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Multiple Sclerosis Progression: a clinical, genetic, and environmental investigation

By

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Abstract

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Doctor of Philosophy in Epidemiology

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Multiple sclerosis (MS) is an autoimmune disease that affects the central nervous system. Disease progression is highly variable, with very few established predictors. This dissertation focuses on several disease progression measures and hypothesized clinical, genetic, and environmental predictors.

Chapter 1 provides a general introduction to MS and highlights the background, significance, and specific aims for each study/chapter.

Chapter 2 focuses on cognitive impairment measured in a MS case-control study nested in the Kaiser Permanente Medical Care Plan, Northern California Region. This study utilizes the modified telephone interview for cognitive status (TICS-M) in a large study of MS cases and healthy controls. The aim of this study is to examine cognitive status, as measured by TICS-M, and investigate potential associations between clinical, environmental, and genetic risk factors for MS susceptibility. This study is the first to implement this brief, phone-administered assessment of cognitive status in MS patients.

Chapter 3 focuses on 52 MS genetic risk variants and their potential associations with the MS Severity Score (MSSS) in a cohort of 7,125 MS cases provided through collaboration with the International Multiple Sclerosis Genetics Consortium (IMSGC). This study aims to test association between the strongest established genetic risk variants and a well-known measure of clinical disability. This study tests each variant individually, as well as two composite scores. While no genetic risk factor is associated with MS disease disability measured using the MSSS, previously established associations between gender and age of onset with MS progression are confirmed. These results substantiate that the genetic associations between susceptibility and progression in MS are distinct.

Chapter 4 focuses on a meta-analysis of smoking and MS disease progression. To date, there is controversy in the literature regarding the effects of smoking on MS progression. This study aims to incorporate all existing studies of smoking and disease progression in MS, as measured by time to transition from relapsing remitting (RR) MS to secondary progressive (SP) MS; time to transition from clinically isolated syndrome (CIS) to clinically definite (CD) MS; time to clinical measures of

ambulatory assistance; mean difference in MSSS; mean difference in expanded disability status scale (EDSS); and two MRI measures. These results support an association between smoking and acceleration of disease progression.

Finally, Chapter 5 highlights the major findings of each chapter, provides a conclusion, and suggested future directions.

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Dedication

I dedicate this dissertation to my husband, Christopher. Your love and support has been essential throughout this entire process. From late night dinners served at my computer, to encouraging me to keep writing, to believing in me; you have always inspired me to be better. I love you so much.

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Chapter 1. Introduction

Multiple sclerosis (MS) is an inflammatory autoimmune disease that affects the central nervous system (CNS). Neurological damage in MS is caused by irreversible demyelination of axons and oligodendroctyes in the brain and spinal cord [1, 2]. Experts believe that MS patients are born with a genetic susceptibility to MS, but require one or more environmental triggers at some point for onset of MS to occur [3, 4]. While there are several identified and confirmed risk factors for MS, much about the etiology of MS is still unknown.

Risk of MS

The World Health Organization has estimated that approximately 1.3 million people have been diagnosed with MS worldwide, with a global incidence of 2.5 per 100,000 persons. Individuals of Northern European descent have a higher risk of MS than individuals from other ethnic/genetic backgrounds [5-7]. In the United States, it has been estimated that one in every 1,000 individuals has MS, while in Sweden the prevalence is estimated at 0.19% [1, 8]. The preponderance of MS is twice as great among women as among men, and the average age of onset tends to be 20-40 years of age [9].

Strong evidence suggests there are both genetic and environmental components to MS onset and progression. There is increased disease concordance among monozygotic twins (~25%) as opposed to dizygotic twins (~5%) or siblings [10], which provides strong evidence for the importance of the genetic component of MS susceptibility. There is strong evidence supporting a role for genetic variation within the major histocompatibility complex (MHC) on chromosome 6p21 in MS. Within the MHC, there appears to be multiple independent susceptibility alleles in class I and class II loci, as well as biologic interactions between susceptibility alleles [11-17]. The strongest genetic risk factor for MS has been identified within the human leukocyte antigen (*HLA*)-*DRB1* locus, specifically, the 15:01 allele [13, 17-20]. Additionally, through the use of internationally pooled data for genome-wide association studies (GWAS) and replication studies, 52 non-MHC variants have been associated with MS onset and confirmed as susceptibility variants [18]. More recently, a candidate gene study identified 48 additional non-MHC risk alleles for MS derived from 14,802 cases and 26,703 controls, for a total of 110 non-MHC risk variants [21].

There are several known environmental risk factors for developing MS: smoking, infectious mononucleosis (IM) or Epstein-Barr virus (EBV) infection, obesity, and low serum levels of vitamin D [22, 23]. Consistently, cigarette smoking has been shown to increase the risk of developing MS. Handel et al. conducted a meta-analysis of 14 studies, which included data from 3,052 cases and 457,619 controls [24]. These data demonstrated that smokers had 1.5 times the risk of developing MS as compared to non-smokers. Individuals who have been infected with EBV are at an increased risk of developing MS [23]. Furthermore, the study by Ascherio et al. showed that individuals who were infected with EBV later in life had an even 2 to 3 fold increased risk of developing MS than those who were infected earlier in life. Childhood and adolescent obesity has been shown to be another risk factor for developing MS in females [25]. Low serum levels of vitamin D have been established as a risk factor for developing MS. The study by Simpson et al. combined 650 published MS prevalence estimates from over 300

published peer-reviewed studies [26]. The investigators confirmed a statistically significant positive association between MS prevalence and latitude globally, after adjusting for *HLA-DRB1* allele frequencies. These findings strongly support a role in the etiology of MS for environmental factors that vary with latitude, the most prominent candidates being ultraviolet radiation (UVR) and low serum levels of vitamin D, which appear to be independent of genetic ancestry.

MS Progression

There are four disease courses in MS: relapsing-remitting (RRMS), secondary progressive (SPMS), primary progressive (PPMS), and progressive relapsing (PRMS) [1]. The majority (~80%) of MS patients begin with a relapsing remitting course, in which they have intermittent attacks, between which they fully recover to baseline health. Generally, as the disease progresses over time, the patients will begin to have persistent symptoms that never fully resolve and eventually transition into secondary progressive. The most common course of the disease involves a series of attacks or exacerbations of symptoms, with periods of remission. Every part of the CNS is at risk of being involved in MS, producing symptoms such as sensory and motor disturbances, as well as cognitive dysfunction. Generally, the diagnosis of MS is made according to the McDonald criteria [27]. The diagnosis criteria include dissemination of the disease in time and space, which require two or more relapses and two or more lesions in the CNS, as documented by MRI. There is no known cure and few effective disease modifying therapies to limit the progression of MS.

There are several ways to measure MS progression. The transition from RRMS to SPMS is a significant milestone in the disease course of the vast majority of MS patients. The severity of the disease can be measured by the time it takes to make that transition: the faster the transition, the more severe the disease. Physical disability is another method of measuring severity. Two scales have been established for measuring MS disability: the expanded disability status scale (EDSS) and MS severity score (MSSS). EDSS is a scale measuring the physical impairments related to each neurological system and was established to measure disease progression: an EDSS of zero indicates no disability, whereas an EDSS of ten indicates death due to MS [28]. The MSSS is a probabilistic algorithm that uses the EDSS and duration of disease, typically measured from the onset of first symptoms to the EDSS exam date, to calculate disease severity [29]. Because MSSS incorporates both disease duration and physical disability, it is favored over EDSS in cross-sectional studies.

Cognitive impairment due to MS affects nearly 70% of individuals with the disease; however, there is great variability in severity and type of impairment [30]. Currently, little is known about the environmental and the genetic risk factors for cognitive impairment in MS. Large scale epidemiologic studies of cognitive impairment in MS are difficult to conduct because cognitive testing is generally lengthy and requires in-person administration [31-33].

To date, very few clinical, genetic, or environmental risk factors have been shown to predict progression of MS. Women are more likely to acquire MS then men and are more likely to have a benign disease course [34, 35]. Additionally, the older the age of onset, the more rapid the progression of disease [36]. However, there is very little evidence of genetic associations with progression of MS. Although a study by Shi et al. showed that carriers of the APOE ε4 genotype had worsening progression of cognitive deficits than non-carriers in a longitudinal

study of MS [37].

Low serum levels of vitamin D have been associated with MS progression. The current literature also suggests that higher serum levels of vitamin D may be protective against both exacerbations of MS [38-41], and development of T2-weighted enhancing lesions in the brain [42]. The study by Simpson et al. found that there was an inverse linear relationship between serum levels of 25-OH-D and the hazard of relapse of MS over the subsequent six months, with a hazard ratio (HR) 0.91 per 10 nmol/l increase in 25-OH-D level [39]. Additionally, exposure to tobacco smoke has been suggested to be associated with MS progression [43-45]. Cigarette smoking has been shown to affect the immune system, both increasing autoimmune reactions and decreasing systemic activity against infections [46]. While biologic plausibility exists for an association between smoking and MS progression, the current literature is not consistent [47].

This dissertation will examine the current literature of MS progression and test three hypotheses. Chapter 2 investigates the association between cognitive impairment, as measured by the modified telephone interview for cognitive status, and genetic markers of MS susceptibility in MS cases seen in the Kaiser Permanente Medical Care Plan, Northern California Region. Chapter 3 investigates the association between physical impairment, as measured by MSSS, and genetic markers of susceptibility in a ten unique datasets provided by the International Multiple Sclerosis Genetics Consortium. Chapter 4 investigates the association between exposure to cigarette smoke and multiple measures of MS progression using previously published results in a meta-analysis. Chapter 5 synthesizes the findings and suggests future directions for research.

Chapter 2. Cognitive impairment in multiple sclerosis assessed using the modified Telephone Interview for Cognitive Status (TICS-M)

ABSTRACT

Background: Cognitive impairment is common in individuals with multiple sclerosis (MS), and can affect social/emotional function, employment status, and quality of life. The application of an easily administered, validated cognitive impairment assessment tool is critical for conducting large studies to help identify clinical, environmental, and genetic factors associated with cognitive outcomes in MS.

Methods: MS cases and controls were identified from Kaiser Permanente Medical Care Plan, Northern California Region. The Modified Telephone Interview for Cognitive Status (TICS-M) was used to evaluate cognitive status. Associations between clinical, established environmental and genetic risk factors and cognitive status in MS cases were studied.

Results: The TICS-M scores were lower among MS cases compared to controls ($p=0.007$); similar results were observed for orientation, registration, and delayed recall sub-scores. Among cases, more severe disease was associated with a lower cognitive score ($p<0.001$). Gender was associated with cognitive impairment; men with MS had significantly lower scores compared to women ($p<0.001$). *HLA-DRB1*15:01* was not associated with cognitive impairment in cases, however, some evidence for an association between cognitive scores derived from the TICS-M in MS cases and non-HLA MS risk variants was observed.

Conclusions: The TICS-M score differed significantly between MS cases and controls. The current study demonstrated the feasibility of using a telephone administered cognitive assessment tool in large epidemiologic study of MS cases. In MS cases, the TICS-M score was associated with level of physical disability and gender. Established genetic risk variants for MS do not appear to play a major role in cognitive impairment, as assessed by the TICS-M.

BACKGROUND

Multiple Sclerosis (MS) is a severe autoimmune inflammatory disease of the central nervous system characterized by irreversible demyelination of axons and formation of lesions in the brain and spinal cord [1, 2]. Symptoms of MS vary greatly, but cognitive impairment may affect as many as 70% of patients [30]. Cognitive deficits can range from memory impairment to reduced visual/spatial processing, and resulting disability can lead to early departure from the work force and diminished self-esteem that can interfere with interpersonal relationships [48-50].

There is a strong genetic contribution to susceptibility of MS [51]; the human leukocyte antigen *(HLA)-DRB1* locus within the major histocompatibility complex (MHC), specifically the *DRB1*15:01* allele, confers the strongest risk [19]. Recent genome-wide association studies and targeted candidate gene approaches have identified 110 non-MHC risk variants for MS [18, 21]. Several environmental factors also confer an individual's risk of developing MS, including childhood obesity, low levels of vitamin D, and exposure to cigarette smoke [22, 23, 25, 52]. However, the genetic and environmental influences on MS clinical outcomes, such as cognitive impairment, are largely unknown.

A full assessment of cognitive function in MS requires neuropsychological examination, involving the use of lengthy questionnaires administered by trained professionals [31-33]; however, this approach is not practical to use in large-scale epidemiologic studies. An easily administered and inexpensive screening tool would make large studies of cognitive health in MS more feasible. However, to date, there is not a validated tool to assess MS cognitive impairment over the telephone.

The current study investigated cognitive status, as measured by a telephone interview using the Modified Telephone Interview for Cognitive Status (TICS-M) assessment tool in a large sample of MS cases and controls. While this tool has yet to be validated in an MS population, it has previously been validated in a healthy elderly population [53]. Additionally, the TICS-M was used to test for possible associations with physical disability and with genetic susceptibility variants in MS cases. By improving our understanding of the etiologic factors contributing to cognitive decline in MS patients, we hope the findings will eventually lead to better therapies and increase overall quality of life for MS patients.

METHODS

Study Population

MS cases and controls were recruited from the Kaiser Permanente Medical Care Plan in Northern California Region (KPNC), an integrated health services delivery system with 3.2 million members in a 22 county service area in Northern California. Comparisons with the general population have shown that the KPNC membership is generally representative with respect to demographic characteristics; although, individuals from impoverished neighborhoods (defined as neighborhoods where >20% of individuals are below the federal US poverty line) are underrepresented among KPNC members [54].

MS cases met well-established disease criteria, as defined by McDonald et al. [27]. MS cases were identified through electronic medical records (EMR), as any KPNC member with at least one outpatient diagnosis of MS by a neurologist (multiple sclerosis, ICD9 code 340.xx); 95% had at least two MS diagnoses by a neurologist at study entry. Controls were randomly selected from current KPNC members without a diagnosis of MS or related condition (optic neuritis, transverse myelitis, or demyelination disease; ICD9 codes: 340, 341.0, 341.1, 341.2, 341.20, 341.21, 341.22, 341.8, 341.9, 377.3, 377.30, 377.39, and 328.82) confirmed through EMR. All participants were 18-69 years of age, white non-Hispanic, and KPNC members at the time of initial contact. Length of membership in KPNC was similar in cases and controls [55]. All study protocols were approved by the Institutional Review Boards (IRB) of KPNC and the University of California, Berkeley, and all participants provided written informed consent.

Exposure and Clinical Data Collection

Study participants completed a detailed computer assisted telephone interview administered by trained staff. Environmental exposure data collected included history of tobacco smoking (ever/never), history of infectious mononucleosis (IM; ever/never), parental and self-education level (7-level scale, doctoral degree=0 to some high school=7), family history of MS (first degree relatives), current body mass index (BMI; kilograms/meters^2), and history of vitamin D supplementation (ever/never) (Table 1). Current and past history of depression were also collected [56]. History of depression was categorized as ever if the participant answered yes to either of the following questions: "Have you ever had a period of at least two weeks when you were bothered most of the day, nearly every day, by feeling depressed, sad, down, or low?" or "Have you ever had a period of at least two weeks when you did not enjoy most things, even things you usually like to do?" and reported having at least four symptoms of depression during that period. Participants who answered no to the proceeding questions but answered yes to one of the following questions: "Have you ever had a period of at least two weeks when you were bothered most of the day, nearly every day, by feeling irritable?" or "By feeling anxious?" and reported having at least five symptoms of depression during that period were also defined as having a history of depression. Depressive periods had to have lasted at least two weeks. Current depression was defined based on answering yes to the question: "Are you currently experiencing an episode of depression?" The current depressive episode was required to last two weeks or longer.

MS cases answered additional questions related to their disease. Age of onset of MS was determined as year of first self-reported symptom, based on the following: "How old were you when you had your first symptoms of MS?" and "What were your first symptoms of MS?" Year for symptom onset was calculated using date of birth provided in the EMR. Age of symptom onset was verified in the EMR when possible. Disease duration was calculated as the time between age of symptom onset and interview date and rounded to the nearest year. Disease course was categorized as relapsing-remitting/secondary progressive or other (which included primary progressive, progressive relapsing or unknown), based on patient history. Cases were asked to recall use of disease modifying therapies (DMT) commonly used to prevent progression of MS. Responses were based on the open-ended question: "Have you ever taken any medications for MS?" Cases were also asked a follow-up question listing all DMTs (i.e. Avonex, Rebif, Betaseron, and Copaxone, others). Self-reported responses were compared to KPNC electronic pharmacy records and were in strong agreement (data not shown).

Disease severity was measured for each MS case using the MSSS [29], and was based on current ambulatory status, as reported by the participant, and disease duration. Self-report of neurological impairment has been previously validated in an MS population [57]. MSSS was analyzed as a continuous variable, and as two dichotomous variables, as previously described [58]. Briefly, a binary MSSS variable was based on the median MSSS value, defined as MSSS ≤5 vs. >5, with a smaller score indicating more benign or 'mild' disease. The second dichotomous variable was based on extreme ends of the MSSS distribution, defined as MSSS <2.5 (benign or 'mild') vs. ≥7.5 ('severe') (Table 1).

Cognitive Score (TICS-M)

Cognitive score was calculated using the TICS-M, administered as part of the computer assisted telephone interview. Each participant was asked 14 questions to assess orientation; registration and free recall; attention and calculation; comprehension; semantic recent memory; language and repetition; and delayed recall. Each of these six areas was also considered as sub-scores of the TICS-M and analyzed individually. The TICS-M was implemented to assess mild cognitive impairment [59]; the questions were previously validated in an elderly population [53], but not validated to assess cognitive impairment due to MS. The scoring method of the TICS-M is the unweighted sum of correct answers (Table 1). The final TICS-M score for each participant was corrected for self-reported education level, as described by Gallo and Breitner [60]. Out of a possible 37 total points, the TICS-M in the current study ranged from 8-36, and was normally distributed in MS cases and in controls (Figure 1A).

Biospecimens and Genotyping

All participants provided biospecimens for DNA extraction; DNA samples were genotyped using Illumina's Human 660K BeadChip; genotypes were also imputed using the 1000 Genome Reference, IMPUTE2 and standard procedures [55]. The 110 non-MHC risk variants were studied [21]. *HLA-DRB1*15:01* was genotyped as previously described [19]. Analyses were restricted to participants who clustered into a homogenous subset based on two dimensions of separation by classical multidimensional scaling, as implemented in PLINK v1.07 [61]. After population outliers were removed, 921 cases and 553 controls were retained for final analysis

Weighted and Unweighted Genetic Risk Score

A weighted genetic risk score (wGRS) [62] was calculated using the natural log of the discovery odds ratios (ORs) for each of 110 non-MHC risk alleles [21] derived from 14,802 cases and 26,703 controls. It was calculated as the product of the weighted odds and the number of risk allele copies at each locus, summed across all variants. The weight for each locus was the natural log of the discovery OR for each allele and was normally distributed (Table 1, Figure 1B). The genetic risk score (GRS) was calculated as the sum of risk allele copies for all 110 variants without weighting. Both wGRS and GRS were analyzed as continuous variables.

