seeking behaviours, or reticence in disclosing a diagnosis may also lead to ascertainment biases resulting in inaccurate estimates of morbidity. Similarly, estimates of the denominator in prevalence calculations involving migrant populations are often problematic, owing to frequent unavailability of population data on immigrant populations in the host country.

1.6.2 Smoking in MS and related disorders

Although tobacco smoking was first investigated as a candidate risk factor for MS nearly 50 years ago, it remained largely unstudied until recently. A meta-analysis of 10 studies that evaluated the risk of MS due to smoking behaviour prior to disease onset found that a positive history of smoking was associated with a 48% increase in risk to develop MS. In further support of a causative role of smoking in the etiology of MS, several studies have demonstrated a dose-response relationship, whereby the number of cigarettes smoked was positively correlated with increasing risk to develop MS.

In addition to influencing susceptibility, smoking may also modify clinical outcomes of MS. Smoking is associated with more rapid accumulation of disability, as well as an increased risk of progressing from CIS to clinically definite MS. Independent studies have also demonstrated a significant increase in risk of progressing to SPMS in RRMS patients who smoked, although one study reported contradictory findings. There are no published studies on the impact of smoking on MS risk or clinical progression in Asian populations.
1.6.3 Other environmental risk factors for MS and related disorders

The ongoing search for environmental risk factors for MS has generated surprisingly few convincing candidates apart from smoking and geographic region. These include sunlight, vitamin D, and Epstein-Barr virus\textsuperscript{253}. Increased maternal exposure to sunlight during gestation\textsuperscript{316}, as well as sun exposure during childhood\textsuperscript{317,318}, adolescence\textsuperscript{319}, and adulthood\textsuperscript{320,321} are all robustly linked to a reduction in MS risk, although interestingly, earlier studies failed to show such an effect\textsuperscript{303,322}. Furthermore, because none of these studies adjusted for sun exposure during different age periods, it is unclear if these are independent associations. A protective effect of vitamin D sufficiency, the presumptive mediator of the sun-exposure effect, is supported by studies demonstrating an inverse relationship between serum 25-hydroxyvitamin D (a precursor metabolite of the biologically active form of vitamin D) levels and MS risk\textsuperscript{323,324}, as well as a study showing vitamin D-regulated expression of the principal risk allele for MS, HLA-DRB1*1501\textsuperscript{325}.

Epstein-Barr virus (EBV) is the only infectious agent for which there is consistent evidence for an association with MS risk\textsuperscript{253}. Nearly all MS patients (99-100\%) are seropositive for antibodies against EBV compared to 84-95\% of healthy controls\textsuperscript{326–330}, suggesting that EBV infection may be a necessary, but not sufficient, antecedent to the development of MS. Other candidate risk factors (\textit{e.g.}, sex hormones, traumatic stress, diet and obesity) have also been investigated, although none of these has consistently yielded evidence of association with MS risk\textsuperscript{253}.
1.6.4 Critical period of environmental risk for MS and related disorders

There is considerable debate as to the specific period during which one is vulnerable to the effects of environmental risk factors for MS. Based on the typical age at which symptoms appear, the risk to develop MS peaks in the third or fourth decade of life and then declines considerably thereafter\textsuperscript{331}. However, substantial variability in the timing of onset, exemplified by a broad range that spans from infancy\textsuperscript{332} to late adulthood\textsuperscript{333}, underscores the challenge in accurately demarcating the lower and upper limits of the at-risk period. Nevertheless, epidemiological research on putative MS risk factors has offered some clues as to the possible critical time points of exposure.

Several lines of evidence point toward the prenatal environment being relevant to MS risk later in life. An excess of MS kinships linked through the maternal lineage in studies of parent-child proband pairs\textsuperscript{334}, multiplex families\textsuperscript{252,335,336}, and mixed-ancestry offspring\textsuperscript{337} is suggestive of a maternal effect. Observation of a month-of-birth effect, whereby MS cases are more likely than controls to have been born during the spring season\textsuperscript{338}, also lends further support to the hypothesis of \textit{in utero} risk acquisition. Some authors have suggested that inherited epigenetic marks, possibly acquired through environmental exposures in previous generations, may influence MS risk\textsuperscript{339}. If such a mechanism were true, the window of environment-mediated risk would necessarily extend to the ancestral period.

The bulk of the evidence implicating childhood and adolescence as the critical period of risk derives from migration studies. The early, influential migration studies in MS demonstrated that migrants who moved before age 15 acquired the MS risk profile of the destination region, but those that migrated thereafter retained the MS risk of their region of origin\textsuperscript{288,340}. These findings
seemed to demarcate age 15 as the point after which the environment did not appreciably modify MS risk. Subsequent studies\textsuperscript{283,296,341}, however, challenged these findings, proposing that the period of vulnerability likely extends into adulthood. On the balance of the evidence to date, it seems rather improbable that the period of labile risk is demarcated sharply at any age; rather, a continuum of susceptibility to environmental risk factors that tapers off during adulthood, is perhaps more plausible.

1.7 Genetic factors associated with MS and related disorders

Marked population differences in prevalence and familial aggregation of MS\textsuperscript{256} prompted many to suspect the involvement of genetic factors in susceptibility to MS long before formal molecular genetic investigations were initiated. Subsequent studies have enlightened our understanding of the complex genetics of MS, and continue to do so at an astounding pace. Despite these remarkable advances, considerably less is known about the influence of genetic factors on MS risk in Asian populations, in large measure due to a relative dearth of studies in these populations. Renewed interest in clarifying the genetic basis of differential MS risk between populations and emerging high-throughput genetic analytic tools offer great promise for continued progress in this field.

1.7.1 Familial aggregation of MS and related disorders

The existence of families with more than one person with MS (multiplex families) in MS has been recognized for nearly a century\textsuperscript{342–344}, but it is only within the last few decades that population-based studies have correlated MS risk in family members with the degree of genetic sharing and ruled out the shared family environment as the major cause of familial clustering\textsuperscript{251}.
Influential work in the Canadian population revealed that the prevalence of MS in adopted siblings of MS cases was more similar to that in the general population than in biological siblings. Studies of recurrence risk in monozygotic twins, first cousins, half-siblings, and full siblings also support the hypothesized link between familial recurrence risk and degree of kinship. In addition, some studies have also revealed a “maternal effect”, whereby genetic susceptibility to MS was more likely to be acquired from the maternal lineage. It is now known that at least 15-20% of MS cases of European ancestry report having a biological relative with MS.

To date, there have been few population-based studies systematically investigating familial recurrence of NMO, despite the fact that familial NMO was reported in the literature as early as 1938. Several studies with selected or consecutive cohorts have captured familial NMO cases, including multigenerational pedigrees, suggesting that familial cases may account for a small but appreciable proportion of NMO cases. A population-based study in Denmark involving 41 NMO patients reported one parent-child pair concordant for NMO, as well as five NMO cases with either a first- or second-degree relative with MS, but not NMO. By one estimation, familial aggregation in NMO occurs at a frequency of 2.8% based on the number of such cases in a combined series of 386 patients ascertained at centres in the US, UK, South Korea, and Brazil.

In contrast to Caucasian populations, multiplex MS pedigrees are exceedingly rare in Asian-ethnic populations. In a national survey involving 1,084 MS cases in Japan, the rate of familial recurrence was <1%. A Chinese study from the same period reported a single case of familial MS among a series of 70 patients. In other case series ranging from 30 to 249 cases from...
clinic-based studies in Malaysia, Taiwan, Thailand, and China, there were no cases reporting positive family history of MS\textsuperscript{54,55,100,114}. However, a recent study in Malaysia reported familial MS in five of 104 cases (5%), although it warrants noting that this patient cohort included ethnic Indians, a population with a greater genetic risk for MS compared to East and Southeast Asians\textsuperscript{355}. With respect to NMO, there have been few case reports of NMO in first-degree relatives of probands in Japan\textsuperscript{356,357}.

1.7.2 Genetic susceptibility factors for MS and related disorders

Early epidemiological observations in multiplex families implied that familial factors, later established to be genetic\textsuperscript{249,251}, were involved in the pathogenesis of MS. The earliest studies to associate a genetic locus with MS nominated human leukocyte antigen (HLA) genes encoding the major histocompatibility complex (MHC) class I antigens on chromosome 6p21.3\textsuperscript{358,359}. Subsequent fine-mapping studies refined this association to the MHC class II gene, HLA-DRB\textsubscript{1}\textsuperscript{360,361}, and specifically, the HLA-DRB\textsubscript{1}*1501 allele on the HLA-DRB\textsubscript{5}*0101—HLA-DRB\textsubscript{1}*1501—HLA-DQA\textsubscript{1}*0102—HLA-DQB\textsubscript{1}*0602 extended haplotype\textsuperscript{362}. The complexities of the relationship between HLA haplotypes and MS susceptibility are now being increasingly understood, with evidence for epistasis, epigenetic effects, and gene-environment interactions recently emerging\textsuperscript{363}.

In contrast, remarkably little progress was made in identifying non-HLA risk alleles until recently. This reality was underscored by several large, family-based, genome-wide linkage studies\textsuperscript{364–368} that unequivocally confirmed the previous HLA association, but were unsuccessful in reliably identifying novel non-HLA risk loci. The complex pattern of inheritance in MS nonetheless suggested that several genes were likely to be involved in its pathogenesis.
This suspicion was confirmed with the emergence of genotyping technologies based on high-density single-nucleotide polymorphisms (SNP), which ushered in a new era of discovery in the genetics of MS.

The first wave of genome-wide association studies in MS leveraged these technical advances to identify over 20 novel, non-HLA susceptibility variants associated with genes primarily related to immune function\textsuperscript{369–374}. In 2011, the International MS Genetics Consortium published the results of a multi-centre, collaborative genetic association study involving 9,772 MS cases and 17,376 controls of European ancestry\textsuperscript{375}. An additional 29 novel susceptibility variants with small-to-modest effects were identified in pooled analysis, mostly nominating genes related to T-cell differentiation\textsuperscript{375}. Importantly, the individual risks associated with all non-HLA risk variants identified to date are small in magnitude (odds ratio: 1.03-1.34) compared to the principal risk variant, \textit{HLA-DRB1*1501} (odds ratio: 3-6)\textsuperscript{363,376}. It has been estimated that non-HLA susceptibility variants collectively account for 20\% of the sibling recurrence risk in MS in Caucasian populations\textsuperscript{376}.

It can be argued that a substantial fraction of genetic susceptibility to MS remains largely unexplained, given the proportion of heritability for which the known risk variants fail to account. A number of hypotheses have been put forward to explain this gap in understanding, including epigenetic mechanisms, as well as gene-gene and gene-environment interactions\textsuperscript{377}. In addition, common variants with very small effects (OR<1.03), which require very large sample sizes to reliably detect, may explain some of this latent heritability. However, experiences in other common, complex disorders suggest that there is an upper limit to the heritability attributable to common variants\textsuperscript{378}. 

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On this basis, the role of low-frequency (minor allele frequency <5%) genetic variants in the pathogenesis of MS warrants further investigation. Recent studies, enabled by the emergence of next-generation sequencing technologies and concurrent expansion of publicly available reference sequence datasets, have begun to address this possibility. Studies in Canadian families with large, multi-generational pedigrees identified rare variants in the coding sequences of the TYK2 and CYP27B1 genes, conferring modest and large (OR=4.7) risk for MS, respectively. Although there are currently no published genetic association studies in MS using whole genome sequence data, studies applying sequence-based analysis enabled by next-generation sequencing hold reasonable promise of identifying additional rare variants associated with MS risk.

Although the bulk of research on genetic susceptibility to MS has been conducted in predominantly Caucasian populations, the first study investigating genetic MS risk in an Asian population was published nearly four decades ago. These early studies presented equivocal evidence of an association of HLA genes with MS in the Japanese population. Subsequent findings with respect to the putative HLA association with MS risk in Asians have generally been conflicting. Studies reporting association of alleles at genes encoding MHC class I and class II antigens are counterbalanced by studies reporting no association of HLA genes with MS susceptibility. Some investigators have considered the possibility that genetic differences might underlie the dichotomy of clinical phenotypes—that is, conventional MS versus NMO—in Asian-ethnic patient populations. Several studies that have accordingly stratified patients by clinical phenotype have reported differential genetic associations specific to clinical phenotype. Although HLA-associations have been rather heterogeneous in
these populations, there is reasonable convergence on association of HLA-DPB1*0501 with opticospinal variants (OSMS and NMO)\textsuperscript{156,398–400,402} and HLA-DRB1*1501 with conventional MS\textsuperscript{396,397}. The results of a recent meta-analysis\textsuperscript{404} of studies in Chinese populations corroborate the finding concerning HLA-DRB1*1501, although the magnitude of excess risk associated with this variant (OR=1.39) was considerably less than that demonstrated in Caucasian populations.

The preponderance of optic involvement in Asians with MS and related disorders prompted genetic screens of the mitochondrial genome, where causative mutations for Leber’s hereditary optic neuropathy localize. However, separate studies in Japanese and Korean populations\textsuperscript{405,406} failed to identify similar mutations associated with MS. Other studies applying a hypothesis-driven, candidate-gene approach have also been conducted in Japanese and Chinese populations. Some have reported polymorphisms associated with MS and related disorders\textsuperscript{407–413}, while others have reported negative findings\textsuperscript{396,414–423}, occasionally in contradiction to previous positive findings\textsuperscript{424}. The few existing studies on genotype-phenotype correlations in Asian populations have reported association of age at onset\textsuperscript{392,408}, CSF OCB status\textsuperscript{149,170,425}, MRI lesion burden\textsuperscript{426}, and disability progression\textsuperscript{392,408} with several genes with roles in immune response. Importantly, none of the variants found to be associated with MS risk or clinical features in Asian populations through candidate-gene studies has been independently replicated.

The relatively scant body of literature on the genetics of MS in Asian-ethnic populations has generated mostly unconfirmed or conflicting findings. Moreover, there have been no genome-wide studies on MS or related disorders in these populations, due in large part to insufficient statistical power afforded by the inevitably smaller case samples. Whereas the role of common genetic variants influencing susceptibility to MS in Caucasian populations is becoming
increasingly clear, there is considerable uncertainty as to their role in historically low-risk ethnic
groups. The question of whether low-frequency or rare variants are implicated in the
pathogenesis of MS and related disorders in Asian-ethnic populations remains unanswered, as
sequencing studies in these populations have not been undertaken to date. While it seems likely
that the genetic load in persons of Asian ancestry is lower than that in Caucasian populations
(based on the rate of familial aggregation alone), heritability of MS and related disorders in
Asians currently remains almost entirely unexplained.

1.8 Research objectives

MS was long considered a disease of Caucasian persons, reflecting its high frequency in
populations of European ancestry and relative scarcity in non-Caucasian populations. As a
consequence of this insular perspective, recognition of MS and research devoted to
understanding its clinical scope and cause in Asian-ethnic populations remained largely
neglected until recently. There is now increasing interest in furthering current understanding of
MS and related disorders in Asian populations.

Nevertheless, the epidemiology, clinical profile, and genetics of MS and related disorders in
populations of Asian ancestry remain poorly understood. For instance, the frequency of these
disorders in Canadians of Asian ancestry is unknown. Whether clinical features and outcomes in
this ethnic group differ from that in Caucasian patients is a matter of debate. Environmental risk
factors for MS that have been thoroughly validated in Caucasian populations, such as smoking,
have not been systematically evaluated in Asian populations. Previous genetic studies seeking
variants associated with susceptibility to MS in Asians have generally been conflicting. These
prevailing uncertainties call attention to the fact that the current body of knowledge with respect to MS and related disorders in Asian populations is comparatively limited.

The objective of the research detailed in this dissertation is to bridge the substantial gaps in knowledge through a comprehensive investigation of MS and related disorders in persons of Asian ancestry in Canada (including migrants and Canadian-born) and China. In so doing, several important research questions are directly addressed:

1. What is the current prevalence of MS and related disorders (CIS, NMO, and NMOSD) in the Asian-ethnic population of British Columbia?
2. Has the incidence of MS and related disorders changed over time in this population?
3. What are the clinical characteristics of MS and related disorders in patients of Asian ancestry?
4. Do clinical outcomes and characteristics of MS and related disorders differ between Asian-ethnic patients versus the general (primarily Caucasian) clinic population?
5. Are environmental factors associated with differential clinical phenotypes (i.e., MS and NMO) in patients of Asian ancestry?
6. Is smoking associated with risk or clinical outcomes of MS and related disorders in populations of Asian ancestry?
7. What is the rate of familial aggregation of MS and related disorders in Asian-ethnic populations?
8. Are genetic variants in MS susceptibility genes validated in Caucasian populations also associated with risk or clinical phenotype of MS and related disorders in populations of Asian ancestry?
This dissertation summarizes several studies spanning a range of disciplines to advance the current body of knowledge pertaining to MS and related disorders in this unique population. Following is a summary of the relevant knowledge gaps addressed by this research and the specific research objectives of the studies summarized in this dissertation.

1.8.1 Frequency of MS and related disorders among Asians in British Columbia

The low frequency of MS in Asia has long been recognized\textsuperscript{86}, but there is now emerging evidence of a possible increase in prevalence and incidence\textsuperscript{61}. The question of whether the population risk of MS is also increasing in Asian populations in other regions warrants consideration. Literature on the epidemiology of MS in Asian-ethnic populations residing in high-prevalence regions outside of their countries of origin is exceptionally sparse.

Chapter 3 of this dissertation explicitly addresses this deficit in knowledge. The objective of this study was to characterize the distribution and frequency of MS and related disorders in persons of Asian ancestry in British Columbia (BC) by determining the current prevalence and temporal trends in the incidence of MS and related disorders, and drawing comparisons to the general population of BC.

1.8.2 Clinical profile of MS and related disorders in Asian populations

The most salient clinical feature of MS and related disorders in Asian-ethnic populations is the high relative frequency of the NMO clinical phenotype\textsuperscript{86}. Previous studies in this ethnic group (discussed in Section 1.5) suggest substantial heterogeneity in the clinical characteristics of MS and related disorders. In light of recent advances in the diagnostic classification of NMO\textsuperscript{23}, it is now recognized that the bulk of the existing clinical descriptions of MS in this population may
be spurious due to historical conflation of NMO and MS. Consequently, a great deal of uncertainty persists with respect to the genuine clinical features of MS in this population. Furthermore, there is a notable dearth of clinical descriptions of MS and related disorders in Asian-ethnic patients from clinical populations in high-prevalence regions where expert MS care is more readily available.

The descriptive research on which Chapter 4 of this dissertation focuses aims to resolve this gap in understanding. The primary objective of this study was to characterize the clinical and paraclinical features of MS and related disorders in patients from clinic populations in Canada and China. In addition, this study aimed to identify factors associated with clinical prognosis and outcomes in patients of Asian ethnic origin.

1.8.3 Comparison of clinical outcomes of MS and related disorders in Asians and Caucasians

There is considerable debate as to whether clinical features and outcomes of MS in Asian-ethnic patients differ from those in Caucasian patients. Earlier studies demonstrating less favourable prognosis in Asian-ethnic patients\textsuperscript{53,55,126} generally subsumed presumptive NMO cases with MS cohorts. Although this practice was consistent with convention at the time\textsuperscript{86}, the conclusions drawn from these influential early studies may nevertheless be unjustified because of more recent clarification in diagnostic classification of opticospinal clinical variants such as NMO. Overall, findings with respect to possible ethnic differences in clinical outcomes have been conflicting. Few studies have applied uniform methods of ascertainment and clinical evaluation to compare ethnic cohorts from the same clinic population.
Chapter 5 of this dissertation explores the role of ethnicity in clinical presentation and outcomes of MS and related disorders. The central objective of this study was to clarify whether there are ethnic differences in the clinical profile of MS in patients obtaining care in the same clinic setting. Specifically, this study conducted in a Canadian clinic population sought to determine whether patients of Asian extraction differed from those in the largely Caucasian general patient population with respect to demographic characteristics, clinical features, and disability progression.

1.8.4 Geography and clinical phenotype in Asians with MS and related disorders

Geographic environment is one of the most firmly established correlates of MS susceptibility\(^{254}\). It stands to reason, based on accumulating evidence suggesting pathological distinction of MS from NMO\(^{16}\), that these clinical phenotypes may also have dissimilar risk factors. Although migration studies have implicated the early-life environment in susceptibility to MS\(^{274}\), few studies have explored the impact of the regional environment on clinical phenotype, particularly with respect to differentiation of MS and NMO.

The work summarized in Chapter 6 examines the role of geographical factors in phenotype differentiation in Canadians of Asian ancestry with MS and related disorders. The objective of this study was to determine whether exposure to factors related to the geographic environment prior to onset of symptoms is associated with clinical phenotype in patients of Asian ancestry.

1.8.5 Smoking and MS susceptibility in Asian populations

Studies conducted in predominantly Caucasian populations have associated smoking with increased MS risk and adverse clinical outcomes. Smoking is in fact one of a number of well-
established environmental factors associated with MS susceptibility in Caucasian populations. By comparison, research on environmental risk factors for MS in Asian-ethnic populations is sparse. Remarkably, there are no published studies examining smoking as a potential risk factor for MS in Asian populations, despite evidence to suggest a concurrent increase in the prevalence of MS and smoking in some regions of Asia, particularly among females.

The analyses detailed in Chapter 7 of this dissertation address this critical gap in knowledge. The objective of this study was to determine whether smoking is associated with susceptibility to MS and related disorders, as well as clinical outcomes thereof, in Asian-ethnic populations in Canada and China.

1.8.6 Genetic susceptibility to MS in Asian populations

The low prevalence of MS in Asian populations is largely ascribed to low genetic susceptibility (or by tautology, increased genetic resistance) to MS. The much lower frequency of positive family history in Asians with MS and related disorders compared to Caucasians corroborates this hypothesis. Nevertheless, genetic susceptibility to MS and related disorders has been scarcely studied in Asian-ethnic populations. Putative genetic risk variants outside of the HLA region have not been broadly studied in this ethnic group.

Chapter 8 of this dissertation examines the role of genetic factors in susceptibility to MS and clinical phenotypes thereof in Asian-ethnic populations. The objectives of this study were to determine the rate of familial MS in Asian patients exposed to the high-risk Canadian environment and the low-risk Chinese environment, and to screen known MS risk genes to
identify rare and common variants associated with risk and clinical phenotype in persons of Asian ancestry.
Chapter 2: Materials and Methods

The research presented in this dissertation applied a multi-disciplinary approach integrating various observational research methods, drawing upon numerous data sources and informative study populations (Figure 2.1). The overall approach to data acquisition and analysis is presented in this chapter. Detailed methodologies for specific studies are provided in the relevant section of the respective chapters.
Figure 2.1. Schematic representation of data sources, types, and analyses.

Major source populations were British Columbia, Canada, and Shanghai. Data were derived from clinical databases, questionnaires, and population-based survey data. Data types included serial and cross-sectional clinical data; family history, ethnicity, and migration data; personal smoking history; and DNA samples. A variety of epidemiological, clinical, environmental, and genetic analytical approaches were applied to these data.
2.1 Case ascertainment

Eligible patients were primarily identified and enrolled through one of two centres specializing in clinical care and research for MS and related disorders: (1) The University of British Columbia (UBC) MS Clinic, located in Vancouver, BC, Canada; and (2) the Institute of Neurology at Huashan Hospital, located in Shanghai, China. Although operating under distinctly different systems of health care delivery, both centres recruited patients for this research using the same set of eligibility criteria for study entry (discussed below) to ensure uniformity in case selection. A combinatorial approach of retrospective and prospective case ascertainment was used to identify existing and newly diagnosed patients, respectively. At both centres, eligible cases were enrolled following retrospective identification through comprehensive screening of the respective clinic registers (which in the case of the case of the UBC MS Clinic, also included linked patient-reported ancestry information). In addition, consecutive eligible cases were enrolled prospectively on initial registration at each clinic.

In addition to these principal source populations, patients fulfilling eligibility criteria were retrospectively identified from the research database of the Canadian Collaborative Project on Genetic Susceptibility to MS (CCPGSMS)\textsuperscript{430}. The CCPGSMS was a longitudinal study that collected data from over 25,000 neurologist-diagnosed MS patients collectively registered at 18 MS clinics in 8 provinces across Canada from 1993 through 2012. Relevant cross-sectional clinical data, family history information, and environmental exposure data from the CCPGSMS were used in this research.

The UBC MS Clinic is the largest tertiary care centre for MS and other IIDDs in BC, overseeing the care of over 10,000 patients since opening on October 1, 1980. In addition to patients seeking
care at the main clinic at UBC Hospital, the UBC MS Clinic database includes patients registered at affiliated clinics located at Royal Jubilee Hospital (Victoria, BC), Kelowna General Hospital (Kelowna, BC), and Prince George Regional Hospital (Prince George, BC). Earlier studies have established that the UBC MS Clinic captures approximately 80% of all patients with MS in BC\textsuperscript{346,431}, and more recent estimates of prevalence in BC\textsuperscript{44} suggest that this capture rate has remained relatively stable over time. Estimates derived from the CCPGSMs indicate that >90% of patients in the UBC MS Clinic database are of full European ethnic origins (Caucasian).

The Institute of Neurology at Huashan Hospital is the main neurological care centre in the Shanghai region. The neurological service at Huashan Hospital oversees the care of more than 200 patients diagnosed with MS or related disorders at in-patient and out-patient clinics.

Inclusion criteria for this research were based on the patient’s neurologist-confirmed diagnosis and self-reported ethnicity. Eligible diagnoses were definite/probable MS, CIS, NMO, and NMOSD by applicable diagnostic criteria for MS\textsuperscript{11,102,184,188,189} or NMO/NMOSD\textsuperscript{16,22,23} in use at the time of diagnosis. All other diagnoses were excluded. Studies detailed in Chapters 3, 4, 5, 7, 8 included all eligible diagnoses, whereas investigations summarized in Chapter 6 were limited to patients diagnosed with MS, NMO, or NMOSD. Eligibility criteria for ethnicity were fulfilled by patients identifying as having full East or Southeast Asian ethnic origins in at least one parental lineage. Ethnicity classification followed guidelines specified by the 2006 Canadian Census Ethnic Origin Reference Guide\textsuperscript{432}. Therefore, the following East or Southeast Asian-ethnic origins, regardless of the country of birth, were eligible: Burmese, Cambodian, Chinese, Filipino, Hmong, Indonesian, Japanese, Khmer, Korean, Laotian, Malaysian, Mongolian, Singaporean, Taiwanese, Thai, Tibetan, Vietnamese, and other East or Southeast Asian-
ethnicities not included elsewhere or otherwise specified. For simplicity, these ethnicities are collectively referred to as “Asian” hereafter and throughout this dissertation. Only patients fulfilling both eligibility criteria pertaining to diagnosis and ethnicity were considered for this research.

The Asian case cohort for studies detailed in Chapters 3, 4, 5, and 6, as well as the genetic association analyses in Chapter 8, were limited to patients with full Asian ethnic origins. However, studies in Chapter 7 and the family history analysis in Chapter 8 additionally included patients with partial ethnic origins as described above. This approach ensured that cases were adequately matched in terms of ethnicity to relevant controls.

Eligible cases registered at the UBC MS Clinic were primarily identified by screening a longitudinal database containing routinely collected ethnicity and family history information obtained through structured, personal interviews with a genetic counselor at the time of initial clinic registration. The database includes information on the place of birth and ethnicity of each patient to the precision of grandparental ethnicity. It is estimated that this database captured >90% of patients diagnosed with probable or definite MS who registered at the UBC MS Clinic between October 1, 1980 and May 15, 2012. Eligible cases were also identified through a manual search of clinic records (including both active and inactive patients) and referral by the patient’s UBC MS Clinic neurologist. Retrospective and prospective case ascertainment was carried out between January 1, 2009, and May 15, 2012. The latter was selected as the cut-off date for case ascertainment because routine collection of ethnicity information on newly registered patients attending the UBC MS Clinic ceased after this date due to unavailability of a genetic counsellor.
Eligible cases in Shanghai were identified through consecutive enrolment of patients attending in-patient and out-patient neurology clinics at Huashan hospital. Patients fulfilling eligibility criteria for diagnosis and ethnicity, as detailed above, were included. Eligibility of all ascertained cases was reviewed and confirmed by a clinical team under the direction of Dr. Zhi-Ying Wu (Fudan University, Shanghai, China). Identification and enrolment of cases through this study site was carried out between March 1, 2012 and December 31, 2013.

Investigations detailed in Chapters 7 and 8 of this dissertation necessitated the acquisition of data from appropriate control cohorts consisting of unrelated individuals from the general population who were frequency-matched to the ethnicity of cases. The initial research plan specified recruitment of unaffected spouses, partners, and friends of eligible cases to comprise the control group. It was reasoned that this source population would ensure that the case and control cohorts would be sufficiently similar with respect to ethnicity and age, thereby minimizing the effect of confounding variables. In addition, this approach was successfully applied in the CCPGSMS. There was an a priori expectation that there would be particular challenges to recruiting unaffected controls due to cultural reservations against participation in health research that has been well-documented in some ethnic minority populations and socio-cultural groups. These challenges were considerably more problematic than initially anticipated; there was an extraordinarily high rate of refusal by prospective control subjects who were personally approached by cases for participation in this research. Similar problems were also encountered when attempting to recruit control subjects in the Shanghai study region. To resolve his shortfall in recruiting ethnicity-matched controls, suitable alternative sources of control data were sought.
to enable completion of the case-control studies in Chapters 7 and 8. These data sources are discussed further in Sections 2.2.4 and 2.2.6, respectively.

2.2 Data sources and acquisition

2.2.1 Prevalence and incidence data

The frequency of MS and related disorders in the Asian-ethnic population of BC was quantified through measures of prevalence and incidence. Prevalence refers to the proportion of diseased individuals at a given point in time in a defined population at risk\textsuperscript{66}. Incidence is a measure of the rate of newly diagnosed individuals during a specified period of time in the at-risk population\textsuperscript{66}. Therefore, the number of incident and prevalent cases is equivalent to the numerator in estimates of the frequency of disease.

Incident and prevalent cases of MS and related disorders in the Asian-ethnic population of BC were identified through the retrospective and prospective ascertainment strategy detailed in Section 2.1. Clinic registration records and the Genetic Research database were manually reviewed for all eligible cases to confirm place of residence in BC at the time of diagnosis. In addition to these resources, the CCPGSMS database was screened to determine whether any cases exited the prevalent cohort through emigration from BC or death.

The number of individuals at risk, representing the denominator in estimates of prevalence and incidence, was determined from reference data published by Statistics Canada\textsuperscript{335–440}. Population estimates in BC within strata of ethnic origins, immigrant status, sex, and age groups were obtained from public-use microdata files of the quinquennial Census of Canada for the years 1986, 1991, 1996, 2001, and 2006. Estimates of the reference population were stratified by sex,
age group (0-14, 15-24, 25-44, 45-64, and ≥65 years), ethnicity (Asian and non-Asian), and immigrant status (Canadian-born and immigrant).