Statistical Analyses

Student's T-test and chi-squared statistic were used to compare the distribution of variables between MS cases and controls (Table 1). Unadjusted and adjusted linear regression models were used to estimate beta values (β) and 95% confidence intervals

(95% CIs). The outcomes of interest were the scores derived from the TICS-M and the six sub-scores. Case-control models used case status as the predictor. Additional covariates modeled were: year of birth, gender, history of smoking and IM, self- and parental education level, current and history of depression, immediate family history of MS, current BMI, vitamin D intake, and *HLA-DRB1*15:01* status. Case-only models individually used the following predictors: MSSS, each of the 110 non-MHC SNPs, wGRS, GRS, and *HLA-DRB1*15:01* status (carrier/non-carrier). Case-only models also included age of onset, disease course, and DMT use. All variables were included in initial models; however, only variables that significantly affected the outcome, after backward stepwise elimination (α-level=0.05), were retained in the final adjusted models. All analyses were conducted using STATA v13.1 (StataCorp, TX). The current study was well powered for all analyses (Table 5).

RESULTS

MS cases were more likely to have ever smoked, have a history of IM, have a family history of MS, have current depression, and have an increased genetic risk burden (*HLA-DRB1*15:01*, wGRS, GRS), as expected (Table 1). The overall average TICS-M score differed significantly between MS cases and controls (Table 1), and this association persisted in multivariable regression models (Table 2A and 2B). On average, the overall TICS-M score was 0.64 points lower in MS cases when compared to controls (p=0.001) (Table 1); results were consistent with models that were adjusted for current depression, history of depression, year of birth, gender, smoking and parental education (β=-0.53, p=0.007) (Table 2A). Further, when MS cases with disease duration less than five years were compared to controls, the association persisted (data not shown). Significant differences were also detected between MS cases and controls when the TICS-M was divided into sub-scores measuring orientation, registration, and delayed-recall (Table 2B).

In the case-only analysis, year of birth, history of smoking, parental education, current depression, disease course, and MSSS were significantly associated with the TICS-M score in MS cases when each variable was considered independently ($p<0.05$, data not shown). The strongest association was observed for gender (β=-1.46, p=<0.001). Results suggest that on average, male MS cases had lower cognitive scores than female cases. Similarly, cases who were older, who were smokers, whose parents had a lower level of education, who were currently depressed, or who had a more progressive/unknown disease course at onset demonstrated lower cognitive scores, when compared to those who were younger, who never smoked, had more highly educated parents, who were not currently depressed, or a relapsing-remitting/secondary-progressive disease course at onset. These variables were subsequently included in case-only models investigating the relationship between disease severity, as measured by MSSS, and the TICS-M score.

In multivariable models, MS cases with more severe disease, as measured by MSSS, had a lower TICS-M score (Table 3). Notably, individuals with very severe MS (MSSS ≥7.5) had a lower TICS-M score compared to those with a benign presentation (MSSS <2.5) after adjustment (β=-1.12, p=0.005). Similar results were observed whether MSSS was considered as either a

continuous or binary variable. The relationship between gender and TICS-M also persisted in all MSSS multivariable models ($\beta_{Continuous}$=-1.32, p<0.001; β_{Binary}=-1.33, p<0.001; $\beta_{Extreme}$=-1.40, p<0.001), where men had a significantly lower TICS-M score than women, even after accounting for disease severity. Consistent associations were also observed between an older age of onset, less parental education, and disease course, with a lower TICS-M score.

Associations between the overall TICS-M score and sub-scores with established genetic risk factors were evaluated in MS cases. No evidence for an association between the wGRS or GRS and cognitive status was observed (data not shown). Similarly, *HLA-DRB1*15:01* was not associated with cognitive status (data not shown). One SNP, rs35967351 within *SLAMF7,* showed evidence of an association with the delayed-recall sub-score after adjustment and correction for multiple testing. Carriers of the risk allele for rs35967351 had a lower overall delayed recall sub-score compared to those who did not carry the risk allele (β=-0.34, p=<0.001) (Figure 2).

DISCUSSION

Cognitive impairment is a frequent and debilitating symptom of MS. Memory impairment is the most common symptom in MS cognitive impairment, however, deficits are also observed in information processing speed, executive functioning, and visual/spatial processing [63]. No single brief assessment tool can test all areas of cognitive function in MS patients. The TICS-M was validated as a telephone administered tool to distinguish between normal cognition, mild cognitive impairment, and dementia in individuals from an otherwise healthy elderly population [59]. The specific areas of cognitive impairment tested in the TICS-M are similar to the types of deficits experienced by MS patients. Therefore, this tool was used to assess cognitive impairment over the phone to a large population of MS cases and controls in KPNC. The assessment is based on the assumption an individual has a high school education; however, scores derived from the TICS-M can be corrected for education level, as previously described [60].

The TICS-M tests six areas of cognition, including orientation; registration and free recall; attention and calculation; comprehension; semantic recent memory; language and repetition; and delayed recall, some of which are commonly impaired in MS patient [30]. While this tool has not been specifically validated for cognitive assessment in patients with MS, there were significant differences observed in this population, even after accounting for education and depression in the models [56]. On average, the TICS-M score was lower in MS cases as compared to controls. These findings persisted when cases were restricted to those with disease duration of less than five years, suggesting that cognitive decline, as measured by TICS-M, may begin before or shortly after onset of other neurologic symptoms.

The TICS-M score was inversely associated with a stable measure of physical disability (MSSS) in this cohort of MS cases. As physical disability increased in MS cases, so did evidence for cognitive impairment, demonstrated by a lower TICS-M score (Table 3). Several clinical variables were also associated with the TICS-M in the case-only analysis, including gender, age of onset, parental education, and disease course. Male cases had more than one point decrease in TICS-M cognitive scores as compared to female cases. Notably, gender was not associated with

the TICS-M score among control individuals when considered separately (data not shown). Previously published work suggests that males have more severe disease progression [35]; results in the current study suggest disease mechanisms contributing to cognitive health in MS may differ by gender as well.

This is the first large study to test whether cognitive impairment, as measured by the TICS-M, varies by *HLA-DRB1*15:01* carrier status in MS cases; no association was observed. Our results are consistent with the lack of an association between *HLA-DRB1*15:01* and other clinical phenotypes that characterize disease progression (i.e. MSSS [64]). Recent research suggests several additional and independent MHC risk variants contribute to MS susceptibility [65]. Additional work is needed to fully exclude the possibility of a relationship between MHC and cognitive disability in MS, as the TICS-M was not designed to test all areas of cognitive impairment in MS. Further, no evidence of an association was observed between the TICS-M score in MS cases and either the wGRS or GRS. Results suggest the cumulative burden of non-MHC MS risk variants does not contribute to this phenotype.

We investigated each of the 110 non-MHC risk variants in MS cases. After correcting for multiple testing, a significant association between rs35967351within the *SLAMF7* gene and cognitive status assessed using the TICS-M was observed. Carriers of the risk allele had lower delayed-recall sub-scores than non-carriers (Figure 2). *SLAMF7* encodes a protein that plays a critical inhibitory role in human monocytes to control pro-inflammatory immune responses and is expressed on Natural Killer cells, a subset of CD8+ T lymphocytes, mature dendritic cells, and activated B cells [66]. Variation within *SLAMF7* has previously been associated with other autoimmune diseases, including systemic lupus erythematous [67]. Among Northern Europeans from the 1000 Genomes study population, several functional SNPs reside within a linkage disequilibrium (LD) block encompassing rs35967351 (approximately 11kb in length), including two splice region variants and 14 missense variants.

The current study had many strengths, including the use of a large sample of MS cases and controls in KPNC. Prevalent cases were studied, including those with very recent symptom onset. Disease course, as indicated by patient histories, suggest our case sample was representative of other established cohorts of MS cases; for example, ~80% of cases were relapsing-remitting at onset [64]. Clinical, environmental, and genetic data were collected for all participants, and analytic models in this analysis controlled for many potential confounders. Two variables captured history of depression and current depression assessed at the same time the TICS-M was administered. Both measures can affect cognitive performance [56], and therefore, were included in the current study. Additionally, other covariates, such as gender and parental education, were also associated with the TICS-M score, and were accounted for in models; therefore, the observed differences between cases and controls are likely due to true cognitive impairment, as measured by TICS-M. The TICS-M scores were corrected for education, as previously described [60]. Self-education, which differed as baseline between MS cases and controls (Table 1), can impact an individual's TICS-M score. In order to account for the baseline difference self-education is was critical to make the correction to the TICS-M scores prior to analysis.

Other commonly used cognitive assessment tools specific for use in MS patients include: Rao Brief Repeatable Neuropsychological Battery (BRNB), Minimal Assessment of Cognitive

Function in MS (MACFIMS), Brief International Cognitive Assessment for MS (BICAMS), and the Mini-Mental State Examination (MMSE). While these assessment tools are highly sensitive and specific for MS-specific cognitive impairment, each requires in-person administration (Table 4). Therefore, these assessment tools cannot be feasible administered in a large scale epidemiologic study, whereas the TICS-M has been utilized successfully in randomized controlled trials and large scale observational studies [68, 69]. These studies by Walker et al. and by Lacruz et al. were conducted in elderly populations testing for normal cognition, mild cognitive impairment, and dementia, which is how the TICS-M assessment tool was validated.

Some limitations must be acknowledged. The TICS-M has not been validated for testing cognitive impairment in MS patients. In order to perform a validation study in MS patients, the TICS-M would need to be validated among other cognitive assessment tools such as the BICAMS. For example, in a representative sample of MS patients, conduct both the BICAMS and TICS-M assessments and compare the results, using the BICAMS as the gold standard. If the TICS-M was able to identify the same level and type of cognitive impairment in MS patients as the BICAMS, then the TICS-M could be used in place of the more time consuming assessment tools.

MS cases and controls in the current study were restricted to white non-Hispanic participants, as defined by genetic ancestry, who were KPNC members; thus, potentially limiting generalizability to non-white populations. The case-only study of cognitive impairment was partly cross-sectional, as the MSSS and the TICS-M score were assessed for each individual at study entry; therefore, it is not possible to establish a temporal relationship between disease severity and cognitive status. However, this was not true for other variables, such as gender and genotype. Several other risk factors that can affect cognitive impairment, such as use of alcohol, comorbidities, and treatments given for symptoms were not controlled for in this analysis.

Further, the TICS-M does not test all aspects of cognitive impairment generally observed in MS patients. For example, while the sub-scores for orientation were significantly different between MS cases and controls, the questions included in the TICS-M cannot comprehensively assess executive functioning. Further, there are no tests for visual/spatial function in the TICS-M. As the TICS-M was not developed to assess cognitive decline due to MS disease processes, the association with the TICS-M score and related sub-scores cannot be attributed to the disease process with certainty.

Another limitation of this study was the possibility that very severe MS patients were not represented in this sample, as they may have been too ill to participate. However, if that was the case, the current associations with disease severity would be biased toward the null, and therefore conservative. Also, although MS patients with early disease (<5 years since onset) were included and differences in the TICS-M were detected, the current study was not designed to determine whether cognitive status was affected prior to onset of other neurologic symptoms.

Early identification of cognitive dysfunction, through the use of a screening tool, and characterization of clinical, environmental, and genetic predictors of poor cognitive health outcomes in MS may have benefits in the clinical setting. To date, there has been limited, but encouraging research showing that behavioral interventions have been successful in improving cognitive function in MS patients [70]. Most studies to date have been small (ranging from 50-

100 participants) and had short follow-up periods. Several randomized trials reported improved memory performance following a behavioral intervention [71, 72]. Additionally, there have been promising results from drug treatment studies. Results have shown increased mental processing speed with increasing doses of l-amphetamine [73], and significantly improved attention process in a double-blind placebo-controlled study giving a single dose of methylphenidate [74]. However, treatment studies so far have had similar limitations, including small size (20-60 on average) and short follow-up periods, and thus require replication.

In summary, an association between lower overall cognitive status, as measured by the TICS-M, and MS was observed in the current study. The current study showed an association between the TICS-M score and case status when comparing MS cases with recent onset and controls. Among MS patients, disease severity was also associated with lower overall TICS-M scores. These results support previous findings that show significant cognitive impairment can occur in patients with MS with little or no apparent physical disability [75]. Further, established genetic risk factors for MS do not appear to play a major role in cognitive health, as measured by the TICS-M. Validation studies are required to determine if the TICS-M can be used to identify cognitive impairment due to the disease process of MS.

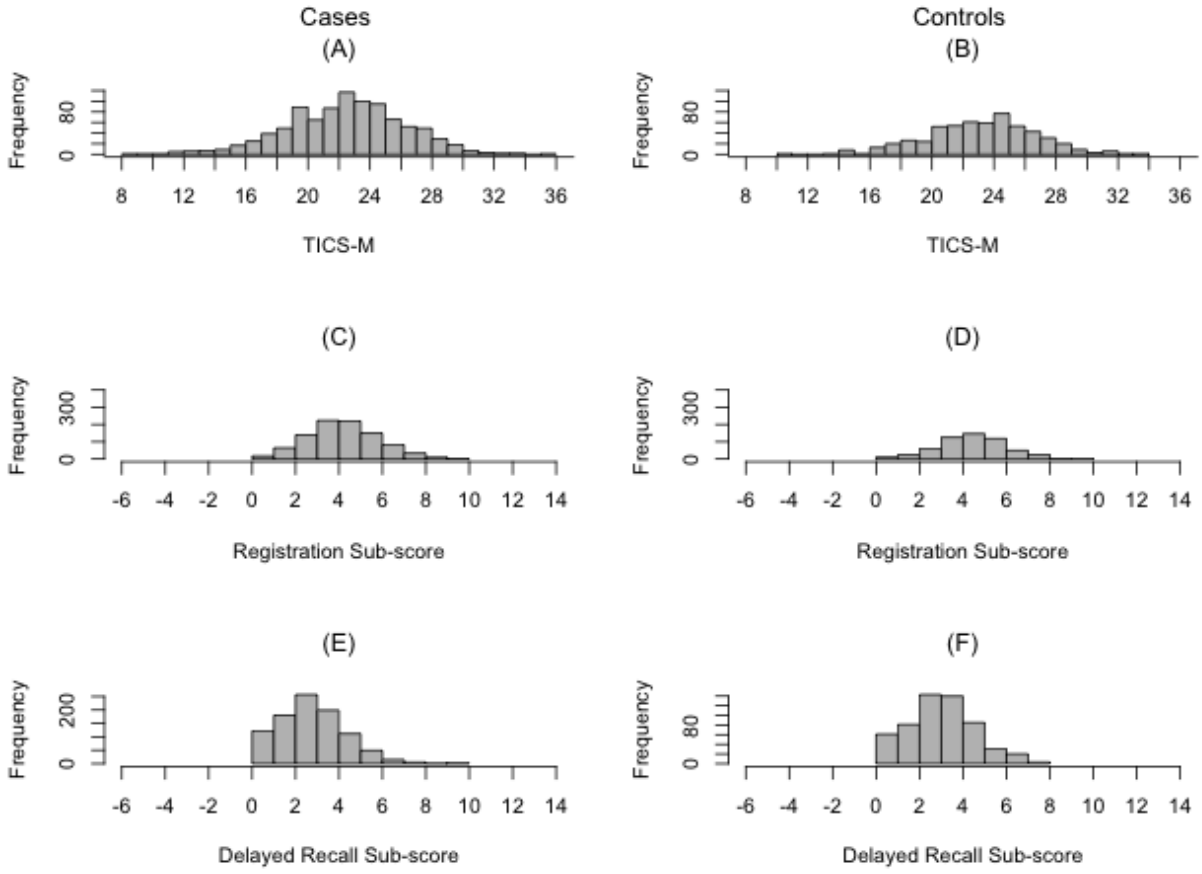
TABLES AND FIGURES

Table 1. Characteristics of the Kaiser Permanente Medical Care Plan, Northern California Region (KPNC) MS Cases and Control Individuals

	Cases Mean (SD)/Frequency (%)		**Controls Mean (SD)/Frequency (%)**		**P-value**
Number of individuals	921		553		--
Cognitive Score (TICS-M)	22.4 (3.8)		23.0 (3.5)		0.001
Orientation	5.5 (1.1)		5.8 (0.7)		<0.001
Registration	4.7 (1.7)		4.9 (1.6)		0.011
Calculation	4.0 (1.5)		4.0 (1.4)		0.953
Comprehension	5.0 (0.3)		5.0 (0.2)		0.038
Language	0.9 (0.2)		0.9 (0.2)		0.328
Delayed-recall	3.2 (1.6)		3.5 (1.6)		0.004
Year of birth	1958 (8.9)		1957 (8.2)		0.151
Female	722 (78.4)		464 (83.9)		0.010
Smoking (ever)	462 (50.2)		262 (40.9)		0.001
IM (ever)	233 (25.6)		75 (13.7)		<0.001
Education	--		--		0.008 **(Self)** 0.138 **(Parental)**
	Self	**Parental**	**Self**	**Parental**	
Some high school	0	26 (2.8)	2 (0.4)	11 (2.0)	--
High school graduate or GED	13 (1.4)	30 (3.3)	5 (0.9)	17 (3.1)	--
Some college or technical/ trade/vocational school	116 (12.6)	272 (29.5)	55 (10.0)	166 (30.0)	--
Associate's degree	357 (38.8)	228 (24.8)	189 (34.2)	110 (19.9)	--
Bachelor's degree	269 (29.2)	212 (23.0)	181 (32.7)	145 (26.2)	--
Master's degree	134 (14.6)	98 (10.6)	95 (17.2)	66 (11.9)	--
Doctoral degree	32 (3 4)	55 (6.0)	26 (4.7)	38 (6.9)	--
Family history of MS	78 (8.5)		12 (2.2)		<0.001
BMI (kg/m2)	26.9 (6.4)		26.9 (5.8)		0.998
Vitamin D supplements (ever)	277 (30.3)		133 (24.4)		0.016
History of depression					0.042
No	416 (45.2)		287 (51.9)		--
Yes	323 (35.1)		173 (31.3)		--
Don't know	182 (19.8)		93 (16.8)		--
Current depression (yes)	98 (10.6)		30 (5.4)		0.001
HLA-DRB1*15:01 **(positive/negative)**	491 (53.3)		156 (28.2)		<0.001
wGRS	11.3 (0.7)		11.1 (0.7)		<0.001
GRS	106.8 (6.5)		105.0 (6.7)		<0.001
MSSS Continuous	3.4 (2.6)		--		--
MSSS Binary (high)	215 (23.8)		--		--
MSSS Extreme (high)	109 (20.1)		--		--
Age of onset (years)	32.0 (9.7)		--		--
Disease duration (years)	12.1 (8.5)		--		--
Disease course			--		--
RRMS/SPMS	764 (83.0)		--		--
Other	156 (17.0)		--		--
DMT (ever)	711 (77.2)		--		--

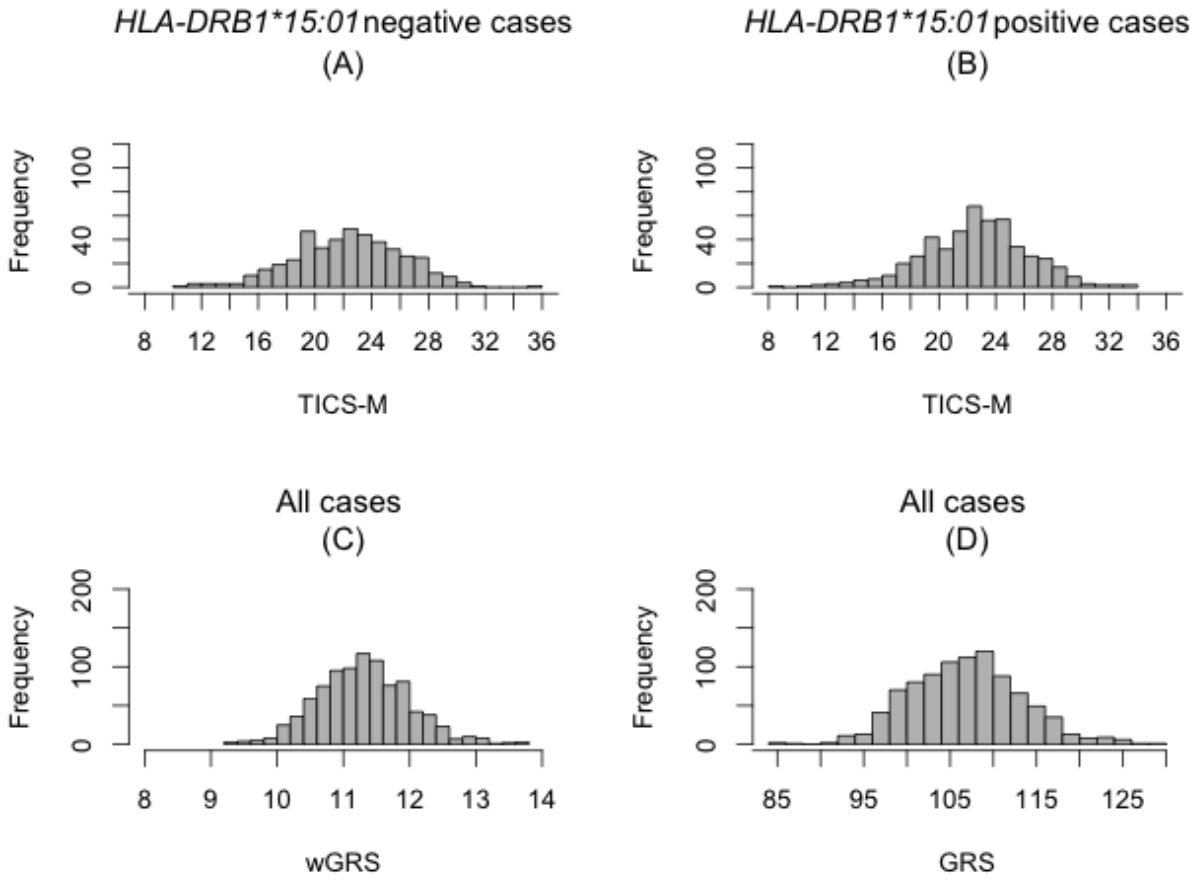
Abbreviations used: SD = standard deviation, TICS-M = modified Telephone Interview for Cognitive Status, IM = infectious mononucleosis, BMI = body mass index, wGRS = weighted genetic risk score, GRS = unweighted genetic risk score, MSSS = MS severity score, RRMS = relapsing remitting MS, SPMS = secondary progressive MS, DMT = disease modifying therapy. Education (self and parental) was coded as 0=doctoral degree to 7=some high school, indicating that less education is a risk factor. Student's T-test compared continuous variables between cases and controls; Chi Squared statistic compared categorical variables.

Figure 1A. Distribution of TICS-M Scores and TICS-M Sub-scores in cases and controls



(A) Distribution of the TICS-M scores in cases; (B) Distribution of the TICS-M scores in controls; (C) Distribution of registration sub-score in cases; (D) Distribution of registration sub-score in controls; (E) Distribution of delayed recall sub-score in cases; and (F) Distribution of delayed recall sub-score in controls.

Figure 1B. Distribution of TICS-M score in *HLA-DRB1*15:01* negative and positive cases, and distribution of the wGRS and GRS in all cases



(A) Distribution of the TICS-M scores in *HLA-DRB1*15:01* negative cases; (B) Distribution of the TICS-M scores in *HLA-DRB1*15:01* positive cases; (C) Distribution of wGRS in all cases; and (D) Distribution of GRS in all cases.

Table 2A. Associations for predictors in multivariable regression models for the TICS-M score in MS cases and controls

Model	β	95% CI	p-value
Case status	-0.53	(-0.9, -0.1)	0.007
Current depression	-0.95	(-1.6, -0.3)	0.006
History of depression	0.01	(0.003, 0.01)	0.001
Year of birth	0.03	(0.01, 0.1)	0.004
Female	-0.95	(-1.4, -0.5)	<0.001
Smoking	-0.46	(-0.8, -0.1)	0.016
Parental education	-0.15	(-0.3, -0.01)	0.033

Case status was used as a predictor for the TICS-M score. On average, MS cases had a -0.53 lower TICS-M score than controls after adjustment. The difference persisted when restricting to MS cases with less than five years of disease duration as compared to controls (data not shown). Backward elimination was used to retain variables in the final models, see Methods for details. Variables in the primary model were coded as: case-control status (0=control), current depression (0=not depressed), history of depression (0=never depressed), year of birth (years), gender (0=female), smoking (0=never smoked), parental education (0=doctoral degree to 7=some high school).

Table 2B. The association between MS case status on the TICS-M sub-scores in multivariable regression models

Model	Case Status β	95% CI	p-value
Orientation	-0.23	(-0.3, -0.1)	<0.001
Registration	-0.17	(-0.3, 0.003)	0.046
Calculation	0.02	(-0.1, 0.2)	0.776
Comprehension	-0.02	(-0.04, 0.01)	0.138
Language	-0.01	(-0.03, 0.02)	0.442
Delayed recall	-0.20	(-0.4, -0.03)	0.022

MS cases had significantly lower orientation, registration, and delayed-recall sub-scores than controls in adjusted models. All models were adjusted for: current and history of depression, year of birth, gender, smoking, self- and parental education. Self-education was added in the sub-score models because there was no correction made for the sub-scores. Self-education was coded as: 0=doctoral degree to 7=some high school.

Table 3. The associations between clinical factors and TICS-M cognitive scores in MS cases in multivariable regression models

Variable	**Model 1 (Continuous)**		**Model 2 (Binary)**		**Model 3 (Extreme)**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
MSSS Continuous	-0.18 (-0.3, -0.1)	<0.001	--	--	--	--
MSSS Binary	--	--	-1.26 (-1.9, -0.7)	<0.001	--	--
MSSS Extreme	--	--	--	--	-1.12 (-1.9, -0.4)	0.005
Gender*	-1.32 (-1.9, -0.7)	<0.001	-1.33 (-1.9, -0.8)	<0.001	-1.40 (-2.2, -0.6)	<0.001
Age of onset*	0.03 (0.01, 0.1)	0.011	0.03 (0.003, 0.1)	0.030	--	--
Parental education*	-0.36 (-0.5, -0.2)	<0.001	-0.34 (-0.5, -0.2)	<0.001	-0.38 (-0.6, -0.2)	0.001
Disease course*	-0.78 (-1.4, 0.1)	0.020	-0.73 (-1.4, -0.1)	0.030	--	--

[y]MSSS was considered as continuous (Model 1), or dichotomous (Model 2 and Model 3); backward elimination was used to retain variables in the final models, see Methods for details. Variables in the initial model were: gender (0=female), age of onset (years), parental education (0=doctoral degree to 7=some high school), and disease course (0=RRMS and SPMS, 1=PPMS, PRMS or Unknown).

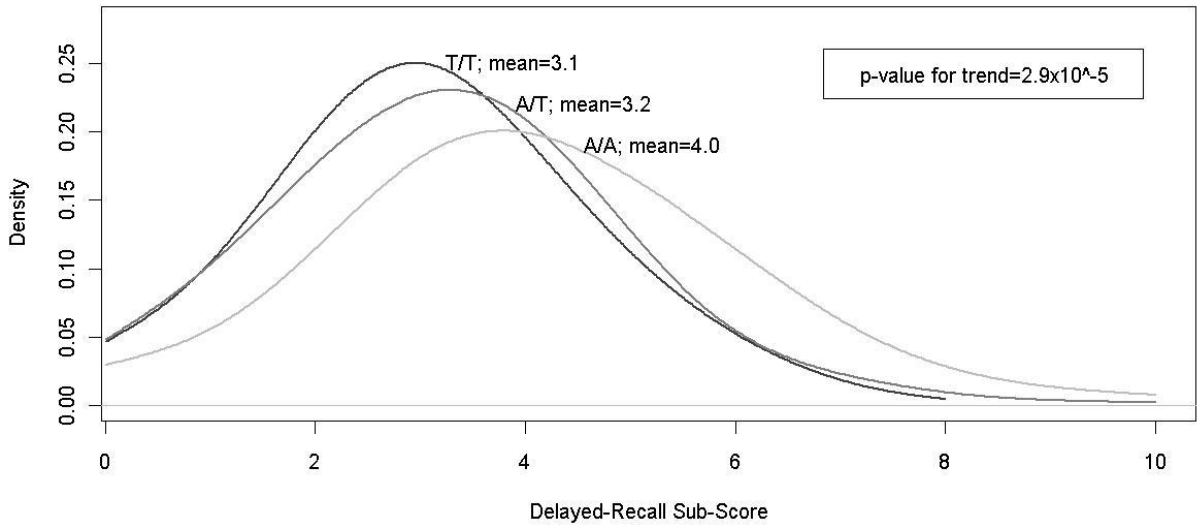
*These results suggest that, on average, men with MS have a lower cognitive score than women with MS; individuals with a later age of onset of MS had a higher cognitive score, therefore individuals with an earlier age of onset have lower cognitive scores; individuals with MS who had parents with less education had a lower cognitive score than individuals with MS who had parents with more education; PPMS, PRMS or Unknown disease course individuals had a lower cognitive score than RRMS or SPMS individuals.

Table 4. Summary of validated MS neuropsychological assessment tools

Name of Assessment Tool	Measures Included in Tool	Aspects of Cognition Assessed	Time to Complete
Minimal Assessment of Cognitive Function in MS (MACFIMS)	PASAT, SDMT, CVLT2, Brief Visuospatial Memory Test (revised), COWAT, Judgment of Line Orientation, and Delis-Kaplan Executive Function System	Processing speed and working memory, learning and memory, executive function, visual-spatial processing, and word retrieval	90 minutes
Rao Brief Repeatable Neuropsychological Battery (BRNB)	PASAT, SDMT, Selective Reminding Test, 10/36 Spatial Recall Test, and COWAT	Sustained attention/concentration, verbal learning and delayed recall, visual-spatial learning, and sematic retrieval	30 minutes
Brief International Cognitive Assessment for MS (BICAMS)	SDMT, Brief Visuospatial Memory Test, and CVLT2	Visual processing speed and working memory, auditory/verbal episodic memory, visual/spatial episodic memory	15 minutes
Mini-Mental State Examination (MMSE)	MMSE	Orientation, registration, attention and calculation, recall and language	10 minutes

This table summarizes the other commonly used cognitive assessment tools validated for MS. The table includes a list of assessments measured within each tool, the areas of cognition assessed, and features of each assessment. Abbreviations used: Paced Auditory Serial Addition Test (PASAT), Symbol Digital Modalities Test (SDMT), California Verbal Learning Test 2 (CVLT2), and Controlled Oral Word Association Test (COWAT).

Figure 2. Case-only analysis of rs35967351 and TICS-M delayed recall sub-score



All 110 susceptibility variants were tested individually in MS cases as genotype predictors of the TICS-M score overall, as well as orientation, registration, and delay-recall sub-scores. Each model was adjusted for education, current and history of depression, year of birth, gender, smoking, MSSS at assessment, disease course, parental education, and age of onset. There was one significant SNP association (rs35967351) after accounting for multiple tests. The figure shows the mean value of delayed-recall sub-score by each genotype for rs35967351 in MS cases. Carriers of the risk allele for rs35967351 had a lower delayed-recall sub-score compared to those who did not (β=-0.34, p=<0.001).

Table 5. Power analyses to detect differences in TICS-M scores for case-control and case-only associations

Analysis	MAF tested	Beta ranges for which there is sufficient power
Case-control status	--	≤-0.5; ≥0.5
Case-only (MSSS continuous)	--	≤-0.14; ≥0.14
Case-only (MSSS binary)	--	≤-0.8; ≥0.8
Case-only (110 MS risk variants)	0.10	≤-0.85; ≥0.85
	0.20	≤-0.65; ≥0.65
	0.30	≤-0.55; ≥0.55
	0.40	≤-0.55; ≥0.55
	0.50	≤-0.50; ≥0.50

The power needed to detect marginal associations for the case-control and case-only analyses were determined. All calculations assumed a two-sided type 1 error rate of 5% (α=0.05), as these analyses were not hypothesis free, but strongly hypothesis driven. All ranges indicate an 80% power to detect β values specified. Therefore, the results and interpretations are in accordance with these established criteria.

Chapter 3. Meta-analysis of multiple sclerosis risk variants and disease severity in 7,125 individuals

ABSTRACT

Objective: Identification of genetic variants that predict severe clinical outcomes in multiple sclerosis (MS) is critical to understanding disease mechanisms and guiding development of effective treatment. We investigated the association between 52 risk variants identified through genome-wide association studies (GWAS) and disease severity in MS.

Methods: Ten unique MS case datasets were analyzed. The MS severity score (MSSS) was calculated using the Expanded Disability Status Scale at study entry and disease duration. MSSS was considered as a continuous and two dichotomous outcomes (median and extreme ends: MSSS $\leq$5 vs. >5 and MSSS <2.5 vs. $\geq$7.5, respectively). SNPs were examined individually and combined in both weighted and unweighted genetic risk scores (wGRS and GRS) for association with disease severity. Random-effects meta-analyses were conducted and adjusted for cohort, gender, age of onset, and *HLA-DRB1*15:01*.

Results: A total of 7,125 MS cases were analyzed. The wGRS and GRS were not strongly associated with disease severity after accounting for cohort, gender, age of onset, and *HLA-DRB1*15:01*. After restricting the analysis to cases with disease duration $\geq$10 years, all associations were null (p-value$\geq$0.50). No single SNP was associated with disease severity after adjusting for multiple testing.

Conclusions: A large meta-analysis of established MS genetic risk variants and MSSS was performed. The results suggest that MS genetic risk variants are not associated with disease severity, after controlling for potential confounders. Underlying genetics determinants of MS disease severity have yet to be identified.

INTRODUCTION

Multiple Sclerosis (MS) is a severe autoimmune inflammatory disease of the central nervous system. Neurological damage in MS is caused by irreversible demyelination of axons and lesion formation [1, 2]. While early disease may manifest as attacks with full recovery, over time MS is progressive and extremely debilitating for the majority of patients [76]. The incidence of MS is twice as great among women, and onset typically occurs during the child-bearing years (20-40 years old) [9]. However, men with MS are at greater risk for developing a more severe phenotype of the disease [34, 35]. Additionally, more rapid progression of disease has been observed among those with onset later in life [36]. On average, within 15 years of diagnosis, 50-60% of patients will require assistance walking, and 70% will not be able to perform normal daily activities, posing tremendous economic and societal burdens [9].

Strong evidence suggests there are genetic and environmental components to the risk of MS. The strongest genetic risk factor for MS is within the human leukocyte antigen (*HLA)-DRB1* locus, specifically the 15:01 allele [19]. There is evidence supporting the presence of additional independent susceptibility alleles within the major histocompatibility complex (MHC) class I and class II regions [14, 15, 17, 77]. However, *HLA-DRB1*15:01* and the extended MHC region genes have not been associated with progression [19, 64]. Environmental risk factors for MS include previous infection with Epstein Barr virus or infectious mononucleosis (IM), tobacco smoke exposure, low serum levels of vitamin D, and childhood obesity [22, 23, 25]. With the exception of tobacco smoke exposure and low serum levels of vitamin D [38, 78], the genetic and environmental influences on MS clinical outcomes, including disease severity are unknown [64].

Through international collaboration, genome-wide association studies (GWAS) followed by replication have identified 52 non-MHC MS risk variants [18]. We investigated the association of disease severity, as measured by MS Severity Score (MSSS), with both a weighted and unweighted genetic risk score (wGRS and GRS), as well as, each of the 52 susceptibility variants in 7,125 MS cases from ten independent cohorts.

MATERIALS AND METHODS

Study Populations

Ten independent and well-characterized MS case datasets were analyzed (Table 1). The analysis included 1,079 white non-Hispanic MS cases recruited from Kaiser Permanente Medical Care Plan in the Northern California Region (KPNC) [55]. The following cases were included as well: 1,019 MS white non-Hispanic cases were recruited from two other clinical sites in the United States (US1 and US2) [64]; 422 MS cases recruited through a population based study in Oslo, Norway (Norway) [79]; 2,348 MS cases recruited through a population based study in Sweden (Sweden) [80]; 890 MS cases from a cohort in Denmark (Denmark) [21]; 485 white non-Hispanic MS cases from a UCSF cohort (UCSF) [81]; 678 MS cases from two cohorts recruited in Italy (Italy1 and Italy2) [18, 82]; and 204 cases from a Tasmanian cohort study (Australia) [83, 84]. Each case included in the meta-analysis fulfilled disease criteria for MS as defined by

McDonald et al. [27]. Classical *HLA-DRB1*15:01* typing was used in the KPNC, Sweden, and UCSF studies. Validated tagging SNPs were used in the US1 and US2, Italy1 and Italy2, and Australia (rs9271366), Norway (rs9270986) and Denmark (rs3135388) studies. Each of these tagging SNPs was tested against the classical typing performed in the KPNC dataset; the correlations (r^2) were 0.99, 0.91, and 0.95, respectively (Table 1).

Standard Protocol Approvals, Registrations, and Patient Consents

Each study protocol was approved by the appropriate Institutional Review Board (IRB) of the participating academic institution. All participants provided written informed consent.

Genotyping and Imputation

All subjects were genotyped using separate platforms: Affymetrix platform using the GeneChip Human Mapping 500K Array set (KPNC and US), TaqMan® OpenArray® Genotyping Technology (Norway), Illumina Infinium HD Custom Array and Illumina Human Quad 660 (Sweden, Denmark, and Italy), and Illumina HumanHap550 Beadchip ©2006 (UCSF). All cohorts, except US and Australia, contained genotyped information for all 52 variants. In US, tagging SNPs were used as proxies for two missing SNPs: rs6693456 tagged rs11581062 (r^2=0.95), and rs8106574 tagged rs1077667 (r^2=0.63). In Australia, 15 SNPs were missing: rs1323292, rs7522462, rs17174870, rs10201872, rs669607, rs12212193, rs17066096, rs13192841, rs354033, rs1520333, rs10466829, rs2119704, rs7200786, rs13333054, rs2425752. Genetic data within 1MB regions around all missing SNPs were available for each patient; therefore, imputation was possible against the 1000 Genomes reference. After imputation, missingness was lower than 2% in all cohorts. However, in order to recover all missing genotypes, the risk allele frequency from each cohort was used to estimate the missing genotypes. The estimation for each individual was made using a multinomial distribution generated from 1000 random samples from each respective cohort. The probability for each of the three possible genotypes was generated. When a single genotype was missing for an individual one sample was drawn randomly from the generated distribution from that cohort. This was done for a total of 146 individuals (KPNC=92, Norway=19, Sweden=22, Denmark=9, UCSF=15, and Italy=8) and a total of 48 SNPs (KPNC=44, Norway=15, Sweden=4, Denmark=2, UCSF=10, and Italy=8).

Weighted and Unweighted Genetic Risk Scores

A weighted genetic risk score (wGRS) was calculated for each MS patient using the natural log of the discovery odds ratios (ORs) as the weight for each of 52 non-MHC risk alleles derived from 9,772 MS cases and 17,376 controls [18], as previously described. Briefly the number of risk alleles for each SNP was multiplied by the weight for that variant, and then the sum across all 52 variants was calculated (Table 1, Figure 1A). [18, 85]. The genetic risk score (GRS) was calculated as the sum of risk allele copies for each SNP without weighting (Table 1, Figure 1B). Both wGRS and GRS were analyzed as continuous variables.