2.2.2 Clinical data

Clinical data used in observational clinical studies in this dissertation were obtained from existing databases and medical records. Longitudinal clinical data analyzed in Chapter 5 were extracted from the clinical research database of the UBC MS Clinic. This database contains prospectively collected, longitudinal data summarizing clinical assessments for all visits to the clinic by patients who have provided informed consent for use of their personal health data for research (>95% of patients attending the clinic). All personally identifying information is removed from the dataset prior to any release of data for research applications. The database includes MS neurologist-generated data pertaining to a wide range of clinical and paraclinical variables acquired through standardized case report forms (Appendix A.1) for each clinician-patient interaction at the UBC MS Clinic. Most patients (>85%) attending the clinic undergo follow-up assessment on an annual (or more frequent) basis. Patients with more active or aggressive disease are typically examined in follow-up more frequently, usually on a semi-annual basis.

Clinical data were stratified by patient ethnicity into Asian and non-Asian patient cohorts through record linkage of database and study identification numbers. In addition, manual review of charts was performed for subjects in the Asian patient cohort to clarify discrepant data, acquire additional clinical information using standardized case report forms, and confirm accuracy of the diagnostic classification. With respect to the latter, complete clinical records were reviewed under the supervision of Dr. Anthony Traboulsee to classify diagnosis according
to current diagnostic criteria for MS\textsuperscript{189}, NMO\textsuperscript{23}, and NMOSD\textsuperscript{16}. Clinical data acquired through February 28, 2013, were extracted from the clinical research database for this research.

Additionally, cross-sectional clinical data for Asian-ethnic cases registered at MS Clinics in Canada (for analyses detailed in Chapter 4) were extracted from the CCPGSMS database. In addition to patients ascertained at the UBC MS Clinic, Asian-ethnic patients fulfilling eligibility criteria were identified at 11 other MS clinics in the CCPGSMS network. Clinical data associated with these cases were extracted from medical records and coded into standardized case report forms (Appendix A.2) by trained CCPGSMS research staff at the respective clinics.

Clinical data for patients ascertained through Huashan Hospital were acquired retrospectively following enrolment in the study. Relevant clinical data were extracted from personal health records by manual chart review and coded into translated, standardized case report forms (Appendix A.2) by a collaborating physician (Dr. Gui-Xian Zhao). Accuracy and consistency of these data were further reviewed by a supervising physician (Dr. Zhi-Ying Wu) with expertise in MS and related disorders in Asian populations. Clinical data for this cohort were collected between March 1, 2012, and December 31, 2013.

2.2.3 Geography and ethnicity data

Data on ethnicity, geographic origins, and migration history for the study presented in Chapter 6 of this dissertation were obtained from eligible patients ascertained at the UBC MS Clinic (detailed in Section 2.1). Patient-reported data were obtained through standardized questionnaires (Appendix B.1) administered over telephone or in person. Family members or friends were encouraged to provide language translation as necessary. The majority of
questionnaires were administered between January 1, 2009, and May 15, 2012. However, patients who participated in the CCPGSMS prior to enrolment in this study (n=20) had been previously administered the questionnaires by CCPGSMS staff between January 1, 1993 and December 31, 2008. The acquisition and coding of questionnaire data were guided by standardized questionnaires adapted from the CCPGSMS. The questionnaires used in work detailed in Chapter 6 specifically queried respondents on ethnic origins, place of birth, places of residence, and years of migration, the latter including both moves between countries and internal migration events (Appendix B.1).

2.2.4 Smoking exposure data

Personal smoking history data studied in Chapter 7 of this dissertation were similarly obtained using standardized questionnaires (Appendix B.2), as detailed above (Section 2.2.4). Eligible patients were asked whether they had ever smoked cigarettes, and if responding affirmatively, were asked to provide a detailed account of the frequency and total number of cigarettes smoked during patient-defined age intervals in their lifetime. An estimate of whether subjects had smoked at least 100 cigarettes to date was derived from the detailed personal smoking history above. Smoking exposure data were also obtained from eligible cases ascertained at 11 other Canadian MS clinics participating in the CCPGSMS (detailed in Section 2.1) for this study. Personal smoking history from these additional participants were obtained comparably, using the same standardized questionnaires as those administered to cases ascertained from the UBC MS Clinic population.

For cases ascertained at Huashan Hospital, translated versions of the smoking exposure questionnaires (Appendix B.2) that were adapted for use in the Shanghai population (translated
to Simplified Chinese) were jointly developed and validated in close collaboration with colleagues at Huashan Hospital and Fudan University (Shanghai, China). Interviewers received training specific to the administration of this questionnaire to ensure uniformity in the acquisition of patient-reported data between research sites. Primary and back-translated questionnaire data from a random sample ($n=10$) of Shanghai-resident patients registered through Huashan Hospital were compared and independently evaluated by two raters proficient in English and Chinese to confirm accuracy and validity of English-Chinese translation in the adapted questionnaires.

Primary data on smoking exposure in ethnicity-matched healthy controls could not be obtained specifically for this study due to unanticipated and irresolvable challenges to recruiting control subjects from this population (discussed in greater detail in Section 2.1). To address this problem, suitable alternative data from healthy controls were sought. After careful consideration, weighing a number of factors including comparability and reliability of the data, suitability of the source population, and availability of the data, the best approach was to obtain smoking exposure data from existing sources specific to each population. For the Canadian population, control data were drawn from contemporaneous cycles of the population-based Canadian Community Health Survey (CCHS) through Statistics Canada. For the Shanghai population, recently published data in the peer-reviewed literature reporting sex- and age-specific smoking prevalence in this region$^{441}$ were used for comparative analysis in this study.

The CCHS is an ongoing, cross-sectional, voluntary national survey administered biannually as a joint initiative of the Canadian Institute for Health Information and Statistics Canada since 2000. The survey collects demographic information and data on health status and health determinants from a randomly selected sample of persons aged 12 years and older in all provinces and
territories of Canada. Survey data are collected from approximately 130,000 respondents per two-year cycle, using a sampling method that aims to achieve adequate coverage of all 115 administrative health regions in Canada and proportional representation of every province and territory based on population density. Survey data are weighted on socio-demographic variables, such that CCHS data released by Statistics Canada are representative of the survey population (i.e., Canadians aged 12 years and older residing in private-dwellings) for the cycle during which the data were acquired. Sample weighting is performed by Statistics Canada using the bootstrap re-sampling method. The socio-demographic component of the survey includes specific questions pertaining to the ethnic origins, thereby enabling extraction of data from Asian-ethnic groups relevant to this work, as well as sex and age of the respondent. The core content of the CCHS, which is invariant from one cycle to the next, queries smoking status through measures comparable to the questionnaire used in this study. Specific to this study, survey respondents are asked whether they have smoked 100 or more cigarettes in their lifetime.

For this study, CCHS data were acquired through a custom tabulation request submitted to the Client Services Department of Statistics Canada. A specific application to access these data was necessary because CCHS data specifying ethnic origins are suppressed in publicly available data files in compliance with privacy legislation in the Statistics Act. Requested data used in this work were received on March 7, 2014. Population estimates of smoking exposure rates in Canadians identifying as having primarily East or Southeast Asian ethnic origins were generated by Statistics Canada from survey data collected during CCHS cycles 2007 through 2012. This interval was selected to correspond with the period during which the majority of self-reported data on smoking history were collected from eligible cases in this study, thereby obviating
potential cohort effects. It also warrants emphasizing that the control CCHS data on smoking history were obtained through methodologically comparable means to those in the patient cohort of this study. For both cases and controls, self-reported data were acquired through standardized questionnaires administered in-person or via telephone by trained personnel using scripted prompts. In addition, both questionnaires queried lifetime smoking history using equivalent operational definitions.

For case-control comparisons of smoking in the Shanghai population, estimates of smoking in this region were obtained from a recent community-based survey in the Changqiao region of Shanghai, China. In this population-based survey, a random sample of 1,500 respondents was selected from a total of 2,100 surveyed residents between the ages of 13 and 84 years. Sampling was performed using a multi-stage proportional random sampling approach to minimize clustering effects. Similar to the approach applied to cases in the present research, data were acquired by standardized questionnaires administered by trained research personnel. Data from this community-based survey were deemed to be an appropriate comparator on account of its comparability to the Shanghai case cohort, particularly with respect to age, ethnic origins, and region of residence of the source population, as well as the period and method of data acquisition. Moreover, the operational definition of positive smoking history in the survey was equivalent to that employed in the present study.

2.2.5 Family history data

Patient-reported family history data analyzed as part of the study presented in Chapter 8 were also obtained through standardized questionnaires (Appendix B.3) administered to Asian-ethnic patients ascertained at the UBC MS Clinic and 11 other MS clinics across Canada, as well as
patients ascertained at Huashan Hospital in Shanghai, China (detailed in Section 2.1). Participating patients in both populations were asked if they were aware of any other member of their family who has been clinically investigated for or diagnosed with MS or related disorder. Patients were specifically queried with respect to known family history of MS, ON, or any other neurological disorders. In addition, detailed family histories were collected with respect to the nuclear family of patients. As appropriate, additional clinical documentation was obtained to confirm instances of positive family history of MS or related disorder reported by the index case. Accordingly, medical records pertaining to diagnosis were sought for putatively affected family members using a release-of-information form that was endorsed by the relevant family member and subsequently forwarded to his/her physician.

2.2.6 Genetic sequence data

DNA samples were obtained from patients agreeing to provide a blood or saliva sample for the genetic study presented in Chapter 8. Whole blood samples (10 ml) drawn into ethylene-diamine-tetra-acetate Vacutainer (BD Canada, Mississauga, ON) collection tubes were obtained by routine venipuncture. Saliva samples were obtained using the Oragene DNA OG-500 self-collection and DNA stabilization kit (DNA Genotek, Kanata, ON). Blood and saliva samples were maintained at ambient temperature in their collection vessel for a period not exceeding 24 hours or 14 days, respectively, after which they were placed in storage at -80°C. Genomic DNA was extracted and purified from whole blood and stabilized saliva samples through automated or manual techniques, respectively: blood samples were processed with the Autogen FLEX STAR system according to the manufacturer’s protocols (AutoGen, Inc., Hilliston, MA), and saliva samples were processed manually using the prep-IT purification reagent per manufacturer’s
protocols (DNA, Genotek, Kanata, ON). Purified DNA samples were stored at -80°C until processing for genetic analysis.

Genetic variants in the coding regions (exons) and sequences flanking the intron-exon junctions of 61 candidate genes (Appendix C) previously validated as susceptibility genes in a large, multi-centre genetic association study in Caucasian populations were identified in cases using next-generation exome sequencing. Exome amplicon libraries were generated with the Ion AmpliSeq™ Exome Kit (Life Technologies, Burlington, ON), according to the manufacturer’s instructions. Sequencing was performed on approximately 100 ng of genomic DNA from Asian-ethnic MS and NMO patients ascertained at the UBC MS Clinic using the Ion Proton™ System on Ion PI™ Sequencing 200 Kit v2 (Life Technologies, Burlington, ON) according to the manufacturer’s protocols. Samples were screened to ensure adequate sequencing coverage and read length in the 61 analyzed genes, as well as across the overall sample. Those failing to reach a threshold of 50-fold mean read depth and mean read length of 50 bases were rejected and sequencing was repeated as necessary. Raw data from sequencing runs were transmitted to the manufacturer’s Proton™ Torrent Server for remote processing, where it was aligned to the human reference genome (National Center for Biotechnology Information human reference genome NCBI37.4) and converted to base calls with the Torrent Suite™ Software. Variant detection and variant calling was also performed using this integrated software suite. Functional annotation of variants (e.g., synonymous, nonsense, missense, read-through, or frame-shift) was performed with the ANNOVAR software package, and variants were additionally annotated with a reference SNP (rs) number from the dbSNP repository (dbSNP Build 137) and allele frequencies from the 1000 Genomes Project East Asian Ancestry Phase 1 exome dataset.
As discussed in Section 2.1, acquisition of DNA samples from unrelated control subjects from the same source population from which cases were ascertained was unsuccessful due to unexpectedly high rates of refusal by eligible control participants. It was therefore necessary to pursue an alternate source of genetic sequence data to which cases could be compared. Comparison of cases against an ethnically comparable cohort in the 1000 Genomes Project was deemed to be the most appropriate option on the balance of accessibility, volume, and quality of the sequence data.

The East Asian Ancestry cohort of the 1000 Genomes Project Phase 1 dataset was used in this study. This resource includes high-coverage (50-100x), targeted exome data acquired from 286 subjects representing three Asian-ethnic populations: Han Chinese from Beijing \( (n=97) \), Han Chinese from Southern China \( (n=100) \), and Japanese from Tokyo \( (n=89) \). Amalgamation of these populations into a unified ancestry group was justified by the 1000 Genomes Project Consortium on the basis of demonstrable genetic similarity between the populations. Specifically, indices of genetic variability between the populations, based on differentiation in frequency of common variants, were acceptably low to validate aggregation of the data.

Importantly, the ethnicities encompassed by the populations in the 1000 Genomes East Asian Ancestry cohort overlap considerably with the Asian ethnic origins of the patient samples included in this study, thereby enabling ethnicity-matched comparisons of cases and controls.

### 2.3 Statistical analysis

The analytical workflow for all studies in this dissertation began with a descriptive statistical approach involving visual inspection of the data to characterize features of its distribution \( (e.g., \) normality, skewing, and outliers). Summary statistics for all variables of interest were computed
accordingly: Continuous variables were summarized as mean values with 95% confidence intervals (CI) if normally distributed, and as median values with inter-quartile ranges (IQR) if non-normally distributed or derived from a small sample (n<50). Categorical (ordinal and nominal) variables were summarized as counts or proportions.

The inferential statistical tests used in this dissertation were specific to the analyses involved in each study and are therefore discussed in greater detail in their respective chapters. For the general case of univariate, two-sample comparisons, the following approaches were used:
Continuous variables were compared with a *t* test if normally distributed, or a Wilcoxon rank sum (Mann-Whitney U) test if non-normally distributed. Categorical variables were compared with a chi-square test or, in circumstances where individual cell counts were <25, a Fisher exact test. All tests were two-tailed unless otherwise specified, and *p* <0.05 was considered to be statistically significant. For analyses involving multiple comparisons, Bonferroni correction was applied, and in such cases, adjusted *p*-values were reported. All statistical analyses were performed using the R Statistical Package version 3.0.2 for Windows (R Foundation for Statistical Computing, Vienna, Austria).
Chapter 3: Frequency of MS and related disorders in the Asian-ethnic population of British Columbia

3.1 Introduction

The frequency of MS and related disorders is characterized by striking global variation. Regional differences in the burden of disease have been ascribed to presumptive population differences in both genetic and environmental risk. Relative to global regions predominantly inhabited by persons of Caucasian ancestry, studies in Asia have historically reported remarkably low MS incidence and prevalence. Until recently, in fact, there was near universal agreement that prevalence was less than 2 cases per 100,000 population. By way of comparison, this is a rate that is some 100-fold less than that in the Canadian population. Similarly, most studies examining MS frequency in immigrant Asian populations in North America corroborated the notion of intrinsically low risk of MS and related disorders in this ethnic group.

Changes in the global epidemiology of MS are now being recognized. A growing body of literature suggests that incidence, prevalence and female preponderance of MS are increasing. This trend likely extends to populations in Asia, as emerging data from epidemiological studies in the region suggest that the current prevalence and incidence of MS are greatly increased over historical estimates. The sum of these observations points to a widespread temporal increase in environmental risk for MS and related disorders, as it is unlikely that the entirety of this increase is attributable to improvements in case ascertainment. However, it is unclear whether this pattern of increasing risk also applies to Asian-ethnic populations residing in high-prevalence regions such as Canada.
The objectives of this study were to determine the prevalence of MS and related disorders in persons of full Asian ancestry residing in BC, and to evaluate temporal trends in incidence in this population relative to the general BC population.

3.2 Methods

3.2.1 Case ascertainment and classification

Prevalent and incident cases of MS and related disorders in BC were identified through the UBC MS Clinic (detailed in Sections 2.1 and 2.2.1). This study included clinical records for all patient visits to the clinic for the 25-year period beginning January 1, 1986 and ending December 31, 2010. A run-in period of approximately five years, separating the clinic’s initial opening and the start of retrospective observation, was selected to allow case ascertainment to stabilize during the initial years of operation of the UBC MS Clinic.

Patients were categorized by ethnicity and assigned to Asian or non-Asian cohorts (Section 2.2.1). Patients with partial Asian ancestry were not included as a separate cohort in this study due to a relative paucity \( n = 13 \) of such cases and a lack of available reference population data for this heterogeneous at-risk population. Thus, patients with partial Asian ancestry were subsumed in the non-Asian population cohort. For subgroup analysis, the Asian patient cohort was further stratified by birthplace (immigrant status) into Canadian-born and immigrant sub-cohorts, as detailed in Section 2.2.3.

In addition to definite MS, this study included patients diagnosed with NMO, NMOSD, and CIS. This inclusive ascertainment approach was appropriate, in light of changing practices in diagnosis and coding of these disorders during the period of observation in this study. Although
clinical records for all of the Asian patients were individually reviewed to specifically confirm the diagnosis of NMO and NMOSD (Section 2.2.2), an analogous case-by-case review of non-Asian patients was not feasible due to the large size of this cohort (n>6,000). However, based on previous estimates\textsuperscript{85}, patients with NMO or NMOSD were likely to account for a negligible fraction (<2%) of cases in the non-Asian cohort. Prevalence and incidence estimates in the Asian-ethnic population were also analyzed within MS and NMO diagnostic subgroups, with the latter including NMOSD.

Prevalent cases were defined as individuals resident in BC with a diagnosis of MS, NMO, NMOSD, or CIS on prevalence day (December 31, 2010). Incident cases were those who received an initial eligible diagnosis at the UBC MS Clinic during the years of interest (1986-2010). It was necessary to select diagnosis, as opposed to symptom onset, for this study as the incident event due to onset data being unavailable for some cases. To adjust for incomplete ascertainment of MS cases in BC through the UBC MS Clinic (Section 2.1), all prevalent and incident case totals were divided by a constant scaling factor of 80%.

### 3.2.2 Standardized prevalence and incidence

Prevalence within strata of age and sex of were calculated as the number of extant cases living in BC on prevalence day divided by the corresponding at-risk population. In this study, estimation of prevalence was limited to the Asian-ethnic population due to unavailability of death and residency data for cases in the non-Asian cohort. Crude, stratum-specific prevalence rates were subsequently standardized to the 2006 population of Canada by the direct method\textsuperscript{446}, yielding standardized prevalence estimates for males and females in the Asian-ethnic population.
Annual incidence rates within strata of sex and age were calculated as the number of cases with an initial eligible diagnosis during the year divided by the estimated at-risk population for each year from 1986 through 2010. Annual, sex-specific incidence rates were also standardized to the 2006 population of Canada by the direct method. Direct standardization adjusted for differences in population structure (e.g., age and sex distribution), thereby enabling direct comparisons of incidence between different five-year intervals. Standardized, five-year mean annual incidence rates were then calculated to enable comparison of incidence between intervals during which few incident MS cases were documented.

3.2.3 Statistical analyses

Due to the limited number of observed events, particularly in the Asian cohort, a conditional test suitable for a range of sample sizes was used to evaluate differences in prevalence and incidence between groups. Differences in the mean annual incidence of MS and related disorders were evaluated between non-Asian and Asian cohorts. In addition, prevalence within the latter cohort was compared between sub-groups defined by immigrant status. To compare incidence and prevalence estimates, normal approximation to the binomial distribution was applied using a z-test when the number of observed events was sufficiently large, such that variance was ≥5. An exact binomial test was used when variance was <5. Interval estimates, expressed as 95% confidence intervals (CI) for prevalence and incidence densities were estimated with an exact Poisson distribution.

These statistical analyses were carefully chosen to accommodate the relative infrequency of MS and related disorders in the Asian-ethnic population compared to the general population. The
suitably conservative statistical approach applied to this study was robust to biases from small sample sizes and minimized the probability of a Type I error.

3.3 Results

3.3.1 Reference population

Between 1986 and 2006, the BC population grew by 43% from 2.85 million to 4.08 million. During this period, the Asian subpopulation increased more than three-fold, from 157,550 to 543,783 (Figure 3.1). Rapid expansion of this subpopulation coincided with robust immigration from Asia, particularly from 1986 to 1995, during which the immigrant Asian population in BC increased more than two-fold (Figure 3.1). Growth in the non-Asian BC population was consistent with national population growth trends, increasing from 2.69 million to 3.53 million.
Figure 3.1. Population growth trends in British Columbia (1986-2006).

Annual population estimates for (A) the overall population and (B) the Asian-ethnic population of BC were derived from population estimates in the Census of Canada, 1986-2006.
3.3.2 Patient demographics

A total of 8,894 patients seen at the UBC MS Clinic prior to prevalence day (December 31, 2010) were screened. For analysis of incidence, 2,801 patients were excluded on the basis of unspecified or ineligible diagnosis \((n=1,191)\) or first diagnosis occurring outside of the study period \((n=1,610)\). Thus, a total of 6,093 incident cases were eligible, including 90 Asian cases \((1\%)\) and 6,003 non-Asian cases \((99\%)\). Among Asian cases, 53 \((59\%)\) were MS, 31 \((34\%)\) were NMO, and 6 \((7\%)\) were CIS.

Both patient cohorts were characterized by female preponderance (Table 3.1), which was greater among Asian patients compared to non-Asian patients (sex ratio: 5.4 vs. 2.6; \(p<0.012\)). This disparity was largely driven by a significantly greater female-to-male ratio in Asians with NMO \((p=0.026)\). Although the mean age at symptom onset did not differ significantly between the Asian and non-Asian patient cohorts \((33.2\text{ years}, 95\% \text{ CI: 30.6-35.8 vs. 33.7 years, 95\% CI: 33.4-34.1}; p=0.685)\), Asian patients were on average younger at diagnosis \((37.2\text{ years}, 95\% \text{ CI: 34.4-40.0 vs. 42.1 years, 95\% CI: 41.8-42.4}; p<0.001)\). Consequently, the interval from onset to diagnosis was considerably shorter in Asians \((4.0\text{ years}, 95\% \text{ CI: 2.8-5.3})\) compared to non-Asians \((7.7\text{ years}, 95\% \text{ CI: 7.5-8.0}); p<0.001\) (Table 3.1).
### Table 3.1. Clinical and demographic characteristics of Asian and non-Asian patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Non-Asian</th>
<th>Asian</th>
<th></th>
<th></th>
<th>( p ^ b )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total ( a )</td>
<td>MS</td>
<td>NMO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases, ( n )</td>
<td>6,003</td>
<td>90</td>
<td>53</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, ( n ) (%)</td>
<td>4,355 (72.5)</td>
<td>76 (84.4)</td>
<td>43 (81.1)</td>
<td>28 (90.3)</td>
<td></td>
</tr>
<tr>
<td>Male, ( n ) (%)</td>
<td>1,648 (27.5)</td>
<td>14 (15.6)</td>
<td>10 (18.9)</td>
<td>3 (9.7)</td>
<td></td>
</tr>
<tr>
<td>Female-male ratio</td>
<td>2.6</td>
<td>5.4</td>
<td>4.3</td>
<td>9.3</td>
<td>0.012</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset ( c ), mean years (95% CI)</td>
<td>33.7 (33.4-34.1)</td>
<td>33.2 (30.6-35.8)</td>
<td>31.3 (28.0-34.5)</td>
<td>35.2 (30.8-39.7)</td>
<td>0.685</td>
</tr>
<tr>
<td>Diagnosis, mean years (95% CI)</td>
<td>42.1 (41.8-42.4)</td>
<td>37.2 (34.4-40.0)</td>
<td>34.9 (31.6-38.2)</td>
<td>40.3 (35.2-45.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diagnostic Delay ( c ), mean years (95% CI)</td>
<td>7.7 (7.5-8.0)</td>
<td>4.0 (2.8-5.3)</td>
<td>3.7 (2.3-5.0)</td>
<td>5.2 (2.3-8.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Patients were diagnosed at the UBC MS Clinic between January 1, 1986 and December 31, 2010, inclusive.

CI, confidence interval

\( a \) Total comprises MS and related disorders (including CIS)

\( b \) \( p \)-values refer to comparisons between Asian and non-Asian total cohorts

\( c \) Data were available in all Asian cases and 4,336 non-Asian cases
3.3.3 Prevalence

The prevalence of MS and related disorders in the Asian-ethnic population of BC was 16.51 per 100,000 (95% CI: 13.59-20.05). When stratified by diagnosis, the prevalence of MS (9.97 per 100,000, 95% CI: 7.66-12.76) was approximately twice that of NMO (5.06 per 100,000, 95% CI: 3.46-7.13) ($p=0.028$) (Table 3.2). A significant sex disparity in prevalence was observed, with females being affected three times more frequently than males ($p<0.001$). This difference was most pronounced among those diagnosed with NMO, for which standardized prevalence in females (9.05 per 100,000, 95% CI: 6.11-12.94) was 10 times that in males (0.90 per 100,000, 95% CI: 6.11-12.94) ($p<0.001$).

Stratification by place of birth revealed marked cohort differences in prevalence between Canadian-born and immigrant Asians in BC (Table 3.3). The prevalence of MS and related disorders overall was more than four times greater among Canadian-born Asians (51.33 per 100,000, 95% CI: 36.34-70.32) compared to the immigrant Asian subpopulation (11.90 per 100,000, 95% CI: 9.22-5.11) ($p<0.001$). This imbalance was greatest in MS, for which cohorts were separated a $>5$-fold difference ($p<0.001$), but was also apparent in NMO, characterized by a $>3$-fold difference in prevalence ($p<0.001$).
Table 3.2. Prevalence of MS and related disorders in the Asian population of British Columbia in 2010.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MS and related disorders</strong></td>
<td>16.51 (13.59-20.05)</td>
<td>25.38 (20.31-31.47)</td>
<td>7.27 (4.44-11.19)</td>
</tr>
<tr>
<td>MS</td>
<td>9.97 (7.66-12.76)</td>
<td>13.80 (10.14-18.35)</td>
<td>5.98 (3.42-9.68)</td>
</tr>
<tr>
<td>NMO/NMOSD</td>
<td>5.06 (3.46-7.13)</td>
<td>9.05 (6.11-12.94)</td>
<td>0.90 (0.11-3.23)</td>
</tr>
</tbody>
</table>

Prevalence rates are expressed as cases per 100,000 and age-standardized to the 2006 general population of Canada. 95% confidence intervals are indicated in parentheses.
Table 3.3. Prevalence of MS and related disorders in the Asian population of British Columbia in 2010.

<table>
<thead>
<tr>
<th></th>
<th>Canadian-born</th>
<th>Immigrant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Female</td>
</tr>
<tr>
<td>MS and related disorders</td>
<td>51.33 (36.34-70.32)</td>
<td>78.63 (53.07-112.44)</td>
</tr>
<tr>
<td>MS</td>
<td>32.92 (21.70-47.74)</td>
<td>46.23 (28.61-70.72)</td>
</tr>
<tr>
<td>NMO/NMOSD</td>
<td>13.90 (5.57-28.64)</td>
<td>25.14 (9.23-54.80)</td>
</tr>
</tbody>
</table>

Prevalence rates are expressed as cases per 100,000 and age-standardized to the 2006 general population of Canada; 95% confidence intervals are indicated in parentheses.
3.3.4 Incidence

The 25-year mean annual incidence of MS and related disorders in the non-Asian population of BC (Figure 3.2A; Table 3.4) was 10.21 per 100,000 (95% CI: 9.99-10.45), approximately 10-fold greater than in the Asian population (1.02 per 100,000, 95% CI: 0.85-1.23) ($p<0.001$) (Figure 3.2B; Table 3.4). Ethnic differences in MS incidence trends were underscored by relative stability in the non-Asian population contrasting with increasing incidence in the Asian-ethnic population (Table 3.4). In the non-Asian population, the incidence of MS and related disorders remained relatively constant from 1986-1990 (10.41 per 100,000, 95% CI: 9.87-10.97) to 2006-2010 (9.91 per 100,000, 95% CI: 9.46-10.39) ($p<0.151$) (Figure 3.2A, Table 3.4). In contrast, the mean annual incidence of MS and related disorders doubled in the BC Asian-ethnic population (Figure 3.2B, Table 3.4) from 1.04 per 100,000 (95% CI: 0.52-1.86) to 2.02 per 100,000 (95% CI: 1.55-2.58) ($p<0.005$).
Figure 3.2. Incidence of MS and related disorders in British Columbia (1986-2010).

Five-year, mean annual incidence rates are shown for the (A) non-Asian and (B) Asian subpopulations. Incidence was age-standardized to the 2006 general population of Canada. Error bars indicate 95% confidence intervals.
Table 3.4. Incidence of MS and related disorders in the Asian and non-Asian populations of British Columbia (1986-2010).

<table>
<thead>
<tr>
<th>Year</th>
<th>Non-Asian</th>
<th></th>
<th>Asian</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Female</td>
<td>Male</td>
<td>Total</td>
</tr>
<tr>
<td>1986-2010</td>
<td>10.21 (9.99-10.45)</td>
<td>14.49 (14.11-14.88)</td>
<td>5.75 (5.51-6.01)</td>
<td>1.02 (0.85-1.23)</td>
</tr>
<tr>
<td>1986-1990</td>
<td>10.41 (9.87-10.97)</td>
<td>14.42 (13.56-15.35)</td>
<td>6.21 (5.63-6.86)</td>
<td>1.04 (0.52-1.86)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>9.83 (9.34-10.35)</td>
<td>13.54 (12.74-14.39)</td>
<td>5.97 (5.44-6.56)</td>
<td>0.40 (0.15-0.88)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>10.92 (10.42-11.44)</td>
<td>16.04 (15.20-16.92)</td>
<td>5.58 (5.08-6.12)</td>
<td>0.75 (0.44-1.20)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>9.86 (9.27-10.49)</td>
<td>14.22 (13.29-15.21)</td>
<td>5.32 (4.71-6.00)</td>
<td>0.82 (0.43-1.39)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>9.91 (9.46-10.39)</td>
<td>14.13 (13.37-14.92)</td>
<td>5.52 (5.04-6.03)</td>
<td>2.02 (1.55-2.58)</td>
</tr>
</tbody>
</table>

Five-year, mean annual incidence rates are expressed as cases per 100,000 and age-standardized to the 2006 general population of Canada; 95% confidence intervals are indicated in parentheses.
Stratification of the Asian cohort by sex and diagnosis revealed that the rising incidence was driven by a sharp increase in the number of incident female MS cases, particularly between 2001 and 2010 (Figure 3.3A; Table 3.5). In this group, incidence increased from 0.71 per 100,000 (95% CI: 0.23-1.65) to 2.08 per 100,000 (95% CI: 1.43-2.91) between 1986 and 2010 ($p<0.004$). However, this increase was driven primarily by changing incidence between the 2001-2005 and 2006-2010 intervals. In contrast, the incidence of NMO in the Asian-ethnic population of BC during the same period remained relatively stable (Figure 3.3B; Table 3.5).
Figure 3.3. Incidence of MS and NMO in the Asian population of British Columbia (1986-2010).

Five-year mean annual incidence rates for (A) MS and (B) NMO were age-standardized to the 2006 general population of Canada. Error bars indicate 95% confidence intervals.
Table 3.5. Incidence of MS and NMO in the Asian population of British Columbia (1986-2010).

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th></th>
<th></th>
<th>NMO</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Female</td>
<td>Male</td>
<td>Total</td>
<td>Female</td>
</tr>
<tr>
<td><strong>1986-2010</strong></td>
<td>0.59 (0.45-0.74)</td>
<td>0.85 (0.64-1.11)</td>
<td>0.31 (0.16-0.54)</td>
<td>0.39 (0.27-0.53)</td>
<td>0.69 (0.48-0.96)</td>
</tr>
<tr>
<td><strong>1986-1990</strong></td>
<td>0.64 (0.26-1.32)</td>
<td>0.71 (0.23-1.65)</td>
<td>0.57 (0.07-2.05)</td>
<td>0.40 (0.08-1.17)</td>
<td>0.79 (0.16-2.30)</td>
</tr>
<tr>
<td><strong>1991-1995</strong></td>
<td>0.26 (0.08-0.61)</td>
<td>0.36 (0.07-1.06)</td>
<td>0.15 (0.00-0.86)</td>
<td>0.14 (0.00-0.79)</td>
<td>0.28 (0.01-1.54)</td>
</tr>
<tr>
<td><strong>1996-2000</strong></td>
<td>0.45 (0.22-0.81)</td>
<td>0.54 (0.22-1.12)</td>
<td>0.35 (0.07-1.03)</td>
<td>0.30 (0.11-0.65)</td>
<td>0.59 (0.22-1.28)</td>
</tr>
<tr>
<td><strong>2001-2005</strong></td>
<td>0.26 (0.08-0.61)</td>
<td>0.37 (0.08-1.09)</td>
<td>0.14 (0.00-0.79)</td>
<td>0.55 (0.24-1.09)</td>
<td>0.72 (0.26-1.57)</td>
</tr>
<tr>
<td><strong>2006-2010</strong></td>
<td>1.19 (0.84-1.64)</td>
<td>2.08 (1.43-2.91)</td>
<td>0.26 (0.05-0.77)</td>
<td>0.60 (0.36-0.95)</td>
<td>1.08 (0.63-1.73)</td>
</tr>
</tbody>
</table>

Five-year, mean annual incidence rates are expressed as cases per 100,000 and age-standardized to the 2006 general population of Canada; 95% confidence intervals are indicated in parentheses.
Stratification by place of birth revealed a significant increase in MS incidence among females in both Canadian-born and immigrant Asian subpopulations (Table 3.6). Between 1986 and 2010, the mean annual incidence of MS doubled from 2.43 per 100,000 (95% CI: 0.50-7.08) to 4.97 per 100,000 (95% CI: 2.57-8.70) in Canadian-born Asian females ($p<0.003$) and increased more than six-fold from 0.23 per 100,000 (95% CI: 0.23-1.27) to 1.49 per 100,000 (95% CI: 0.92-2.27) in immigrant Asian females ($p<0.001$). Between 1986 and 2010, the mean age at migration to Canada among Asian immigrant patients (25.8 years; 95% CI: 22.3-29.3 years) did not change significantly ($p<0.203$).
Table 3.6. Incidence of MS and NMO in the Canadian-born and immigrant Asian population of British Columbia (1986-2010).

<table>
<thead>
<tr>
<th></th>
<th>Canadian-born</th>
<th>Immigrant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS Female</td>
<td>MS Male</td>
</tr>
<tr>
<td>1986-2010</td>
<td>2.54 (1.65-3.76)</td>
<td>0.76 (0.25-1.77)</td>
</tr>
<tr>
<td>1986-1990</td>
<td>2.43 (0.50-7.08)</td>
<td>0 (0-0.00)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>1.98 (0.41-5.78)</td>
<td>0.56 (0.01-3.10)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>1.03 (0.13-3.73)</td>
<td>2.01 (0.05-11.21)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>2.16 (0.26-7.80)</td>
<td>0 (0-0.00)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>4.97 (2.57-8.70)</td>
<td>0.93 (0.11-3.35)</td>
</tr>
</tbody>
</table>

Five-year, mean annual incidence rates are expressed as cases per 100,000 and age-standardized to the 2006 general population of Canada. 95% confidence intervals are indicated in parentheses.
3.4 Discussion

From the perspective of epidemiological inquiry, BC is unique in that it is home to both a European-ethnic majority population with high MS risk and a large ethnic minority population of Asian extraction with relatively low baseline MS risk. In fact, the latter ethnic group accounted for over 15% of the BC population in the 2006 Census of Canada\textsuperscript{440}. This is the first study in Canada to evaluate the prevalence and incidence of MS and related disorders in the Asian-ethnic subpopulation.

Asian populations are generally regarded as being relatively resistant to MS, presumably due to protective genetic factors, as well as lower environmental risk. The findings reported here corroborate this notion, as the prevalence of MS and related disorders in the Asian-ethnic population of BC was approximately 15 times less than that in the general population of BC\textsuperscript{44}. Although such a disparity in prevalence is consistent with the existing literature\textsuperscript{40,43}, the prevalence in this population was considerably greater than that historically reported in Asia (<2 per 100,000), and greater still than that more recently reported in Hong Kong, Taiwan, and South Korea (2.96-4.8 per 100,000)\textsuperscript{62-64}. Recent epidemiological surveys conducted in Japan\textsuperscript{57-61} reported prevalence rates of 7.7-16.2 per 100,000, similar to that in this study, although it warrants noting that these prevalence estimates are among the highest in Asia. Taken together, the findings of this study suggest that the burden of MS in the Asian-ethnic population of BC is likely intermediary to that in Asia and Canada.

The present study was the first to estimate systematically the prevalence of NMO and related disorders in an ethnic population in Canada. The prevalence of NMO and its spectrum disorders in this population (5.06 per 100,000, 95% CI: 3.46-7.13) was more than five times that recently
reported in Japan (0.9 per 100,000)\textsuperscript{61} and, in fact, was among the highest reported in any study to date. An epidemiological survey in the predominantly Caucasian population of Denmark yielded a similar prevalence estimate (4.4 per 100,000)\textsuperscript{88}, suggesting an important role of the regional environment in shaping NMO risk. Furthermore, the greater sex disparity in the prevalence of NMO observed in this study is consistent with the near-universal finding of greater female predilection of NMO/NMOSD relative to MS\textsuperscript{24}.

A large disparity in the frequency of MS and related disorders was also noted between Canadian-born Asians and those who immigrated to Canada. This was true for the pooled analysis of all diagnoses, as well as within the MS and NMO diagnostic subgroups. These observations suggest potential relevance of the early-life (prior to migration) regional environment in modifying susceptibility. Early migration studies in MS demonstrated similar findings, whereby adolescence was the approximate cut-off point up to which MS susceptibility was modifiable by a high-risk environment\textsuperscript{288,340}. The fact that this disparity in prevalence differed in magnitude between MS and NMO subgroups implies that the excess risk conferred by the regional environment in Canada may be more important for MS than NMO. This hypothesis is formally explored in Chapter 6 of this dissertation.

The 10-fold difference in MS incidence between the non-Asian (predominantly Caucasian) and Asian populations in this study was in keeping with the higher baseline risk in Caucasian populations. A recent study in US military veterans reported a considerably smaller disparity in MS incidence between Asian and non-Asian sub-populations in the US\textsuperscript{449}. Authors of that study reported a three-fold difference in MS incidence in the overall US veteran population compared to those self-identifying as Asian or Pacific Islander. Notably, the reported incidence rates
among Asians in the US veteran study were remarkably high in general. The highly selected nature of the study population in the US study (i.e., military personnel) is one possible explanation for this seeming discrepancy.

In the present study, the standardized incidence of MS in Asians was higher than that recently reported in northern Japan, a region noted for having one of the highest reported MS incidence rates in East Asia. Similar to that study, increasing incidence of MS in females amid relative stability in the rates of NMO was noted in the present analysis. Collectively, these observations imply temporal elevation in MS risk in females in both populations. Given the brevity of this period, constitutional genetic changes can be reasonably ruled out. The authors of the Japanese study ascribe this epidemiological trend to “westernization” of the Japanese environment, lucid characterization of which warrants further study.

There are some important methodological limitations worth noting in this study. Foremost among these is the possibility of incomplete case ascertainment. Given the clinic-based, referral-dependent approach used in this study, incident and prevalent cases seeking care through services outside of the UBC MS Clinic would have been overlooked. Cases with mild or atypical clinical presentation would be particularly vulnerable to being excluded in this way. Incomplete ascertainment would have resulted in an underestimate of prevalence and incidence of MS and related disorders in this population. It is also important to note that the estimated province-wide case capture rate (80%) on which this adjustment was based was itself derived from previous studies at the UBC MS Clinic that examined ascertainment rates in the general population of BC. These estimates were not specific to any particular ethnic group. It may therefore be suggested that the scaling factor may not be applicable to the Asian-ethnic population of BC, given that the
vast majority of members of this ethnic group reside in the Metro Vancouver region, rather than being broadly distributed across the province\textsuperscript{440}.

Other circumstances leading to ascertainment biases in this ethnic group, particularly those changing over time, also warrant consideration. For instance, historical under-recognition of MS and related disorders in persons of Asian ancestry may have discouraged referral by primary care-providers of putative cases to MS clinics in the earlier time periods in this study. Similarly, limited public awareness of these disorders in previous years, particularly in non-Caucasian populations in which MS is rare, may have been an additional impediment to care-seeking by such individuals. The possibility that overall reticence among new immigrants with regard to voluntary care-seeking may have diminished over time—whether due to evolving attitudes toward Western medicine, decreasing language barriers, or other factors—should also be considered, as all of these circumstances would be compatible with the data in the present study.

It may therefore be argued that the increasing incidence of MS in Asians simply reflects advances in case ascertainment in this emerging patient population. For instance, improvements in the sensitivity of diagnosis, increases in referral to MS specialists, or a lower threshold of care-seeking among Asians would all be expected to cause an apparent rise in incidence. These explanations, however, may not entirely account for the differential trends in incidence, as systemic improvements in MS diagnosis or changes in patterns of referral would reasonably impact observed MS incidence rates in the overall population, as well as incidence of NMO in the Asian subpopulation. Furthermore, relative stability over time in the length of diagnostic delay in Asian patients challenges the notion that changing patterns in care-seeking are primarily driving the increasing frequency of MS in this population. Moreover, increasing MS incidence in
Asian immigrants was concurrent with only modest overall changes in the mean age at immigration. Therefore, changing age structure in this subpopulation can also be reasonably ruled out as the primary explanation for the upward trend in MS incidence.

Nevertheless, these findings are, to varying degrees, conducive to differing explanations. On one hand, the increase in MS incidence in the Asian-ethnic population may be a spurious trend, perhaps more readily ascribed to temporal changes in case ascertainment. In support of this view is the precipitous nature of the increasing incidence, which was largely restricted to the period between 2001 and 2010, and was noticeably more abrupt than that reported in studies in Asia\textsuperscript{60,61}. This sharp rise in MS incidence is atypical compared to most epidemiological observations of increasing incidence in populations\textsuperscript{42} and is more consistent with geographically restricted epidemics of MS\textsuperscript{450}.

On the other hand, the observed increase in MS incidence in the Asian-ethnic population may reflect, to some extent, a genuine increase in risk. Supportive evidence in this regard includes the fact that the increase in MS frequency was restricted to the female subpopulation, which coheres with observations in several other populations\textsuperscript{42}. Moreover, the female-specificity of this trend challenges the notion that referral biases owing to increasing recognition of MS and related disorders in Asians among healthcare professionals would adequately explain the present findings, as improvements in identifying and diagnosing Asian-ethnic cases would likely also extend to males. The fact that diagnostic delay did not appreciably decrease over time (as would be predicted by improvements to case ascertainment) in Asian patients lends further support to the view that the present findings do not merely reflect ascertainment biases.
A major implication of an actual increase in MS incidence is that a female-specific environmental risk factor may be emerging in this population. Viable candidates to this end include known risk factors for MS\textsuperscript{253}, such as vitamin D deficiency and tobacco smoking. It is conceivable that these factors could impart differential risk based on ethnic differences in exposure level or interaction with genetic factors.

Tobacco smoking in Asian countries, for instance, follows a pattern consistent with our observations, exhibiting increasing use among young adults in Asian populations, particularly in females\textsuperscript{451–453}. If similar trends were present in the Asian-ethnic population of BC, smoking behaviour could be a major explanatory factor. This hypothesis is further explored in Chapter 7. Vitamin D deficiency is also plausibly implicated as a possible factor underlying this trend, as it is known that darker skin pigmentation, such as those in some Asian-ethnic groups, is associated with lower serum levels of vitamin D\textsuperscript{454}. Excess MS risk associated with vitamin D deficiency may therefore be amplified in these individuals by reduced penetration of UVB radiation (needed to promote vitamin D biosynthesis), particularly in the northern latitudes of Canada. Asian cultural mores favouring avoidance of sun exposure by females also corroborate this hypothesis.

It is must be emphasized that the aforementioned interpretations of the data are not mutually exclusive. It is plausible that the trends observed in the present study may be attributable to a combination of a genuine increase in MS incidence and improvements to case ascertainment. Given the relative infrequency of MS and related disorders in this population, as well as the unusual abrupt and recent nature of this apparent increase in incidence, further investigations are recommended. For instance, prospective studies surveying trends in the incidence of MS and
related disorders in the Asian-ethnic population of Canada may bring further clarity to this intriguing epidemiological phenomenon.

Nonetheless, this study features a number of methodological strengths. To optimize comprehensive and uniform case ascertainment, cases were identified from the principal MS clinic in BC, including its network of affiliated clinics serving the province’s major urban centres. Furthermore, the publicly funded, universal healthcare system in Canada, which is accessible to all permanent BC residents irrespective of socioeconomic status, greatly minimized the risk of referral bias. Additionally, trends in incidence were evaluated using reasonably conservative statistical analysis that was appropriate to the disparate cohort sample sizes encountered in this study.

Although other explanations such as ascertainment biases cannot be ruled out, these findings are suggestive of a possible female-specific, temporal increase in the risk of MS and related disorders in the Asian-ethnic population of BC. Restriction of an environmental risk factor to a demographic subset would have profound implications for disease prevention and understanding etiology, as it implies the existence of an in situ modifiable risk factor. Further work will be needed to establish the authenticity of this epidemiological trend and to characterize the putative risk factor(s) involved.
Chapter 4: Clinical profile of MS and related disorders in Asians

4.1 Introduction

The longstanding notion of MS as a disease restricted to Caucasian populations is increasingly challenged by growing recognition of MS and related disorders in other ethnic groups. The emergence of these disorders in historically under-recognized populations is perhaps no more readily apparent than in Asians. Clinical awareness of MS in this ethnic group has increased considerably in the past decade. Despite this encouraging trend, studies characterizing the clinical features of MS and related disorders in Asian-ethnic patients are comparatively sparse.

The most salient clinical feature distinguishing MS and related disorders in patients of Asian ancestry from Caucasian patients is the dichotomy of clinical phenotypes: Conventional clinical presentation is seen in approximately two-thirds of patients, whereas NMO-like clinical presentation, featuring optic nerve and spinal cord predilection, is seen in approximately one-third of patients. The latter clinical phenotype is exceptionally rare in Caucasian patient populations, typically comprising less than 2% of patients. NMO and its diverse clinical variants are characteristically more aggressive than conventional forms of MS. MS and NMO clinical phenotypes are, in fact, dissimilar in many other respects as well.

Thus, lucid description of MS and related disorders in Asian-ethnic populations demands that cases be accurately classified according to diagnosis. Nevertheless, few studies have fulfilled this condition, despite major recent advances in nosological distinction of MS and NMO. Therefore, accurate clinical descriptions of MS and NMO in Asian-ethnic patients remain elusive, being encumbered by historical conflation of MS and NMO. In addition, considerable
heterogeneity in methodologies and study populations of past research in this population has also resulted in substantial disagreement with respect to fundamental clinical features. Together, these issues have impeded efforts to establish a consensus clinical description of MS and related disorders in patients of Asian ancestry. Consequently, present understanding of factors associated with clinical outcomes in this patient population is also very limited.

The objective of the present study is to provide a comprehensive clinical account of MS and related disorders in Asian-ethnic patients ascertained from clinic settings in Canada and China. Particular emphasis was placed on analyses of clinical outcomes and factors related to prognosis in patients stratified by clinical phenotype, as well as geographical location, in order to address shortcomings of previous studies in this ethnic group. The bulk of existing research in Asian-ethnic patients have given inadequate attention to the distinct clinical patterns of MS and NMO and failed to consider possible regional differences in clinical profile. Notably, there have been no published studies to date on clinical characteristics of MS and related disorders in patients of Asian ancestry in Canada. Moreover, the body of literature on prognostic indicators in patients of Asian ancestry is virtually non-existent.

4.2 Methods

4.2.1 Case ascertainment and clinical characterization

This was a retrospective cohort study analyzing the clinical profile of MS and related disorders in patients of Asian ancestry from two distinct clinic populations. Eligible patients in Canada were primarily identified from the UBC MS Clinic through the ascertainment protocols detailed in Section 2.1. In addition, eligible Asian cases from 11 other Canadian MS clinics were also
identified by screening the CCPGSMS database. Eligible patients from a population in Shanghai, China, were enrolled through a collaborating neurological centre at Huashan Hospital, as detailed in Section 2.1.

This study included patients fulfilling eligibility criteria related to ethnicity and diagnosis. With regard to the former, patients reporting full East or Southeast Asian ancestry were considered eligible. Patients with partial Asian ancestry were excluded from the present analysis in order to enable direct comparisons of Canadian and Chinese patient cohorts matched to ethnicity. All included patients were diagnosed with an eligible MS spectrum disorder, which included definite or probable MS, NMO/NMOSD, and CIS. Patients were diagnosed by an MS-specialist neurologist at their respective clinics.

Cross-sectional clinical data were obtained from each patient by retrospective review of clinical records conducted at the respective clinic site following enrolment. Data were recorded in standardized case report forms to ensure comparability between clinic sites across Canada and in China. This study analyzed clinical data corresponding to assessments up to August 30, 2013. Relevant data on the following clinical and demographic variables were extracted from medical records: sex, maternal and paternal ethnic origins, age at last clinical follow-up, age at symptom onset, age at diagnosis, diagnostic classification, clinical course, and most recent EDSS score. In addition, the Multiple Sclerosis Severity Score (MSSS)\textsuperscript{455}, a validated disease severity index based on percentile rank of EDSS within strata of disease duration, was determined for all patients.
4.2.2 Statistical analyses

Demographic and clinical characteristics of the Canadian and Chinese patient cohorts were compared with a Fisher exact test for categorical variables, \( t \)-test for continuous variables, and Wilcoxon rank sum test for discrete quantitative variables. Subgroup analyses included comparison of clinical and demographic features between Canadian and Chinese patient cohorts stratified by clinical phenotype.

To investigate factors related to prognosis, two clinical outcomes—progressive course and disease severity—were evaluated in the Chinese and Canadian patient cohorts, as well as in the pooled cohort and subgroups of clinical phenotypes. Patients with progressive course at any stage of disease (i.e., PPMS, SPMS, or PRMS) were considered to be progressive, whereas those with an exclusively benign or relapsing course were considered not to be progressive. Disease severity was represented by the MSSS, a continuous outcome variable derived from the EDSS score and disease duration\(^{455}\). These outcomes were selected as indices of clinical prognosis, as they have consistently been associated with disability progression in long-term, longitudinal cohort studies\(^{110,456}\). Moreover, these outcome measures were particularly well suited to the present analyses due to the cross-sectional nature of the data and the possibility of large cohort disparities in clinical follow-up owing to regional differences in access to care.

Factors associated with progressive clinical course were evaluated with multivariable logistic regression to account for potential confounding. In this analysis, progressive course was modeled as a function of several predictor covariates, selected \textit{a priori} on the basis of evidence from previous natural history studies\(^{110,457}\) or from evidence of independent association in simple logistic regression models. CIS cases were excluded from this analysis, as this clinical variant,
by definition, precludes progressive clinical course. Covariates included in the multivariable logistic regression model were as follows, with the reference level listed first in parentheses: diagnosis (MS, NMO), sex (female, male), age at onset, and disease duration. The magnitude of association of modeled covariates with progressive course was reported as odds ratios with 95% confidence intervals.

Association of clinical and demographic factors with disease severity was evaluated with multivariable linear regression, in which the MSSS index was modeled as a response variable dependent on predictor covariates of diagnosis, sex, and age at onset (selected as above). Disease duration was excluded as a covariate for disease severity because MSSS is defined in terms of disease duration\textsuperscript{455}. The covariate-adjusted effect of each predictor variable was reported as the regression coefficient, $\beta$, with corresponding 95% confidence intervals.

4.3 Results

4.3.1 Patient characteristics

In total, 246 patients with full Asian-ethnic origins fulfilled selection criteria, including 114 cases from MS clinics in Canada and 132 cases from Huashan Hospital in Shanghai, China. Of 114 cases in the Canadian cohort, 95 were ascertained through the UBC MS Clinic, while 19 were registered at 11 other MS clinics across Canada. The majority of cases were diagnosed with MS (67.5%), followed by NMO/NMOSD (30.1%) and CIS (2.4%). Clinical and demographic characteristics of the cohorts are shown in Table 4.1. Cohorts were similar with respect to sex ratio, age at last clinical assessment, and disease severity as indicated by the MSSS. The Chinese cohort exhibited modest trends toward later onset, greater relative frequency of NMO/NMOSD,
lower frequency of primary progressive course, and lower EDSS disability level, although none of the differences was significant. Disease duration was significantly greater in the Canadian cohort (6.0 vs. 3.0 years, $p<0.001$).
Table 4.1. Clinical and demographic characteristics of Asian patients ascertained at clinics in Canada and Shanghai, China.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
<th>( p ^{a} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients, ( n )</strong></td>
<td>246</td>
<td>114</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td><strong>Sex, ( n (%) )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>190 (77.2)</td>
<td>85 (74.6)</td>
<td>105 (79.5)</td>
<td>0.365</td>
</tr>
<tr>
<td>Male</td>
<td>56 (22.8)</td>
<td>29 (25.4)</td>
<td>27 (20.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, median years (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>30.0 (24.0-41.0)</td>
<td>29.0 (24.0-37.5)</td>
<td>32.0 (24.8-42.3)</td>
<td>0.076</td>
</tr>
<tr>
<td>At last assessment</td>
<td>38.5 (30.0-50.3)</td>
<td>39.5 (31.0-51.0)</td>
<td>38.0 (27.0-49.3)</td>
<td>0.183</td>
</tr>
<tr>
<td><strong>Diagnosis, ( n (%) )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>166 (67.5)</td>
<td>83 (72.8)</td>
<td>83 (62.9)</td>
<td>0.092</td>
</tr>
<tr>
<td>NMO</td>
<td>74 (30.1)</td>
<td>27 (23.7)</td>
<td>47 (35.6)</td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>6 (2.4)</td>
<td>4 (3.5)</td>
<td>2 (1.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical course at onset, ( n (%) ) (^{b})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>164 (91.6)</td>
<td>61 (87.1)</td>
<td>103 (94.5)</td>
<td>0.101</td>
</tr>
<tr>
<td>Progressive</td>
<td>15 (8.4)</td>
<td>9 (12.9)</td>
<td>6 (5.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease duration, median years (IQR)</strong></td>
<td>4.0 (1.0-8.0)</td>
<td>6.0 (3.0-11.8)</td>
<td>3.0 (1.0-5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDSS, median (IQR)</td>
<td>2.5 (1.0-4.5)</td>
<td>3.0 (1.5-6.0)</td>
<td>2.0 (1.0-4.0)</td>
<td>0.087</td>
</tr>
<tr>
<td>MSSS, median (IQR)</td>
<td>3.86 (2.14-6.86)</td>
<td>4.30 (2.39-7.30)</td>
<td>3.34 (1.77-6.46)</td>
<td>0.273</td>
</tr>
</tbody>
</table>

\(^{a}\) \( p \)-values refer to comparisons between the Canadian Asian cohort and the Shanghai cohort.

\(^{b}\) Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range

EDSS, Expanded Disability Status Scale

MSSS, Multiple Sclerosis Severity Score
When analysis was restricted to MS cases, Canadian and Chinese patients were remarkably similar across most clinical and demographic characteristics (Table 4.2). However, when only NMO/NMOSD cases were considered, cohort differences emerged with respect to age, disease duration, and disability level (Table 4.3). The Canadian NMO patients were significantly older (49.0 vs. 38.0 years, \( p=0.037 \)), had greater disease duration (9.0 vs. 3.0 years, \( p<0.001 \)), and had considerably higher disability level (EDSS 5.0 vs. 2.0, \( p=0.020 \)). The MSSS disease severity index was greater in the Canadian cohort, although not significantly (Table 4.3).
**Table 4.2. Clinical and demographic characteristics of MS patient cohorts.**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
<th>( p ^{a} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients, ( n )</strong></td>
<td>166</td>
<td>83</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td><strong>Sex, ( n (%) )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>119 (71.7)</td>
<td>59 (71.1)</td>
<td>60 (72.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>47 (28.3)</td>
<td>24 (28.9)</td>
<td>23 (27.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, median years (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>30.0 (24.0-39.0)</td>
<td>29.0 (23.0-36.0)</td>
<td>32.0 (25.0-40.0)</td>
<td>0.149</td>
</tr>
<tr>
<td>At last assessment</td>
<td>38.0 (30.0-50.0)</td>
<td>37.0 (31.0-50.0)</td>
<td>38.0 (28.5-48.5)</td>
<td>0.682</td>
</tr>
<tr>
<td><strong>Clinical course at onset, ( n (%) ) ( ^{b} )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>126 (89.4)</td>
<td>49 (84.5)</td>
<td>77 (92.8)</td>
<td>0.165</td>
</tr>
<tr>
<td>Progressive</td>
<td>15 (10.6)</td>
<td>9 (15.5)</td>
<td>6 (7.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease duration, median years (IQR)</strong></td>
<td>4.0 (1.0-8.8)</td>
<td>6.0 (3.0-10.0)</td>
<td>3.0 (1.0-6.0)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>EDSS, median (IQR)</strong></td>
<td>2.5 (1.0-4.5)</td>
<td>2.5 (1.5-5.0)</td>
<td>2.0 (1.0-4.0)</td>
<td>0.350</td>
</tr>
<tr>
<td><strong>MSSS, median (IQR)</strong></td>
<td>4.21 (1.78-7.13)</td>
<td>4.08 (2.32-4.63)</td>
<td>4.21 (1.61-7.65)</td>
<td>0.832</td>
</tr>
</tbody>
</table>

\( ^{a} \) \( p \)-values refer to comparisons between the Canadian Asian cohort and the Shanghai Chinese cohort.

\( ^{b} \) Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range

EDSS, Expanded Disability Status Scale

MSSS, Multiple Sclerosis Severity Score
### Table 4.3. Clinical and demographic characteristics of NMO/NMOSD patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients, n</strong></td>
<td>74</td>
<td>27</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>69 (93.2)</td>
<td>25 (92.6)</td>
<td>44 (93.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>5 (6.8)</td>
<td>2 (7.4)</td>
<td>3 (6.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, median years (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>32.0 (25.0-44.0)</td>
<td>31.5 (27.3-40.8)</td>
<td>33.0 (24.0-45.0)</td>
<td>0.872</td>
</tr>
<tr>
<td>At last assessment</td>
<td>44.0 (30.0-51.0)</td>
<td>49.0 (38.0-53.5)</td>
<td>38.0 (26.0-50.0)</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Clinical course at onset, n (%)^b</strong></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Relapsing</td>
<td>38 (100)</td>
<td>12 (100)</td>
<td>26 (100)</td>
<td></td>
</tr>
<tr>
<td>Progressive</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease duration, median years (IQR)</strong></td>
<td>3.0 (1.75-6.50)</td>
<td>9.0 (4.8-13.5)</td>
<td>3.0 (1.0-4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>EDSS, median (IQR)</strong></td>
<td>2.5 (1.5-5.0)</td>
<td>5.0 (2.5-6.5)</td>
<td>2.0 (1.5-4.0)</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>MSSS, median (IQR)</strong></td>
<td>3.34 (2.44-6.17)</td>
<td>5.41 (2.97-8.01)</td>
<td>2.64 (2.01-4.82)</td>
<td>0.067</td>
</tr>
</tbody>
</table>

^a p-values refer to comparisons between the Canadian Asian cohort and the Shanghai Chinese cohort.

^b Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range

EDSS, Expanded Disability Status Scale

MSSS, Multiple Sclerosis Severity Score
4.3.2 Factors associated with clinical outcomes

Clinical and demographic predictors of progressive disease course and disease severity were examined with logistic and linear regression, respectively. In the overall patient cohort, NMO diagnosis was associated with a nearly 10-fold decrease in the risk of primary or secondary progression relative to MS (OR=0.11, \( p=0.035 \)) (Table 4.4). Similarly, males were at a significantly greater risk to develop progressive clinical course (OR=2.60, \( p=0.034 \)) (Table 4.4). Stratification of cases by diagnosis into MS and NMO subgroups revealed that this association was driven exclusively by an excess of male MS cases with progressive clinical course (Table 4.5). In contrast to these findings, neither diagnosis nor sex was associated with disease severity, as indicated by the MSSS (Table 4.6). However, later age at onset was associated with a modest increase in MSSS (\( \beta=0.10, p<0.001 \)) in both the Canadian and Chinese patient cohorts, as well as in both MS (\( \beta=0.11, p<0.001 \)) and NMO (\( \beta=0.09, p=0.024 \)) subgroups (Table 4.7).
Table 4.4. Association of demographic and clinical factors with progressive disease course.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>NMO</td>
<td>0.11 (0.01-0.56)</td>
<td>0.035</td>
<td>0.12 (0.01-0.84)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Male</td>
<td>2.60 (1.07-6.33)</td>
<td>0.034</td>
<td>1.69 (0.52-5.60)</td>
</tr>
<tr>
<td>Age at onset</td>
<td>1.02 (0.98-1.06)</td>
<td>0.242</td>
<td>1.05 (0.99-1.11)</td>
</tr>
<tr>
<td>Disease duration</td>
<td>1.03 (0.98-1.08)</td>
<td>0.261</td>
<td>1.02 (0.96-1.10)</td>
</tr>
</tbody>
</table>

OR, odds ratio

CI, confidence interval
Table 4.5. Association of demographic and clinical factors with progressive disease course in MS and NMO patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>NMO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Male</td>
<td>2.58 (1.06-6.30)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Age at onset</strong></td>
<td>1.02 (0.98-1.06)</td>
<td>0.371</td>
</tr>
<tr>
<td><strong>Disease duration</strong></td>
<td>1.03 (0.97-1.08)</td>
<td>0.301</td>
</tr>
</tbody>
</table>

OR, odds ratio
CI, confidence interval
Table 4.6. Association of demographic and clinical factors with disease severity.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MS</td>
<td>0.95 (-3.22 – 5.13)</td>
<td>0.652</td>
<td>1.14 (-4.89 – 7.16)</td>
<td>0.708</td>
<td>0.56 (-5.50 – 6.61)</td>
<td>0.855</td>
</tr>
<tr>
<td>NMO</td>
<td>0.14 (-4.17 – 4.46)</td>
<td>0.948</td>
<td>1.07 (-5.27 – 7.40)</td>
<td>0.738</td>
<td>-0.55 (-6.73 – 5.64)</td>
<td>0.861</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Male</td>
<td>-0.74 (-2.04 – 0.55)</td>
<td>0.259</td>
<td>-0.69 (-2.38 – 1.01)</td>
<td>0.422</td>
<td>-0.99 (-3.18 – 1.20)</td>
<td>0.371</td>
</tr>
<tr>
<td><strong>Age at onset</strong></td>
<td>0.10 (0.06 – 0.14)</td>
<td>&lt;0.001</td>
<td>0.10 (0.03 – 0.17)</td>
<td>0.007</td>
<td>0.10 (0.04 – 0.16)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

β, linear regression coefficient
CI, confidence interval
--. reference level
Table 4.7. Association of demographic and clinical factors with disease severity in MS and NMO patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th></th>
<th>NMO</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Male</td>
<td>-0.78 (-2.17 – 0.62)</td>
<td>0.272</td>
<td>-0.34 (-5.91 – 5.23)</td>
<td>0.902</td>
</tr>
<tr>
<td>Age at onset</td>
<td>0.11 (0.05 – 0.16)</td>
<td>&lt;0.001</td>
<td>0.09 (0.01 – 0.16)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

β, linear regression coefficient
CI, confidence interval
--, reference level
4.4 Discussion

In the present study, patients of full Asian ancestry diagnosed with MS or related disorders were examined in case series ascertained at clinics in Canada and Shanghai, China. The clinical profile in these geographically distinct but ethnically comparable populations was remarkably similar. Relative proportions of MS, NMO/NMOSD, and CIS were similar between the cohorts, as were measures in fundamental demographic and clinical characteristics, including sex ratio, age at onset, clinical course at onset, and indices of disability and severity.