Multiple Sclerosis Severity Score

MSSS is a probabilistic algorithm that uses the Expanded Disability Severity Scale (EDSS) to calculate disease severity and duration of disease, which was defined as time between first

symptom and EDSS assessment [29]. MSSS was analyzed as a continuous variable and as two dichotomous variables, as previously described [64]. Briefly, a binary MSSS variable was based on the median MSSS value, defined as MSSS ≤5 vs. >5, with a smaller score indicating more benign or 'mild' disease. The second variable was based on extreme ends of the MSSS distribution, defined as MSSS <2.5 (benign or 'mild') vs. ≥7.5 ('severe') (Table 1, Figure 1C).

Statistical Analyses

All ten datasets were included in a random-effects meta-analysis. Random-effects meta-analysis allows for heterogeneity across studies due to inherent differences and/or differential biases among each cohort, unlike fixed-effects models, which assume a single common effect underlies each study. A random-effects meta-analysis is generally more conservative, generating wider confidence interval and larger p-values [86]. Weighted and unweighted GRS, as well as all 52 risk variants, were tested with the three MSSS outcomes in meta-analyses. Additionally, analyses restricted to cases with a disease duration greater than or equal to ten years were conducted. Both adjusted linear and logistic regression models were used to estimate adjusted beta values (β), odds ratios (ORs), and 95% confidence intervals (95% CIs). The meta-analysis was adjusted for gender, age of onset, and *HLA-DRB1*15:01*. All analyses were conducted in STATA v13.1 (StataCorp, TX). The current study had sufficient power for all analyses (Table 2).

RESULTS

All 7,125 individuals were included in the meta-analysis of the three MSSS outcomes (Table 1). The distributions of MSSS were similar across all cohorts (Figure 1C) and similar to those in other cohorts reported in the literature [29]. The gender distribution was also very similar across cohorts, with a 3:1 female to male ratio; KPNC had the highest proportion of females (80.4%), and Italy had the lowest proportion of females (64.3%) (Table 1). Age of onset was similar among all cohorts and normally distributed, ranging from 30.9 years of age in Denmark to 35.3 years of age in Australia (Table 1, Figure 1D). On average, disease duration was 11.4 years across all cohorts and normally distributed, ranging from 9.5 years in UCSF to 16.2 years in Norway (Table 1, Figure 1E). Lastly, the distribution of *HLA-DRB1*15:01*, while not as similar across the cohorts, was typical of established genetic patterns in the literature (Table 1). MS cases of Northern European decent were more likely to be *HLA-DRB1*15:01* positive than cases in the Italy cohort [87]. All wGRS and GRS were similar across cohorts (Figure 1A and 1B).

Meta-analyses accounting for the random effects of cohort were used. Gender, age of onset, and *HLA-DRB1*15:01* were all analyzed as fixed effects. After adjustment, some evidence for association with wGRS and GRS was observed for the MSSS outcomes (Table 3A). However, after restricting the data set to individuals who had ten years or more of disease, no significant associations remained (Table 3B). Gender and age of onset were consistently associated with MSSS in all three models, even after restricting to individuals with ten years or more of disease (all p-values<0.001) (Tables 3A and 3B). Male gender and a later age of onset were associated with more severe disease. *HLA-DRB1*15:01* was not associated with MSSS in any of the models.

Each of the 52 risk variants was tested individually in a random-effects model adjusting for gender, age of onset, and *HLA-DRB1*15:01* in the entire cohort and in the restricted cohort with a disease duration of ten years or more (Tables 4A and 4B). In the entire cohort, two risk variants showed evidence of positive association with increased disease severity for all three MSSS outcomes: rs874628 and rs650258, within *MPV17L2* and 44kb upstream of *CD6*, respectively. All associations were in the same direction as the wGRS and GRS. However, after accounting for multiple testing, no single variant remained significant.

DISCUSSION

A meta-analysis of established MS genetic risk factors and disease severity was performed. This is the largest cohort used to evaluate the association between 52 established risk variants and MS disease severity. Ten independent MS patient datasets consisting of 7,125 individuals with confirmed MS were studied. The hypothesis that one or more known MS risk variants are associated with MS disease severity characterized by MSSS was tested. Genetic factors that influence disease susceptibility were not shown convincingly to impact disease severity.

The individuals included in this analysis are representative of the international MS population with regard to gender distribution, average age of onset, and proportion of *HLA-DRB1*15:01* positive individuals. Male gender and an older age of onset were consistently associated with more severe disease, as consistent with previous studies [88]. While *HLA-DRB1*15:01* was associated with risk of MS, it was not associated with MS disease severity in this study or previous studies [89].

There is accumulating evidence that both genetic and environmental factors contribute to MS progression. Findings from studies of other neurodegenerative diseases, such as Alzheimer Disease and Parkinson's Disease, have linked disease progression with specific genetic markers, raising the possibility that MS progression also has a genetic component [90, 91], given evidence for neurodegeneration and inflammation in MS [92]. However, to date, there has been limited success identifying any underlying genetic mechanisms contributing to disease severity or clinical phenotypes in MS. The study by Kalinick et al. showed that a subset of the same risk variants investigated here was not associated with clinical and MRI outcomes in recently diagnosed MS cases [85, 93]. These findings suggest that the genetics underlying MS susceptibility and progression are not likely to substantially overlap.

There is evidence in MS animal models to suggest that risk alleles have an impact on progression [94]. The experimental autoimmune encephalomyelitis (EAE) model, which mirrors an inflammatory autoimmune disease of the central nervous system in rats, has offered numerous experimental insights into MS. When genetically dissected into high resolution quantitative trait loci, *Eae25* and *Eae29* have been shown to influence both susceptibility and progression [95, 96]. Additionally, differential expression of an interleukin 2 (*IL2*) repressor, in the gene *ZEB1*, result in EAE severity changes [97]. Similarly, congenic rats with *Eae18b* locus have been shown to develop milder disease, with decreased demyelination and reduced recruitment of inflammatory cells to the brain [98]. However, parallel findings have not been observed in humans.

The current study has many strengths. First, the MSSS has been favored over EDSS as a way to capture disease severity in MS because it accounts for disease duration, as well as ambulatory debilitation [29]. The MSSS incorporates disease duration, to account for time, which is advantageous when EDSS measurements are taken cross-sectionally. Moreover, the analysis that used MSSS extremes reduced the possibility of misclassification because it compared individuals with very benign disease and individuals with very severe disease, excluding individuals in the middle of the MSSS measurement spectrum and who are most likely to be misclassified. While using individuals in only the extreme categories of MSSS will reduce the overall number of individuals analyzed, there were nearly 4,000 individuals with MS who remained in this analysis.

Analyses were also restricted to individuals with ten or more years disease duration to ensure the stability of the MSSS measurements. MSSS requires at least one year of duration before the EDSS can be used in the algorithm to calculate the score [29]. Median time to requiring unilateral assistance ranges from 15-30 years, based on current estimates [88]. Conservatively, the current study used disease duration near the median disease duration for the overall dataset (Table 1), but before the early end of the transition to requiring unilateral assistance or an EDSS of six. When the data were restricted by disease duration ($\geq$5 years, $\geq$10 years, $\geq$15 years and $\geq$20 years), wGRS and GRS were not associated with any of the three MSSS outcomes (Figure 2A and 2B).

There are several limitations in this study. The current study can address only physical disease severity because the MSSS only incorporates physical measurements of disability. No follow-up was conducted with any of the cases to look at the longitudinal effects of progression. There is potential selection bias if cases with mild disease are more willing to be in research studies than those with severe disease (or vice versa). However, over 1,100 individuals in the highest or "severe" MSSS phenotype who participated in the current study (Table 1), which offers a sufficient representation of cases with severe disease. The current dataset includes 52 non-MHC MS risk variants [18] and not a larger list including the most recently identified non-MHC MS risk variants from a targeted autoimmune candidate gene study [21]. However, the 52 non-MHC MS risk variants were identified through a GWAS of MS cases and controls, and not through a more targeted autoimmune candidate gene study, therefore these original variants are likely stronger genetic markers of MS susceptibility (average OR for 52 non-MHC variants is 1.19 versus average OR for new 48 non-MHC variants is 1.09) [18, 21].

In summary, the current study of 7,125 individuals does not support a strong role for association between individual wGRS or GRS and MSSS. The current study is among the first studies to investigate the association of wGRS and individual established non-MHC MS risk variants on this important MS phenotype, and is the largest meta-analysis of this topic to date.

TABLES AND FIGURES

Table 1. Characteristics of white non-Hispanic MS cases included in the meta-analysis

	Mean (SD) or Frequency (%)								
	KPNC	US	Norway	Sweden	Denmark	UCSF	Italy	Australia	Meta-analysis
Number of cases	1,079	1,019	422	2,348	890	485	678	204	7,125
MSSS Continuous	3.37 (2.6)	3.28 (2.6)	4.59 (3.0)	4.61 (2.8)	4.48 (2.6)	2.98 (2.4)	4.57 (2.9)	4.12 (2.7)	4.09 (2.8)
MSSS Binary (high)	255 (23.6)	261 (25.6)	180 (42.7)	1,045 (44.5)	370 (41.6)	101 (20.8)	295 (43.5)	73 (35.8)	2,580 (36.2)
MSSS Extreme (high)	129 (19.6)	103 (17.3)	101 (40.2)	474 (41.2)	136 (36.8)	31 (10.5)	150 (40.7)	30 (28.6)	1,154 (30.4)
Female	868 (80.4)	779 (76.5)	309 (73.2)	1,702 (72.5)	614 (69.0)	333 (68.7)	436 (64.3)	147 (72.1)	5,188 (72.8)
Age of Onset	31.94 (9.8)	31.03 (8.5)	32.66 (9.4)	34.60 (10.7)	30.92 (8.9)	33.47 (9.3)	32.50 (10.5)	35.25 (10.2)	32.85 (10.0)
*HLA-DRB1*15:01* (positive)	581 (53.9)	567 (55.6)	243 (57.6)	1,366 (58.2)	534 (60.0)	224 (46.2)	189 (27.9)	120 (58.8)	3,824 (53.7)
Disease duration	12.23 (8.5)	12.05 (8.5)	16.16 (10.5)	9.71 (8.6)	12.23 (7.8)	9.54 (9.1)	11.38 (8.5)	15.67 (10.2)	11.44 (8.9)
wGRS	6.75 (0.5)	6.59 (0.5)	6.84 (0.5)	6.74 (0.5)	6.68 (0.5)	6.71 (0.5)	6.85 (0.5)	6.72 (0.5)	6.73 (0.5)
GRS	56.72 (4.4)	55.77 (4.4)	57.11 (4.4)	56.44 (4.5)	56.38 (4.5)	56.46 (4.3)	57.81 (4.5)	56.47 (4.6)	56.55 (4.5)
Genotyping platform	Affymetrix GeneChip Human Mapping 500K Array set	Affymetrix GeneChip Human Mapping 500K Array set	TaqMan® OpenArray® Genotyping Technology	Immunochip - Illumina Infinium HD Custom Array and Illumina Human Quad 660	Immunochip - Illumina Infinium HD Custom Array	Illumina HumanHap550 Beadchip circa 2006	Illumina Human Quad 660	Illumina Infinium Hap370 CNV array	--

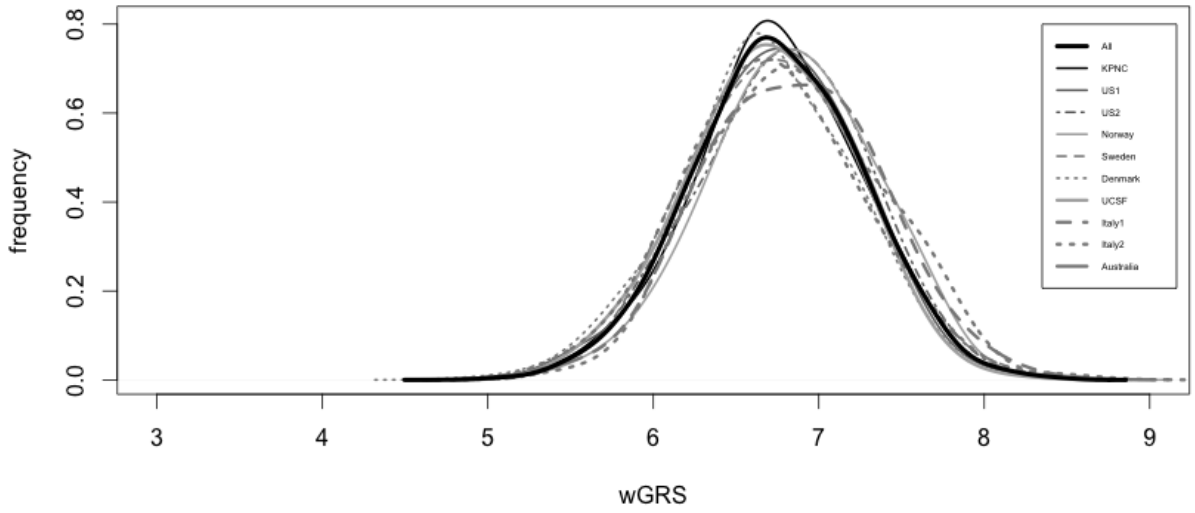
MSSS is presented as a continuous variable, and as two dichotomous variables. The first, binary MSSS variable was based on the median MSSS value, defined as MSSS ≤5 vs. >5, with a smaller score indicating a more benign phenotype. The second variable was based on extreme ends of the MSSS distribution, defined as MSSS <2.5 vs. ≥7.5.

*HLA-DRB1*15:01* tag SNP if classical HLA typing was not available: US and Italy cohorts used rs9271366 as a tag SNP, Norway used rs9270986 as a tag SNP, and Denmark used rs3135388 as a tag SNP.

All cohorts were contained data at all 52 individual SNP locations, except the US and Australian cohorts. The US cohort used the following SNPs as tagging SNPs: rs6685440 tags rs11581062; and rs8106574 tags rs1077667. The Australian cohort imputed the following SNPs: rs1323292, rs7522462, rs17174870, rs10201872, rs669607, rs12212193, rs17066096, rs13192841, rs354033, rs1520333, rs10466829, rs2119704, rs7200786, rs13333054, rs2425752.

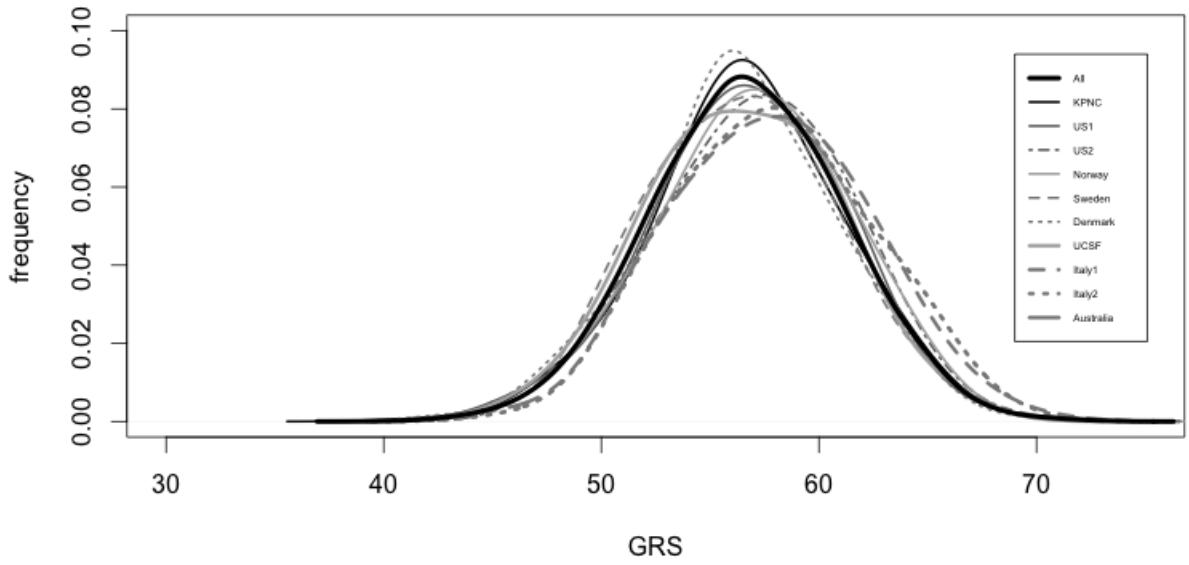
The US cohort contains two cohorts: US1 and US2. The Italy cohort contains two cohorts: Italy1 and Italy2. These cohorts are combined in Table 1, but analyzed separately in the meta-analysis.

Figure 1A. Density plot of wGRS by the ten cohorts



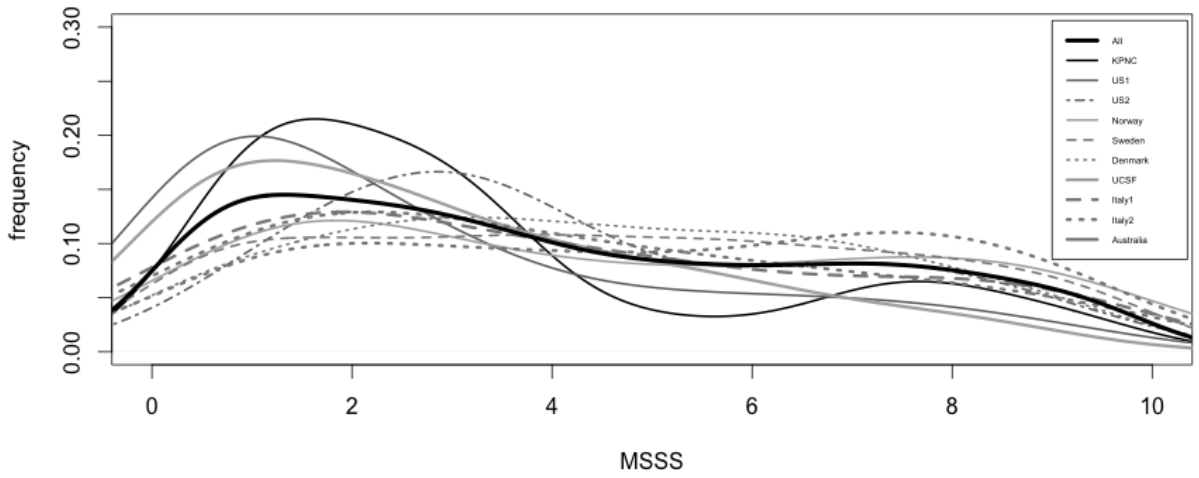
The density plot includes the frequency of all wGRS by ten cohorts and the total 7,125 individuals with MS.

Figure 1B. Density plot of GRS by the ten cohorts



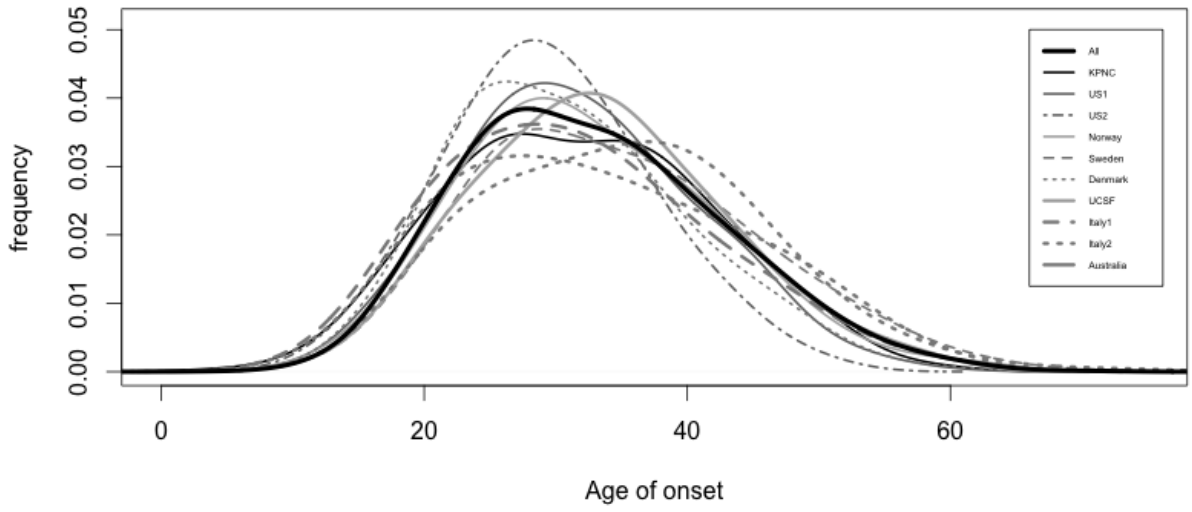
The density plot includes the frequency of all GRS by ten cohorts and the total 7,125 individuals with MS.

Figure 1C. Density plot of MSSS by the ten cohorts



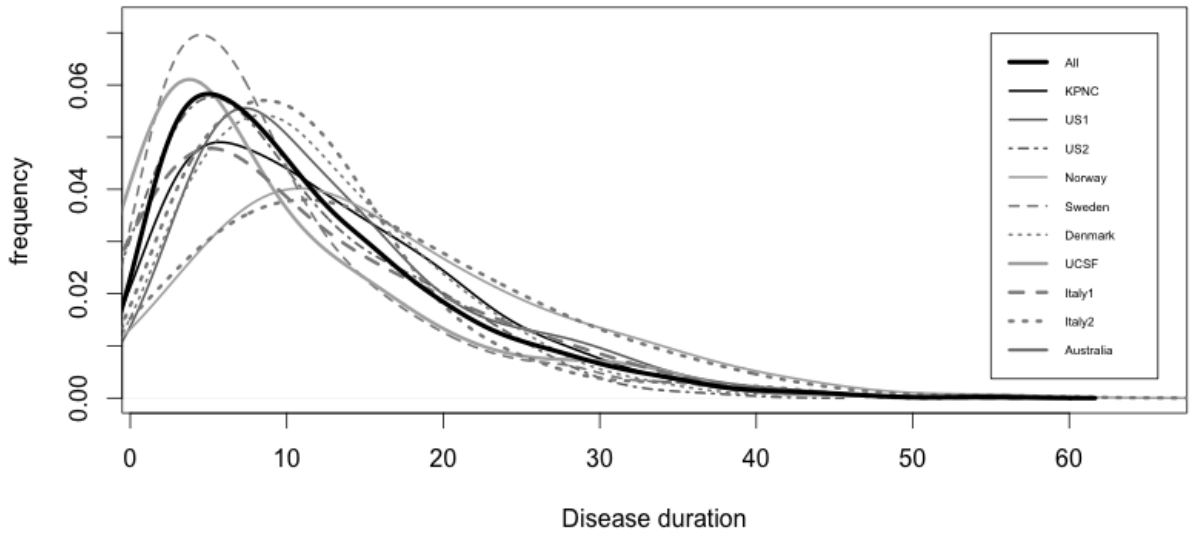
The density plot includes the frequency of all MSSS by ten cohorts and the total 7,125 individuals with MS.

Figure 1D. Density plot of age of onset by the ten cohorts



The density plot includes the frequency of all age of onset by ten cohorts and the total 7,125 individuals with MS.

Figure 1E. Density plot of disease duration by the ten cohorts



The density plot includes the frequency of all disease duration by ten cohorts and the total 7,125 individuals with MS.

Table 2. Power analysis

Analysis			<80% power	Sample size
All cases	wGRS	MSSS continuous	-0.2 to 0.2	7,125
		MSSS binary	0.87 to 1.15	7,125
		MSSS extreme	0.82 to 1.22	3,795
	GRS	MSSS continuous	-0.025 to 0.025	7,125
		MSSS binary	0.98 to 1.02	7,125
		MSSS extreme	0.97 to 1.03	3,795
Cases with ≥10 years duration	wGRS	MSSS continuous	-0.3 to 0.3	3,437
		MSSS binary	0.8 to 1.3	3,437
		MSSS extreme	0.7 to 1.4	1,957
	GRS	MSSS continuous	-0.03 to 0.03	3,437
		MSSS binary	0.97 to 1.03	3,437
		MSSS extreme	0.96 to 1.04	1,957

The power needed to detect associations for the meta-analyses were determined. All calculations assumed a two-sided type 1 error rate of 5% (α=0.05), as these analyses were hypothesis driven. All ranges indicate an 80% power to detect β or OR within the range. Therefore, the results and interpretations are in accordance with these established criteria.

Table 3A. Meta-analysis results for weighted and unweighted genetic risk scores and MSSS in 7,125 MS cases

	MSSS Outcome					
	Continuous		**Binary**		**Extreme***	
	β (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
wGRS (unadjusted)	0.08 (-0.1, 0.2)	0.220	1.05 (1.0, 1.2)	0.363	1.10 (1.0, 1.3)	0.168
wGRS (adjusted)	0.13 (0.01, 0.3)	0.030	1.08 (1.0, 1.2)	0.093	1.20 (1.0, 1.4)	0.016
Gender	-0.57 (-0.7, -0.4)	<0.001	0.72 (0.6, 0.8)	<0.001	0.54 (0.5, 0.6)	<0.001
Age of Onset	0.06 (0.05, 0.07)	<0.001	1.04 (1.0. 1.0)	<0.001	1.07 (1.1, 1.1)	<0.001
*HLA-DRB1*15:01*	-0.02 (-0.1, 0.1)	0.763	0.98 (0.9, 1.1)	0.648	0.97 (0.8, 1.1)	0.748
GRS (unadjusted)	0.01 (-0.004, 0.02)	0.179	1.01 (1.0, 1.0)	0.316	1.01 (1.0, 1.0)	0.111
GRS (adjusted)	0.02 (0.002, 0.03)	0.027	1.01 (1.0, 1.0)	0.088	1.02 (1.0, 1.0)	0.013
Gender	-0.57 (-0.7, -0.4)	<0.001	0.72 (0.6, 0.8)	<0.001	0.54 (0.5, 0.6)	<0.001
Age of Onset	0.06 (0.05, 0.07)	<0.001	1.04 (1.0. 1.0)	<0.001	1.07 (1.1, 1.1)	<0.001
*HLA-DRB1*15:01*	-0.02 (-0.1, 0.1)	0.773	0.98 (0.9, 1.1)	0.656	0.98 (0.8, 1.1)	0.762

All βs and ORs are from the adjusted random effects models. Unadjusted and adjusted results are shown. Adjusted models are adjusted for gender, age of onset and *HLA-DRB1*15:01.* All βs and ORs for the adjusted variables are also listed above.
*A total of 3,795 individuals with MS are included in this analysis.

Table 3B. Meta-analysis results for weighted and unweighted genetic risk scores and MSSS in MS cases with disease duration greater than or equal to ten years

	MSSS Outcome					
	Continuous		**Binary**		**Extreme***	
	β (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
wGRS (unadjusted)	-0.02 (-0.2, 0.2)	0.785	0.98 (0.9, 1.1)	0.804	0.94 (0.8, 1.1)	0.523
wGRS (adjusted)	0.03 (-0.1, 0.2)	0.714	1.02 (0.9, 1.2)	0.741	0.99 (0.8, 1.2)	0.947
Gender	-0.64 (-0.8, -0.4)	<0.001	0.66 (0.6, 0.8)	<0.001	0.54 (0.4, 0.7)	<0.001
Age of Onset	0.05 (0.04, 0.06)	<0.001	1.03 (1.0. 1.0)	<0.001	1.05 (1.0, 1.1)	<0.001
*HLA-DRB1*15:01*	0.13 (-0.1, 0.3)	0.159	1.09 (0.9, 1.3)	0.260	1.20 (1.0, 1.5)	0.115
GRS (unadjusted)	-0.001 (-0.02, 0.02)	0.941	1.00 (1.0, 1.0)	0.856	1.00 (1.0, 1.0)	0.766
GRS (adjusted)	0.01 (-0.01, 0.3)	0.586	1.00 (1.0, 1.0)	0.712	1.00 (1.0, 1.0)	0.820
Gender	-0.64 (-0.8, -0.4)	<0.001	0.66 (0.6, 0.8)	<0.001	0.54 (0.4, 0.7)	<0.001
Age of Onset	0.05 (0.04, 0.06)	<0.001	1.03 (1.0. 1.0)	<0.001	1.05 (1.0, 1.1)	<0.001
*HLA-DRB1*15:01*	0.13 (-0.1, 0.3)	0.157	1.09 (0.9, 1.3)	0.259	1.20 (1.0, 1.5)	0.113

All βs and ORs are from the adjusted random effects models. Unadjusted and adjusted results are shown. Adjusted models are adjusted for gender, age of onset and *HLA-DRB1*15:01.* All βs and ORs for the adjusted variables are also listed above.
*A total of 1,957 individuals with MS are included in this analysis.

Table 4A. Meta-analysis results for 52 SNPs and MSSS in 7,125 MS cases

Chr	Gene	SNP	MSSS Outcome					
			Continuous		Binary		Extreme*	
			β (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value
1	*MMEL1*	rs4648356	-0.07 (-0.17, 0.02)	0.144	0.97 (0.90, 1.05)	0.450	0.91 (0.81, 1.03)	0.139
	EVI5	rs11810217	0.06 (-0.03, 0.16)	0.206	1.05 (0.97, 1.14)	0.271	1.09 (0.97, 1.22)	0.153
	VCAM1	rs11581062	0.05 (-0.05, 0.14)	0.323	1.04 (0.97, 1.13)	0.241	1.03 (0.91, 1.15)	0.654
	CD58	rs1335532	0.07 (-0.07, 0.21)	0.324	1.05 (0.93, 1.17)	0.444	1.08 (0.91, 1.28)	0.351
	RGS1	rs1323292	-0.11 (-0.23, 0.01)	0.062	0.88 (0.80, 0.97)	0.010	0.95 (0.82, 1.10)	0.475
	C1orf106	rs7522462	-0.09 (-0.19, 0.01)	0.085	0.93 (0.86, 1.01)	0.076	0.89 (0.79, 1.01)	0.075
2	No gene	rs12466022	0.05 (-0.05, 0.15)	0.341	1.03 (0.95, 1.12)	0.488	1.04 (0.92, 1.17)	0.539
	PLEK	rs7595037	0.05 (-0.03, 0.14)	0.232	1.02 (0.95, 1.10)	0.545	1.08 (0.97, 1.21)	0.164
	MERTK	rs17174870	-0.003 (-0.11, 0.10)	0.944	0.98 (0.91, 1.07)	0.706	1.02 (0.90, 1.15)	0.802
3	*SP140*	rs10201872	0.01 (-0.10, 0.13)	0.815	0.98 (0.89, 1.08)	0.687	1.00 (0.87, 1.15)	0.985
	EOMES	rs11129295	0.02 (-0.07, 0.11)	0.638	1.00 (0.93, 1.08)	0.984	1.01 (0.90, 1.12)	0.926
	No gene	rs669607	0.09 (-0.003, 0.18)	0.051	1.06 (0.98, 1.14)	0.124	1.10 (0.99, 1.23)	0.076
	CBLB	rs2028597	0.06 (-0.10, 0.22)	0.493	1.03 (0.90, 1.17)	0.677	0.98 (0.81, 1.19)	0.876
	TMEM39A	rs2293370	-0.10 (-0.22, 0.02)	0.090	0.91 (0.83, 1.01)	0.068	0.91 (0.79, 1.05)	0.183
	CD86	rs9282641	-0.05 (-0.20, 0.11)	0.560	0.97 (0.86, 1.11)	0.687	0.97 (0.80, 1.18)	0.755
	IL12A	rs2243123	0.01 (-0.08, 0.11)	0.821	1.02 (0.94, 1.10)	0.691	1.01 (0.89, 1.13)	0.905
5	*IL7R*	rs6897932	0.004 (-0.10, 0.10)	0.932	1.02 (0.94, 1.11)	0.610	0.98 (0.87, 1.11)	0.760
	PTGER4	rs4613763	0.06 (-0.06, 0.18)	0.307	1.09 (0.99, 1.20)	0.084	1.11 (0.96, 1.29)	0.154
	IL12B	rs2546890	0.05 (-0.03, 0.14)	0.236	1.00 (0.93, 1.08)	0.897	1.11 (0.99, 1.24)	0.063
6	*BACH2*	rs12212193	0.03 (-0.06, 0.11)	0.558	1.01 (0.94, 1.08)	0.803	1.03 (0.93, 1.15)	0.577
	THEMIS	rs802734	0.03 (-0.07, 0.12)	0.579	1.02 (0.94, 1.10)	0.666	1.05 (0.93, 1.18)	0.403
	MYB	rs11154801	0.08 (-0.01, 0.17)	0.080	1.06 (0.99, 1.15)	0.095	1.08 (0.97, 1.21)	0.154
	IL22RA2	rs17066096	0.09 (-0.01, 0.18)	0.091	1.06 (0.98, 1.15)	0.144	1.16 (1.03, 1.31)	0.014
	No gene	rs13192841	-0.02 (-0.12, 0.08)	0.709	0.99 (0.91, 1.07)	0.774	0.96 (0.85, 1.09)	0.524
	TAGAP	rs1738074	-0.03 (-0.12, 0.06)	0.500	0.99 (0.92, 1.07)	0.870	0.97 (0.87, 1.08)	0.538
7	*ZNF746*	rs354033	-0.03 (-0.13, 0.07)	0.560	0.99 (0.91, 1.08)	0.855	0.94 (0.83, 1.06)	0.312
8	*IL7*	rs1520333	-0.01 (-0.10, 0.09)	0.892	1.04 (0.96, 1.13)	0.303	0.98 (0.87, 1.10)	0.719
	MYC	rs4410871	0.06 (-0.04, 0.16)	0.246	1.05 (0.96, 1.14)	0.282	1.04 (0.92, 1.18)	0.541
	PVT1	rs2019960	0.05 (-0.05, 0.15)	0.321	1.03 (0.95, 1.12)	0.488	1.06 (0.93, 1.20)	0.375
10	*IL2RA*	rs3118470	0.05 (-0.04, 0.14)	0.298	0.98 (0.91, 1.06)	0.623	1.11 (0.99, 1.24)	0.069
	ZMIZ1	rs1250550	0.04 (-0.05, 0.13)	0.371	1.05 (0.97, 1.13)	0.244	1.09 (0.97, 1.22)	0.137
	HHEX	rs7923837	0.02 (-0.07, 0.11)	0.692	1.02 (0.95, 1.10)	0.564	0.99 (0.88, 1.10)	0.801
11	*CD6*	rs650258	0.11 (0.02, 0.20)	0.017	1.09 (1.01, 1.18)	0.024	1.16 (1.03, 1.29)	0.012
12	*TNFRSF1A*	rs1800693	-0.02 (-0.11, 0.07)	0.629	0.99 (0.92, 1.06)	0.776	0.97 (0.87, 1.08)	0.596
	CLECL1	rs10466829	0.02 (-0.06, 0.11)	0.611	0.99 (0.92, 1.07)	0.838	1.07 (0.96, 1.19)	0.234
	CYP27B1	rs12368653	-0.04 (-0.12, 0.05)	0.415	0.98 (0.91, 1.05)	0.613	0.96 (0.86, 1.07)	0.431
	ARL61P4	rs949143	0.07 (-0.03, 0.16)	0.164	1.02 (0.95, 1.10)	0.545	1.08 (0.97, 1.22)	0.161
14	*ZFP36L1*	rs4902647	0.06 (-0.03, 0.14)	0.188	1.04 (0.97, 1.12)	0.288	1.08 (0.97, 1.20)	0.149
	BATF	rs2300603	-0.05 (-0.15, 0.05)	0.310	0.96 (0.88, 1.04)	0.342	0.98 (0.87, 1.11)	0.794
	GALC	rs2119704	-0.01 (-0.19, 0.17)	0.934	1.02 (0.88, 1.18)	0.771	0.92 (0.74, 1.14)	0.438
16	*CLEC16A*	rs7200786	0.02 (-0.06, 0.11)	0.591	1.02 (0.95, 1.09)	0.668	1.06 (0.95, 1.18)	0.278
	IRF8	rs13333054	0.10 (-0.01, 0.20)	0.070	1.09 (1.00, 1.18)	0.048	1.12 (0.98, 1.27)	0.085
17	*STAT3*	rs9891119	-0.02 (-0.11, 0.07)	0.633	1.00 (0.93, 1.07)	0.933	1.00 (0.89, 1.11)	0.945
18	*MALT1*	rs7238078	-0.06 (-0.16, 0.04)	0.237	0.95 (0.88, 1.04)	0.266	0.95 (0.84, 1.08)	0.437
19	*TNFSF14*	rs1077667	-0.04 (-0.16, 0.07)	0.468	0.95 (0.87, 1.04)	0.284	1.00 (0.87, 1.15)	0.977
	TYK2	rs8112449	-0.01 (-0.10, 0.09)	0.845	0.98 (0.90, 1.05)	0.533	0.93 (0.82, 1.04)	0.196
	MPV17L2	rs874628	0.13 (0.02, 0.23)	0.017	1.10 (1.01, 1.20)	0.029	1.18 (1.03, 1.34)	0.013
	DKKL1	rs2303759	0.06 (-0.04, 0.15)	0.243	1.08 (1.00, 1.17)	0.053	1.04 (0.92, 1.17)	0.513
20	*CD40*	rs2425752	0.03 (-0.13, 0.06)	0.517	0.96 (0.88, 1.04)	0.274	0.97 (0.86, 1.09)	0.580
	CYP24A1	rs2248359	0.07 (-0.02, 0.16)	0.131	1.05 (0.97, 1.13)	0.199	1.05 (0.94, 1.17)	0.393
22	*MAPK1*	rs2283792	0.06 (-0.03, 0.14)	0.189	1.04 (0.97, 1.12)	0.277	1.08 (0.97, 1.20)	0.177

Chr	Gene	SNP	MSSS Outcome					
			Continuous		Binary		Extreme*	
			β (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value
22	*SCO2*	rs140522	-0.02 (-0.11, 0.08)	0.708	1.00 (0.92, 1.08)	0.993	1.06 (0.94, 1.19)	0.362

These marginal models tested the association between the three MSSS phenotypes and all 52 individual SNPs Cohort was used as a random effect in the models.

ǂ All βs and ORs are from the adjusted models. Models are adjusted for gender, age of onset, *HLA-DRB1*15:01*, and cohort.

*A total of 3,795 individuals with MS are included in this analysis.