The relative proportion of cases with MS and NMO clinical phenotypes was similar to that commonly observed in previous case series in Asian populations. Most such studies have reported an approximately 2-3:1 ratio of MS to NMO. Prior to this study, the relative proportions of MS and NMO had not been reported in any Asian patient population residing in a high-prevalence region. That the dichotomy of clinical phenotype was also observed among Asian patients residing in Canada, a region in which NMO is exceedingly rare, strongly suggests that the relative enrichment of NMO in Asians may be a universal feature of the disease in this ethnic group, irrespective of the environment. Similarity in the demographic and clinical features of MS and related disorders between patient populations in Canada and China also supports the notion of relative uniformity of the disease in this ethnic group across diverse environments.

Given the intrinsic nature of the chief features of MS and related disorders in this population, it is eminently plausible that genetic factors underlie this observation. However, culturally pervasive environmental factors that are ubiquitous in native and migrant Asian populations cannot be
ruled out. Further studies will be needed to establish the basis of clinical uniformity across Asian populations in dissimilar environments.

Similar to observations in case cohorts from predominantly Caucasian populations\(^{123,459–462}\), male sex and later onset of symptoms were associated with an increased risk of progressive clinical course and greater disease severity in Asian patients in the present study. These findings suggest that prognostic factors such as sex and age at initial symptoms may be broadly applicable to patients across different populations. However, few studies to this end have been conducted in non-Caucasian populations, and this is among the first studies to investigate prognostic factors for MS and related disorders in patients of Asian ancestry. The collective findings of this study and previous investigations in other Caucasian populations support the notion of pathophysiological distinction of MS and related disorders between males and females. A variety of genetic, epigenetic, and environmental factors underlying sex differences in clinical prognosis have been proposed in this regard\(^{463}\), although characterization of these mechanisms remains elusive.

On the other hand, the association of later age at onset with greater disability are more amenable to explanation. Aging is itself correlated with a decline in mobility, even in healthy individuals. However, in persons with MS, the effects of aging further compound the functional deterioration accompanying the disease, as well as the ability to cope with such changes\(^{464}\). NMO, which is characterized by later onset and more rapid disability accrual compared to MS, comprised approximately 30% of cases in this study. It therefore warrants consideration that the correlation between age at onset and disease severity may have been driven, in part, by relative enrichment of NMO. However, this association cannot be explained solely by the presence of NMO cases, as
demonstrated by a residual effect of age after adjustment for diagnosis in the multivariable analysis.

The cross-sectional nature of the clinical data is the chief limitation of this study. Unavailability of longitudinal clinical data in the Chinese case series precluded time-dependent analysis of disability progression, which would adjust for varying duration of clinical follow-up between cohorts. However, the MSSS disease severity index, which takes disease duration into account, was intended to address these concerns. The MSSS has previously been validated as a predictive index of disease progression over time\textsuperscript{456}.

Small sample sizes have historically been a formidable obstacle to characterizing the clinical profile of MS and related disorders accurately in Asian populations owing to the rarity of these disorders in this ethnic group. This study profiling clinical aspects of MS and related disorders in patients of Asian ancestry is among the largest of its kind in terms of the total number cases. Moreover, this was the first study to characterize and compare clinical profiles in distinct Asian populations from regions of divergent environmental risk. Few previous studies in Asian populations have differentiated NMO clinical variants from conventional MS. Indeed, the rigorous diagnostic classification, which enabled clinical phenotypes to be factored into the analysis, was a key methodological strength of this study.

A multi-centre approach was used in this study to clarify the clinical characteristics of MS and related disorders in persons of Asian ancestry. Observation of overall similarity in the clinical profile of these disorders between patient cohorts from regions with distinct profiles of environmental risk and health care landscape suggests relative uniformity of clinical characteristics in this ethnic group. On this basis, it may reasonably be proposed that clinical
practice guidelines could be standardized across Asian patient populations from differing regions. This is particularly topical amid the ongoing expansion of Asian-ethnic populations in regions with high MS risk, such as North America and Europe. In addition, identification of prognostic factors associated with progressive disease course and disease severity in the present study may guide clinical management of MS and related disorders in Asian patients, particularly in the newly diagnosed. Although these findings contribute to the burgeoning but comparatively incomplete body of literature on the clinical features of MS and related disorders in Asian populations, additional large-scale cohort studies will be needed to move toward a more complete understanding of the clinical scope of these disorders in this emerging patient population.
Chapter 5: Role of ethnicity in clinical outcomes of MS and related disorders in Asians

5.1 Introduction

Early MS research in Asian populations was influential in shaping initial and enduring beliefs concerning clinical outcomes in this ethnic group. In addition to the conspicuous dichotomy of major clinical phenotypes (i.e., MS versus NMO), many of these studies in Asian patients painted a prognostically calamitous clinical picture. These early studies featured MS cases with fulminant symptoms, more aggressive clinical course, and unfavourable clinical outcomes compared to the typical clinical presentation in Caucasian patients. Although some contemporary and later studies challenged the earlier findings, the perception that MS and related disorders were more malignant in Asian-ethnic patients generally did not subside. Moreover, subsequent studies substantiating the notion of overall poor prognosis in Asian patients lent further credence and durability to these earlier claims.

With recent and ongoing advances in the nosology of NMO, it is now increasingly clear in retrospect that many of these earlier studies subsumed patients exhibiting NMO-like clinical presentation (for instance, OSMS) with those presenting with conventional MS. It warrants noting that consolidation of these clinical variants as a single nosological entity (i.e., simply as “MS”) was in fact consistent with best practices at the time. Indeed it is only with recent advances in nosology that we can assuredly maintain that NMO and NMOSD are distinct from conventional MS in many respects. Furthermore, the majority of patients previously classified as OSMS would very likely fulfill modern criteria for NMO or NMOSD. Because the clinical
outcomes in NMO and NMOSD are generally worse than in MS, it stands to reason that historical conflation of these disorders in Asian populations may have resulted in a distorted, if not wholly inaccurate, clinical picture. Presumptive ethnic differences in clinical outcomes of MS are therefore open to doubt, given that the true clinical profile of conventional MS in Asian patients remains equivocal.

The objective of this study was to determine whether clinical outcomes of MS and related disorders differ in patients of Asian ancestry versus those in a general (predominantly Caucasian) clinic population at a Canadian MS clinic after clinical phenotype has been taken into account. There have been no studies to date in a high-risk population comparing clinical outcomes of MS in Asian-ethnic patients to those in non-Asian patients from the same clinic population.

5.2 Methods

5.2.1 Case ascertainment and acquisition of clinical data

Differences in the clinical features and outcomes of MS and related disorders between patients of Asian ancestry relative to the general clinic population were evaluated through a retrospective cohort study analyzing prospectively collected, longitudinal clinical data. All clinical data included in this study were extracted from the UBC MS Clinic clinical research database, a detailed description of which is provided in Section 2.2.2. The database was screened to select patients with eligible diagnoses, which were classified according to the following diagnostic rubric: (1) MS: probable or definite MS; (2) NMO: definite NMO or NMOSD; and (3) Possible MS: CIS and other clinical presentations consistent with MS, but otherwise not meeting diagnostic criteria. Clinical records for all eligible patients with clinical assessments performed
at the UBC MS Clinic between October 1, 1980, and February 28, 2013, were included in the present analysis. Relevant data pertaining to the following demographic and clinical variables were analyzed: sex, age at onset, age at diagnosis, age at assessment, diagnosis, year of initial assessment, clinical course, relapse rate, serial EDSS scores, and DMT indication status. MSSS disease severity index was derived according to previously described methods\textsuperscript{455} for all patients with at least one EDSS score.

Patients were assigned to either the Asian or non-Asian cohort on the basis of their self-reported ethnic origins, the classification of which is detailed in Section 2.1. For this study, the Asian cohort was restricted to patients with full Asian ancestry. Patients with partial Asian ancestry were excluded. All other patients were assigned to the non-Asian cohort, which in the present analysis served as the comparator, representative of the general patient population. In subgroup analyses, cohorts were further divided into subgroups defined by clinical phenotype (i.e., MS or NMO) and era of clinical follow-up, in which analysis was restricted to patients initially assessed after 1995. The aim of these secondary analyses was to identify diagnosis-specific and cohort effects. The latter consideration was necessary because it was recognized \textit{a priori} that the overwhelming majority of patients in the Asian cohort were clinically evaluated within the last two decades; in contrast, clinic visits by patients in the non-Asian cohort were distributed more evenly over the follow-up period.

In addition, retrospective chart review to extract relevant paraclinical data was performed on a broader cohort of Asian-ethnic patients assessed at the UBC MS Clinic prior to December 31, 2013. Due to the later ascertainment cut-off date, this cohort included several patients that were not encompassed in the Asian patient cohort. Comparative analysis for the following paraclinical
laboratory investigations, where available, were performed in MS and NMO subgroups of this larger cohort: CSF oligoclonal banding, NMO-IgG serology, initial brain MRI, and spinal cord MRI.

5.2.2 Statistical analyses

Cohorts were compared with respect to clinical and demographic characteristics. Differences were assessed with a Fisher exact test for categorical variables, $t$-test for continuous variables, and Wilcoxon rank sum test for discrete quantitative variables. In addition, MS and NMO subgroups in the Asian cohort were likewise compared to identify clinical and demographic differences between the major clinical phenotypes in patients of Asian ancestry.

Clinical outcomes with respect to disability accrual were compared between cohorts. The primary outcome in this study was the time elapsed from symptom onset to first clinical visit in which an EDSS score of 6.0 or greater was assigned. An EDSS score of 6.0, which corresponds to a level of disability in which a walking aid (such as a cane or crutches) is required to ambulate 100 metres, was selected for this study on account of its functional relevance and overall temporal stability (discussed in Section 1.5.3). Cohort differences in the time to reach EDSS 6.0 were evaluated by time-to-event survival analysis using the Kaplan-Meier estimator method in order to adjust for considerable variation in the duration of clinical follow-up among cases. Survival analysis was performed in all patients with an eligible diagnosis, as specified above, as well as in a subgroup limited to patients with a diagnosis of MS. To adjust for potential temporal cohort effects, additional subgroup analyses were performed within a subset of patients initially assessed after 1995; this was the year during which the first efficacious disease-modifying therapy for MS was licensed in Canada. Patients who did not reach EDSS 6.0 disability during
clinical follow-up period were right-censored at the time of their final clinical assessment. Left-censored patients initially presenting to clinic with an EDSS $\geq 6.0$ were included in the present analysis by assigning the date of initial clinical assessment as the event time. Cohort differences in survival functions were evaluated by the Mantel-Cox log-rank test.

Clinical and demographic factors associated with the rate of disability accrual to EDSS 6.0 was examined with multivariable Cox proportional hazards regression. Time to event was modeled as a function of several predictor covariates, which were selected and fitted to the regression model through step-wise addition. The following covariates, with the reference level listed first in parentheses, were included in the regression model: ethnic group (non-Asian, Asian), diagnosis (MS, NMO/NMOSD), sex (female, male), age at onset, year of initial clinical assessment, and clinical course at onset (relapsing, progressive). Cox proportional hazards regression modeling was also performed in the MS diagnostic subgroup to identify determinants of disability progression specific to MS. Covariates associated with the rate of disability accrual in regression models were summarized with hazard ratios and 95% confidence intervals.

5.3 Results

5.3.1 Patient characteristics

A total of 5,535 patients fulfilling selection criteria, of which 89 were of full Asian ancestry and 5,446 were non-Asian, were included in this study. A substantial majority of cases in the overall patient cohort were diagnosed with MS (91.1%), followed distantly by CIS (7.5%). In the Asian cohort, however, MS accounted for just 60.7% ($n=54$) of cases, while NMO and NMOSD collectively comprised 30.3% of cases ($n=27$), and CIS comprised 9.0% of cases ($n=8$). Clinical
and demographic characteristics of the Asian and non-Asian patient cohorts are shown in Table 5.1. Although cohorts were similar in terms of age at onset, clinical course at onset, MSSS disease severity index, and indication rate of first-line therapies, several notable differences were observed, particularly with respect to age, disease duration, and diagnostic delay. At last clinical assessment, non-Asian patients were considerably older (50.4 vs. 42.3 years, \( p<0.001 \)), had longer disease duration (16.1 vs. 9.6 years, \( p<0.001 \)), and longer interval from onset to diagnosis (7.0 vs. 4.2 years, \( p<0.001 \)) relative to Asian patients. These observation were consistent with Asian patients occupying a more recent period of clinical follow-up, which was substantiated by a later median year of initial clinical assessment, some 9 years later than in the non-Asian cohort \( (p<0.001) \). Of 89 Asian cases, 74 (83.1%) were initially assessed at the UBC MS Clinic after 1995. Cohorts also differed with respect to clinical features such as relapse rate and disability. Annualized relapse rate was significantly higher in Asian patients (0.501 vs. 0.376, \( p=0.006 \)), as was the indication rate of second-line therapies (22.5% vs 3.1%, \( p<0.001 \)). However, EDSS scores for disability were higher in the non-Asian cohort (3.5 vs. 2.5, \( p<0.001 \)).
Table 5.1. Clinical and demographic characteristics of Asian and non-Asian patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Asian</th>
<th>Non-Asian</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, ( n )</td>
<td>89</td>
<td>5,446</td>
<td></td>
</tr>
<tr>
<td>Sex, ( n ) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>71 (79.8)</td>
<td>3,947 (72.5)</td>
<td>0.150</td>
</tr>
<tr>
<td>Male</td>
<td>18 (20.2)</td>
<td>1,499 (27.5)</td>
<td></td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>30.0 (23.1-40.2)</td>
<td>31.6 (25.2-39.8)</td>
<td>0.288</td>
</tr>
<tr>
<td>At last assessment</td>
<td>42.3 (34.1-51.1)</td>
<td>50.4 (41.4-58.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diagnosis, ( n ) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>54 (60.7)</td>
<td>4,991 (91.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NMO/NMOSD</td>
<td>27 (30.3)</td>
<td>47 (0.9)</td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>8 (9.0)</td>
<td>408 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Clinical course at onset, ( n ) (%)(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>76 (95.0)</td>
<td>4,619 (88.4)</td>
<td>0.076</td>
</tr>
<tr>
<td>Progressive</td>
<td>4 (5.0)</td>
<td>607 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Disease duration, median years (IQR)</td>
<td>9.6 (4.9-13.9)</td>
<td>16.1 (9.1-25.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Annualized relapse rate, mean (IQR)(^a)</td>
<td>0.501 (0.15-0.63)</td>
<td>0.376 (0.078-0.407)</td>
<td>0.006</td>
</tr>
<tr>
<td>Diagnostic delay, median years (IQR)</td>
<td>4.2 (1.4-8.4)</td>
<td>7.0 (2.9-14.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDSS, median (IQR)</td>
<td>2.5 (1.5-5.5)</td>
<td>3.5 (2.0-6.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>MSSS, median (IQR)</td>
<td>3.45 (1.16-6.58)</td>
<td>4.35 (1.80-7.40)</td>
<td>0.162</td>
</tr>
<tr>
<td>Therapy, ( n ) (%)(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-line</td>
<td>31 (34.8)</td>
<td>1,869 (34.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Second-line</td>
<td>20 (22.5)</td>
<td>169 (3.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^a\)Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range; EDSS, Expanded Disability Status Scale; MSSS, Multiple Sclerosis Severity Score
Stratification by diagnosis enabled investigation of cohort differences specific to MS.
Observations remained largely unchanged when analysis was restricted to MS patients, with cohort differences in age, disease duration, relapse rate, diagnostic delay, EDSS, and second-line treatment indication remaining intact (Table 5.2). However, when analysis was restricted to patients with initial clinical assessment after 1995, cohort differences diminished further, and cohorts were not significantly different in key clinical domains such as EDSS disability level, MSSS disease severity index, and rate of treatment indication for first-line therapies (Table 5.2).
Table 5.2. Clinical and demographic characteristics of MS patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>All MS patients</th>
<th>Initially assessed after 1995</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asian</td>
<td>Non-Asian</td>
</tr>
<tr>
<td>Patients, n</td>
<td>54</td>
<td>4,991</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40 (74.1)</td>
<td>3,623 (72.6)</td>
</tr>
<tr>
<td>Male</td>
<td>14 (25.9)</td>
<td>1,368 (27.4)</td>
</tr>
<tr>
<td>Ratio (F/M)</td>
<td>2.86</td>
<td>2.65</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>28.4 (22.4-37.5)</td>
<td>31.3 (25.0-39.3)</td>
</tr>
<tr>
<td>At last assessment</td>
<td>41.6 (34.5-49.9)</td>
<td>50.6 (41.9-58.9)</td>
</tr>
<tr>
<td>Clinical course at onset, n (%) b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>46 (92.0)</td>
<td>4,321 (88.9)</td>
</tr>
<tr>
<td>Progressive</td>
<td>4 (8.0)</td>
<td>542 (11.1)</td>
</tr>
<tr>
<td>Disease duration, median years (IQR)</td>
<td>8.5 (5.1-14.0)</td>
<td>16.7 (9.8-25.5)</td>
</tr>
<tr>
<td>Annualized relapse rate, mean (IQR) b</td>
<td>0.43 (0.14-0.61)</td>
<td>0.346 (0.076-0.386)</td>
</tr>
<tr>
<td>Diagnostic delay, median years (IQR)</td>
<td>3.7 (1.2-7.7)</td>
<td>7.2 (3.1-14.2)</td>
</tr>
<tr>
<td>EDSS, median (IQR)</td>
<td>2.0 (1.5-4.5)</td>
<td>3.5 (2.0-6.5)</td>
</tr>
<tr>
<td>MSSS, median (IQR)</td>
<td>3.34 (1.04-5.93)</td>
<td>4.35 (1.92-7.54)</td>
</tr>
<tr>
<td>Therapy, n (%) b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-line</td>
<td>27 (50.0)</td>
<td>1,835 (36.8)</td>
</tr>
<tr>
<td>Second-line</td>
<td>6 (11.1)</td>
<td>146 (2.9)</td>
</tr>
</tbody>
</table>

* p-values refer to comparisons between the respective Asian and non-Asian patient cohorts.

b Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range; EDSS, Expanded Disability Status Scale; MSSS, Multiple Sclerosis Severity Score
Differences between MS and NMO subgroups in the Asian cohort were also examined (Table 5.3). Relative to MS, NMO patients exhibited trends toward greater female preponderance (sex ratio: 12.5 vs. 2.86, \( p=0.074 \)), older age at onset (36.0 vs. 28.4 years, \( p=0.084 \)), older age at last assessment (48.4 vs. 41.6 years, \( p=0.091 \)), longer diagnostic delay (7.3 vs. 3.7 years, \( p=0.056 \)), and greater MSSS disease severity index (5.28 vs. 3.34, \( p=0.071 \)). Disability level at last follow-up, as indicated by median EDSS scores, was also greater in NMO compared to MS (3.5 vs. 2.0, \( p=0.038 \)).

Paraclinical studies routinely used in diagnosing MS and NMO were examined specifically in patients of Asian ancestry (Table 5.4). MS cases exhibited low yield of CSF oligoclonal banding (51.9\%) and abnormal initial brain MRI (70.5\%). NMO-IgG seropositivity was noted in only 38.1\% of NMO cases but was highly specific for this diagnosis, being observed in none of the tested MS cases. In addition, spinal cord lesions extending across three or more vertebral segments on MRI were highly sensitive and specific for NMO, being present in 75\% of NMO cases, but only 4.3\% of MS cases (\( p<1\times10^{-6} \)).
Table 5.3. Clinical and demographic characteristics of Asian MS and NMO patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>NMO</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>54</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40 (74.1)</td>
<td>25 (92.6)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (25.9)</td>
<td>2 (7.4)</td>
<td></td>
</tr>
<tr>
<td>Ratio (F/M)</td>
<td>2.86</td>
<td>12.50</td>
<td>0.074</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>28.4 (22.4-37.5)</td>
<td>36.0 (26.0-44.9)</td>
<td>0.084</td>
</tr>
<tr>
<td>At last assessment</td>
<td>41.6 (34.5-49.9)</td>
<td>48.4 (39.3-55.2)</td>
<td>0.091</td>
</tr>
<tr>
<td>Clinical course at onset, n (%) a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>46 (92.0)</td>
<td>24 (100)</td>
<td>0.297</td>
</tr>
<tr>
<td>Progressive</td>
<td>4 (8.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Disease duration, median years (IQR)</td>
<td>8.5 (5.1-14.0)</td>
<td>12.1 (7.9-14.5)</td>
<td>0.273</td>
</tr>
<tr>
<td>Annualized relapse rate, mean (IQR) a</td>
<td>0.43 (0.14-0.61)</td>
<td>0.64 (0.18-0.66)</td>
<td>0.946</td>
</tr>
<tr>
<td>Diagnostic delay, median years (IQR)</td>
<td>3.7 (1.2-7.7)</td>
<td>7.3 (3.2-10.0)</td>
<td>0.056</td>
</tr>
<tr>
<td>EDSS, median (IQR)</td>
<td>2.0 (1.5-4.5)</td>
<td>3.5 (2.5-7.0)</td>
<td>0.038</td>
</tr>
<tr>
<td>MSSS, median (IQR)</td>
<td>3.34 (1.04-5.93)</td>
<td>5.28 (2.65-7.93)</td>
<td>0.070</td>
</tr>
<tr>
<td>Therapy, n (%) a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-line</td>
<td>27 (50.0)</td>
<td>4 (14.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Second-line</td>
<td>6 (11.1)</td>
<td>14 (51.9)</td>
<td>0.120</td>
</tr>
</tbody>
</table>

a Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range

EDSS, Expanded Disability Status Scale

MSSS, Multiple Sclerosis Severity Score
Table 5.4. Paraclinical characteristics of Asian MS and NMO patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>NMO</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>72</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>CSF oligoclonal banding, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14 (51.9)</td>
<td>5 (23.8)</td>
<td>0.075</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (48.1)</td>
<td>16 (76.2)</td>
<td></td>
</tr>
<tr>
<td>NMO-IgG, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0 (0.0)</td>
<td>8 (38.1)</td>
<td>0.011</td>
</tr>
<tr>
<td>Negative</td>
<td>15 (100.0)</td>
<td>13 (61.9)</td>
<td></td>
</tr>
<tr>
<td>Brain MRI (initial scan), n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>43 (70.5)</td>
<td>4 (13.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal/non-specific</td>
<td>18 (29.5)</td>
<td>26 (86.7)</td>
<td></td>
</tr>
<tr>
<td>LESCL on MRI, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2 (4.3)</td>
<td>27 (75.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>45 (95.7)</td>
<td>9 (25.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range

CSF, cerebrospinal fluid

NMO-IgG, Anti-aquaporin-4 autoantibody serology

LESCL, longitudinally extensive spinal cord lesion
5.3.2 Disability progression

Cohort differences in the rate of disability accrual from onset was studied using Kaplan-Meier survival analysis in which the first occurrence of EDSS 6.0 disability was selected as the endpoint. A total of 84 Asian and 4,756 non-Asian patients with at least one EDSS score during clinical follow-up were included in the analysis. Median survival in the Asian cohort (22.0 years, 95% CI: 15.0-29.1) was similar to that in the non-Asian cohort (22.1 years, 95% CI: 21.5-22.9). However, visual inspection of the overall survival curves of each cohort revealed that EDSS 6.0-free survival was lower in the Asian cohort for nearly all time points during the first 28 years of disease (Figure 5.1). The overall difference in the survival function of each cohort, while modest in magnitude, was in fact significant by log-rank test ($p=0.0288$). Survival analysis restricted to patients with initial clinical assessment after 1995 reinforced this finding. In this subgroup analysis on 70 Asian and 2,302 non-Asian patients, median survival in the Asian cohort (15.0 years, 95% CI: 12.1-27.5) was considerably less than that in non-Asians (25.5 years, 95% CI: 23.9-28.1, $p=0.0031$).
Patients at risk, $n$

<table>
<thead>
<tr>
<th>Years</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian</td>
<td>84</td>
<td>62</td>
<td>32</td>
<td>16</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-Asian</td>
<td>4,756</td>
<td>3,932</td>
<td>2,967</td>
<td>2,004</td>
<td>1,284</td>
<td>753</td>
<td>403</td>
<td>183</td>
<td>80</td>
<td>28</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 5.1. Disability progression from onset to EDSS 6 in Asian and non-Asian patients with MS and related disorders.

Survival functions are shown as solid, black lines for the Asian cohort and broken, grey lines for the non-Asian cohort. Vertical hash marks indicate right-censoring.
To explore whether observed cohort differences could be explained by a greater proportion of cases with NMO clinical phenotype in the Asian cohort, survival analysis was restricted to MS patients. A total of 52 Asian and 4,425 non-Asian patients were included in this analysis. Median EDSS 6.0-free survival in Asian patients (24.2 years, 95% CI: 22.0-27.5) was similar to that in non-Asian patients (22.0 years, 95% CI: 21.4-22.9), and the overall survival functions (Figure 5.2) did not differ significantly between cohorts ($p=0.46$). Moreover, when analysis was further circumscribed to patients with an initial clinical assessment after 1995, median EDSS 6.0-free survival among Asian patients (27.9 years, 95% CI: 11.1-27.5) remained similar to that in non-Asians (25.8 years, 95% CI: 23.9-28.1), and overall survival functions were not significantly different ($p=0.10$).
Patients at risk, \( n \)

<table>
<thead>
<tr>
<th>Years</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian</td>
<td>52</td>
<td>39</td>
<td>21</td>
<td>11</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-Asian</td>
<td>4,425</td>
<td>3,751</td>
<td>2,842</td>
<td>1,923</td>
<td>1,238</td>
<td>731</td>
<td>394</td>
<td>178</td>
<td>77</td>
<td>26</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 5.2. Disability progression from onset to EDSS 6 in Asian and non-Asian patients with conventional MS.

Survival functions are shown as solid, black lines for the Asian cohort and broken, grey lines for the non-Asian cohort. Vertical hash marks indicate right-censoring.
5.3.3 Determinants of disability progression

Factors associated with the rate of disability progression to EDSS 6.0 were examined with multivariable Cox regression models (Table 5.5). Notably, Asian ethnicity was not associated with an increase in hazard to reach EDSS 6.0 after adjustment for clinical phenotype (diagnosis), age at onset, sex, and year of initial clinical assessment. On the other hand, NMO clinical phenotype (HR=2.86, 95% CI: 1.72-4.75, \( p<0.0001 \)), male sex (HR=1.16, 95% CI: 1.06-1.28, \( p<0.0021 \)), later age at onset (HR=1.02, 95% CI: 1.01-1.02, \( p<0.0001 \)), and progressive clinical course (HR=1.83, 95% CI: 1.62-2.07, \( p<0.0001 \)) were all factors associated with increased hazard of reaching the disability endpoint. The year of initial clinical assessment was negatively associated with hazard to reach EDSS 6.0 (HR=0.95, 95% CI: 0.95-0.96, \( p<0.0001 \)). When regression analysis was confined to patients with initial clinical assessment after 1995, findings were not appreciably modified from those in the analysis in the complete patient cohort.
Table 5.5. Association of demographic and clinical factors with disability progression from onset to EDSS 6.0 in patients with MS and related disorders.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Initially assessed after 1995</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Asian</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1.02 (0.65-1.60)</td>
<td>0.915</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>NMO</td>
<td>2.86 (1.72-4.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.16 (1.06-1.28)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age at onset (per year)</td>
<td>1.02 (1.01-1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year of first assessment (per year)</td>
<td>0.95 (0.95-0.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical course at onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Progressive</td>
<td>1.83 (1.62-2.07)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HR, hazard ratio

--, reference level
5.4 Discussion

There is considerable debate as to whether clinical features and outcomes of MS and related disorders in patients of Asian ancestry differ from those in the general patient population in a high-risk region. To date, few studies have compared clinical outcomes in Asian and non-Asian patients from the same clinic population with uniform methods of ascertainment and clinical assessment. Prior to this work, no such studies had been conducted in a region with high MS prevalence. Here, a retrospective cohort design was used in the comparative analysis of clinical differences between patients of Asian ancestry and those from a general (predominantly Caucasian) clinic population in Canada. In keeping with expectation, the relative proportions of MS and NMO clinical phenotypes in Asian and non-Asian patients differed significantly: NMO comprised >30% of Asian patients but was exceptionally rare (<1%) among non-Asians. This disparity in the relative abundance of NMO is a long-recognized clinical observation that is conclusively established in the literature.

Direct comparison of cohort characteristics between Asian patients with MS and NMO confirmed that the latter is distinguished by greater female preponderance, later onset, and a higher level of disability. Moreover, analysis of paraclinical studies confirmed that NMO in this ethnic group is characterized by a lower frequency of CSF oligoclonal banding and abnormal initial brain MRI. Among paraclinical findings, the low diagnostic sensitivity of CSF OCB for MS and NMO-IgG for NMO in Asian patients was noteworthy, particularly when compared against typical yields in Caucasian populations. The markedly lower frequency of OCB in MS and IgG seropositivity in NMO in Asian-ethnic patients in the present analysis is in keeping with a number of previous studies in Asian patients in which CSF OCB status and NMO-IgG
seropositivity\textsuperscript{468,469} were reported in MS and NMO patients, respectively. Taken together, these findings suggest that diagnostic sensitivity of paraclinical studies such as CSF OCB and NMO-IgG may be characterized by ethnic differences.

A more intriguing finding was that, apart from the above conspicuous differences in relative frequencies of clinical phenotypes, Asian and non-Asian patients were remarkably similar with respect to several fundamental demographic and clinical characteristics, particularly when clinical phenotypes and disease duration were taken into account. There were no significant cohort differences in sex ratio, age at onset, or clinical course at onset. Small, albeit significant, cohort differences in relapse frequency and disability level were observed, even when analysis was restricted to MS. Disparities in disability level, disease severity score, and indication rate for first-line therapies, however, were markedly diminished in magnitude and were not significant when analysis was restricted to patients with an initial clinical assessment after 1995, indicative of a temporal cohort effect. Moreover, the pace of progression to EDSS 6.0 disability level was similar between MS patient cohorts when NMO cases were excluded from analysis.