Table 4B. Meta-analysis results for 52 SNPs and MSSS MS cases with disease duration greater than or equal to ten years

Chr	Gene	SNP	MSSS Outcome					
			Continuous		Binary		Extreme*	
			β (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value
1	*MMEL1*	rs4648356	-0.06 (-0.20, 0.08)	0.379	1.01 (0.90, 1.13)	0.912	0.86 (0.73, 1.03)	0.097
	EVI5	rs11810217	0.05 (-0.08, 0.19)	0.445	1.03 (0.92, 1.15)	0.633	1.07 (0.91, 1.26)	0.433
	VCAM1	rs11581062	-0.10 (-0.24, 0.04)	0.148	0.91 (0.81, 1.02)	0.091	0.90 (0.76, 1.06)	0.211
	CD58	rs1335532	-0.02 (-0.23, 0.19)	0.845	1.02 (0.86, 1.20)	0.851	0.91 (0.71, 1.16)	0.439
	RGS1	rs1323292	-0.15 (-0.32, 0.02)	0.088	0.85 (0.74, 0.97)	0.017	0.87 (0.70, 1.08)	0.193
	C1orf106	rs7522462	0.02 (-0.13, 0.17)	0.778	1.04 (0.93, 1.17)	0.481	0.99 (0.83, 1.19)	0.954
2	No gene	rs12466022	0.04 (-0.10, 0.19)	0.562	1.02 (0.90, 1.14)	0.800	1.03 (0.87, 1.24)	0.710
	PLEK	rs7595037	0.01 (-0.12, 0.14)	0.902	0.97 (0.88, 1.08)	0.629	1.07 (0.91, 1.25)	0.438
	MERTK	rs17174870	0.003 (-0.15, 0.15)	0.997	0.99 (0.88, 1.12)	0.908	1.06 (0.89, 1.27)	0.503
3	*SP140*	rs10201872	0.05 (-0.11, 0.22)	0.522	0.98 (0.86, 1.13)	0.815	1.10 (0.90, 1.35)	0.348
	EOMES	rs11129295	0.03 (-0.10, 0.16)	0.634	0.98 (0.88, 1.09)	0.652	1.00 (0.85, 1.18)	0.973
	No gene	rs669607	0.06 (-0.07, 0.19)	0.379	1.07 (0.96, 1.19)	0.205	1.05 (0.90, 1.22)	0.558
	CBLB	rs2028597	0.14 (-0.09, 0.37)	0.232	1.15 (0.96, 1.39)	0.131	1.07 (0.81, 1.40)	0.644
	TMEM39A	rs2293370	-0.04 (-0.21, 0.13)	0.635	0.97 (0.85, 1.11)	0.628	0.97 (0.80, 1.19)	0.799
	CD86	rs9282641	-0.08 (-0.30, 0.15)	0.502	0.96 (0.80, 1.15)	0.670	0.93 (0.71, 1.21)	0.578
	IL12A	rs2243123	0.09 (-0.05, 0.23)	0.225	1.03 (0.92, 1.15)	0.633	1.06 (0.90, 1.26)	0.487
5	*IL7R*	rs6897932	-0.07 (-0.21, 0.08)	0.375	0.98 (0.87, 1.10)	0.709	0.91 (0.77, 1.09)	0.300
	PTGER4	rs4613763	-0.01 (-0.19, 0.17)	0.876	1.08 (0.94, 1.25)	0.295	1.02 (0.82, 1.27)	0.863
	IL12B	rs2546890	0.07 (-0.06, 0.19)	0.324	0.99 (0.89, 1.10)	0.874	1.14 (0.97, 1.33)	0.110
6	*BACH2*	rs12212193	0.02 (-0.10, 0.15)	0.714	0.99 (0.89, 1.09)	0.777	1.00 (0.86, 1.17)	0.984
	THEMIS	rs802734	0.01 (-0.13, 0.15)	0.877	1.01 (0.90, 1.13)	0.885	1.03 (0.87, 1.22)	0.760
	MYB	rs11154801	0.06 (-0.07, 0.19)	0.373	1.07 (0.96, 1.19)	0.213	1.04 (0.89, 1.21)	0.657
	IL22RA2	rs17066096	0.03 (-0.12, 0.17)	0.717	1.04 (0.93, 1.17)	0.515	1.10 (0.92, 1.30)	0.292
	No gene	rs13192841	-0.12 (-0.26, 0.02)	0.102	0.90 (0.80, 1.01)	0.066	0.89 (0.74, 1.06)	0.183
	TAGAP	rs1738074	-0.03 (-0.16, 0.10)	0.666	1.03 (0.91, 1.13)	0.794	0.90 (0.77, 1.06)	0.203
7	*ZNF746*	rs354033	-0.004 (-0.15, 0.14)	0.959	1.03 (.91, 1.16)	0.657	0.87 (0.73, 1.04)	0.134
8	*IL7*	rs1520333	-0.02 (-0.17, 0.12)	0.729	1.02 (0.91, 1.14)	0.770	0.93 (0.79, 1.11)	0.441
	MYC	rs4410871	0.02 (-0.12, 0.17)	0.745	1.01 (0.90, 1.14)	0.839	1.03 (0.86, 1.23)	0.774
	PVT1	rs2019960	0.08 (-0.07, 0.23)	0.283	1.04 (0.92, 1.18)	0.484	1.11 (0.92, 1.33)	0.278
10	*IL2RA*	rs3118470	0.08 (-0.05, 0.21)	0.240	0.98 (0.88, 1.09)	0.726	1.11 (0.94, 1.30)	0.220
	ZMIZ1	rs1250550	-0.02 (-0.16, 0.12)	0.776	1.02 (0.92, 1.14)	0.713	1.00 (0.85, 1.17)	0.970
	HHEX	rs7923837	0.02 (-0.11, 0.15)	0.742	1.02 (0.92, 1.14)	0.681	1.04 (0.89, 1.21)	0.657
11	*CD6*	rs650258	0.11 (-0.03, 0.24)	0.126	1.08 (0.926, 1.20)	0.197	1.12 (0.96, 1.32)	0.160
12	*TNFRSF1A*	rs1800693	-0.02 (-0.15, 0.11)	0.801	1.00 (0.90, 1.11)	0.998	0.98 (0.84, 1.15)	0.844
	CLECL1	rs10466829	-0.11 (-0.24, 0.02)	0.086	0.89 (0.80, 0.98)	0.020	0.89 (0.76, 1.04)	0.141
	CYP27B1	rs12368653	-0.01 (-0.14, 0.12)	0.871	0.99 (0.89, 1.10)	0.860	0.98 (0.84, 1.15)	0.823
	ARL61P4	rs949143	0.003 (-0.13, 0.14)	0.971	0.98 (0.88, 1.10)	0.771	1.04 (0.88, 1.22)	0.634
14	*ZFP36L1*	rs4902647	0.03 (-0.10, 0.16)	0.630	1.00 (0.90, 1.11)	0.989	1.01 (0.87, 1.18)	0.896
	BATF	rs2300603	-0.01 (-0.16, 0.14)	0.882	1.01 (0.90, 1.14)	0.881	0.98 (0.82, 1.16)	0.786
14	*GALC*	rs2119704	-0.05 (-0.31, 0.22)	0.725	0.98 (0.80, 1.21)	0.860	0.86 (0.63, 1.16)	0.328
16	*CLEC16A*	rs7200786	0.06 (-0.07, 0.19)	0.370	1.07 (0.92, 1.18)	0.202	1.09 (0.94, 1.28)	0.260
	IRF8	rs13333054	0.05 (-0.09, 0.20)	0.474	1.04 (0.92, 1.17)	0.524	1.13 (0.95, 1.35)	0.161
17	*STAT3*	rs9891119	-0.07 (-0.20, 0.06)	0.316	0.94 (0.85, 1.05)	0.286	0.93 (0.80, 1.10)	0.407
18	*MALT1*	rs7238078	-0.04 (-0.19, 0.11)	0.605	0.95 (0.84, 1.07)	0.406	0.95 (0.79, 1.14)	0.565
19	*TNFSF14*	rs1077667	-0.07 (-0.23, 0.10)	0.412	0.96 (0.84, 1.09)	0.513	0.95 (0.78, 1.16)	0.630
	TYK2	rs8112449	0.01 (-0.13, 0.14)	0.941	1.02 (0.91, 1.14)	0.781	0.96 (0.81, 1.14)	0.643
	MPV17L2	rs874628	0.18 (0.02, 0.33)	0.023	1.13 (1.00, 1.27)	0.059	1.22 (1.01, 1.46)	0.039
	DKKL1	rs2303759	0.03 (-0.11, 0.18)	0.644	1.09 (0.97, 1.22)	0.130	0.97 (0.81, 1.15)	0.710
20	*CD40*	rs2425752	0.01 (-0.13, 0.15)	0.923	0.99 (0.88, 1.10)	0.797	1.02 (0.85, 1.21)	0.851

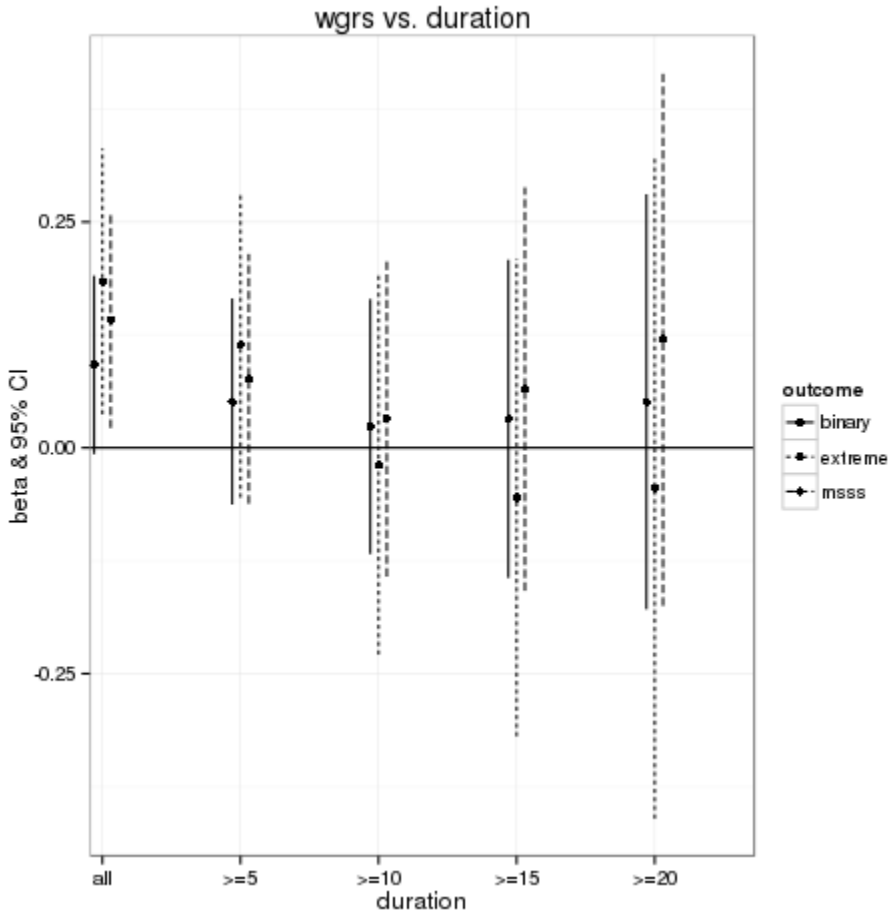
Chr	Gene	SNP	MSSS Outcome					
			Continuous		Binary		Extreme*	
			β (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value	OR (95% CI)ǂ	p-value
20	*CYP24A1*	rs2248359	0.08 (-0.05, 0.22)	0.215	1.10 (0.99, 1.22)	0.089	1.05 (0.89, 1.23)	0.559
22	*MAPK1*	rs2283792	0.11 (-0.01, 0.24)	0.083	1.05 (0.95, 1.16)	0.366	1.11 (0.95, 1.30)	0.173
	SCO2	rs140522	-0.02 (-0.16, 0.12)	0.821	1.01 (0.91, 1.13)	0.801	1.04 (0.88, 1.23)	0.604

These marginal models tested the association between the three MSSS phenotypes and all 52 individual SNPs Cohort was used as a random effect in the models.

ǂ All βs and ORs are from the adjusted models. Models are adjusted for gender, age of onset, *HLA-DRB1*15:01*, and cohort.

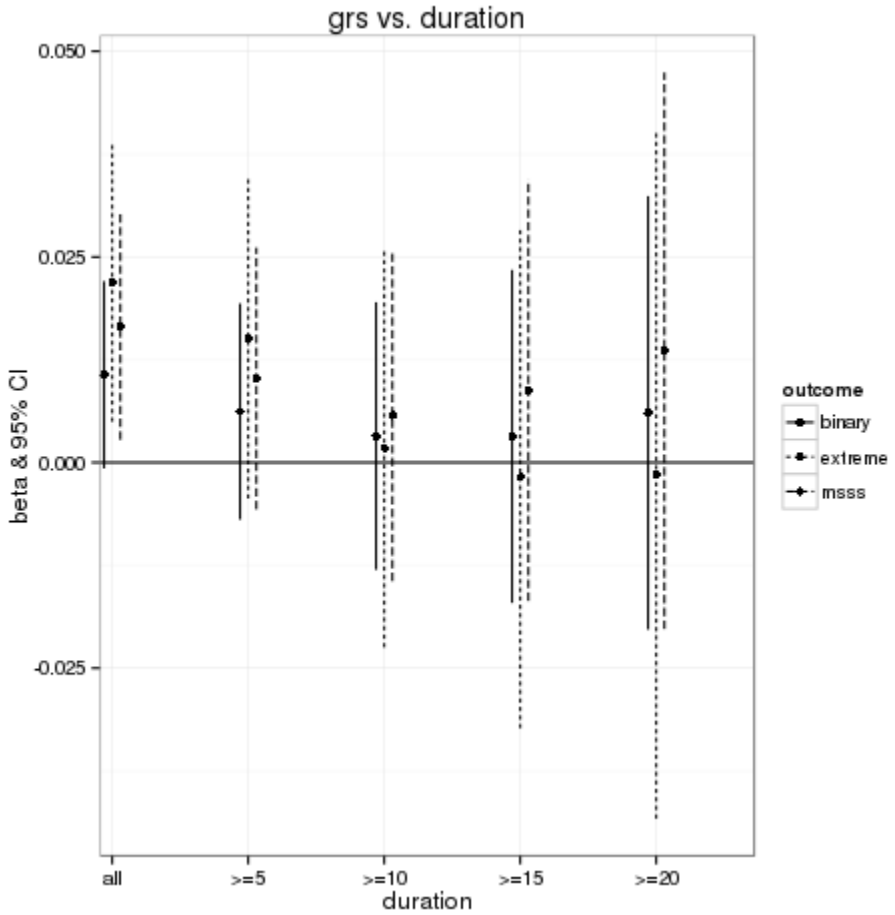
*A total of 1,957 individuals with MS are included in this analysis.

Figure 2A. Association between wGRS and MSSS in MS cases stratified by disease duration



Total number of individuals in the all category for MSSS continuous and binary associations = 7,125; >=5 years = 5,424; >=10 years = 3,437; >=15 years = 2,084; >=20 years = 1,187. Total number of individuals in the all category for the extreme associations = 3,795; >=5 years = 3, 016; >=10 years = 1,957; >=15 years =1,170; >=20 years =657. MSSS binary and extreme associations were log transformed to match the scale for the continuous measure.

Figure 2B. Association between GRS and MSSS in MS cases stratified by disease duration



Total number of individuals in the all category for MSSS continuous and binary associations = 7,125; >=5 years = 5,424; >=10 years = 3,437; >=15 years = 2,084; >=20 years = 1,187. Total number of individuals in the all category for the extreme associations = 3,795; >=5 years = 3, 016; >=10 years = 1,957; >=15 years =1,170; >=20 years =657. MSSS binary and extreme associations were log transformed to match the scale for the continuous measure.

Chapter 4. The association between cigarette smoking and multiple sclerosis progression: a meta-analysis

ABSTRACT

Background: Multiple sclerosis (MS) is a leading cause of disability in young adults, but environmental and genetic factors affecting the clinical progression are largely unknown. A previous meta-analysis in 2011, suggested that smoking was an important risk factor for MS progression, but other studies have been published more recently. To account for the heterogeneity of MS progression, there are more outcome measurements of MS incorporated in this meta-analysis.

Objective: To review, analyze, and summarize existing data on smoking and the risk of more severe and rapid MS progression.

Methods: An extensive literature search was undertaken to identify all studies that investigated the association between exposure to cigarette smoking and MS progression. Fifteen studies met inclusion criteria and were included. Summary measures of association were calculated using a random-effects model.

Results: Smokers were nearly two times as likely to transition to SPMS as non-smokers over the same time period (summary risk ration (SRR)=1.93; p-value=0.013); smokers were also more likely to require unilateral ambulatory assistance as non-smokers over the same period of time with the disease (SRR=1.32; p-value=0.042), and smokers had a higher T2 lesion load than non-smokers over the course of the observation period (summary mean difference=0.18; p-value<0.001).

Conclusions: These results suggest that smoking is not only a risk factor for MS, but also associated with MS progression. Smoking cessation efforts may benefit MS cases throughout the course of the disease.

INTRODUCTION

Multiple sclerosis (MS) is a severe autoimmune disease affecting the central nervous system (CNS). MS is the second leading cause of neurological disability in young and middle-aged adults [5]. There have been advances in treatment of the symptoms of MS, but the prognosis for MS patients remains poor [99]. MS disease progression is extremely variable, with few known environmental and genetic predictors. About 60% of individuals with MS will require assistance walking after 20 years with the disease, and extremely severe MS occurs in 5-10% of MS patients [100]. There is very little explanation as to why some individuals are wheel-chair bound shortly after diagnosis, while others remain virtually symptom free for many years. The heterogeneity in the clinical course of MS is poorly understood, but been suggested to be the result of variation in the extent of demyelination [101]. The majority of MS patients follow a disease course known as relapsing remitting MS (RRMS). This disease course includes flares of symtpoms that last a short time (typically days or weeks), after which patients recover to baseline health. After the relapsing course, patients transition to a more progressive form of disease with no defined relapses, only the steady progression of symptoms, known as secondary progressive MS (SPMS) [102]. This transition typically occurs within 15-30 years of disease onset [88]. Several predictors of MS susceptibility have been investigated in connection with progression; however, few of the same predictors of MS risk also predict MS progression [52]. Higher levels of circulating of vitamin D in serum have been found to be associated with a lower number of relapses in MS patients [39-41, 103]. Tobacco smoke is an avoidable exposure that has been suggested to worsen MS progression [43-45], but conflicting evidence exists [47].

While the exact underlying mechanism is not known, there is biological plausibility supporting a connection between tobacco smoke exposure and MS progression. Cigarette smoking has been shown to affect the immune system, both increasing autoimmune reactions and decreasing systemic activity against infections [46]. Additionally, chronic exposure to cigarette smoke or nicotine causes T cell unresponsiveness, which may contribute to the immunosuppressive and anti-inflammatory properties of cigarette smoke/nicotine [104]. Nicotine-induced immunosuppression may result from its direct effects on lymphocytes, indirectly through its effects on the neuroendocrine system, or both. In animal models, nicotine has also been shown to affect the blood brain barrier by increasing permeability, which may be involved in the disease process [105]. Therefore, tobacco smoke and/or nicotine may be responsible for the further inflammatory and immunosuppression suspected to be involved in the progression of MS by suppressing the regulation of T-cells, mainly through production of Th1 and Th17 cytokines [106].

A connection between exposure to tobacco smoke and accelerated disease progression has been observed in other autoimmune diseases. For example, in rheumatoid arthritis, number of pack years of cigarette smoking was significantly associated with rheumatoid factor seropositivity, radiographic erosions, and nodules, which are all characteristic of more severe disease [107]. In patients with systemic sclerosis, smoking has been shown to have a significant negative effect on almost all vascular, gastrointestinal, and respiratory outcomes [108]. Additionally, exposure to cigarette smoking has been shown to have an association with disease progression in other neurodegenerative diseases. For example, in Alzheimer's disease, active smokers were significantly younger at death and studies of higher levels of smoking were

associated with shorter interval between onset and death [109]. However, in Parkinson's disease, the evidence suggests that smoking is protective against disease progression, as patients who smoked have been shown to have a longer survival time than expected, which may suggest a modulating effect of smoking on the neural pathways that are affected by dopamine [110, 111]

Transition from RRMS to SPMS is a significant milestone in the disease course of MS. While most individuals who have the relapsing disease course will transition to the progressive form, the speed of that transition offers insight into the severity of the disease. Similarly, the speed of transition from clinically isolated syndrome (CIS) to clinically definite MS (CDMS), may be predictive of disease severity. There are several ways to measure physical disability in MS patients, including the expanded disability status scale (EDSS) and MS severity score (MSSS). EDSS is a scale measuring physical impairments in each neurological system. EDSS was established to measure disease progression in MS patients: an EDSS of zero correlates with no disability, whereas an EDSS of ten is death due to MS [28]. An EDSS of six correlates to an MS patient requiring unilateral ambulatory assistance, such as a cane or crutch. MSSS is a probabilistic algorithm that uses the EDSS to calculate disease severity and duration of disease, typically measured from the onset of first symptoms to the EDSS exam date [29]. Brain and spinal cord magnetic resonance imaging (MRI) is used to measure the physical impact of the demyelination in the CNS. T2-weighted (T2) lesion load is the total impact of demyelination in the CNS, whereas contrast enhancing lesion (CEL) load is the measurement of active or recent demyelination in the CNS. There is controversy in the literature whether or not lesion volume is correlated with clinical disability and progression [112, 113].

In order to measure MS progression, the current meta-analysis will use eight outcomes: transition from RRMS to SPMS, transition from CIS to CDMS, time to EDSS 4 and 6, mean difference of EDSS, mean difference of MSSS, mean difference of T2 lesion load, and mean difference of CEL load. The only prior meta-analysis on this topic suggested that smoking was associated with a faster transition from relapsing remitting (RR) MS to secondary progressive (SP) MS, although failed to reach statistical significance [24]. The study by Handel et al. measured only one outcome of progression in MS. The aim of the current study is to establish the relationship between exposure to cigarette smoking and MS progression measured using the eight outcomes defined above.

MATRIALS AND METHODS

Ethics approval

Ethical approval was not required for this study.

Literature Search

PubMed was searched for relevant studies using the following terms: smoking or smok*, multiple sclerosis, phenotype and/or progression. There was no time period or study design restriction; however, only studies published in English, published in peer-review journals, and conducted with humans were considered. The database was last searched on September 1st, 2014.

No additional studies were identified through the review of the selected studies.

Exposure and Outcome Definitions

Smoking was defined in two ways: ever versus never and current versus never. All but one study used self-reported smoking history [114]. Eight different measures of MS progression were tested: transition from RRMS to SPMS, transition from CIS to CDMS, time to EDSS 4, time to EDSS 6, mean difference in EDSS, mean difference in MSSS, mean difference inT2 lesion load, and mean difference in CEL load.

Statistical Analysis

Relative measures of association and 95% confidence intervals (95% CI) were extracted from the literature for the transition from RRMS to SPMS, transition from CIS to CDMS, time to EDSS 4 and time to EDSS 6 analyses. All relative measures were log transformed and 95% CI were used to calculate standard errors prior to conducting the meta-analyses. Number of smokers and non-smokers and the respective means and standard deviations were extracted from the included studies for analyses of mean differences in: MSSS, EDSS, T2 lesion load, and CEL. Each meta-analysis was performed using a random effects model to account for heterogeneity of study results. Eight analyses were performed to account for different measurements of MS progression (Table 1). Summary risk ratios (SRR) and summary mean difference (SMD) were reported. Egger and Begg-Mazumdar p-values were used to assess publication bias. All analyses were conducted in STATA v13.1 (StataCorp, TX).

RESULTS

Sixty-nine articles were identified from the electronic database search. After further review of the abstracts for content, 21 studies remained. Six of the studies were excluded because the outcome of progression or measure of association used was unique and no other studies were available to make a statistical comparison. Therefore, 15 studies with a total of 8,871 MS and CIS patients remained (Table 1 and Figure 1). Studies included prospective and retrospective cohort studies and cross-sectional studies from 13 different countries. When exact results were not reported in the primary literature, corresponding authors were contacted and provided the missing necessary data [45, 114-116].

Time to event

The first analysis conducted replicated the results from the only prior meta-analysis examining smoking and MS progression, as measured by transition from RRMS to SPMS [24]. The current study incorporated newly published results (Roudbari [117]) and previously published results (Hernan [43], Sundstrom [44], Healy [45], Koch [47]), and found that smokers were nearly two times as likely as non-smokers to transition from RRMS to SPMS in the same time period (SRR=1.93; 95%CI=1.15, 3.25; p-value=0.013) (Table 2 and Figure 2A). The second analysis examined the transition from CIS to CDMS (Di Pauli [118], Horakova [114], and Arikanoglu [116]). These results showed that exposure to cigarette smoking were not associated with progression from CIS to CDMS (Table 2). The third analysis tested the association between

exposure to cigarette smoking and time to EDSS 4 (Koch [47] and Manouchehrinia [119]), but no association was found (Table 2). The fourth analysis tested the association between smoking and time to EDSS 6 (Koch [47], D'hooghe [120], Manouchehrinia [119], and Weiland [115]). These results suggest that smokers were more likely than non-smokers to reach EDSS 6 in the same time period (SRR=1.32; 95%CI=1.01, 1.72; p-value=0.042) (Table 2 and Figure 2B).

A previous study of 806 RRMS patients showed that time to a sustained EDSS 6 was typically the point at which these patients transitioned to SPMS [121]. Therefore, the current study combined the studies that reported time to transition from RRMS to SPMS and results from studies that reported time to EDSS 6. The pooled meta-analysis found an association between exposure to cigarette smoking and disease progression. Results showed that smokers were more likely to progress than non-smokers in the same time period (SRR=1.63; 95%CI=1.27, 2.08; p-value<0.001) (Table 3 and Figure 3). While the study by Koch et al. was included in both sub-analyses above, only one result was included in the pooled analysis, so as to not double count data.

Clinical measurements

The fifth analysis tested the pooled mean difference of MSSS between smokers and non-smokers (Sena [122], Pittas [83], and Maghzi [123]). The study by Sundstrom et al. was excluded from the final analysis as the reported results did not include the variance among smokers and non-smokers. These results suggested that smokers may have a slightly increased MSSS as compared to non-smokers, but the difference did not achieve statistical significance (Table 2). Similarly, the sixth analysis tested the pooled mean difference in EDSS between smokers and non-smokers (Sena [122], Pittas [83], Horakova [114], and Arikanoglu [116]). The studies by Healy et al. and Zivadinov et al. were excluded from the final analysis as the reported results included medians and interquartile ranges (IQR) rather than mean and standard deviations. These results suggest that smoking is not associated with EDSS (Table 2).

MRI measurements

The seventh and eighth analyses examined differences in MRI outcomes, T2 lesion load (Zivadinov [124], Healy [45], Horakova [114], and Arikanoglu [116]) and CEL load (Zivadinov [124] and Horakova [114]). The results show smokers have a higher T2 lesion load as compared to non-smokers (SMD=0.17; 95%CI=0.09, 0.26; p-value<0.001) (Table 2 and Figure 1C). However, exposure to cigarette smoke was not associated with increased CEL load (Table 2).

DISCUSSION

The current study is the most comprehensive meta-analysis to date and data from nearly 9,000 MS patients were included. The current study updated the prior meta-analysis of cigarette smoking and MS progression, and included analyses testing the association between cigarette smoking and additional progression measures. Due to the heterogeneity of the disease, there is no single measurement that encompasses all aspects of MS progression. Therefore, the current meta-analysis uses eight measures of MS progression to test the association with exposure to cigarette smoking. These results support an association between cigarette smoking and MS

progression, as measured by a decreased time to transition between RRMS to SPMS, increased time to EDSS 6, and increased T2 lesion load.

Three studies were not incorporated in the current analysis because the reported results were not compatible with the remaining results found in the literature. The study by Sundstrom et al. was not included in the mean difference of MSSS, however, the mean MSSS in ever smokers was reported as 5.2, whereas the mean in never smokers was reported as 3.2, with a p-value of 0.042. These results have the same magnitude and direction of association as the other reported results in the current meta-analysis. If a measurement of variance had been included by Sundstrom et al., the inclusion of this study in the current meta-analysis would have added more evidence that exposure to cigarette smoking was associated with a difference in mean of MSSS. Additionally, the studies by Healy et al. and Zivadinov et al. were not included when calculating the mean difference of EDSS because only medians and IQR were reported. Healy et al. reported a median (IQR) of 2.0 (2.5) in ever smokers and 1.5 (2.0) in never smokers. Likewise, in the study by Zivadinov et al., the median (IQR) in ever smokers was 3.0 (2.0) and 2.5 (2.5) in never smokers. Both of these results are in agreement with the magnitude and direction of association as the other results included in the current meta-analysis. If the studies by Healy et al. and Zivadnov et al. had been included in the current meta-analysis, they would have added more evidence that exposure to cigarette smoking was associated with mean difference of EDSS.

Six studies were found in the literature that each analyzed a unique measure of MS progression, and therefore could not be included in the current study because no comparable measure of MS progression was available for a meta-analysis. The study by Emre et al. found that 16 of 21 MS patients who were current smokers had transient deterioration of their motor skills immediately after smoking, with the effect lasting for ten minutes [125]. The study by Turner et al. found that higher levels of physical pain were associated with a higher likelihood of current smoking within a population of MS patients within the Veterans Affairs system in the United States (OR=1.12 (1.02-1.20)) [126]. However, that same study also reported that current smokers were more likely to self-report better physical health but poorer mental health than non smokers (OR=1.03 (1.01-1.15); OR=0.95 (0.94-0.97), respectively). The study by Staff et al. found that among MS patients with severe cognitive impairment, 67% were smokers, which is substantially higher than the national and MS population prevalences of smoking at the time [127].

The study by Gholipour et al. reported that MS patients who smoked were more likely to have “sustained malignant status of disease” than non-smokers [128]. This measurement of severe versus benign disease course was a unique measurement of MS progression, but was consistent with the results reported in the current meta-analysis showing that progressive disease in MS is more likely in smokers than non-smokers. The study by Marrie et al. found that ever smokers had an increased odds of reporting a co-morbid autoimmune disease (CAD) before MS onset (OR=1.22 (1.08, 1.38)) and an increased risk of developing a CAD after MS onset (HR=1.23 (1.08, 1.41) [129]. Lastly, the study by Manouchehrinia et al. found that within a cohort of MS patients, the risk of death was higher in smokers than in never smokers within the same time period (HR=2.70 (1.59, 4.58) and 1.30 (0.72, 2.32), respectively) [130]. Overall, while these six studies could not be included quantitatively in this meta-analysis, qualitatively each found that smoking was associated with progression of MS.

One limitation of the current study is that all but one of the included studies relied on self-reported smoking history as the primary exposure of interest. The study by Marrie et al. found that depressed patients were 50% less likely to be reliable when reporting smoking status as compared to non-depressed patients [131]. None of the studies in the current meta-analysis controlled for current depression; therefore, the results may be differentially misclassified. If depressed smokers were less like to reliably report their smoking status than non-depressed smokers, there may be misclassification of the exposure of cigarette smoking. But the study by Marrie et al. also reported that the differences in agreement between depressed and non-depressed MS patients were small and did not affect the reliability of other study variables, such as disease duration or disability status.

Another limitation of the current study is the inability to utilize all data reported in the literature. In the eight analyses in the current study, the p-value for the test of heterogeneity is significant in all but three analyses (Table 2), which indicates that the results from the included studies are not similar and should not be combined. Relapse rate or the number of relapses a patient experiences is another measure of disease activity. Currently, number and frequency of relapses have not been associated with disease progression in RRMS patients [101]; however, relapses are experienced by the vast majority of MS patients. Four of the studies included in this meta-analysis also reported findings about relapses and smoking [83, 114, 115, 122]. However, because these results were not consistently reported, it was impossible to combine them in a meta-analysis. Qualitatively, the available studies suggests that smoking may not be associated with number of relapses or relapse rate among MS patients, offering further evidence that relapse rate and overall disease progression may results from different biologic mechanisms.

While the current meta-analysis found smoking was associated with decreased time to transition from RRMS to SPMS, the association of smoking and time to transition from CIS to CDMS remained inconclusive. If smoking was associated with an accelerated progression toward SPMS, then it would be expected that other major transitions and disease milestones should be consistent. The magnitude of the association was smaller in the association between cigarette smoking and transition from CIS to CDMS results, but the direction of association was similar. Perhaps the current meta-analysis did not have the power to detect an association as the CIS to CDMS meta-analysis had 418 individuals, as compared to the 2,530 individuals included in the RRMS to SPMS meta-analysis. Additionally, the time between CIS diagnosis and CDMS confirmation tends to be shorter than the transition time between RRMS to SPMS, 1-3 years versus 15-30 years, respectively [88, 99, 132]. Perhaps the biological effect of smoking takes longer to take exhibit results than the short transition period between CIS and CDMS.

Much of the previous literature concerning smoking and MS progression refers to the study by Koch et al. as evidence against of an association between exposure to cigarette smoking and MS progression, as measured by transition from RRMS to SPMS. Roughly one quarter of the initially contacted individuals with MS did not return the questionnaire, and if those participants were more likely to be smokers and have progressive disease, then the reported results may have been biased. This information was not attainable, but may explain the difference between results found in the study by Koch et al., and the studies by all other investigators included in the current meta-analysis. Furthermore, dropping this study from the meta-analysis substantially decreases the heterogeneity (p-value>0.850, data not shown).

The pooled results in Table 3, which tested the association between cigarette smoking and MS progression, as measured by transition from RRMS to SPMS and time to EDSS 6, offered more evidence that cigarette smoking is associated with MS progression. Time to sustained EDSS 6 can be used as a surrogate of time to SPMS [121]. Therefore, both of these measures of MS progression used in this pooled meta-analysis are similar clinically. The pooled meta-analysis included 3,667 individuals and eight studies, which provided more evidence that cigarette smoking and disease progression in MS are associated.

In conclusion, the current study is the most extensive meta-analysis of smoking and MS progression of date. All included studies were of high quality and included a large sample of MS patients. The current study assessed MS progression with eight measures of progression, attempting to account for the heterogeneity of the disease. The results support an association between cigarette smoking and MS progression, as measured by a decreased time to transition between RRMS to SPMS, time to EDSS 6 and T2 lesion load. However, not all measures of MS progression were found to be associated with smoking. Further research is needed to more directly assess the timing and overall dose of smoking exposure and disease progression.

TABLES AND FIGURES

Table 1. Studies included in the current meta-analysis

Authors	Year	Study population	Study type	Number of patients	Smoking definition	Type of Progression
Hernan et al.*	2005	General Practice Research Database	Prospective cohort	179	Ever	RRMS to SPMS
Koch et al.*	2007	Groningen MS Database	Prospective cohort	271	Ever	RRMS to SPMS, time to EDSS of 4 and EDSS 6
Sundstrom et al.*	2008	Vasterbottom County in Sweden	Cross-sectional	122	Ever	RRMS to SPMS
Di Pauli et al.	2008	MS Outpatient Clinics at the Clinical Department of Neurology in Austria	Retrospective cohort	129	Ever	CIS to CDMS
Sena et al.	2009	MS outpatient Clinic at the Neurology Service of Centro Hospitalar de Lisboa-Central	Cross-sectional	205	Ever	Mean difference in MSSS and EDSS
Zivadinov et al.	2009	Baird MS Center	Cross-sectional	368	Ever	T2 lesion load, CEL load
Pittas et al.	2009	Southern Tasmania	Prospective cohort	198	Ever and current	Mean difference in MSSS and EDSS
Healy et al.*	2009	Partners MS Center, Boston, MA, USA	Cross-sectional with follow-up	1,465	Ever	RRMS to SPMS, mean difference in MSSS, T2 lesion load
Maghzi et al.	2011	Isfahan Multiple Sclerosis Society	Cross-sectional	516	Ever	Mean difference in MSSS
D'hooghe et al.	2011	Flemish MS society in Belgium	Cross-sectional	1,372	Current	Time to EDSS 6
Manouchehrinia et al.	2013	East Midlands MS specialist clinic database at Nottingham University Hospital	Cross-sectional	895	Ever	Time to EDSS of 4 and EDSS 6
Roudbari et al.	2013	MS Society in Guilan, Iran	Cross-sectional	400	Ever	RRMS to SPMS
Horakova et al.	2013	SET Study - multi-center (Prague)	Prospective cohort	194	Current	CIS to CDMS, mean difference in EDSS, T2 lesion load, CEL load
Arikanoglu et al.	2013	Neurology Clinic, Istanbul University Medical School	Prospective cohort	95	Ever	CIS to CDMS, mean difference in EDSS, T2 lesion load
Weiland et al.	2014	Web 2.0 Platforms - international	Cross-sectional	2,469	Current	Time to EDSS 6

The 15 studies included in all meta-analyses are listed by first author's last name. The year of publication is listed. The study population used in each study is described. The study designed used in each study is listed. The total

number of individuals used in the smoking verses MS progression outcome is listed. The manner in which smoking was categorized in the study is listed. The meta-analysis in which the study was included is listed. Most studies were used in several analyses to incorporate all comparable results reported.
*These studies were used in the study by Handel et al. in 2011.