Collectively, these results lend credence to the assertion that clinical outcomes of conventional MS (that is, excluding NMO and its spectrum disorders) in Asian-ethnic patients are essentially equivalent to those in Caucasian patients. These findings are consistent with a number of earlier studies in Asian populations suggesting overall similarity between the ethnic groups\textsuperscript{31,51,97,98}, but contradict other studies demonstrating a more rapidly debilitating and commonly fatal disease among Asians\textsuperscript{53,55,126}.

The bulk of the early published research on clinical outcomes of MS in Asian populations did not adequately distinguish between MS and NMO. Consequently, patients with clinical presentation
consistent with NMO were subsumed in MS cohorts. In light of the findings in the present study, it is reasonable to speculate that the marked clinical differences observed between Asian and Caucasian patients in previous studies were chiefly driven by disparities in the relative frequency of NMO, a clinical variant typified by a demonstrably more pernicious natural history.

Further corroborating this notion was the finding that Asian-ethnicity was not associated with disability progression per se in multivariable Cox regression models of disability progression among MS patients. Diagnosis of NMO, on the other hand, was associated with a nearly three-fold increase in hazard to reach the disability endpoint. Moreover, risk factors for clinical progression previously established in Caucasian populations (e.g., age at onset, sex, progressive course) were in fact more robustly predictive of clinical trajectory than ethnicity in regression analysis among MS patients. Taken together, these results again suggest that clinical outcomes of MS, at least with respect to the rate of disability accrual to EDSS 6.0, may be more similar in Asian and Caucasian patients than previously proposed, once diagnosis of NMO is properly taken into account.

Survival analysis with EDSS 6.0 disability level as the endpoint revealed a median survival time of approximately 24 years in the Asian cohort when MS alone was considered. This estimate is in line with findings in a recent study in a Korean MS cohort that used the same disability endpoint in survival analysis. Median event-free survival in the Korean study, which also carefully excluded NMO cases in the analysis of MS cases, was 20 years. The nominal difference in median survival among ethnically similar patients in these studies can be reasonably ascribed to regional differences in a variety of extrinsic factors, such as frequency and accuracy of clinical assessment, access to care, and perhaps even environmental exposures.
modifying disease course. Tobacco smoking and nutritional factors are plausible candidates in regard to the latter. Median event-free survival in the present analysis was also consistent with recent estimates derived from large cohort studies in predominantly Caucasian populations in North America and Europe, falling squarely within the combined range of 15 to 32 years reported across six studies collectively encompassing over 10,000 patients.

Some limitations of the present study warrant mentioning. Although NMO and NMOSD were carefully and accurately differentiated from MS in the Asian patient cohort through rigorous review of individual medical records, equivalent classification of NMO variants was unfeasible in the non-Asian cohort due to the large number of cases. Diagnostic classification in the latter cohort was therefore based on the status indicated in the clinical research database, which prior to 2008 did not include a specific code for NMO. As a result, it is probable that perhaps as many as 100 non-Asian NMO cases assessed prior to 2008 were either misclassified as not having MS or as having opticospinal variant MS, the latter generally being coded as MS. Consequently, these de facto NMO cases would have been included among MS cases in the present analyses involving the non-Asian cohort. However, the number of such spurious cases would have been so small as to be materially inconsequential during analysis, given the rarity of NMO (<2% of patients attending MS clinics) in Caucasian populations. The small number of misclassified cases in the Caucasian cohort would, in effect, be diluted by the approximately 5,000 legitimate MS cases.

Multivariable regression analysis of factors associated with disability progression confirmed the existence of a temporal cohort effect, whereby patients with more recent clinical evaluations experienced more favourable clinical outcomes in general. A similar inverse relationship
between the pace of disability progression and time of evaluation was evident in a recent review of several large cohort studies on long-term clinical outcomes of MS\textsuperscript{110}. This cohort effect initially presented a methodological concern because Asian patients were, on average, assessed more recently than non-Asian patients. To mitigate possible confounding arising from a temporal cohort effect, subgroup analysis was performed in cohorts restricted to patients with first clinical evaluation after 1995. Additionally, statistical adjustment for this cohort effect was achieved by incorporating the year of initial clinical assessment as a covariate in Cox proportional hazard regression models.

The findings presented here challenge the prevailing notion that clinical outcomes of MS, particularly with respect to disability progression, are worse in patients of Asian ancestry. Few studies to date have applied longitudinal methods such as Kaplan-Meier survival analysis to characterize long-term clinical outcomes of MS in Asian case series. Indeed, this is the first study to directly compare clinical features and outcomes of MS between Asian-ethnic patients and non-Asian patients with high risk from the same clinic population. Several methodological strengths lend additional weight to these findings. Foremost, methods of case ascertainment and clinical evaluation were uniformly applied to both the Asian-ethnic and comparator cohort. Particularly relevant to this study, clinical follow-up assessment, including the diagnostic work-up and determination of EDSS scores, was performed by the same clinic team for both cohorts. Further underscoring the overall uniformity of evaluation between cohorts was the largely equivalent access to and quality of care afforded to all patients in the publicly-funded, universal healthcare system of Canada. Equal access to medically necessary care ensured that economic
barriers to accessing healthcare, which may be particularly problematic in some immigrant populations\textsuperscript{471–473}, did not unduly influence clinical outcomes in either cohort.

Finally, scrupulous diagnostic classification of Asian cases in this study, including careful exclusion of NMO cases in MS-only subgroup analyses, enabled valid cohort comparisons within subgroups defined by clinical phenotype. The principal shortcoming of the vast majority of previous clinical studies of MS and related disorders in Asian populations is that this diagnostic heterogeneity had not being taken into proper account. The findings reported here, bolstered by a methodologically rigorous approach, cast doubt on previous conclusions of MS being substantively more clinically aggressive in patients of Asian ethnic origins. This study has potentially important clinical implications, as it expands the evidence basis for accurate prognostication and clinical management in this emerging patient population.
Chapter 6: Association of geography with clinical phenotype in Asians with MS and related disorders

6.1 Introduction

It has long been known that the geographic location of residence early in life is associated with the risk to develop MS later in life. Latitude is indeed one of the most consistent predictors of susceptibility to MS, evinced time and again in MS studies of global prevalence and migration. On the other hand, the influence of geographic factors on clinical phenotype is less clear. No previous studies have explored the role of the regional environment on clinical phenotype of MS and related disorders in patients of Asian ancestry. Apart from ethnicity, the determinants of clinical phenotype are, as yet, unknown.

In the present study, exposure to factors related to the region of residence in the pre-onset period were examined in MS and NMO/NMOSD patients of Asian ancestry residing in BC. The objective of this retrospective study was to determine whether environmental factors related to the geographic region of residence are associated with clinical phenotype of MS and related disorders. The Asian patient population on which this study focused was especially informative for three reasons: (1) The large proportion of cases with NMO-like presentation enabled an analysis based on the dichotomous clinical phenotypes; (2) the relative homogeneity in ethnicity of this cohort obviated the need to adjust for ethnicity as a confounding variable; and (3) the large proportion of patients who migrated from Asia to Canada enabled a wide range of the exposure variable—geographic region of residence prior to symptom onset—to be evaluated in this study. For these reasons, this population was well suited to the present investigation.
6.2 Methods

6.2.1 Case ascertainment, and data collection

Eligible cases were retrospectively and prospectively identified from the UBC MS Clinic as previously described in Section 2.1. Since the primary objective of this study was to explore the impact of the macro-geographic environment on clinical phenotype, only diagnoses consistent with either conventional MS (probable or definite MS) or NMO (definite NMO or NMOSD) were included in the primary analysis. Patients were stratified by clinical phenotype into MS and NMO cohorts, as described previously (Section 2.2.2). Diagnostic classification was performed blinded to place of birth or migration history. Relevant data on ethnic origins and personal history of places of residence were acquired through standardized questionnaires as detailed in Section 2.2.3. This study was limited to patients with full Asian ethnic origins, the rationale for which is discussed in Section 2.1.

6.2.2 Statistical analysis

Logistic regression was used to model clinical phenotype as a function of several predictor covariates. Selection of covariates incorporated in the final multivariable logistic regression model was informed by preliminary simple logistic regression analyses in which the outcome was modeled independently as a function of individual candidate covariates. In the final multivariable logistic regression model, clinical phenotype was modeled as a function of the nominated demographic and geographic covariates. Covariates included in the model were as follows, with the range of levels indicated in parentheses and the first level as the reference: sex (male, female), age (per year), place of birth (Canada, other), place of symptom onset (Canada,
other), age at immigration to Canada (per year), and duration of residence in Canada prior to onset of symptoms (0, 1-10, 11-20, 21-30, 31-40, and ≥41 years). The magnitude of association of modeled covariates with clinical phenotype was reported as odds ratios with 95% confidence intervals.

6.3 Results

6.3.1 Patient clinical and demographic characteristics

Of the 92 patients fulfilling inclusion criteria, 59 (64%) were classified as MS and 33 (36%) were NMO (Table 6.1). There was a greater proportion of females in the NMO cohort (sex ratio: 10.0 vs. 3.2), although the difference did not reach significance ($p=0.099$). Median age at symptom onset was greater in NMO (33 years, interquartile range (IQR): 26-45) than MS (28 years, IQR: 22-37) ($p=0.066$), as was median age at last clinical follow-up (47 years, IQR: 38-55 vs. 40 years, IQR: 34-48; $p=0.025$). Median disease duration at last follow-up did not differ significantly between cohorts (9 years, IQR: 5-15 vs. 7 years, IQR: 4-14; $p=0.444$). There was a trend toward greater frequency of progressive course at onset in MS cases compared to NMO (8.5% vs. 0%; $p=0.156$).

Median EDSS score at last follow-up was greater in NMO cases (3.0 vs. 2.0; $p=0.023$). Due to limited availability of serial EDSS scores in some patients, disability level was also evaluated with the MSSS disease severity index. Median MSSS was greater in NMO (5.53, IQR: 2.72-8.25) compared to MS (3.95, IQR: 0.76-6.54) ($p=0.025$) (Table 6.1).
Table 6.1. Demographic and clinical characteristics of MS and NMO patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>MS</th>
<th>NMO</th>
<th>p a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases, n</td>
<td>92</td>
<td>59</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>75 (81.5)</td>
<td>45 (76.3)</td>
<td>30 (90.9)</td>
<td>0.099</td>
</tr>
<tr>
<td>Male</td>
<td>17 (18.5)</td>
<td>14 (23.7)</td>
<td>3 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At symptom onset, median (IQR)</td>
<td>30 (23-40)</td>
<td>28 (22-37)</td>
<td>33 (26-45)</td>
<td>0.066</td>
</tr>
<tr>
<td>At last follow-up, median (IQR)</td>
<td>43 (34-50)</td>
<td>40 (34-48)</td>
<td>47 (38-55)</td>
<td>0.025</td>
</tr>
<tr>
<td>Disease duration, median (IQR)</td>
<td>8 (4-14)</td>
<td>7 (4-14)</td>
<td>9 (5-15)</td>
<td>0.444</td>
</tr>
<tr>
<td>Disease duration, median (IQR)</td>
<td>8 (4-14)</td>
<td>7 (4-14)</td>
<td>9 (5-15)</td>
<td>0.444</td>
</tr>
<tr>
<td>Disease duration, median (IQR)</td>
<td>8 (4-14)</td>
<td>7 (4-14)</td>
<td>9 (5-15)</td>
<td>0.444</td>
</tr>
<tr>
<td>Clinical course at onset</td>
<td>0.156</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing, n (%)</td>
<td>87 (94.6)</td>
<td>54 (91.5)</td>
<td>33 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Progressive, n (%)</td>
<td>5 (5.4)</td>
<td>5 (8.5)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Disability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDSS, median (IQR)</td>
<td>2.5 (1.5-6.0)</td>
<td>2.0 (1.5-5.5)</td>
<td>3.0 (2.5-6.5)</td>
<td>0.023</td>
</tr>
<tr>
<td>MSSS, median (IQR)</td>
<td>4.5 (1.2-7.3)</td>
<td>3.9 (0.8-6.5)</td>
<td>5.5 (2.7-8.2)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

IQR, interquartile range

EDSS, Expanded Disability Status Scale

MSSS, Multiple Sclerosis Severity Score

*p-values refer to comparisons between MS and NMO cohorts.

Categorical variables were evaluated with Fisher exact test and continuous variables were evaluated with Wilcoxon rank sum test.
6.3.2 Patient ethnicity and geographic characteristics

The majority of patients were of full Chinese ethnic origin (72%), while the remainder were of other East Asian or Southeast Asian ancestries, including Filipino (8%), Japanese (8%), Korean (5%), Vietnamese (3%), Taiwanese (2%), and multiple Asian ethnicities (2%) (Table 6.2). Approximately two-thirds of patients were immigrants born outside of Canada (Table 6.2). The majority of these patients were born in one of the following countries: Hong Kong (31%), China (20%), the Philippines (8%), Vietnam (8%), Taiwan (8%), or South Korea (7%). A greater proportion of NMO cases were born outside of Canada (76% vs. 58%), although this difference was not significant \( p<0.113 \).
Table 6.2. Ethnic origins and region of birth of MS and NMO patient cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>MS</th>
<th>NMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases, n</td>
<td>92</td>
<td>59</td>
<td>33</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>66</td>
<td>43 (72.9)</td>
<td>23 (69.7)</td>
</tr>
<tr>
<td>Filipino</td>
<td>7</td>
<td>7 (11.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Japanese</td>
<td>7</td>
<td>3 (5.1)</td>
<td>4 (12.1)</td>
</tr>
<tr>
<td>Korean</td>
<td>5</td>
<td>2 (3.4)</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>Vietnamese</td>
<td>3</td>
<td>2 (3.4)</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>Taiwanese</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Multiple Asian</td>
<td>2</td>
<td>2 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Country of birth, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>33</td>
<td>25 (42.4)</td>
<td>8 (24.2)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>18</td>
<td>12 (20.3)</td>
<td>6 (18.2)</td>
</tr>
<tr>
<td>China</td>
<td>12</td>
<td>5 (8.5)</td>
<td>7 (21.2)</td>
</tr>
<tr>
<td>Philippines</td>
<td>5</td>
<td>5 (8.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>5</td>
<td>4 (6.8)</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>Taiwan</td>
<td>5</td>
<td>3 (5.1)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>South Korea</td>
<td>4</td>
<td>1 (1.7)</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>4 (6.8)</td>
<td>6 (18.2)</td>
</tr>
</tbody>
</table>
6.3.3 Factors associated with clinical phenotype

After adjustment for sex and age at onset, duration of residence in Canada prior to onset was associated with clinical phenotype (Table 6.3). In particular, intervals of 11-20 years (OR=0.09; 95% CI: 0.01-0.43; p=0.005), 21-30 years (OR=0.08; 95% CI: 0.01-0.39; p=0.004), and 31-40 years (OR=0.06; 95% CI: 0.00-0.72; p=0.043) of residence in Canada were associated with conventional MS (Table 6.3). Onset of symptoms prior to immigrating to Canada (OR=7.23; 95% CI: 1.86-36.42; p=0.007), older age at symptom onset (OR=1.04; 95% CI: 1.00-1.08; p=0.049), and older age at immigration to Canada (OR=1.07; 95% CI: 1.01-1.13; p=0.019) were associated with NMO (Table 6.3).
Table 6.3. Association of demographic and geographic factors with clinical phenotype.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>NMO</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>45 (76.3)</td>
<td>30 (90.9)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (23.7)</td>
<td>3 (9.1)</td>
<td>0.32 (0.07-1.09)</td>
<td>0.094</td>
</tr>
<tr>
<td><strong>Age at onset, median (IQR)</strong></td>
<td>28 (22-37)</td>
<td>33 (26-45)</td>
<td>1.04 (1.00-1.08)</td>
<td>0.049</td>
</tr>
<tr>
<td><strong>Place of birth, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>25 (42.4)</td>
<td>8 (24.2)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>34 (57.6)</td>
<td>25 (75.8)</td>
<td>1.93 (0.72-5.49)</td>
<td>0.201</td>
</tr>
<tr>
<td><strong>Place of onset, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>56 (94.9)</td>
<td>24 (72.7)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (5.1)</td>
<td>9 (27.3)</td>
<td>7.23 (1.86-36.42)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Age at migration, median (IQR)</strong></td>
<td>22 (14-30)</td>
<td>30 (18-41)</td>
<td>1.07 (1.01-1.13)</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>Time in Canada prior to onset, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 years</td>
<td>3 (5.1)</td>
<td>9 (27.3)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>1-10 years</td>
<td>13 (22.0)</td>
<td>10 (30.3)</td>
<td>0.30 (0.05-1.42)</td>
<td>0.146</td>
</tr>
<tr>
<td>11-20 years</td>
<td>18 (30.5)</td>
<td>5 (15.2)</td>
<td>0.09 (0.01-0.43)</td>
<td>0.005</td>
</tr>
<tr>
<td>21-30 years</td>
<td>20 (33.9)</td>
<td>4 (12.1)</td>
<td>0.08 (0.01-0.39)</td>
<td>0.004</td>
</tr>
<tr>
<td>31-40 years</td>
<td>4 (6.8)</td>
<td>1 (3.0)</td>
<td>0.06 (0.00-0.72)</td>
<td>0.043</td>
</tr>
<tr>
<td>≥41 years</td>
<td>1 (1.7)</td>
<td>4 (12.1)</td>
<td>0.92 (0.06-26.46)</td>
<td>0.952</td>
</tr>
</tbody>
</table>

IQR, inter-quartile range

--: reference level
6.4 Discussion

The present study illustrates involvement of geography-associated factors in determining clinical phenotype of MS and related disorders in patients of Asian ancestry. In this population, greater duration of residence in Canada prior to symptom onset was associated with MS, whereas onset of symptoms prior to immigrating to Canada and older age at symptom onset were associated with NMO. Patients who had resided in Canada between 11 and 40 years prior to onset were approximately 10 times more likely to be diagnosed with MS versus NMO. Conversely, those with symptom onset prior to moving to Canada were at 7-fold greater odds of being diagnosed with NMO. In addition, each one-year increase in age at onset was associated with a 5% increase in risk of NMO diagnosis.

These findings imply that factors in the Canadian environment predispose to MS whereas those in the Asian countries of origin predispose to NMO. This model of differential environmental influence on clinical phenotype coheres with established data on the relative frequency of MS and NMO in populations around the world. In predominantly Caucasian populations, the prevalence of NMO is 1-2% that of MS\textsuperscript{85}. In non-Caucasian populations considered to be at low risk for MS, however, the relative frequencies of MS and NMO are more similar. For instance, in Asian populations, NMO typically accounts for 15-40\% of cases\textsuperscript{467}.

These findings are also consistent with observations from previous studies examining shifts in the ratio of MS and NMO in populations undergoing urbanization and other environmental changes. For instance, longitudinal surveillance in Japan demonstrated a temporal increase in the incidence of MS (but not NMO), concurrent with increasing Western influences on society in Japan\textsuperscript{61}. Studies in the French West Indies have also shown similar epidemiological patterns,
whereby the frequency of MS has steadily increased amid relative stability in the incidence of NMO. Changing patterns of exposure to putative environmental risk factors for MS—for example, sun exposure, improvements to hygiene, and transient exposure to risk factors encountered in France among return-immigrants—were cited as possible reasons underlying this trend\textsuperscript{286}.

The factors accounting for the relative prominence of NMO in non-Caucasian patient populations remain poorly understood. Genetic factors are likely to be explanatory to some extent, given that the relative ratio of NMO to MS in certain populations appears to be more tightly linked to ethnicity than the early-life environment. This is, for example, evidenced in the present study by the relatively high proportion of NMO cases among Asian-ethnic patients born and reared in Canada: In total, 24\% of Canadian-born patients had clinical presentation consistent with NMO clinical phenotype, substantially surpassing the expected fraction (1-2\%) in a typical North American clinic population\textsuperscript{85}. In addition, a number of studies have identified genetic associations specific to NMO\textsuperscript{402,413,474–476}.

These results suggest that the regional environment in the period preceding symptom onset may also have a detectable influence on clinical phenotype in Asian populations. Greater pre-onset exposure to the Canadian environment was associated with MS, whereas exposure during the same period to the Asian regional environment was associated with NMO. The mechanism underlying this association is unclear. However, given the environment-associated pattern of phenotype differentiation, one might speculate that the relevant risk factors have contrasting exposure profiles in the respective geographic environments of Canada and Asia. Although the extent of the literature on environmental risk factors for NMO is limited, several environmental
exposures associated with MS fit this model of divergent geographic distribution. Overall solar UV exposure, for instance, is generally greater in the populated areas of East and Southeast Asia relative to the higher latitudes of most urban centres in Canada. Similarly, the prevalence of Epstein-Barr virus infection in adolescents is substantially greater in Asia and other regions with low MS prevalence than in countries with predominantly Caucasian populations. Tobacco smoking, which has also been associated with increased MS risk, also exhibits a similar pattern of differential exposure rates between Canada and many regions of Asia.

In the present study, factors related to the regional environment during the pre-onset period were associated with clinical phenotype in patients of Asian ancestry residing in Canada. These findings highlight the potential prognostic value of non-conventional risk factors in Asian-ethnic patients presenting with demyelinating syndromes in which the clinical phenotype (that is, MS or NMO) has not yet declared itself. Furthermore, these data guide future studies seeking the etiology of these disorders. Additional studies examining the role of candidate environmental risk factors on clinical outcomes in patients of Asian ancestry, particularly with respect to NMO, will be needed to advance the present understanding of the mechanisms underlying these observations.
Chapter 7: Association of smoking with risk and clinical phenotype of MS and related disorders in Asian populations

7.1 Introduction

Although the etiology of MS remains uncertain, genetic and environmental factors have important pathogenic roles. With regard to the latter, tobacco smoking is among the most firmly established environmental risk factors for MS. Smoking is in fact associated with both an increased risk to develop MS and more rapid clinical progression in persons with MS\textsuperscript{304}. The prevalence of tobacco smoking varies considerably around the world, with the highest current rates in the Western Pacific region inclusive of several East and Southeast Asian countries\textsuperscript{428}. Despite emerging evidence suggestive of a simultaneous increase in the prevalence of MS\textsuperscript{60,61} and smoking\textsuperscript{427,478} in many regions of Asia, the effect of smoking on MS risk and progression has not previously been investigated in any Asian population.

The objective of the present study was to determine whether a positive history of smoking is associated with MS susceptibility and adverse clinical outcomes in persons of Asian ethnic origin. This question was explored in distinct Asian-ethnic populations in Canada and Shanghai, China. This is the first investigation of smoking as a putative risk factor and modifier of clinical outcomes in MS and related disorders in Asian populations.
7.2 Methods

7.2.1 Study participants and data acquisition

Cases were retrospectively and prospectively identified from the UBC MS Clinic (Vancouver, Canada) and Huashan Hospital (Shanghai, China) as described in Section 2.1. Patients with full Asian ethnic origins in at least one parental lineage and with a diagnosis of MS, NMO, NMOSD, or CIS were eligible. For this particular study, additional eligible cases ascertained at one of 11 other Canadian MS clinics in the CCPGSMS network were also included, provided that they fulfilled inclusion criteria above. Data on smoking exposure were collected from all cases using the same standardized questionnaire (Appendix B.2) and method of administration (Section 2.2.4), except with differences in language of administration, as appropriate.

For this study, positive history of smoking was defined as having ever smoked 100 or more cigarettes. In addition to being equivalent to variable definitions of ever-smoking in the control datasets, this threshold was justified on the basis of accumulating evidence supporting a dose-response effect of tobacco smoking on MS risk\textsuperscript{305–309}. It was therefore reasoned that a definition of positive smoking history that excluded individuals with very limited exposure—for example, those who may have briefly experimented with tobacco smoking—was necessary to avoid spurious conclusions.

Relevant smoking exposure data from ethnicity-matched control subjects from the general population were obtained from either Statistics Canada or the published literature, the details of which are provided in Section 2.2.4. Specifically, smoking exposure data in the general Asian-ethnic populations of Canada and Shanghai, China, were respectively drawn from the CCHS and
a population-based study on smoking prevalence in Shanghai, China\textsuperscript{441}. In both control cohorts, the numbers of individuals in the survey population with positive or negative smoking history were derived from responses to a survey item asking respondents if they had smoked 100 or more cigarettes in their lifetime.

\textbf{7.2.2 Statistical analyses}

The impact of smoking on the risk to develop MS and related disorders was analyzed separately in the Canadian and Shanghai cohorts, with eligible diagnoses pooled to comprise the case cohort in each population. In addition, subgroup analyses specific to MS and NMO were also performed. Association of smoking was evaluated with multivariable logistic regression in which disease status was modeled as a function of smoking history, as well as sex and age to adjust for possible confounding. Association of smoking with MS risk in regression models was expressed as covariate-adjusted odds ratios and 95\% CIs.

To explore the impact of smoking on clinical outcomes of MS and related disorders in Asians, regression analyses were performed within the case cohort. As in the above analyses, clinical outcomes were modeled on covariates of sex, age, and smoking status in multivariable regression models. The primary outcomes in these analyses were disease severity (as represented by the MSSS index) and progressive clinical course, which were modeled using linear and logistic regression, respectively.
7.3 Results

7.3.1 Patient characteristics

Smoking exposure data were available from a total of 202 Asian-ethnic patients fulfilling eligibility criteria, including 70 cases in the Canadian cohort and 132 cases in the Chinese cohort. Clinical and demographic characteristics of the pooled and individual cohorts are presented in Table 7.1. Cohorts were similar with respect to most evaluated characteristics, including the relative proportion of clinical phenotypes, sex ratio, mean age, clinical course at onset, disability level as measured by the EDSS, and disease severity as measured by the MSSS. Modest differences were observed in terms of age at symptom onset and disease duration. Chinese cases were generally older at onset (32.0 vs. 27.0 years, \( p=0.033 \)) and had shorter disease duration (3.0 vs. 5.5 years, \( p=0.001 \)) relative to Canadian Asian-ethnic cases.
Table 7.1. Clinical and demographic characteristics of case cohorts from Canada and Shanghai, China.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
<th>( p^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, ( n )</td>
<td>202</td>
<td>70</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Sex, ( n ) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>157 (77.7)</td>
<td>52 (74.3)</td>
<td>105 (79.5)</td>
<td>0.478</td>
</tr>
<tr>
<td>Male</td>
<td>45 (22.3)</td>
<td>18 (25.7)</td>
<td>27 (20.5)</td>
<td></td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>30.0 (23.0-41.0)</td>
<td>27.00 (22.3-35.5)</td>
<td>32.0 (24.8-42.3)</td>
<td>0.033</td>
</tr>
<tr>
<td>At interview</td>
<td>38.0 (28.3-50.8)</td>
<td>38.5 (29.3-51.8)</td>
<td>38.0 (27.0-49.3)</td>
<td>0.540</td>
</tr>
<tr>
<td>Diagnosis, ( n ) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>131 (64.9)</td>
<td>48 (68.6)</td>
<td>83 (62.9)</td>
<td>0.339</td>
</tr>
<tr>
<td>NMO</td>
<td>66 (32.7)</td>
<td>19 (27.1)</td>
<td>47 (35.6)</td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>5 (2.5)</td>
<td>3 (4.3)</td>
<td>2 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Clinical course at onset, ( n ) (%)  ( b )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>138 (93.9)</td>
<td>35 (92.1)</td>
<td>103 (94.5)</td>
<td>0.696</td>
</tr>
<tr>
<td>Progressive</td>
<td>9 (6.1)</td>
<td>3 (7.9)</td>
<td>6 (5.5)</td>
<td></td>
</tr>
<tr>
<td>Disease duration, median years (IQR)</td>
<td>3.0 (1.0-7.0)</td>
<td>5.5 (2.0-10.3)</td>
<td>3.0 (1.0-5.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>EDSS, median (IQR)</td>
<td>2.0 (1.0-4.0)</td>
<td>2.5 (1.5-5.0)</td>
<td>2.0 (1.0-4.0)</td>
<td>0.396</td>
</tr>
<tr>
<td>MSSS, median (IQR)</td>
<td>3.69 (1.79-6.35)</td>
<td>3.81 (2.30-6.19)</td>
<td>3.34 (1.77-6.46)</td>
<td>0.818</td>
</tr>
</tbody>
</table>

\( a \) \( p \)-values refer to comparisons between the Canadian Asian cohort and the Shanghai cohort.

\( b \) Data not available for all cases. Proportions calculated from total cases with available data.

IQR, inter-quartile range

EDSS, Expanded Disability Status Scale

MSSS, Multiple Sclerosis Severity Score
7.3.2 Smoking prevalence in cases and controls

Smoking rates in the case and control populations are shown in Table 7.2 and Table 7.3. Overall smoking prevalence in the Shanghai population was significantly greater than in the Canadian Asian-ethnic population (28.4% vs. 21.3%, \( p<0.001 \)). This difference was entirely attributable to a remarkably high frequency of tobacco smoking among males in Shanghai (53%), particularly among those aged 40 to 59 years (66.7%). Differential rates of smoking between males and females were apparent in both populations, although the disparity in smoking rates between males and females was demonstrably greater in the Shanghai population (53.4% vs. 2.2%) than in the Canadian Asian population (35.8% vs. 8.5%).