Table 2. Meta-analyses results from eight measures of MS progression

Meta-analysis	Number of individuals	Pooled measure of association (95% CI)	p-value	Test for heterogeneity
RRMS to SPMS	2,437	1.93 (1.15, 3.25)	0.013	0.004
CIS to CDMS	418	1.54 (0.89, 2.67)	0.124	0.036
Time to EDSS 4	1,166	1.15 (0.81, 1.64)	0.428	0.069
Time to EDSS 6	5,007	1.32 (1.01, 1.72)	0.042	0.009
Mean difference in MSSS	2,384	0.12 (-0.03, 0.28)	0.122	0.143
Mean difference in EDSS	692	0.08 (-0.31, 0.47)	0.684	0.017
T2 lesion load	2,122	0.17 (0.09, 0.26)	<0.001	0.926
CEL load	562	1.98 (-1.62, 5.58)	0.280	<0.001

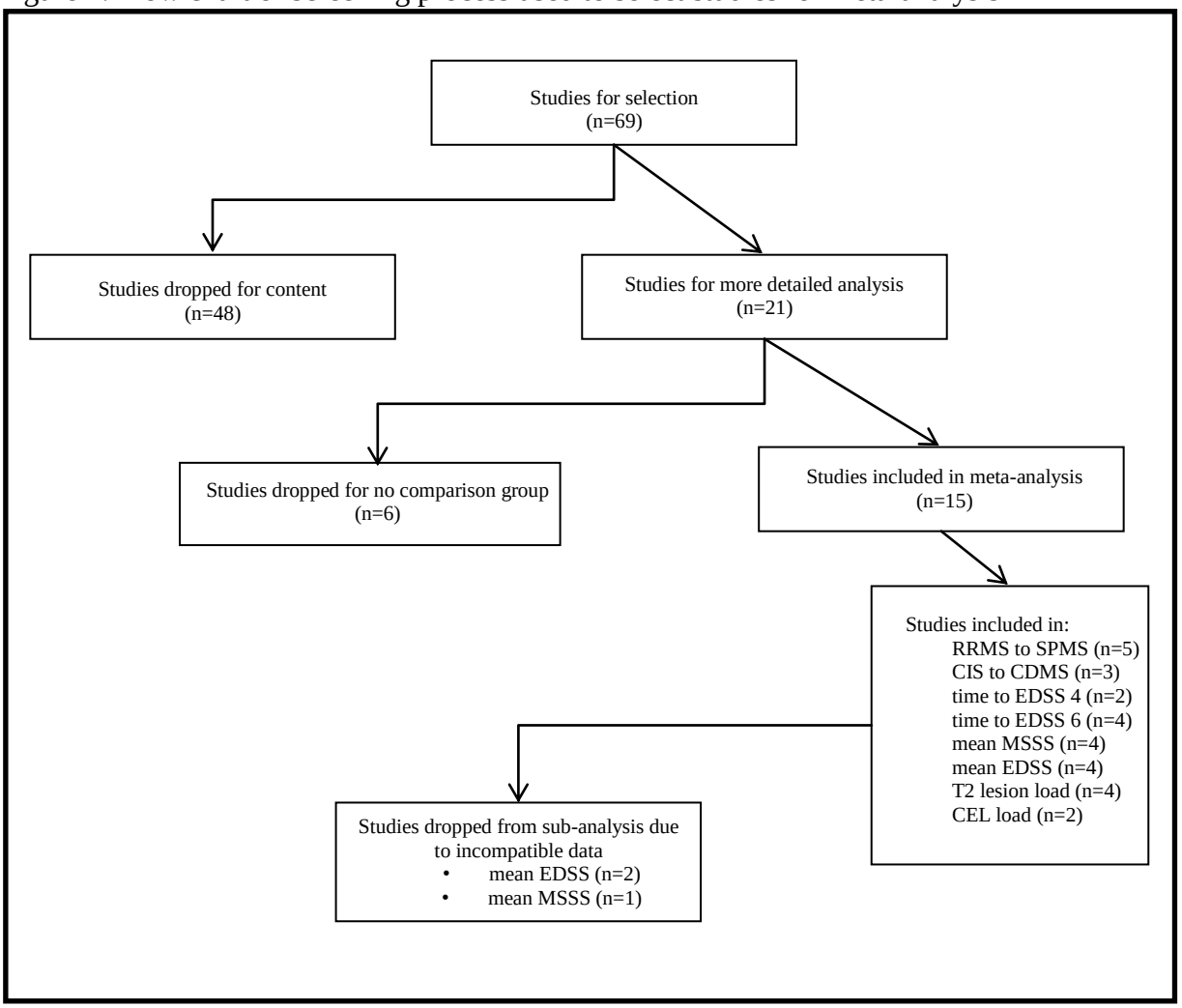
All meta-analysis results from eight MS progression outcomes are listed above with number of individuals included. The pooled measure of association with the 95% CI and p-value are listed. All p-values for the test for heterogeneity are listed.

Table 3. Pooled meta-analysis: transition from relapsing remitting multiple sclerosis (RRMS) to secondary progressive multiple sclerosis (SPMS) and time to expanded disability status scale (EDSS) of six

Study	Reported measure of association (95% CI)	Weight (%)
Sundstrom et al 2008	2.10 (1.1, 4.0)	8.7
Roudbari et al 2013	2.25 (1.3, 3.9)	10.4
Healy et al 2009	2.50 (1.4, 4.4)	10.1
Koch et al 2007	0.89 (0.6, 1.3)	13.8
Hernan et al 2005	3.60 (1.3, 9.9)	4.7
Weiland et al 2014	1.90 (1.4, 2.5)	16.8
D'hooghe et al 2011	1.35 (1.0, 1.8)	16.9
Manouchehrinia et al 2013	1.25 (1.0, 1.5)	18.9
Summary RR	**1.63 (1.3, 2.1)**	**100**

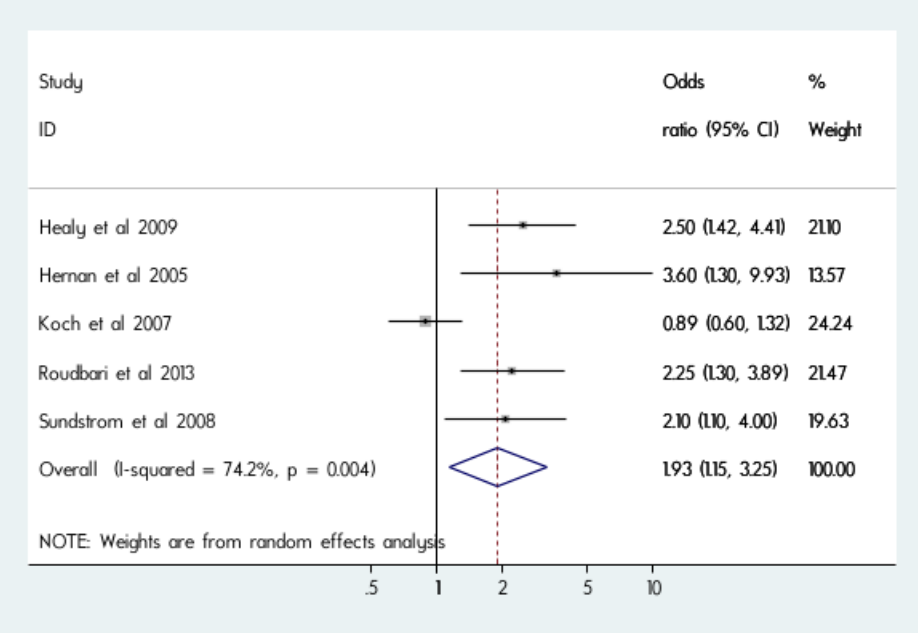
These results combine the studies that report time to transition from RRMS to SPMS and time to EDSS 6. The reported measure of associate from each study is listed, along with the 95% CI. The weight describes the weight given to each study in the meta-analysis, which is reflective of the sample size. There are 7,173 individuals with MS included in this analysis.

Figure 1. Flow chart of screening process used to select studies for meta-analysis



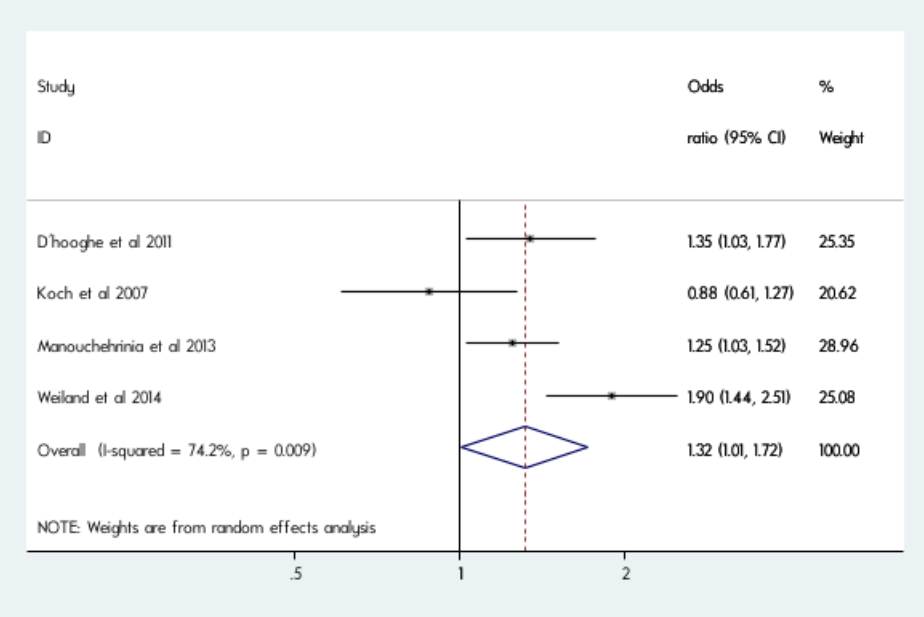
Abbreviations include: RRMS for relapsing remitting multiple sclerosis (MS); SPMS for secondary progressive MS; CIS for clinically isolated syndrome; CDMS for clinical definite MS; EDSS for expanded disability status scale; MSSS for MS severity score; and CEL for contrast enhancing lesion.

Figure 2A. Forest plot for relapsing remitting multiple sclerosis (RRMS) to secondary progressive multiple sclerosis (SPMS)



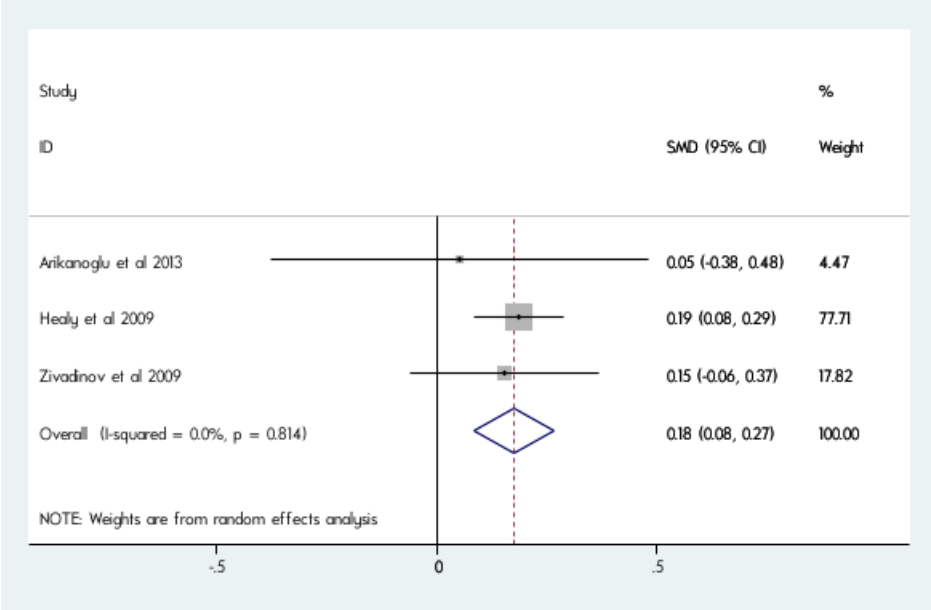
This figure is the forest plot from the meta-analysis measuring the association between smoking and transition time from RRMS to SPMS. The summary risk ratio (and 95% CI) is 1.93 (1.15, 3.25).

Figure 2B. Forest plot for time to expanded disability status scale (EDSS) of six



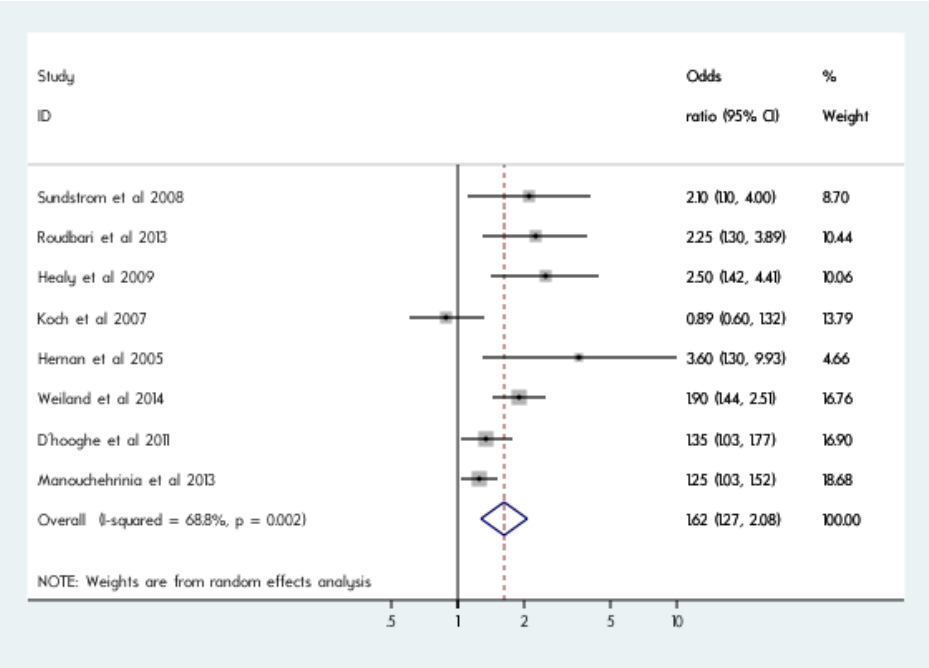
This figure is the forest plot from the meta-analysis measuring the association between smoking and time to EDSS 6. The summary risk ratio (and 95% CI) is 1.32 (1.01, 1.72).

Figure 2C. Forest plot for T2 lesion load



This figure is the forest plot from the meta-analysis measuring the association between smoking and T2 lesion load. The summary mean difference (and 95% CI) is 0.17 (0.09, 0.26).

Figure 3. Forest plot for pooled results for relapsing remitting multiple sclerosis (RRMS) to secondary progressive multiple sclerosis (SPMS) and time to expanded disability status scale (EDSS) of six



This figure is the forest plot from the pooled meta-analysis measuring the association between smoking and transition time from RRMS to SPMS and time to EDSS 6. The summary risk ratio (and 95% CI) is 1.63 (1.3, 2.1).

Chapter 5. Conclusion

Multiple sclerosis (MS) is a severe autoimmune disease of the central nervous system (CNS). Demyelination of nerve cells in the brain and spinal cord cause diverse symptoms, which can include diminished sensation, weakened motor control, and/or cognitive impairment [1, 2]. The World Health Organization has estimated that approximately 1.3 million people have been diagnosed with MS worldwide, with a global incidence of 2.5 per 100,000 persons. There are clinical, genetic, and environmental risk factors for developing MS; however, very few confirmed risk factors for MS progression. This dissertation focused on investigating MS progression and possible associations with cognitive impairment, with established genetic risk factors for MS, and with exposure to cigarette smoking.

Summary of Findings

In Chapter 2, this dissertation investigated the difference of cognitive impairment, as measured by the modified telephone interview for cognitive status (TICS-M), between MS cases and controls recruited from the Kaiser Permanente Medical Care Plan in Northern California Region (KPNC). On average, the overall TICS-M score was lower in MS cases when compared to controls (β=-0.64, p=0.001); results were consistent with models that were adjusted for current depression, history of depression, year of birth, gender, smoking and parental education (β=-0.53, p=0.007). The TICS-M score was also associated with disease severity, as measured by the MS severity scale (MSSS), in MS cases. On average, MS cases with a higher MSSS had a lower TICS-M score, even after controlling for gender, age of onset, parental education, and disease course. Notably, individuals with very severe MS (MSSS $\geq$7.5) had a lower TICS-M score as compared to those with a benign presentation (MSSS <2.5) after adjustment (β=-1.12, p=0.005).

In Chapter 3, this dissertation investigated the association between 52 genetic risk variants located outside the major histocompatibility complex (MHC) and disease severity, as measured by MSSS, in 7,125 MS cases. Ten independent datasets of cases were analyzed. A weighted genetic risk score (wGRS) and an unweighted genetic risk score (GRS) were calculated using the established 52 non-MHC risk alleles [18], and these genetic scores were also tested for an association with MSSS. Random-effects meta-analyses accounting for the random effects of dataset were used. Gender, age of onset, and *HLA-DRB1*15:01* were all analyzed as fixed effects. After adjustment, all three measurements of MSSS were suggestive of an association with wGRS and GRS. However, after restricting the data set to individuals who had ten years or more of disease, no significant associations remained (wGRS: $\beta_{continuous}$=0.03, p=0.714; OR_{binary}=1.02, p=0.743; $OR_{extreme}$=0.98, p=0.859; and GRS: $\beta_{continuous}$=0.01, p=0.577; OR_{binary}=1.00, p=0.698; $OR_{extreme}$=1.00, p=0.885). Gender and age of onset were consistently associated with MSSS in all three models, even after restricting to individuals with ten years or more of disease (all p-values<0.001). Male gender and a later age of onset were associated with more severe disease. *HLA-DRB1*15:01* was not associated with MSSS in any of the models.

In Chapter 4, this dissertation investigated the association between exposure to cigarette smoking and several markers of MS progression in a meta-analysis. The results indicated that smokers were nearly two times as likely to transition from the relapsing remitting phase MS (RRMS) to the secondary progressive phase of MS (SPMS) as non-smokers in the same time period (summary risk ratio (SRR)=1.93; 95%CI=1.15, 3.25; p-value=0.013). A second analysis

found that smokers were more likely to reach an expanded disability status score (EDSS) of six than non-smokers in the same time period (SRR=1.32; 95%CI=1.01, 1.72; p-value=0.042). As time to transition from RRMS to SPMS is a similar progression marker as time to EDSS of six, a pooled meta-analysis was conducted testing the association between exposure to cigarette smoking and disease progression. Results showed that smokers were more likely to progress to a disabled phenotype faster than non-smokers in the same time period (SRR=1.63; 95%CI=1.27, 2.08; p-value<0.001). Additionally, magnetic resonance imaging (MRI) progression outcomes were tested. These results showed smokers have a higher T2-weighted lesion load as compared to non-smokers (summary mean difference=0.17; 95%CI=0.09, 0.26; p-value<0.001).

Conclusions and Further Directions

MS disease progression is heterogeneous and clinically difficult to measure with a single scale, score, or measurement tool. This dissertation looked at several physical and cognitive markers of MS disease progression to comprehensively understand risk factors associated with a "severe" phenotype of MS or a more rapid disease progression. The identification of modifiable or treatable risk factors would allow individuals diagnosed with MS to impact the severity of their disease.

In Chapter 2, this dissertation found that TICS-M was able to distinguish between MS cases and controls in KPNC, even when the analysis was restricted to individuals with a disease duration less than 5 years. But validation studies are needed to confirm TICS-M is suitable to detect cognitive impairment in MS cases. If TICS-M can detect cognitive impairment just as well as other validated measurements, then it would be preferred due to the ease of use over the telephone and quick administration of the questionnaire. The ability to conduct large epidemiologic studies of cognitive impairment in MS is essential. Nearly 70% of MS cases will suffer from cognitive symptoms over the course of the disease [30]. Therefore, the impact of this research would be significant. The earlier cognitive impairment can be identified, the more successful treatment interventions may be. But an easily administered, validated assessment tool to measure cognitive impairment in MS cases is the first step.

In Chapter 3, this dissertation found that the genetic variants that make an individual susceptible to develop MS are not the same genetic variants that are associated with MS disease progression. There was only one marker of MS progression measured in this chapter. MSSS only captured the physical disability caused by MS. A possible future direction for this work could include determining if cognitive impairment or MRI measurements are associated with MS genetic risk variants. Additionally, a larger list of non-MHC risk variants could be compiled to test the association with MSSS and other MS progression markers. Studies designed specifically to identify the genetics of MS progression are important.

In Chapter 4, this dissertation found that exposure to cigarette smoking was associated with MS disease progression, as observed by a quicker transition from RRMS to SPMS, a quicker sustained level of disability that required ambulatory assistance, and MRI evidence of more demyelination in the brain among smokers as compared to non-smokers. Smoking is a modifiable behavior, and with more evidence suggesting that smoking may cause a more severe phenotype of MS, the design of smoking cessation programs for MS cases are crucial.

Taken together, these chapters demonstrate the need for further research of MS progression. Some established environmental risk factors for developing MS, such as smoking, appear to be associated with markers of disease progression as well; whereas this dissertation did not find that genetic risk factors for developing MS also confer risk of MS disease progression. The development of validated, easily administered tools to measure multiple markers of MS progression on a large epidemiologic scale are necessary to further this research.

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