Among cases, smoking was again more prevalent among males than females in both the Canadian (55.6% vs. 21.2% \( p=0.015 \)) and Chinese (37.0% vs. 2.9%, \( p<0.001 \)) cohorts (Table 7.2; Table 7.3). Stratification of cases by diagnostic classification revealed differences in smoking rates between MS and NMO subgroups. Positive history of smoking was significantly more frequent in MS cases compared to NMO cases in both the Canadian (37.5% vs. 10.5%, \( p=0.039 \)) and Chinese (14.5% vs. 0%, \( p=0.004 \)) case cohorts (Table 7.2; Table 7.3).
Table 7.2. Prevalence of smoking in Asian-ethnic controls and cases (MS and related disorders) in Canada.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Controls Total</th>
<th>Males</th>
<th>Females</th>
<th>Cases Total</th>
<th>Males</th>
<th>Females</th>
<th>MS</th>
<th>NMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤19</td>
<td>5,517 / 231,056</td>
<td>4,195 / 124,511</td>
<td>1,322 / 106,545</td>
<td>0 / 1</td>
<td>0 / 0</td>
<td>0 / 1</td>
<td>0 / 1</td>
<td>0 / 0</td>
</tr>
<tr>
<td></td>
<td>(2.4)</td>
<td>(3.4)</td>
<td>(1.2)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td></td>
<td>(22.9)</td>
<td>(33.9)</td>
<td>(12.8)</td>
<td>(31.4)</td>
<td>(45.5)</td>
<td>(25.0)</td>
<td>(37.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>40-59</td>
<td>164,868 / 672,068</td>
<td>139,131 / 296,944</td>
<td>25,737 / 375,124</td>
<td>10 / 31</td>
<td>5 / 7</td>
<td>5 / 24</td>
<td>8 / 19</td>
<td>2 / 11</td>
</tr>
<tr>
<td></td>
<td>(24.5)</td>
<td>(46.9)</td>
<td>(6.9)</td>
<td>(32.3)</td>
<td>(71.4)</td>
<td>(20.8)</td>
<td>(42.1)</td>
<td>(18.2)</td>
</tr>
<tr>
<td>≥60</td>
<td>69,046 / 270,511</td>
<td>57,526 / 122,090</td>
<td>11,520 / 148,421</td>
<td>0 / 3</td>
<td>0 / 0</td>
<td>0 / 3</td>
<td>0 / 1</td>
<td>0 / 2</td>
</tr>
<tr>
<td></td>
<td>(34.3)</td>
<td>(47.1)</td>
<td>(7.8)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>394,714 / 1,849,684</td>
<td>311,403 / 870,123</td>
<td>83,311 / 979,561</td>
<td>21 / 70</td>
<td>10 / 18</td>
<td>11 / 52</td>
<td>18 / 48</td>
<td>2 / 19</td>
</tr>
<tr>
<td></td>
<td>(21.3)</td>
<td>(35.8)</td>
<td>(8.5)</td>
<td>(0.30)</td>
<td>(55.6)</td>
<td>(21.2)</td>
<td>(37.5)</td>
<td>(10.5)</td>
</tr>
</tbody>
</table>

Smoking prevalence is presented as the number of subjects reporting a positive history of smoking (numerator) as a proportion of the total sample within the respective strata (denominator), with the percentage indicated in parentheses.
Table 7.3. Prevalence of smoking in controls and cases (MS and related disorders) in Shanghai, China.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Controls</th>
<th></th>
<th>Controls</th>
<th></th>
<th>Controls</th>
<th></th>
<th>Controls</th>
<th></th>
<th>Controls</th>
<th></th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Males</td>
<td>Females</td>
<td></td>
<td>Total</td>
<td>Males</td>
<td>Females</td>
<td></td>
<td>Total</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>≤19</td>
<td>1 / 99 (1.0)</td>
<td>1 / 47 (2.1)</td>
<td>0 / 52 (0.0)</td>
<td></td>
<td>1 / 10 (10.0)</td>
<td>1 / 3 (33.3)</td>
<td>0 / 7 (0.0)</td>
<td></td>
<td>1 / 6 (16.7)</td>
<td>0 / 4 (0.0)</td>
<td></td>
</tr>
<tr>
<td>20-39</td>
<td>76 / 336 (22.6)</td>
<td>74 / 173 (42.8)</td>
<td>2 / 163 (1.2)</td>
<td></td>
<td>8 / 60 (13.3)</td>
<td>5 / 14 (35.7)</td>
<td>3 / 46 (6.5)</td>
<td></td>
<td>7 / 38 (18.4)</td>
<td>0 / 20 (0.0)</td>
<td></td>
</tr>
<tr>
<td>40-59</td>
<td>246 / 706 (34.8)</td>
<td>242 / 366 (66.7)</td>
<td>3 / 340 (0.9)</td>
<td></td>
<td>4 / 54 (7.4)</td>
<td>4 / 10 (40.0)</td>
<td>0 / 44 (0.0)</td>
<td></td>
<td>4 / 35 (11.4)</td>
<td>0 / 19 (0.0)</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>103 / 359 (28.7)</td>
<td>92 / 179 (51.4)</td>
<td>11 / 180 (6.1)</td>
<td></td>
<td>0 / 8 (0.0)</td>
<td>0 / 0 (0.0)</td>
<td>0 / 8 (0.0)</td>
<td></td>
<td>0 / 4 (0.0)</td>
<td>0 / 4 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>426 / 1,500 (28.4)</td>
<td>409 / 765 (53.4)</td>
<td>16 / 735 (2.2)</td>
<td></td>
<td>13 / 132 (9.8)</td>
<td>10 / 27 (37.0)</td>
<td>3 / 105 (2.9)</td>
<td></td>
<td>12 / 83 (14.5)</td>
<td>0 / 47 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

Smoking prevalence is presented as the number of subjects reporting a positive history of smoking (numerator) as a proportion of the total sample within the respective strata (denominator), with the percentage indicated in parentheses.
7.3.3 Association of smoking with MS risk

Association of smoking with the risk to develop MS and related disorders was evaluated in the Asian-ethnic populations of Canada and Shanghai, China, in separate multivariable logistic regression models (Table 7.4, Table 7.5). After adjustment for sex and age, positive history of smoking was associated with a >2-fold increased risk for MS and related disorders (OR=2.29, 95% CI: 1.29-3.94, p=0.003) in the Asian-ethnic population of Canada. A diagnosis-specific effect was evident on stratification of cases by diagnosis. Smoking was strongly associated with MS risk (OR=3.15, 95% CI: 1.64-5.88, p<0.001), but not NMO (OR=0.82, 95% CI: 0.12-3.10, p=0.800) in separate multivariable regression analyses. In contrast, smoking was not associated with susceptibility to MS and related disorders in the Shanghai population (OR=0.74, 95% CI: 0.35-1.51, p=0.419), nor was it specifically associated with MS or NMO in this population.
Table 7.4. Association of smoking with the risk of MS and related disorders in persons of Asian ancestry in Canada.

<table>
<thead>
<tr>
<th></th>
<th>MS and related disorders</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>MS only</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases, n (%)</td>
<td>Controls, n (%)</td>
<td>OR (95% CI)</td>
<td>p</td>
<td>Cases, n (%)</td>
<td>Controls, n (%)</td>
<td>OR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>52 (74.3)</td>
<td>979,561 (53.0)</td>
<td>--</td>
<td></td>
<td>34 (70.8)</td>
<td>979,561 (53.0)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (25.7)</td>
<td>870,123 (47.0)</td>
<td>0.30 (0.16-0.52)</td>
<td>&lt;0.001</td>
<td>14 (29.2)</td>
<td>870,123 (47.0)</td>
<td>0.31 (0.16-0.60)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤19 years</td>
<td>1 (1.4)</td>
<td>231,056 (12.5)</td>
<td>--</td>
<td></td>
<td>1 (2.1)</td>
<td>231,056 (12.5)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>20-39 years</td>
<td>35 (50.0)</td>
<td>676,049 (36.5)</td>
<td>9.40 (2.01-167.56)</td>
<td>0.028</td>
<td>27 (56.2)</td>
<td>676,049 (36.5)</td>
<td>6.56 (1.37-117.68)</td>
<td>0.066</td>
</tr>
<tr>
<td>40-59 years</td>
<td>31 (44.3)</td>
<td>672,068 (36.3)</td>
<td>8.28 (1.77-147.65)</td>
<td>0.038</td>
<td>19 (39.6)</td>
<td>672,068 (36.5)</td>
<td>4.65 (0.95-83.97)</td>
<td>0.136</td>
</tr>
<tr>
<td>≥60 years</td>
<td>3 (4.3)</td>
<td>270,511 (14.6)</td>
<td>1.99 (0.25-40.27)</td>
<td>0.553</td>
<td>1 (2.1)</td>
<td>270,511 (14.6)</td>
<td>0.60 (0.02-15.35)</td>
<td>0.722</td>
</tr>
<tr>
<td><strong>Smoked</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>49 (70.0)</td>
<td>1,454,970 (78.7)</td>
<td>--</td>
<td></td>
<td>30 (62.5)</td>
<td>1,454,970 (78.7)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (30.0)</td>
<td>394,714 (21.3)</td>
<td>2.29 (1.29-3.94)</td>
<td>0.003</td>
<td>18 (37.5)</td>
<td>394,714 (21.3)</td>
<td>3.15 (1.64-5.88)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

OR, odds ratio
CI, confidence interval
--., reference level
Table 7.5. Association of smoking with the risk of MS and related disorders among Chinese in Shanghai, China.

<table>
<thead>
<tr>
<th></th>
<th>MS and related disorders</th>
<th></th>
<th>MS only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases, n (%)</td>
<td>Controls, n (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>105 (79.5)</td>
<td>735 (49.0)</td>
<td>--</td>
</tr>
<tr>
<td>Male</td>
<td>27 (20.5)</td>
<td>765 (51.0)</td>
<td>0.28 (0.16-0.46)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤19 years</td>
<td>10 (7.6)</td>
<td>99 (6.6)</td>
<td>--</td>
</tr>
<tr>
<td>20-39 years</td>
<td>60 (45.5)</td>
<td>336 (22.4)</td>
<td>1.89 (0.96-4.10)</td>
</tr>
<tr>
<td>40-59 years</td>
<td>54 (40.9)</td>
<td>706 (47.1)</td>
<td>0.82 (0.41-1.77)</td>
</tr>
<tr>
<td>≥60 years</td>
<td>8 (6.1)</td>
<td>359 (23.9)</td>
<td>0.23 (0.09-0.61)</td>
</tr>
<tr>
<td><strong>Smoked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>119 (90.2)</td>
<td>1,075 (71.7)</td>
<td>--</td>
</tr>
<tr>
<td>Yes</td>
<td>13 (9.8)</td>
<td>425 (28.3)</td>
<td>0.74 (0.35-1.51)</td>
</tr>
</tbody>
</table>

OR, odds ratio
CI, confidence interval
-- , reference level
7.3.4 Association of smoking with clinical outcomes of MS

The relationship between smoking and clinical outcomes of MS and related disorders was also evaluated in the pooled patient cohort, as well as in the constituent Canadian and Chinese patient cohorts. After adjustment for age, sex, and disease duration in multivariable logistic regression models (Table 7.6), a trend toward increased risk of progressive clinical course was observed in patients with a positive history of smoking (OR=3.50, 95% CI: 0.91-12.72, \( p=0.059 \)). Separate analyses within each cohort revealed that this association was primarily driven by findings in the Canadian cohort (OR=5.60, 95% CI: 0.73-63.83, \( p=0.112 \)). Stratification by diagnosis did not reveal any associations specific to MS or NMO.

In multivariable linear regression models investigating the effect of smoking on disease severity (Table 7.7), positive history of smoking was not significantly associated with the MSSS index after adjustment for age and sex, although a non-significant trend was apparent (\( \beta=1.30, 95\% \text{ CI: } -0.39 \text{ – } 2.99, p=0.131 \)). Again, stratification of cases by diagnostic classification did not expose any diagnosis-specific effects.
Table 7.6. Association of smoking with the risk of progressive course in Asians with MS and related disorders.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td><strong>Age (per year)</strong></td>
<td>1.02 (0.96-1.07)</td>
<td>0.509</td>
<td>1.02 (0.92-1.13)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5.43 (1.52-20.65)</td>
<td>0.010</td>
<td>11.22 (1.43-146.53)</td>
</tr>
<tr>
<td><strong>Disease duration (per year)</strong></td>
<td>0.99 (0.89-1.09)</td>
<td>0.876</td>
<td>0.97 (0.80-1.17)</td>
</tr>
<tr>
<td><strong>Smoked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>--</td>
<td></td>
<td>--</td>
</tr>
<tr>
<td>Yes</td>
<td>3.50 (0.91-12.72)</td>
<td>0.059</td>
<td>5.60 (0.73-63.83)</td>
</tr>
</tbody>
</table>

OR, odds ratio
CI, confidence interval
--., reference level
Table 7.7. Association of smoking with disease severity in Asians with MS and related disorders.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Canada</th>
<th>China</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ (95% CI)</td>
<td>$p$</td>
<td>$\beta$ (95% CI)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Male</td>
<td>-1.28 (-2.94 – 0.37)</td>
<td>0.128</td>
<td>-1.16 (-3.53 – 1.21)</td>
</tr>
<tr>
<td><strong>Age (per year)</strong></td>
<td>0.04 (-0.00 – 0.09)</td>
<td>0.054</td>
<td>0.01 (-0.06 – 0.09)</td>
</tr>
<tr>
<td><strong>Smoked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Yes</td>
<td>1.30 (-0.39 – 2.99)</td>
<td>0.131</td>
<td>1.46 (-0.71 – 3.63)</td>
</tr>
</tbody>
</table>

$\beta$, linear regression coefficient

CI, confidence interval

--, reference level
7.4 Discussion

Smoking is one of the best-established environmental factors associated with susceptibility to MS. Virtually all of the current evidence associating smoking with MS risk derives from studies in predominantly Caucasian populations; no such studies in Asian populations have been published to date. In the present study, case-control analysis of smoking as a risk factor was performed in distinct Asian-ethnic populations in Canada and China. After adjustment for age and sex, smoking was associated with a >2-fold increase in risk of MS and related disorders in Canadians of Asian ancestry. Cohort stratification by diagnosis revealed that this effect was specific to MS, and excess risk of MS due to smoking was more than 3-fold. Interestingly, smoking was not associated with susceptibility to MS and related disorders in the Shanghai Chinese population.

The findings in the Canadian Asian-ethnic population are in keeping with the expanding body of literature demonstrating smoking as an important environmental risk factor for MS. A recent population-based, case-control study in MS cases and spouse controls from across Canada reported a modest increase in MS risk (OR=1.32) associated with smoking, which was similar in magnitude to the overall risk attributed to smoking (OR=1.48) in a recent meta-analysis. The present study is particularly noteworthy, given that it is the first investigation of smoking as a risk factor for MS in an Asian-ethnic population. A small cohort study in an immigrant Iranian population in Canada reported an extraordinarily large effect of smoking on MS risk (OR=17.0); however, smoking exposure data for controls in that study were derived from a domestic Iranian population, which may not have been accurately representative of smoking patterns among Iranians living in Canada. Taken together with previous studies, the results
reported here suggest that smoking may also influence MS risk in low-prevalence ethnic groups. While it remains possible that smoking might impart differential risk across ethnic groups—this is implied by the broad range of the effect size associated with smoking in studies from different populations—further studies in other non-Caucasian populations are needed to systematically investigate this hypothesis.

Paradoxically, smoking was not associated with MS susceptibility in the Shanghai population. This finding challenges the notion of smoking as a universal risk factor for MS, but does not invalidate it per se. A plausible explanation for this seeming discrepancy is the surprisingly low prevalence of smoking among females (2.2%) in the general Shanghai population, resulting in lower statistical power to detect modest effects associated with smoking. The rate of smoking in this population was particularly low among females in age brackets for which baseline MS susceptibility is greatest. For example, smoking prevalence in females aged 20-39 years and 40-59 years was 1.2% and 0.9%, respectively. A strong sociocultural taboo against smoking by women that persists in most of Asia highlights the possibility that reporting bias may have resulted in an underestimate of smoking among females in this population.

Moreover, the possibility that other environmental factors in China might have interacted with smoking exposure to abrogate or attenuate the effect of the latter factor on MS risk cannot be ruled out, as environmental interactions were not the focus of the present study. It is plausible, for instance, that the background risk for MS in the Shanghai environment was sufficiently low to prevent overall risk from crossing a critical threshold necessary for MS to manifest, even with the additional risk conferred by smoking.
An intriguing observation in this study was that smoking was associated with MS but not NMO in the Canadian cohort. Despite similarities in clinical presentation, MS and NMO are believed to be pathologically distinct\textsuperscript{16}, and the differential influence of smoking on susceptibility demonstrated here would strongly imply distinction in pathogenesis. At present, the etiology of NMO is less clear than that of MS, as environmental risk factors for NMO have been largely unexplored. This is the first study to evaluate smoking as a possible risk factor for NMO. The findings presented here preliminarily suggest that smoking may not be an important risk factor for NMO, although additional studies in larger cohorts, including in Caucasians, will certainly be needed to exhaustively investigate this hypothesis.

Smoking is widely recognized to influence clinical outcomes of MS and related disorders. Because studies investigating this relationship have been exclusively conducted in predominantly Caucasian populations, the generalizability of this association to patients of other ethnicities remains uncertain. In the present study, smoking was associated with progressive clinical course (OR=3.50) and a higher disease severity index ($\beta=1.30$) in patients of Asian ancestry from clinics in China and Canada, although these results fell short of formal statistical significance. These trends are consistent with the majority of previous studies demonstrating an overall deleterious effect of smoking on disease course in MS patients\textsuperscript{310–314}. Further, these data lend support to the hypothesis that the injurious effects of smoking on MS progression extend to non-Caucasian patient populations. Additional larger studies exploring this relationship in other ethnic cohorts are therefore warranted.

This study did have some limitations. The relatively small case sample size, particularly within the NMO subgroups, precluded the detection of small effects. This limitation was most evident
in the Chinese cohort, wherein baseline smoking rates among females in the control population were very low, which limited the power to detect effects of small magnitude. Similarly, the number of NMO cases in each cohort was relatively small and likely insufficiently powered to detect modest effects related to smoking. However, the striking paucity of NMO cases reporting a positive history of smoking (2 of 66 cases in the combined Canadian and Chinese NMO cohorts) casts considerable doubt on smoking as a major risk factor for NMO in this ethnic group.

As detailed in Section 2.1, control subjects matched to ethnicity were not enrolled in parallel with cases. Therefore, control data on smoking prevalence in the general population were obtained through distinct channels from cases (detailed in Sections 2.2.4). Although the potential for selection biases arising from this approach cannot be ruled out, relevant considerations were carefully weighed when selecting the control cohorts in order to minimize the impact of any such biases. First, control cohorts were matched to their respective case cohorts in terms of ethnic origins, region of residence, and time period of data acquisition. Second, self-reported data were acquired from cases and controls through similar methods, both through standardized questionnaires administered via telephone or in person. Moreover, smoking exposure was operationalized with equivalent definitions in both cohorts. Therefore, smoking status was less vulnerable to misclassification or recall bias. However, the possibility of reporting bias arising from sociocultural perceptions concerning smoking, particularly among females, cannot be ruled out. Lastly, estimates of smoking prevalence in both cohorts were population-based, thereby minimizing sampling biases. In particular, the CCHS data were validated by Statistics Canada as representing an accurate and recent estimate of smoking in the Asian-ethnic population of Canada.
This was the first study to examine smoking as a risk factor for MS in populations of Asian ancestry and the first study to investigate the relationship between smoking and NMO risk. The findings presented here are particularly topical, given the considerable attention being paid to the role of smoking in the onset and progression of MS through recent research. In light of ongoing increases in the incidence of MS in regions of Asia with increasingly widespread tobacco use, consideration of smoking as a causal factor underlying this trend is warranted. Although smoking was not associated with MS risk in the Shanghai population examined in this study, an effect of smoking on MS risk in domestic Asian populations cannot be ruled out, especially when taking into account the much lower prevalence of smoking among females in China compared to other Asian countries such as Japan\textsuperscript{428}. Further research in other populations, particularly in those historically considered to be at low risk for MS, will be needed to confirm smoking as a universal risk factor for MS onset and clinical progression.
Chapter 8: Association of genetic factors with risk and clinical phenotype of MS and related disorders in Asians

8.1 Introduction

The incidence, prevalence, and rate of familial aggregation of MS and related disorders are universally low in Asian populations compared to Caucasian populations. The consistency of these observations has been widely accepted as circumstantial evidence of very low genetic susceptibility to these disorders in Asian populations, owing to a reduced genetic risk burden, a profusion of protective factors, or a combination thereof. Nevertheless, relatively few studies have directly investigated the genetic basis for reduced MS risk in persons of Asian ancestry. By and large, these studies have been limited in quantity and scope, primarily focusing on identifying common risk variants in single candidate genes or limited genomic regions, such as the HLA. Notably, most of these studies were conducted prior to widespread recognition of nosological distinction of clinical phenotypes in patients of Asian ancestry.

The overall results of these studies have generally been conflicting, and none has stood up to further scrutiny by replication. This is in stark contrast to the current state of genetic research on MS in Caucasians. In total, over 60 common risk variants, in addition to the established principal risk alleles in the HLA, have been validated by genome-wide association studies in predominantly Caucasian populations. Similar genome-wide approaches to identify common risk variants in Asian populations are virtually impossible, as the rarity of these disorders in this population is at considerable odds with the technical necessity of large sample sizes (n>2000) to
afford adequate power to detect variants with modest effects\textsuperscript{481}. Studies seeking to identify disease associations with highly-penetrant rare variants are, in many respects, less vulnerable to the pitfalls of underpowered sample sizes afflicting genome-wide association studies screening common variants. Recent studies in Caucasian populations in Canada have identified rare genetic variants associated with increased MS susceptibility\textsuperscript{380–382}. Rare-variant genetic screens, which rely on high-resolution, deep-coverage sequence data acquired through next-generation sequencing, are contingent on the availability of reference sequence data from an ethnically similar population. Although such data (at the exome level) for Asian populations are now publicly available, there have been no studies to date examining the possible role of rare genetic variants in the pathogenesis of MS.

The objectives of the present study were to determine the rate of familial aggregation of MS and related disorders in Asian-ethnic populations and to identify variants associated with susceptibility and clinical phenotype in this ethnic group. This study addresses a critical knowledge gap in the etiology of MS and related disorders in Asian populations and explores the genetic pathways involved in the pathogenesis of MS and NMO in this under-recognized population.

8.2 Methods

8.2.1 Characterization of familial aggregation

Patients of Asian ancestry with a diagnosis of MS or related disorders (probands) were ascertained from three source populations, as previously detailed in Section 2.1: the UBC MS Clinic (Vancouver, Canada), the CCPGSMS (Canada), and Huashan Hospital (Shanghai, China).
Probands were stratified by country of residence into Canadian and Chinese cohorts. Probands were administered standardized questionnaires (Section 2.2.5) to obtain family history information with respect to diagnosis of MS, NMO, NMOSD, or CIS in biological relatives. Familial aggregation was defined as having a fourth-degree or more closely related relative with MS or related disorders. The frequency of familial aggregation was compared in Chinese and Canadian cohorts, as well as between subgroups restricted to probands with full Asian ancestry and partial Asian ancestry in the latter cohort. Proportions were compared with a two-sided Fisher exact test.

8.2.2 Analysis of risk variants

The exons of 61 established candidate risk genes for MS (Appendix C) were sequenced, as detailed in Section 2.2.6. These genes were specifically targeted in this analysis to optimize variant discovery while maintaining adequate statistical power. Sequencing was performed in 19 MS and 14 NMO patients with full self-reported East or Southeast Asian-ethnic origins ascertained at the UBC MS Clinic. All variants identified in the coding regions and flanking sequences of the selected candidate genes were assessed for differences in allele frequencies between cases and 286 Asian-ethnic controls matched to self-reported ethnicity. Data for the latter cohort were acquired from the 1000 Genomes Project (Section 2.2.6). Cohort differences in allele frequency were assessed with a two-sided Fisher exact test with Bonferroni correction for multiple comparisons. Case-control analysis was limited to comparisons of allelic frequencies, as genotype data were unavailable in controls.
8.2.3 Genetic associations with clinical phenotype

To identify genes associated with differential clinical phenotypes, association analysis comparing variants in MS versus NMO subgroups was performed. On account of the limited number of cases available for sequencing, genetic association with clinical phenotype was analyzed at the level of the gene using the Sequence Kernel Association Test (SKAT)\textsuperscript{482}, which affords more power than single-variant association tests, especially for low-frequency variants. SKAT assesses all variants grouped by genes for association with a phenotype of interest (in this case, the clinical phenotype as indicated by diagnosis). The test also adjusts for potentially confounding covariates and is suitable for detecting effects in either direction of the phenotype, in addition to being robust to the effects of neutral variants, which introduce statistical noise and diminish power.

SKAT analysis was performed using the SKAT plug-in\textsuperscript{483} for the R Statistical Package version 3.0.2 for Windows (R Foundation for Statistical Computing, Vienna, Austria). Potential group differences in sex ratio were adjusted for by incorporation of sex as a covariate in the SKAT model. Analysis was restricted to variants in exons of the a priori selected candidate genes (Appendix C). Bonferroni correction for multiple comparisons was applied to output $p$-values from SKAT analysis.

8.3 Results

8.3.1 Familial aggregation

Family history information was available for 271 patients of Asian ancestry, including 132 patients identified through Huashan Hospital, and 139 patients in Canada ascertained through the
UBC MS Clinic (n=93) or other CCPGSMS sites (n=46). Of the 25 patients with partial Asian-ethnic origins, Asian ancestry was limited to the paternal lineage in 15 cases and to the maternal lineage in 10 cases. All such patients were from the Canadian cohort.

In total, familial aggregation of MS, NMO, NMOSD, or CIS was reported in seven (2.6%) cases. Specifically, three probands had a first-degree affected relative, one proband had a second-degree affected relative, two probands had a third-degree affected relative, and one proband had a fourth-degree affected relative. Familial aggregation, however, was restricted to patients diagnosed with MS, being noted in 7 of 190 MS cases (3.7%), but in none of the 75 NMO cases (Table 8.1). Moreover, all cases with positive family history were from the Canadian cohort. The rate of familial recurrence of MS and related disorders was significantly greater in the Canadian Asian-ethnic cohort than in the Chinese cohort (5% vs. 0%, p=0.015). This disparity was driven by an excess of MS cases with positive family history in Canadian patients with partial Asian ancestry (n=7) compared to the Chinese MS cohort (20.8% vs. 0%, p<0.001). In five of these seven cases, the parental lineage with the affected relative was Caucasian. Among cases with an affected first degree relative (n=3), family history was associated with the parent of Caucasian ethnic origins. Taken together, these observations suggest that familial MS risk in persons of mixed Asian-Caucasian ancestry does not originate exclusively from the Caucasian genetic lineage.
Table 8.1. Frequency of familial cases of MS and related disorders in Asian patients ascertained at clinics in Shanghai, China and Canada.

<table>
<thead>
<tr>
<th>MS and related disorders</th>
<th>China</th>
<th>Canada</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Full Asian</td>
<td>Partial Asian</td>
<td>p&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cases, n</td>
<td>132</td>
<td>139</td>
<td>114</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Positive family history, n (%)</td>
<td>0 (0.0)</td>
<td>7 (5.0)</td>
<td>2 (1.8)</td>
<td>5 (20.0)</td>
<td>0.015</td>
</tr>
<tr>
<td>MS</td>
<td>83</td>
<td>107</td>
<td>83</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Cases, n</td>
<td>0 (0.0)</td>
<td>7 (6.5)</td>
<td>2 (2.4)</td>
<td>5 (20.8)</td>
<td>0.019</td>
</tr>
<tr>
<td>Positive family history, n (%)</td>
<td>47</td>
<td>28</td>
<td>27</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Positive family history, n (%)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<sup>a</sup> <sup>p</sup>-values refer to comparison of Chinese cohort versus entire Canadian cohort

<sup>b</sup> <sup>p</sup>-values refer to comparison of Chinese cohort versus Canadian cohort with full Asian ancestry

<sup>c</sup> <sup>p</sup>-values refer to comparison of Chinese cohort versus Canadian cohort with partial Asian ancestry
8.3.2 Variants associated with susceptibility and clinical phenotype

A total of 33 cases (27 females, 6 males) with full Asian-ethnic origins ascertained at the UBC MS Clinic provided DNA samples for genetic analysis of variants associated with susceptibility and clinical phenotype. Demographic and clinical characteristics of this case cohort are presented in Table 8.2. Clinical presentation and diagnosis were consistent with conventional MS in 19 cases and NMO in 14 cases. Median age at symptom onset and at sample collection was 29 years (range: 8-61) and 45 years (range: 20-62), respectively. Median EDSS disability level was 2.5 (range: 0-7.5).

To evaluate whether variants in established MS risk genes previously confirmed in Caucasian populations are also associated with susceptibility in Asian populations, allele frequencies were compared between pooled cases and ethnically matched controls from the 1000 Genomes Project East Asian Ancestry cohort (n=286). After correction for multiple comparisons, significant associations were observed for rs417276 (OR=5.0; 95% CI: 2.4-10.2; \( p_{corr} < 0.0065 \)) and rs270032 (OR=3.2; 95% CI: 1.8-5.6; \( p_{corr} < 0.0367 \)), respectively localizing to the \textit{GALC} and \textit{THEMIS} genes (Table 8.3).
### Table 8.2. Demographic and clinical characteristics of cases included in genetic analysis.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>MS</th>
<th>NMO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases, n (%)</strong></td>
<td>33</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Females</td>
<td>27 (81.8)</td>
<td>14 (73.7)</td>
<td>13 (92.9)</td>
</tr>
<tr>
<td>Males</td>
<td>6 (18.2)</td>
<td>5 (26.3)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td><strong>Age, median years (range)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset</td>
<td>29 (8-61)</td>
<td>29 (17-54)</td>
<td>28.5 (8-61)</td>
</tr>
<tr>
<td>At collection</td>
<td>45 (20-62)</td>
<td>43 (20-58)</td>
<td>49.5 (21-62)</td>
</tr>
<tr>
<td><strong>Course at onset, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing</td>
<td>29 (96.7)</td>
<td>16 (94.1)</td>
<td>13 (100.0)</td>
</tr>
<tr>
<td>Progressive</td>
<td>1 (3.3)</td>
<td>1 (5.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>EDSS, median (range)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 (0-7.5)</td>
<td>2.0 (0-6)</td>
<td>3.75 (1.5-7.5)</td>
</tr>
</tbody>
</table>

EDSS, Expanded Disability Status Scale
Table 8.3. Top variants associated with MS and related disorders in Asian populations.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Chr</th>
<th>Position *</th>
<th>Gene</th>
<th>RA</th>
<th>RAF, cases</th>
<th>RAF, controls</th>
<th>OR (95% CI)</th>
<th>Function</th>
<th>p</th>
<th>p_corr</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs417276</td>
<td>14</td>
<td>88406404</td>
<td>GALC</td>
<td>T</td>
<td>0.24</td>
<td>0.06</td>
<td>5.0 (2.4-10.2)</td>
<td>Intronic</td>
<td>9x10^-6</td>
<td>0.007</td>
</tr>
<tr>
<td>rs270032</td>
<td>6</td>
<td>128176383</td>
<td>THEMIS</td>
<td>A</td>
<td>0.39</td>
<td>0.17</td>
<td>3.2 (1.8-5.6)</td>
<td>Intronic</td>
<td>5x10^-5</td>
<td>0.037</td>
</tr>
<tr>
<td>rs10920089</td>
<td>1</td>
<td>200967490</td>
<td>KIF21B</td>
<td>A</td>
<td>0.59</td>
<td>0.35</td>
<td>2.7 (1.5-4.7)</td>
<td>Intronic</td>
<td>0.0002</td>
<td>0.167</td>
</tr>
<tr>
<td>rs2843401</td>
<td>1</td>
<td>2528133</td>
<td>MMEL1</td>
<td>T</td>
<td>0.70</td>
<td>0.47</td>
<td>2.6 (1.5-4.7)</td>
<td>Intronic</td>
<td>0.0006</td>
<td>0.449</td>
</tr>
</tbody>
</table>

Chr, chromosome
RA, risk allele
RAF, risk allele frequency
OR, odds ratio
CI, confidence interval

\( p_{corr} \) corrected \( p \)-value

*Position based on hg19 and dbSNP Build 137

Variants with uncorrected \( p < 0.001 \) were included in this table.
To identify genes associated with clinical phenotype, SKAT analysis comparing variant frequencies in MS cases to those in NMO cases was performed in the same set of 61 candidate genes. Prior to adjusting for multiple comparisons, five genes (AHI1, MMEL1, C1orf106, CD6, and CD40) met the threshold of significance for association with clinical phenotype. However, after Bonferroni correction, only variants in AHI1 remained significantly associated with clinical phenotype ($p_{corr}=0.0361$) (Table 8.4).
Table 8.4. Top genes associated with clinical phenotype of MS and related disorders in Asian patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Total variants</th>
<th>Exonic variants</th>
<th>Chromosome</th>
<th>( p )</th>
<th>( p_{corr} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{AHI1}</td>
<td>28</td>
<td>8</td>
<td>6q23.3</td>
<td>0.0006</td>
<td>0.0361</td>
</tr>
<tr>
<td>\textit{MMEL1}</td>
<td>34</td>
<td>5</td>
<td>1p36</td>
<td>0.0109</td>
<td>0.70</td>
</tr>
<tr>
<td>\textit{C1orf106}</td>
<td>18</td>
<td>8</td>
<td>1q32.1</td>
<td>0.0313</td>
<td>NA</td>
</tr>
<tr>
<td>\textit{CD6}</td>
<td>12</td>
<td>5</td>
<td>11q13</td>
<td>0.0451</td>
<td>NA</td>
</tr>
<tr>
<td>\textit{CD40}</td>
<td>8</td>
<td>1</td>
<td>20q12-q13.2</td>
<td>0.0472</td>
<td>NA</td>
</tr>
</tbody>
</table>

\( p_{corr} \), corrected p-value

NA, Bonferonni-corrected \( p \)-value >1

Genes with uncorrected \( p \)<0.05 were included in this table.
8.4 Discussion

Although Asian populations are believed to be minimally susceptible to MS and related disorders, the genetic basis for lower risk in this population remains poorly understood. Relatively few studies have examined both common and rare variants associated with susceptibility to MS and related disorders in persons of Asian ancestry; fewer still have examined the role of genetic factors in determining clinical phenotype in this population. Furthermore, the rate of familial aggregation of MS and related disorders in Asians residing in a high-risk region has never previously been reported. The present work addresses these gaps in knowledge through pedigree analysis undertaken in Asian patient cohorts in Canada and Shanghai, China, as well as studies of genetic variation in Asian-ethnic cases and controls.

In the present study, the overall rate of familial recurrence of MS and related disorders among patients in Canada and China with full Asian-ethnic origins was low (0-5%) when compared to rates typically reported in Caucasian populations. In patients of European ancestry, for instance, familial recurrence risk among biological relatives of probands typically falls in a range of 15-20%\textsuperscript{346–348}. However, the rate of familial recurrence observed in this study was largely in line with that reported in Asian populations\textsuperscript{54,55,97,101,114,355}, suggesting overall uniformity of familial recurrence risk in Asian populations.

The fact that the rate of familial MS was significantly greater in the Canadian cohort relative to the Chinese cohort corroborates a modifying effect of the environment on MS risk. However, other factors warrant consideration when appraising the frequency of familial aggregation of MS in the Chinese cohort. For instance, legislation which has been in effect in China since 1979 has restricted the number of offspring, thereby resulting in smaller kinships overall. Consequently,
probands in the Chinese cohort would likely have had fewer at-risk family members in more recent generations compared to the Canadian cohort. In addition, the potential for ascertainment bias must be acknowledged, as MS and related disorders are often under-diagnosed in low-prevalence regions such as China\textsuperscript{65}. Therefore, the possibility that familial recurrence rates may have been underestimated in the Chinese cohort cannot be ruled out.

Familial recurrence was markedly less frequent among Canadians with full Asian ethnic origins compared to those with partial Asian ancestry. The higher rate of familial MS recurrence in the latter cohort (20.8\%) was in fact equivalent to that typically observed in Caucasians, an observation that suggests that non-Asian ancestral risk factors in such persons might outweigh putative protective genetic factors inherited from the Asian parental lineage. However, it must be acknowledged that the number of cases on which these rates were based was relatively small. Thus, additional studies in this unique population to confirm these findings are warranted.

The absence of multiplex pedigrees among NMO cases surveyed in this study suggests that the rate of familial recurrence of NMO is low, and likely less than that of MS. Although studies addressing this hypothesis are scarce, the collective body of literature on NMO appears to support this conclusion\textsuperscript{195,351–353}, and the data reported here are also in keeping with the presumptive rarity of familial aggregation of NMO.

As family-based sequence analysis could not be attempted in any of the multiplex pedigrees identified in this study due to unavailability of DNA samples from concordant or unaffected family members, case-control analysis focusing on variants in 61 candidate genes with \textit{a priori} evidence of association in Caucasian populations was performed. Two intronic variants were significantly associated with susceptibility to MS and related disorders in pooled analysis.
Neither of these variants has previously been reported in association with MS risk in Caucasians. The genes in which the associated variants were identified are involved in immune or neurological function, as were the majority of the 61 candidate genes selected for screening in this study. Mutations in *GALC* are known to cause Krabbe disease (globoid cell leukodystrophy), an autosomal recessive disorder characterized by myelin degeneration. *THEMIS* encodes a protein involved in T-cell maturation. The immune-centric biological roles of these genes are consistent with prevailing understanding of MS as a disease of primary immune dysfunction, and moreover, cohere with the existing literature associating variants in the HLA region with MS susceptibility in Asian populations.

In gene-based SKAT analysis comparing MS and NMO cases, only *AHI1* was significantly associated with differential clinical phenotype. *AHI1* appears to be involved in cortical and cerebellar development; mutations in this gene have previously been associated with Joubert syndrome, an autosomal recessive disorder characterized by ataxia, developmental delay, and hypotonia. Prior to this work, the genetic basis for differential clinical phenotypes in Asians with MS and related disorders had been scarcely studied. Previous research in Asian populations had found differential allelic associations with MS and NMO in the *HLA-DPB1* gene and *HLA-DRB1* gene. The present study is the first to report an association of clinical phenotype in a gene outside of the HLA region, further substantiating a genetic basis for phenotype determination in Asians with MS and related disorders.

The primary limitation of this genetic association study was the small number of cases, which limited the power to detect variants with small to moderate-sized effects and precluded two-phase analyses involving separate discovery and validation cohorts. These shortcomings were
acknowledged at the outset when challenges to participant recruitment were first recognized. Genetic analysis was therefore restricted to the most viable candidate genes, selected using a targeted approach focusing on risk genes with consistent evidence of association with MS in Caucasian populations. It warrants mentioning here that the objective of this study was not to identify risk variants conclusively in Asian-ethnic populations; rather, the aim was to highlight areas of further inquiry with respect to genetic factors influencing susceptibility and clinical phenotype in this population through targeted screening of plausibly relevant candidate genes.

It should also be noted that the exome libraries from which variants in candidate genes were identified were generated and sequenced on platforms that differed between the case and control cohorts. Therefore, platform-specific variants (that is, those that were reliably typed in only cases or controls) were excluded from analysis. Consequently, the possibility that a variant legitimately associated with susceptibility in this population may have been overlooked cannot be excluded. In a similar vein, this study focused on intragenic variants (primarily in coding regions) in candidate genes that were nominated by previous research implicating variants localizing predominantly to intergenic regions of the genome. Therefore, one must bear in mind that previously established risk variants may in fact be conferring excess risk through mechanisms unrelated to the hypothesized functional changes targeted in this study.

Nevertheless, this study was bolstered by a number of methodological strengths and novel findings. Limiting analysis to established risk genes for MS, for instance, was a sensible approach that reasonably balanced the modest power afforded by this case cohort with the prospect of biologically meaningful discovery. Furthermore, by applying a sequence-based screen focusing on coding sequences in these risk genes, the likelihood of identifying potentially
pathogenic variants—that is, those to which functional changes could be readily ascribed and characterized—was optimized.

Finally, the present study addresses a substantial deficit in knowledge with respect to rare and common variation in genomic regions beyond the HLA that are potentially relevant to the risk and clinical presentation of MS and related disorders in Asian populations. This study nominates a number of novel candidates in this regard and lays the foundation for further validation and exploratory studies of genetic susceptibility in this sparsely researched population.
Chapter 9: Conclusions

Although MS was historically viewed as a disease of Caucasian populations, it is now widely considered a global disease, being increasingly recognized in virtually all ethnic groups. Nevertheless, current understanding of the clinical characteristics and risk factors in persons of Asian ancestry lags appreciably behind that in Caucasian populations. Considerable knowledge gaps persist with respect to the epidemiology and clinical profile of MS and related disorders in Asian populations. The overarching objective of the research summarized in this dissertation was to specifically address these deficits in knowledge through a multi-disciplinary approach involving Asian-ethnic patients ascertained at MS clinics in Canada and China.

The study populations on which this research was based were particularly informative, owing to numerous distinctive characteristics that afforded unparalleled opportunities to address unique and insightful research questions. For instance, comparative analyses of NMO in relation to conventional MS were enabled by the relative enrichment of NMO and NMOSD in this ethnic group. In addition, studies probing the differential effect of distinct regional environments on clinical presentation and overall risk were feasible on account of the frequent migration in this population.

A major impetus for this research was the recognition that advancing knowledge in this ethnic group would likely benefit an emerging, under-studied, at-risk population by expanding the body of evidence guiding clinical care and public health policy. This research was also undertaken with the broader goal of clarifying universal mechanisms of susceptibility and clinical variability of MS in mind.
9.1 Frequency of MS and related disorders in the Asian-ethnic population of BC

Although the low frequency of MS and related disorders in Asia is consistently reported, few studies to date have reported prevalence or incidence in Asian-ethnic populations in high-risk regions\textsuperscript{289,295,301,302}. No such estimates have recently been published in the literature. BC has, proportionally, one of the largest Asian-ethnic populations among regions outside of Asia. The enrichment of this ethnic group in the BC population presented a unique opportunity to address this knowledge gap.

9.1.1 Summary of key findings

In Chapter 3, standardized estimates of point prevalence and mean annual incidence over a 25-year period in the BC Asian-ethnic population were derived from retrospective, clinic-based case ascertainment. Prevalence of MS and related disorders among Asians in BC was approximately 15-fold less than that most recently estimated in the general population of BC\textsuperscript{44}, but was several times greater than in Asia. Moreover, prevalence of MS was greater among Canadian-born Asians compared to their immigrant counterparts. The incidence of MS among Asian females in this population appears to have increased during a recent 25-year period, but primarily between 2001 and 2010, amid relative stability in incidence in the non-Asian population.

9.1.2 Analysis and integration of findings

Most previous estimates of the prevalence of MS and related disorders in Asia are less than 2 per 100,000. The majority of these studies subsumed NMO and NMOSD under a broad definition of MS. Therefore, estimates of prevalence in the present study surpass those in Asia by a factor of 2-3 when limiting consideration to MS, but 8-fold when broadening the scope to MS and related
disorders. Taken together, this study places the prevalence of MS and related disorders in the Asian-ethnic population of BC between that of Asia and the general Canadian population.

In the few published studies to estimate prevalence in Asian-ethnic populations residing in regions outside of Asia, prevalence ranged from 2.2 to 6.7 per 100,000\textsuperscript{300,302}. Interestingly, in one of these studies\textsuperscript{302}, performed some 20 years ago in BC, prevalence was approximately 8-fold lower than in the present study. It remains unclear whether this ostensible temporal increase in MS prevalence reflects a genuine increase in disease burden, improvements in ascertainment, referral bias, or a combination thereof. Additional studies are needed to clarify the relative contribution of each of these phenomena to the observed epidemiological trends.

The notably higher prevalence of MS and related disorders in the Asian-ethnic population of BC relative to populations in the countries of origin further substantiate the notion that the risk of MS and related disorders in Canada is exceptionally high. However, possible regional differences in case ascertainment, which would distort estimates of the true incidence and prevalence, must also be acknowledged. For instance, under-diagnosis, disparate access to care, and differential care-seeking are all factors that warrant consideration in explaining apparent differences in the frequency of MS between Asians in BC versus those remaining in their countries of origin. However, given the methodological uniformity in case ascertainment between Asian and non-Asian populations in this study, it stands to reason that prevalence in the Asian-ethnic population of BC is higher than that predicted by typical disparity in rates between Asians and Caucasians in the published literature.

Previous studies comparing MS frequency in native-born and immigrant Asian-ethnic populations in a high-risk region are limited to a single mortality study in ethnic Chinese and
Japanese living in California and Washington state\textsuperscript{289}. However, because mortality ratios were based on only five decedent cases in total, reliable conclusions regarding differential risk between these subpopulations could not be made.

Inequality in the prevalence of MS between the Canadian-born and immigrant Asian-ethnic subpopulations in the present study directs attention to the early-life period prior to migration in attempting to resolve the underlying mechanism of differential risk. Candidate risk factors to this end would be those to which the at-risk populations are differentially exposed during this period, including for example, infectious diseases, nutrition, or sunlight exposure. Future studies seeking the causative factors, however, demand that a wide net be cast, given the broad age range implicated and the litany of possible candidate factors within this period of development. Moreover, because the prenatal period cannot be excluded on the basis of the current body of evidence, a rather broad search window is implicated.

In the present study, an apparent temporal increase in the incidence of MS among Asian-ethnic females in BC was observed. Similar female-specific upward trends in incidence have recently been reported elsewhere\textsuperscript{77–81}, including in Japan\textsuperscript{61}. The sum of the evidence to date indicates that the increase of MS in females is in fact a global trend. The factors underlying this epidemiological pattern, nevertheless, are unknown and remain the subject of considerable speculation. Candidate sex-differential risk factors that have been proposed in this regard include occupational exposures\textsuperscript{42}, urbanization\textsuperscript{491}, vitamin D\textsuperscript{492}, tobacco smoking\textsuperscript{493}, and oral contraceptives\textsuperscript{42}, among others. The widespread nature of this trend across ethnic groups representing a broad range of baseline MS risk suggests that the causative factor may indeed be ubiquitous.
9.1.3 Future research directions

The findings reported in Chapter 3 expose avenues of future research that could help to clarify the complex etiology of MS and related disorders. Larger epidemiological studies exploring the environmental basis of differential MS risk in specific subpopulations are a logical extension of the work presented here. For instance, comparative analysis of environmental exposures in domestic-born versus immigrant Asian cases in high-risk regions may contribute greater insight into the impact of early-life or region-specific environmental factors influencing susceptibility. Likewise, comparison of exposure rates between males and females may shed light on sex-specific environmental risk factors.

9.1.4 Significance of findings

By establishing accurate estimates of prevalence and incidence of MS and related disorders in the Asian-ethnic population of BC, this work contributed a number of novel findings and perspectives. This study was the first to compare trends in the incidence of MS in persons of Asian ancestry relative to the general population in a high-risk region. In addition, this research was the first to distinguish patterns of incidence in domestic-born and immigrant Asian subpopulations. Furthermore, this was the first systematic assessment of NMO prevalence in an Asian-ethnic population outside of Asia. This work expands on the nascent body of literature on MS burden in non-Caucasian ethnic groups, including those residing in traditionally high-risk regions and is particularly topical amid growing recognition of MS as a global disorder.
9.2 Clinical characteristics and outcomes of MS and related disorders in Asians

Atypical clinical presentation of MS and related disorders in Asian patients, characterized by more frequent optic nerve and spinal cord involvement, has long been recognized\textsuperscript{86}. This has in fact been one of the most intriguing features of these disorders in this population, particularly when viewed against the backdrop of research conducted primarily in predominantly Caucasian populations. Nevertheless, clinical aspects of MS and related disorders in patients of Asian ancestry continue to be sparsely researched. Remarkably, prior to this work, no studies characterizing clinical features and disability outcomes had been undertaken in Asian-ethnic patients from a high-risk region. Consequently, the prevailing clinical depiction of MS and related disorders in this ethnic group had been informed primarily by studies from Asia, where clinical recognition and expertise, by comparison, are considerably limited.

Relatively few studies adequately distinguish between MS and NMO in patients of Asian ancestry, as the nosology of MS and related disorders in Asian-ethnic patients was rather imprecise until recently. Therefore, many previous studies examining clinical outcomes in this population subsumed NMO with MS, an approach that was consistent with practices at the time\textsuperscript{86}, but is now considered outmoded. Many of these studies demonstrated marked differences in the clinical characteristics and outcomes of MS in Asian patients\textsuperscript{53,55,126,128,155,156,465}, generally showing poorer prognosis compared to Caucasian patients. Given the profound differences in the clinical features and outcomes of MS compared to NMO\textsuperscript{16,24}, many of these earlier studies demonstrating putative ethnic differences in clinical aspects of MS are now being questioned. The dearth of studies in Asian-ethnic patient populations that give adequate consideration to
clinical phenotypes when evaluating clinical features and outcomes served as the underlying rationale for the retrospective cohort studies in Chapters 4 and 5 of this dissertation.

9.2.1 Summary of key findings

In Asian-ethnic patients ascertained at clinics in Canada and China, MS cases outnumbered NMO by a ratio of 2-3:1. Clinical features in these ethnically similar patient cohorts from substantially different regions were generally alike. Male sex, later onset, and NMO clinical phenotype were predictive of unfavourable clinical outcomes. After carefully excluding NMO and NMOSD, differences in the clinical features and outcomes of MS between patients of Asian ancestry and those of predominantly Caucasian descent were rather unremarkable. In fact, ethnicity itself was not a significant determinant of clinical disability progression when considering MS cases alone.

9.2.2 Analysis and integration of findings

These findings cohere with established paradigms of ethnic differences in the relative frequency of clinical phenotypes of MS: Conventional MS is the dominant and nearly exclusive clinical pattern in Caucasians, whereas MS and NMO both contribute substantively to the clinical spectrum in Asians. The latter pattern is nearly universally reported among case series in Asia. That the clinical dichotomy was observed in both Canadian and Chinese patient cohorts suggests an intrinsic nature to the biological process underlying clinical presentation in patients of Asian ancestry—that is, one that proceeds largely independently from the environment. Modest differences in the MS-NMO ratio between patients residing in Canada versus China, however, suggest that environmental factors might modify the ratio to some extent.
Indeed, in support of this view, there is wide variation in the relative proportions of patients with NMO clinical phenotype in Asian populations, ranging from less than 20% to more than 50% in previous reports\textsuperscript{86}. Geographic correlates of clinical phenotype were the focus of investigations in Chapter 6 (discussed in Section 9.3).

Apart from clinical phenotype (diagnostic classification), prognostic factors for MS and related disorders in patients of Asian ancestry had not been investigated prior to this work. Factors associated with prognosis in this ethnic group were generally concordant with those identified in Caucasian populations\textsuperscript{123,459–462}. The overarching implication of this finding is that the key determinants of clinical outcomes in Asian patients may in fact be the same as those in Caucasian patients. These prognostic factors may therefore represent relatively rigid features of the pathophysiology of MS and related disorders. Collectively, these studies suggest that sex and age at onset are associated with clinical outcomes in ethnically distinct populations sharing a common environment, as well as ethnically similar populations localized to distinct environments. The mechanisms underlying these associations are therefore likely to be universal. On this basis, one may reasonably argue that, in some patients, fundamental demographic features such as age and sex should weigh more heavily on prognostication than should ethnicity, once an accurate diagnosis has been established.

In addition, NMO clinical phenotype was strongly associated with less favourable clinical outcomes overall. These findings, which confirm that clinical phenotype is a major determinant of clinical outcomes, underscore the importance of early and accurate diagnosis in this patient population. Diagnostic clarity is all the more imperative in this ethnic group, as treatment for MS
and NMO differs. In fact, it is now recognized that therapies with established efficacy in MS may be deleterious for NMO\textsuperscript{230-235}.

Findings of similarity in the clinical outcomes of MS between Asian-ethnic and Caucasian patients suggest that previous studies in Asian patients, on account of probable nosological conflation of NMO and MS, may have spuriously concluded that the prognosis of MS is less favourable in this ethnic group. Overall similarity in the clinical trajectory of MS between patients stratified by ethnicity could be interpreted as evidence that the primary factors influencing susceptibility may be distinct from those determining clinical progression. This is implied by the seeming paradox of a large disparity in baseline risk but overall similarity in clinical outcomes between these ethnic groups.

This study does not, however, offer sufficient justification for categorical dismissal of an ethnicity-mediated influence on clinical outcomes. The possibility, for example, that ethnicity-associated effects on clinical outcomes are of small magnitude—that is, below the threshold of detection by a study of this size—must be acknowledged. However, on the basis of the analysis presented in Chapter 5, the classical description of MS being profoundly more aggressive in Asian patients does not stand up to scrutiny; moreover, in the absence of appropriate consideration being given to clinical phenotype, this concept may in fact be inaccurate.

9.2.3 Future research directions

These studies address long-standing uncertainties with respect to the clinical profile of MS and related disorders in patients of Asian ancestry, but in doing so, also expose potential areas of further inquiry. For instance, whether there are ethnicity-specific prognostic indicators remains
unclear. Furthermore, the possibility that there may be ethnicity-associated differences in clinical outcomes that are not readily captured by traditional metrics of disability (i.e., EDSS, MSSS) employed in the this study—for example, cognitive deficits and fatigue—remain largely unexplored. Experiences gleaned from the present work clearly indicate that larger sample sizes will be needed to address these outstanding gaps in knowledge reliably. Given the rarity of MS and related disorders in this ethnic group, a collaborative approach involving multiple clinic populations is imperative to the success of such future studies.

9.2.4 Significance of findings

Characterization of the clinical profile of MS and related disorders in patients of Asian-ethnic origins in Chapters 4 and 5 was, in large measure, enabled by access to patient populations of considerable size from distinct clinic settings in China and Canada—the latter including a non-Asian comparator cohort that was ascertained and evaluated in parallel. Uniformity in case ascertainment and assessment, together with accurate diagnostic classification of cases, contributed to the overall methodological rigor of these studies.

This work was the first comparative analysis of long-term clinical outcomes of MS (with scrupulous exclusion of NMO cases) in Asian and non-Asian patients from a high-risk population. These studies offer the most cogent evidence to date that the clinical profile of conventional MS in Asians is largely similar to that in non-Asians, addressing a long-standing controversy in the field and contributing new knowledge to an inadequately studied area of research. The findings reported here are potentially clinically relevant, as they provide evidential basis for standardization of clinical best practices across ethnicities. Moreover, they enable a vast body of existing knowledge (gleaned from decades of clinical experience in predominantly
Caucasian patient populations) to be applied judiciously to an emerging patient population for which empirical evidence remains sparse.

### 9.3 Environmental factors associated with MS and related disorders in Asians

The environment unquestionably plays an important role in MS and related disorders, as several environmental factors have been associated with susceptibility and clinical outcomes. Although the regional environment has long been recognized as a major determinant of MS susceptibility, its influence on clinical phenotypes is largely unknown. Regional differences in the relative proportions of MS and NMO in Asian populations suggest that geographic factors could potentially influence clinical phenotype. This hypothesis, which was investigated in Chapter 6, had not been addressed by any previous study. Apart from studies of susceptibility involving geography and region of residence, there have been remarkably few studies investigating specific environmental risk factors for MS and related disorders in Asian-ethnic populations.

One such factor of particular interest in Asian populations is tobacco smoking. Apparent correlation of increasing incidence of MS with tobacco smoking in some regions of Asia prompted the study in Chapter 7 investigating smoking as an environmental factor with potential relevance to MS and related disorders in Asian populations. Tobacco smoking, which is one of the best established environmental risk factors for MS in Caucasians, had never previously been studied as a risk factor in Asian-ethnic populations.

#### 9.3.1 Summary of key findings

The results of the retrospective cohort study presented in Chapter 6 suggest that, in Canadian patients of Asian ancestry, longer duration of residence in Canada prior to onset of symptoms
predisposes to the conventional MS phenotype. On the other hand, among immigrant cases, onset of symptoms in the country of origin was associated with NMO phenotype. Case-control studies summarized in Chapter 7 revealed that smoking is associated with a three-fold increase in risk to develop conventional MS in the Asian-ethnic population of Canada. Smoking, however, was not associated with susceptibility to NMO in either population, nor was it associated with MS risk in the Shanghai population. In addition, patients who smoked exhibited a trend toward progressive clinical course and a higher disease severity index.

### 9.3.2 Analysis and integration of findings

Findings of an association between clinical phenotype and the place of residence in the pre-onset period are consistent with existing evidence suggesting that the relative proportions of MS and NMO are amenable to changes in the environment. Studies in Japan, for instance, demonstrated a lower prevalence of OSMS (which we now recognize to overlap considerably with NMO) in the northern region compared to the south \(^{58}\), as well as temporal changes in the ratio of MS and NMO clinical phenotypes \(^{60}\). The putative environmental factors underlying these observations, however, remain a matter of speculation. Prior to the present analysis, no studies seeking the determinants of clinical phenotype in Asian-ethnic patients had been undertaken.

Findings in the present work, along with previous reports from Japan, corroborate the view that the relative frequency of MS and NMO clinical phenotypes in Asians is malleable to environmental influences. Factors related to geography are plausible candidates in this regard. Taken together with evidence of a latitudinal gradient in the MS-NMO ratio in Japan \(^{58}\), findings here suggest that solar UV exposure may be one such factor. This hypothesis is further supported by an abundant body of literature associating MS risk with higher geographic latitude \(^{33–36,38,39}\).
and even more directly, with reduced solar UV exposure\textsuperscript{37}. The observed association of the conventional MS phenotype with the Canadian environment is consistent with this hypothesis, as UV penetration is generally lower in most regions of Canada compared to most populated areas of East and Southeast Asia.

Nevertheless, the relationship between regional environment and predisposition to a particular clinical phenotype may be considerably more complex than this. Longitudinal surveys in Japan, for instance, have also shown a temporal increase in the ratio of MS to NMO within the same geographic region\textsuperscript{60}, an observation that suggests an influence beyond geographic factors alone. Clearly, systematic investigation of potentially relevant factors to this end will be challenging, given the vast number of candidate factors with patterns of exposure consistent with these observations. On synthesizing evidence from this work and previous studies, it may be possible to distill the list of plausible candidates to a more manageable number. Viable candidates may include sun exposure, diet and nutrition, and infectious agents, particularly those that are differentially endemic between regions.

Prior to this work, there were no previous studies examining the relationship between smoking and the risk of MS and related disorders in Asian populations. Studies in predominantly Caucasian populations have demonstrated a robust association between smoking and MS risk, and to a somewhat lesser extent, an association between smoking and adverse clinical outcomes\textsuperscript{304}. The results of the present study, in conjunction with the existing body of knowledge, implicate smoking as a universal risk factor for MS onset and progression.

Moreover, these findings suggest a common mechanism of smoking-mediated MS susceptibility among different ethnic groups. Tobacco smoking is believed to influence immune modulation\textsuperscript{494}.
and initiate oxidative stress, leading to destruction of myelin and axons\textsuperscript{495}. The apparently greater risk conferred by smoking in this population compared to estimates in Caucasian populations implies that smoking may be relatively more impactful in the pathogenesis of MS in populations with lower intrinsic risk. It therefore stands to reason that in populations with low intrinsic risk, environmental exposures such as smoking may weigh more heavily in the balance of factors contributing to overall risk to develop MS. This interpretation is consistent with the prevailing multifactorial model of pathogenesis, but extends it with evidence to suggest that the magnitude of increased susceptibility imparted by a universal risk factor for MS may differ between ethnic groups.

Investigation of the possible link between smoking and the risk of NMO in Asian populations yielded contrasting findings relative to MS. Smoking was not associated with NMO risk in this analysis, a finding that suggests probable differences in the pathogenesis and pathophysiology of MS and NMO. Nosological distinction of MS and NMO is increasingly acknowledged in the literature\textsuperscript{496,497}. This study, therefore, further advances this notion by augmenting existing pathologic and immunologic evidence with perspectives from epidemiology.

9.3.3 Future research directions

These findings lay the groundwork for future research aimed at elucidating the role of the environment in MS and related disorders. Studies seeking the specific environmental determinants of clinical phenotype could draw upon these findings to delineate the temporal and spatial boundaries within which the relevant environmental exposures might operate to influence clinical presentation. However, the fact that such a study would need to be broad in scope is
inescapable. Therefore, studies aimed at narrowing the breadth of candidate risk factors should take priority.

Future research to validate the probable association of smoking with MS risk in Asian populations, especially in domestic Asian populations, is also justified in light of this work. Again, such a study would undoubtedly demand a concerted, multi-centre approach, given the rarity of MS and related disorders in these populations. Studies in ethnically heterogeneous populations would help to clarify whether the risk conferred by smoking is modified by ethnicity. In addition, validation studies to rule out smoking as a risk factor for NMO are also warranted. This study was the first to systematically explore this relationship, but additional larger studies will be needed to confirm these findings.

9.3.4 Significance of findings

The research summarized in Chapters 6 and 7 contribute new understanding to the role of the environment in MS and related disorders in persons of Asian ancestry. This study was the first to investigate environmental determinants of clinical phenotype in Asian-ethnic patients and the only study to evaluate smoking as a risk factor in this population. This work meaningfully advances current understanding with respect to the influence of the environment on susceptibility to MS and related disorders, as well as on clinical presentation thereof in Asian populations. The collective insights gleaned from this work will help to guide ongoing research on the etiology of MS and mechanisms underlying population variability in clinical characteristics and outcomes.
9.4 Genetic factors associated with MS and related disorders in Asians

Infrequent familial recurrence of MS and related disorders in Asian populations has been offered as one line of evidence for lower genetic risk. However, lower familial recurrence in Asia may in part be a consequence of lower background risk in Asia, resulting in fewer cases overall. Prior to this study, familial aggregation of MS in an Asian-ethnic population had not previously been reported in a region with high MS risk such as Canada. In addition, familial recurrence in persons of partial Asian ancestry had not previously been studied either. In general, current understanding concerning the genetics of MS and related disorders in populations of Asian-ethnic origin lags far behind that in populations of European ethnic origin. The body of literature in this regard is limited, particularly with respect to studies investigating non-HLA genes. Indeed the majority of genes associated with MS in Caucasian populations have not been screened in populations of Asian ancestry. Furthermore, few studies have explored the role of genetic factors in clinical phenotype determination, despite emerging evidence of pathological and immunological distinction of MS and NMO. Research presented in Chapter 8 of this dissertation was initiated to address these knowledge gaps.

9.4.1 Summary of key findings

The overall rate of familial MS in this ethnic group was low. An intriguing finding was that the rate of familial aggregation of MS and related disorders, which was established through patient report and subsequently confirmed through medical documentation, was greater among Asian patients in Canada than in China. Rates of familial recurrence in Canadian patients with mixed Asian-European ancestry were higher still.
In the analysis of 61 established candidate genes, variants in two genes with immunological and neurological function (GALC and THEMIS) were significantly associated with MS and related disorders in this population. In comparative analysis of MS and NMO cases, a gene involved in cerebellar and cortical development, AHI1, was associated with differential clinical phenotype in patients of Asian ancestry.

9.4.2 Analysis and integration of findings

The findings here are consistent with published reports from Asia universally reporting low familial recurrence of MS and NMO\textsuperscript{54,55,97,100,101,114}. Notably, there are no other studies from high-risk regions outside of Asia to which valid comparisons of the present findings can be made with respect to familial recurrence in persons of partial Asian ancestry.

The low frequency of multiplex pedigrees in Asian-ethnic populations, which in the present study was observed in regions with contrasting background risk, suggests that heritable factors play a lesser role in the risk to develop MS and related disorders in Asians. By the same reasoning, heritable factors are perhaps even less important in NMO. On the other hand, greater frequency of familial MS observed in Asians living in Canada versus those in China points to an appreciable influence of the environment on risk. Disparities in the rate of familial recurrence in these otherwise ethnically similar populations likely reflect substantial inequalities in background risk due to environmental factors.

Nevertheless, factors unrelated to the environment but relevant to the rate of diagnosis—including clinical awareness, care-seeking behaviour, and access to care—cannot be ruled out. The possibility of differential case ascertainment is particularly worthy of consideration, given
that MS and related disorders may be under-diagnosed in low-risk regions such as China. The rather striking observation of frequent familial recurrence among patients with partial Asian ancestry (a rate that was in fact comparable to that typically reported in Caucasian populations) supports the notion that heritable risk factors inherited from the high-risk non-Asian ancestry group might confer considerable risk on their own.

In Asian-ethnic populations, findings to date from studies investigating risk variants in non-HLA genes have been inconsistent. The majority of these studies have been narrow in scope, generally being limited to screens of selected polymorphisms in single candidate genes. This is the first study to investigate putative genetic association of several non-HLA genes with the risk to develop MS and related disorders in Asians. The risk variants identified in this study have not previously been reported in other studies of MS, either in Caucasians or Asians. These findings independently validate previous studies implicating GALC and THEMIS as genes involved in the pathogenesis of MS.

Identification of novel risk variants in genes previously established in Caucasian populations as being associated with MS susceptibility can be interpreted as evidence of common molecular mechanisms underlying pathogenesis in Asian and Caucasian populations. Indeed, among genes with established relevance to MS risk, those with putative immunological roles are enriched. These findings support the hypothesis that the prevailing autoimmune model of MS pathogenesis may be broadly applicable across different ethnic groups.

The genetic mechanisms underlying the phenotypic dichotomy of clinical presentation in Asian-ethnic patients are poorly understood. Prior to this work, few studies had directly addressed this question in a well-defined cohort of MS and NMO cases. Notably, none of these studies
surveyed genes outside of the HLA region. Association of \textit{AHI1} with differential clinical phenotypes in this study complements existing evidence of differential HLA associations with MS\cite{396,397} and NMO\cite{156,398,400,402} in Asian populations.

The putative role of \textit{AHI1} in cortical and cerebellar development supports its plausibility as a biologically relevant determinant of clinical phenotype, in light of recognized disparities in cortical and cerebellar involvement in MS compared to NMO\cite{141,145,178}. Furthermore, implication of a gene involved in neurodevelopment highlights the possibility that mechanisms distinct from primary immune dysfunction—for example, primary parenchymal degeneration—might contribute to the clinical and pathological features that distinguish MS and NMO. This concept warrants further consideration, particularly given its striking parallels to an emerging model of MS as a primary neurodegenerative disease\cite{105}.

\subsection*{9.4.3 Future research directions}

Future research drawing upon and extending the findings in the present work should include genetic screens in substantially larger cohorts. Such studies hold considerable promise with respect to identifying additional variants associated with risk and clinical phenotype in this ethnic group, especially those associated with a modest increase in risk. Furthermore, with increasing accessibility of genome-wide sequencing technologies, studies implicating rare variants associated with susceptibility and clinical presentation in this population are also reasonably within grasp. It is worth emphasizing once again that studies based on larger Asian-ethnic case cohorts will undoubtedly entail extensive collaboration between multiple centres, given the rarity of these disorders in Asians.
9.4.4 Significance of findings

The present study was the first to characterize familial recurrence of MS and related disorders in Asians residing in a high-risk region and in patients with mixed Asian-European ethnic origins. The findings illustrate the relative importance of the environment in modifying recurrence risk in populations with low genetic predisposition to MS and the possibility that Asian and non-Asian ancestral genetic factors may contribute unequally to familial MS risk. In addition, the genetic association study in Chapter 8 was the first to simultaneously screen several established MS risk genes outside of the HLA region in an Asian-ethnic population. Two novel risk variants were identified using this approach. Comparative analysis of MS and NMO cases led to the identification of a non-immunological gene associated with clinical phenotype in patients of Asian ancestry. Collectively, these findings inject valuable new insights into an area of research that has to this point remained inadequately explored.

9.5 Concluding remarks

The research presented here advances the current state of knowledge with respect to MS and related disorders in Asians by contributing novel findings across multiple research disciplines. This work represents the most comprehensive investigation of the clinical, epidemiological, and genetic features of MS and related disorders undertaken to date in persons of Asian ancestry. Moreover, these studies are the first to include both cases ascertained from a clinic population in Asia in addition to clinics from a high-risk region (i.e., Canada). This unprecedented, multi-centre, multi-regional approach enabled a broad range of research questions to be addressed, which in turn generated several important findings.
9.5.1 Strengths and limitations

Some limitations of this work warrant consideration. The threat of sampling bias is an obligatory concern for studies in which participation of eligible subjects is incomplete. With specific reference to the environmental and genetic epidemiological studies in Chapters 7 and 8, which respectively were based on patient-reported data and volunteered biological samples, the possibility remains that included cases may not have been representative of the overall at-risk population. Such sampling biases were largely unavoidable due to the voluntary nature of data acquisition in these studies. This is an acknowledged limitation of this study. However, preliminary analysis confirmed that the cases included in these studies did not differ significantly from non-participating eligible cases in terms of key demographic and clinical characteristics.

The intrinsic limitations of secondary data sources, a number of which were critical to addressing several research questions in this study, also warrant a brief discussion. This work, for example, drew heavily from clinical data repositories including the UBC MS Clinic research database and patient medical records, as well as smoking exposure data from the CCHS and genetic variant frequency data from the 1000 Genomes Project. Foremost among concerns regarding the application of secondary data to clinical and epidemiological research is the fact that such data were not collected with the specific objectives of this particular research in mind. Therefore, given the fixed nature of these datasets, the capacity to obviate or adjust for confounding, biases, or missing data relevant to this study was limited. Although statistical adjustment for confounding was performed where appropriate by incorporating possible confounding variables as covariates in regression modeling, final appraisal of the findings of this research should give appropriate weight to the overall limitations of these secondary data sources.
Finally, although it was anticipated *a priori* that the number of eligible cases in these studies would be considerably smaller than in analogous studies in a high-risk ethnic group, it is worth noting that small sample sizes in these studies imposed a number of methodological constraints that precluded the detection of small effects, analysis of interaction between variables, and some subgroup analyses due to limitations in statistical power. However, it merits noting that each of the individual studies comprising this work yielded significant findings.

Furthermore, the methodological strengths of this research serve as a counterpoint to the above limitations. Foremost among these was scrupulous classification of diagnosis and clinical phenotype. Accurate diagnostic classification of all cases according to current, validated diagnostic criteria and nosological definitions by neurologists with expertise in these disorders was a principal strength of this study, particularly when weighed against previous studies in this ethnic group. The experience to date in this special population is a reminder of the pitfalls of studies that fall short in regard to careful nosological distinction of MS and NMO clinical phenotypes. This study suggests that the ongoing controversy with respect to the true clinical profile of MS in Asian patients is largely a consequence of previous studies failing to account for the range of clinical phenotypes in this ethnic group. The diagnostic rigor built into the methodology of this study enabled a more accurate account of clinical and epidemiological features, particularly that of conventional MS, in this population.

Notwithstanding the aforementioned limitations concerning sample size, a notable strength of this research was the systematic and exhaustive approach applied to ascertaining eligible cases in an otherwise under-recognized patient population. Comprehensive case ascertainment was facilitated by identification of cases through the UBC MS Clinic network, which included four
hospitals across the province, enabling identification of an estimated 80% of all persons with MS and related disorders in BC. Furthermore, this research was undertaken in a universal healthcare setting, thereby assuaging concerns of inadequate case ascertainment due to economic and other barriers to accessing care—a major limitation in previous studies in Asian countries with privately funded health care systems. It warrants emphasizing that, in terms of the number of cases analyzed, this research was the largest study of its kind involving Asian-ethnic patients in a high-prevalence region outside of Asia.

9.5.2 Key contributions to the field

This research generated a number of important new insights concerning the epidemiology and clinical profile of MS. For instance, this work provides the only recent estimate of prevalence and incidence of MS and related disorders in an Asian-ethnic population in a high-prevalence region. In addition, this is the first study to compare directly long-term clinical outcomes in patients of Asian ancestry to a predominantly Caucasian patient cohort ascertained from the same clinic population. Furthermore, it is the only such comparative analysis that adequately distinguished clinical variants typically observed in Asian patients. This study, therefore, is the most methodologically rigorous comparison of clinical outcomes in this ethnic group to date.

The work presented in this dissertation represents the first systematic investigation of the genetic and environmental basis of dichotomous clinical phenotypes in patients of Asian ancestry. More specifically, it includes the first study to examine the relationship between tobacco smoking and the risk of MS and related disorders in Asian-ethnic populations. In addition, this research is the first known application of exome sequencing to evaluate variants associated with MS and NMO in Asian-ethnic populations.
This research focused on an ethnic group that has historically been under-represented in MS research and for which the existing body of knowledge pertaining to MS and related disorders is under-developed. This work directly addresses major knowledge gaps across several disciplines with respect to the MS spectrum in persons of Asian ancestry. In addition to several novel contributions to the field in the way of concrete findings that augment the burgeoning body of literature, this research also advances the field by drawing attention to an under-recognized patient population. This work, therefore, increases awareness of MS and related disorders in persons of Asian ancestry within the lay, scientific, and medical communities, and in so doing, advances efforts to improve diagnosis and clinical management in this emerging patient population.

Ethnic Asians comprise a rapidly expanding proportion of the Canadian population. Commensurate growth in the corresponding MS patient population is all but certain, particularly in light of a recognized increase in the incidence of MS in Canada. This reality underscores the importance and topicality of research in this special population. Moreover, this work establishes a demonstrably useful paradigm upon which future studies in other common complex disorders featuring differential risk between ethnic groups may be modeled.
References


397. Ono, T. *et al.* Molecular analysis of HLA class I (HLA-A and -B) and HLA class II (HLA-DRB1) genes in Japanese patients with multiple sclerosis (Western type and Asian type). *Tissue Antigens* **52**, 539–542 (1998).


444. 1000 Genomes Consortium. 1000 Genomes Project. at <http://www.1000genomes.org/>


Appendices

Appendix A  Case report forms used to acquire clinical data

A.1  Longitudinal clinical data collection form (neurologist-generated data)
<table>
<thead>
<tr>
<th>Disease Modifying Therapy</th>
<th>Present</th>
<th>Start Date (mm-yy)</th>
<th>End Date (mm-yy)</th>
<th>Reason for Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaseron</td>
<td></td>
<td></td>
<td></td>
<td>pregnant</td>
</tr>
<tr>
<td>Rebsib 22</td>
<td></td>
<td></td>
<td></td>
<td>side-effects</td>
</tr>
<tr>
<td>Rebsib 44</td>
<td></td>
<td></td>
<td></td>
<td>non-responder</td>
</tr>
<tr>
<td>Copaxone</td>
<td></td>
<td></td>
<td></td>
<td>other</td>
</tr>
<tr>
<td>Betaseron</td>
<td></td>
<td></td>
<td></td>
<td>pregnant</td>
</tr>
<tr>
<td>Rebsib 22</td>
<td></td>
<td></td>
<td></td>
<td>side-effects</td>
</tr>
<tr>
<td>Rebsib 44</td>
<td></td>
<td></td>
<td></td>
<td>non-responder</td>
</tr>
<tr>
<td>Copaxone</td>
<td></td>
<td></td>
<td></td>
<td>other</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td></td>
<td></td>
<td></td>
<td>pregnant</td>
</tr>
<tr>
<td>Tysabii</td>
<td></td>
<td></td>
<td></td>
<td>side-effects</td>
</tr>
<tr>
<td>Azathioprine</td>
<td></td>
<td></td>
<td></td>
<td>non-responder</td>
</tr>
<tr>
<td>Cyclophosphor</td>
<td></td>
<td></td>
<td></td>
<td>other</td>
</tr>
<tr>
<td>Other O&amp;M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical Trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td></td>
<td></td>
<td></td>
<td>pregnant</td>
</tr>
<tr>
<td>Tysabii</td>
<td></td>
<td></td>
<td></td>
<td>side-effects</td>
</tr>
<tr>
<td>Azathioprine</td>
<td></td>
<td></td>
<td></td>
<td>non-responder</td>
</tr>
<tr>
<td>Cyclophosphor</td>
<td></td>
<td></td>
<td></td>
<td>other</td>
</tr>
<tr>
<td>Other O&amp;M</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Clinical Trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Followup Visit

#### Visit Date (dd-mm-yyyy)

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<thead>
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<th>Symptons</th>
<th>Steroids</th>
<th>Remission</th>
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<tbody>
<tr>
<td></td>
<td>ON</td>
<td>p.o.</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>spcs</td>
<td>i.v.</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td>be</td>
<td>cur</td>
<td>incomplete</td>
</tr>
<tr>
<td></td>
<td>scpbe</td>
<td>be</td>
<td>cur</td>
</tr>
<tr>
<td></td>
<td>bpbe</td>
<td>be</td>
<td>cur</td>
</tr>
<tr>
<td></td>
<td>spcsbe</td>
<td>be</td>
<td>cur</td>
</tr>
<tr>
<td></td>
<td>spcsbe</td>
<td>be</td>
<td>cur</td>
</tr>
</tbody>
</table>

*Disease Course/Impact (Kurtzke's Extended Disability Scale)*

- FS Pyramidal Tract
- FS Cerebellum
- FS Brainstem
- FS Sensation
- FS Bladder/Bowel
- FS Vision
- FS Cognition

Walking Distance > 500 m: yes | no

Maximum Walking Distance with Aid (in m):

EDSS Score:

*If possible, please note FS (in particular with EDSS < 4.0)*

**McDonald Criteria**

- Definite MS 1
- Definite MS 2
- Definite MS 3
- Definite MS 4

**Possible MS**

- Poss MS 1
- Poss MS 2
- Poss MS 3
- Poss MS 4

**Not MS**

- CIS
- NMO
- Myelitis
- ADEM
- Balo
- Other

**MS Diagnosis**

- Current Disease Course:
  - CIS
  - RRMS
  - SPMS
  - PPMS
- Not MS

**Date of Diagnosis (mm-yyyy)**

**Disease Modifying Therapy**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Start Date (mm-yyyy)</th>
<th>Start Date (mm-yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaseron</td>
<td>present</td>
<td>post</td>
</tr>
<tr>
<td>Reinf 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reinf 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avonex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copaxone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other

**Reason for Withdrawal**

- pregnant
- side-effects
- non-responder
- other
A.2 Cross-sectional clinical data collection forms (English and Chinese)
### Kurtzke Score

**Kurtzke Score**  
Date of most recent assessment:  
Kurtzke score at most recent assessment:  
Description of disability:  

### Current status at most recent assessment

- **Relapse**  
- **Progressive**  
- **Stable**  

**Date of most recent assessment:**

### Disposition Investigations

<table>
<thead>
<tr>
<th>Test</th>
<th>Date Performed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT Scan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood immune function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Tests (specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Cognitive Impairment

- **Yes [ ] No [x] Not Noted in Chart [ ]**

  If YES, indicate:
  - Onset (month/year)
  - Severity (if known)

### Depression

- **Yes [ ] No [x] Not Noted in Chart [ ]**

  If YES, indicate:
  - Onset (month/year)
  - Severity (if known)
  - Treatment (if known)

### Attempted Suicide

- **Yes [ ] No [x] Not Noted in Chart [ ]**

  If YES, indicate date (year) if known.

---

**Extended Kurtzke Score [最新病程扩展 Kurtzke 评分]**

- **Latest Evaluation Date:**
- **Latest Kurtzke Score (EDSS):**
- **Disability Overview:**

### 在最近评估时病人的状况

- **Relapse [ ] Progressive [ ] Stable [ ]**

### 对病人最近的处置

<table>
<thead>
<tr>
<th>Test</th>
<th>Date Performed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT Scan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood immune function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Tests (specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Cognitive Impairment [认知功能障碍]:

- Yes [ ] No [x] Not Noted in Chart [ ]

  If YES, please note:
  - Onset (month/year)
  - Severity (if known)

### Depression [精神状态]:

- Yes [ ] No [x] Not Noted in Chart [ ]

  If YES, please note:
  - Onset (month/year)
  - Severity (if known)

### Attempted Suicide [自杀行为]:

- Yes [ ] No [x] Not Noted in Chart [ ]

  If YES, please note:
  - Onset (month/year)
  - Severity (if known)
Appendix B  Standardized questionnaires used to acquire patient-reported data

B.1  Demographics, geography, and ethnicity data collection forms (English and Chinese)
EThiCtiTy oF BOllOgiCAL PATiENTS
A. Mother of Index Case:
Place of Birth: ____________________________
(Town/Province/State/Country)
Primary Language(s) Spoken at Home as a Child:

What is the ethnicity of your biological MOTHER?
Specify Details: ____________________________

A. (i) Maternal Grandmother’s (Grandmother of Index Case) Family:
Ethnic Origin: ____________________________

Place of Birth: ____________________________
(Town/Province/State/Country)
Deceased: YES NO ____________________________
If NO, Current Residence:
(Town/Province/State/Country)

A. (ii) Maternal Grandfather’s (Grandfather of Index Case) Family:
Ethnic Origin: ____________________________

Place of Birth: ____________________________
(Town/Province/State/Country)
Deceased: YES NO ____________________________
If NO, Current Residence:
(Town/Province/State/Country)
B. Father of Index Case:

Place of Birth: ____________________________ (Town/Province/State/Country)

Primary Language(s) Spoken at Home as a Child: ____________________________

What is the ethnicity of your biological FATHER?

specify details: ____________________________

A. (i) Paternal Grandmother's (Grandmother of Index Case) Family:

Ethnic Origin: ____________________________

Place of Birth: ____________________________ (Town/Province/State/Country)

Deceased: YES ______ NO ______

If NO, Current Residence: ____________________________ (Town/Province/State/Country)

A. (ii) Paternal Grandfather's (Grandfather of Index Case) Family:

Ethnic Origin: ____________________________

Place of Birth: ____________________________ (Town/Province/State/Country)

Deceased: YES ______ NO ______

If NO, Current Residence: ____________________________ (Town/Province/State/Country)
## B.2 Smoking history data collection form (English and Chinese)

**C. Smoking**

1. Have you ever smoked cigarettes at all? **YES**! **NO**!

*If YES, fill out the personal exposure history table below.*

<table>
<thead>
<tr>
<th>From Age</th>
<th>To Age</th>
<th>Amount of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Smoke Daily ________ cigarettes/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smoke Occasionally ________ cigarettes/week</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>From Age</th>
<th>To Age</th>
<th>Amount of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Smoke Daily ________ cigarettes/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smoke Occasionally ________ cigarettes/week</td>
</tr>
</tbody>
</table>

| Smoked 200 cigarettes in lifetime? **YES**! **NO**! |

---

**如果吸烟，填写下面的个人吸烟史表格。**

<table>
<thead>
<tr>
<th>Year</th>
<th>Amount of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>每天吸________香烟每一天</td>
</tr>
<tr>
<td></td>
<td>每周吸________香烟每星期</td>
</tr>
<tr>
<td></td>
<td>每月吸________香烟每月</td>
</tr>
<tr>
<td></td>
<td>每年吸________香烟每年</td>
</tr>
<tr>
<td></td>
<td>每周吸________香烟每星期</td>
</tr>
<tr>
<td></td>
<td>每月吸________香烟每月</td>
</tr>
<tr>
<td></td>
<td>每年吸________香烟每年</td>
</tr>
</tbody>
</table>

---

259
B.3 Family history data collection forms (English and Chinese)
FATHER (父亲)

<table>
<thead>
<tr>
<th>Disease Code</th>
<th>T</th>
<th>N</th>
<th>??</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Sclerosis</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>MS Diagnosis?</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Optic Neuropathy</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Lupus</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Thyroid Disease</td>
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<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Schizophrenia</td>
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<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Depression</td>
<td>T</td>
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<td>??</td>
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</tr>
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<td>Anemia</td>
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</tr>
<tr>
<td>Epilepsy</td>
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Comments:

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FATHER (父亲)

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<th>N</th>
<th>??</th>
<th>X</th>
</tr>
</thead>
<tbody>
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<td>??</td>
<td>X</td>
</tr>
<tr>
<td>MS Diagnosis?</td>
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<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Lupus</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Thyroid Disease</td>
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<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Schizophrenia</td>
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</tr>
<tr>
<td>Depression</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Anemia</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Diabetes</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Insulin Required</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
<tr>
<td>Tourette's Syndrome</td>
<td>T</td>
<td>N</td>
<td>??</td>
<td>X</td>
</tr>
</tbody>
</table>
### Biological Brothers and Sisters

**Biological Full Name (Mother and Father's Brothers and Sisters)**

Include all brothers and sisters of the index case, including any who died at a young age. (From oldest to youngest)

**Sibling Number:** __  **Total Number of Full Siblings:** __

**Given Name(s):**

**Last Name:**

For females, maiden name: __________

**Sex:** Male □  Female □

**Date of Birth:** (Month) / (Day) / (Year)  **Place of Birth:** (City, Province, Country)

**Age & Cause of Death (if deceased):**

**City/Province/Country of Residence:**

**Does this sibling have any children?** Yes □  No □  No Info □

**Does this sibling have any of the following diseases - circle the correct code**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Y</th>
<th>N</th>
<th>?</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Sclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS Diagnosed?</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Lupus</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Thyroid Disease</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Nephritis</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Juvenile Diabetes</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Adult Onset Diabetes</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Insulin Required?</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Perinatal Anemia</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Rheumatoid Colitis</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Depression</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Suicide</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Deseretea</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Bipolar Disorder</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Myasthenia Gravis</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Parkinson's Disease</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Other Neurological Disorder</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
</tbody>
</table>

**Comments:**

---

**Sibling Number:** __  **Total Number of Full Siblings:** __

**Given Name(s):**

**Last Name:**

**Sex:** Male □  Female □

**Date of Birth:** (Month) / (Day) / (Year)  **Place of Birth:** (City, Province, Country)

**Age & Cause of Death (if deceased):**

**City/Province/Country of Residence:**

**Has this sibling had any of the following diseases - circle the correct code**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Y</th>
<th>N</th>
<th>?</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS Diagnosed?</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Malignant Disease</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Skin Cancer</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Gastric Cancer</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Neurological Disorders</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
<tr>
<td>Other Neurological Disorders</td>
<td>Y</td>
<td>N</td>
<td>?</td>
<td>X</td>
</tr>
</tbody>
</table>

**Comments:**

---

262
BIOLOGICAL CHILDREN

Do you have any biological children? YES ☑ NO ☑

Fill out the following for all liveborn children of the index case, including
any who died at a young age. (From oldest to youngest)

Child Number: _____ Total Number of Biological Children: _____

Given Name(s): ____________________________

Last Name: ____________________________

For Females, Maiden Name: ____________________________

Sex: Male ☑ Female ☑

Date of Birth: (Month) (Day) (Year) _____ Place of Birth: (City, Province, Country)

Residence of child's mother at conception: ____________________________

Residence of child's mother during pregnancy: ____________________________

Age & Cause of Death (if deceased): ____________________________

City/Province/Country of Residence: ____________________________

Does this child have any of the following diseases? Circle the correct code
(Y = YES, N = NO, ?? = UNSURE, X = NO INFO)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Y</th>
<th>N</th>
<th>??</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Sclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS Diagnosed? Yes/No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optic Neuritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitiligo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid Disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult Onset Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin required?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others neurological disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments: ____________________________

______________________________

亲生子女

你有亲生子女吗？ 有 ☑ 无 ☑

填写每位患病的亲生子女，包括较小年纪就去世的，从年长到年幼

子女编号： _____ 亲生子女的总数： _____

名： ____________________________

姓： ____________________________

如果是女性，原姓： ____________________________

性： 男 ☑ 女 ☑

生目： (月) (日) (年) 出生地：(城镇，省，国家)

孩子母亲受孕时居住地： ____________________________

孩子母亲怀孕期间居住地： ____________________________

(如果死亡)死亡年龄和原因： ____________________________

居住的城镇/省/国家： ____________________________

该子女有无下列疾病 — 选择正确代码

(X = 有  N = 无  ?? = 不确定  X = 没有资料)

<table>
<thead>
<tr>
<th>疾病描述</th>
<th>X</th>
<th>N</th>
<th>??</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS诊断了没</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>视神经炎</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>神经性脊椎症</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>非典型关节炎</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>白内障</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>甲状腺症</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>青少年糖尿病</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>婴儿型糖尿病</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>低血小板</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>重症肌无力症</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>小脑萎缩</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

补充： ____________________________

______________________________
<table>
<thead>
<tr>
<th>Affected Relative Details</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First Name:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last (and/or Maiden) Name:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (circle one) Male Female Male Female Male Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of Birth (Best Estimate): (month/day/year) (month/day/year) (month/day/year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &amp; Cause of Death (if dead):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relationship Details:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>City/Country of Residence:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the following circle the correct code (Y = YES, ?? = UNSURE)

Multiple Sclerosis

<table>
<thead>
<tr>
<th>Can be contacted?</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>所波及的亲属详情</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>名:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>性别（选择） 男 女 男 女 男 女</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>生日 (月/日/年) (月/日/年) (月/日/年)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>年龄及死因 （如果已死亡）:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>与你关系:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>居住地 (城市/村庄):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

下面请选择正确的代号 (Y = 是， ?? = 不确定)

是否患病

<table>
<thead>
<tr>
<th>m9 明显症状</th>
<th>Y ?? Y ?? Y ??</th>
<th>Y ?? Y ?? Y ??</th>
</tr>
</thead>
<tbody>
<tr>
<td>可以触摸上吗?</td>
<td>可以 不可以 可以 不可以</td>
<td>可以 不可以</td>
</tr>
</tbody>
</table>
Appendix C  Candidate MS risk genes screened in genetic analysis

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI1</td>
<td>Abelson helper integration site 1</td>
<td>6q23.3</td>
</tr>
<tr>
<td>ARL6IP4</td>
<td>ADP-ribosylation-like factor 6 interacting protein 4</td>
<td>12q24.31</td>
</tr>
<tr>
<td>BACH2</td>
<td>BTB and CNC homology 1, basic leucine zipper transcription factor 2</td>
<td>6q15</td>
</tr>
<tr>
<td>BATF</td>
<td>Basic leucine zipper transcription factor, ATF-like</td>
<td>14q24.3</td>
</tr>
<tr>
<td>C1orf106</td>
<td>Chromosome 1 open reading frame 106</td>
<td>1q32.1</td>
</tr>
<tr>
<td>CBBLB</td>
<td>Cbl proto-oncogene B, E3 ubiquitin protein ligase</td>
<td>3q13.11</td>
</tr>
<tr>
<td>CD37</td>
<td>CD37 molecule</td>
<td>19q13.3</td>
</tr>
<tr>
<td>CD40</td>
<td>CD40 molecule, TNF receptor superfamily member 5</td>
<td>20q12-q13.2</td>
</tr>
<tr>
<td>CD58</td>
<td>CD58 molecule</td>
<td>1p13</td>
</tr>
<tr>
<td>CD6</td>
<td>CD6 molecule</td>
<td>11q13</td>
</tr>
<tr>
<td>CD80</td>
<td>CD80 molecule</td>
<td>3q13.3-q21</td>
</tr>
<tr>
<td>CD86</td>
<td>CD86 molecule</td>
<td>3q21</td>
</tr>
<tr>
<td>CIITA</td>
<td>Class II, major histocompatibility complex, transactivator</td>
<td>16p13</td>
</tr>
<tr>
<td>CLEC16A</td>
<td>C-type lectin domain family 16, member A</td>
<td>16p13.13</td>
</tr>
<tr>
<td>CLECL1</td>
<td>C-type lectin-like 1</td>
<td>12p13.31</td>
</tr>
<tr>
<td>CXCR5</td>
<td>Chemokine (C-X-C motif) receptor 5</td>
<td>11q23.3</td>
</tr>
<tr>
<td>CYP24A1</td>
<td>Cytochrome P450, family 24, subfamily A, polypeptide 1</td>
<td>20q13</td>
</tr>
<tr>
<td>CYP27B1</td>
<td>Cytochrome P450, family 27, subfamily B, polypeptide 1</td>
<td>12q14.1</td>
</tr>
<tr>
<td>DKKL1</td>
<td>Dickkopf-like 1</td>
<td>19q13.33</td>
</tr>
<tr>
<td>EOMES</td>
<td>Eomesodermin</td>
<td>3p24.1</td>
</tr>
<tr>
<td>EVI5</td>
<td>Ecotropic viral integration site 5</td>
<td>1p22.1</td>
</tr>
<tr>
<td>GALC</td>
<td>Galactosylceramidase</td>
<td>14q31</td>
</tr>
<tr>
<td>GPR65</td>
<td>G protein-coupled receptor 65</td>
<td>14q31-q32.1</td>
</tr>
<tr>
<td>HHEX</td>
<td>Hematopoietically expressed homeobox</td>
<td>10q23.33</td>
</tr>
<tr>
<td>ICAM3</td>
<td>intercellular adhesion molecule 3</td>
<td>19p13.3-p13.2</td>
</tr>
<tr>
<td>IL12B</td>
<td>Interleukin 12B</td>
<td>5q31.1-q33.1</td>
</tr>
<tr>
<td>IL12RB1</td>
<td>Interleukin 12 receptor, beta 1</td>
<td>19p13.1</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>Gene Name</td>
<td>Chromosome</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>IL22RA2</td>
<td>Interleukin 22 receptor, alpha 2</td>
<td>6q23.3</td>
</tr>
<tr>
<td>IL2RA</td>
<td>Interleukin 2 receptor, alpha</td>
<td>10p15-p14</td>
</tr>
<tr>
<td>IL7</td>
<td>Interleukin 7</td>
<td>8q12-q13</td>
</tr>
<tr>
<td>IL7R</td>
<td>Interleukin 7 receptor</td>
<td>5p13</td>
</tr>
<tr>
<td>IRF8</td>
<td>Interferon regulatory factor 8</td>
<td>16q24.1</td>
</tr>
<tr>
<td>KIF21B</td>
<td>Kinesin family member 21B</td>
<td>1q32.1</td>
</tr>
<tr>
<td>MALT1</td>
<td>Mucosa associated lymphoid tissue lymphoma translocation gene 1</td>
<td>18q21</td>
</tr>
<tr>
<td>MANBA</td>
<td>Mannosidase, beta A, lysosomal</td>
<td>4q24</td>
</tr>
<tr>
<td>MAPK1</td>
<td>Mitogen-activated protein kinase 1</td>
<td>22q11.21</td>
</tr>
<tr>
<td>MERTK</td>
<td>c-Mmer proto-oncogene tyrosine kinase</td>
<td>2q14.1</td>
</tr>
<tr>
<td>MMEL1</td>
<td>Membrane metallo-endopeptidase-like 1</td>
<td>1p36</td>
</tr>
<tr>
<td>MPV17L2</td>
<td>MPV17 mitochondrial membrane protein-like 2</td>
<td>19p13.11</td>
</tr>
<tr>
<td>MYB</td>
<td>v-myb avian myeloblastosis viral oncogene homolog</td>
<td>6q22-q23</td>
</tr>
<tr>
<td>MYC</td>
<td>v-myc avian myelocytomatosis viral oncogene homolog</td>
<td>8q24.21</td>
</tr>
<tr>
<td>NFKB1</td>
<td>Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1</td>
<td>4q24</td>
</tr>
<tr>
<td>PLEK</td>
<td>Pleckstrin</td>
<td>2p13.3</td>
</tr>
<tr>
<td>RGS1</td>
<td>Regulator of G-protein signaling 1</td>
<td>1q31</td>
</tr>
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<td>RPS6KB1</td>
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