MULTIPLE SCLEROSIS JOURNAL | MSJ

Environmental Factors, Genetics, and Epigenetics in MS Susceptibility and Clinical Course

Mendelian randomization in multiple sclerosis: A causal role for vitamin D and obesity?

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Multiple Sclerosis Journal

2018, Vol. 24(1) 80–85

DOI: 10.1177/1352458517737373

***Abstract*:** The etiology of multiple sclerosis (MS) involves a complex interplay of genetic and environmental factors. Epidemiologic studies have furthered our understanding of these risk factors but remain limited by residual confounding and potential for reverse causation, particularly in MS where time of disease onset is not known. Mendelian randomization (MR) uses genetic variants to study the causal effect of modifiable exposures on an outcome. This method avoids some of the limitations of classical epidemiology and can strengthen causal inference. Here, we introduce the basic concepts of MR and review its contributions to the field of MS. Indeed, several studies using MR have now provided support for a causal role for low vitamin D level and obesity in the development of MS.

Keywords: Multiple sclerosis, Mendelian randomization, genetic epidemiology, vitamin D, obesity

Date received: 7 May 2017; accepted: 29 June 2017

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Introduction

Several genetic and environmental factors which increase the risk of multiple sclerosis (MS) have been identified.[1] Although their individual effects are small, in combination they explain some of the risk for MS in the general population.[2] The genetic factors conferring the highest risk lie within the human leukocyte antigen (HLA) complex. Large genome-wide association studies (GWAS) have reported an additional ~110 non-HLA variants associated with various immunological processes.[3] Still, there is ample evidence that environmental influences play a key role in MS etiology.[1] Some of the better established risk factors include Epstein–Barr virus (EBV) infection, cigarette smoking, low vitamin D level, reduced sun exposure, and obesity.

Accurate appraisal of environmental risk factors is critical to understand disease etiology, enable prevention strategies, and perhaps identify novel therapeutic targets. Epidemiologic studies have been crucial in this effort. However, they are susceptible to limitations that may bias the association between exposure and disease, complicating causal inference. Many modifiable health determinants are strongly related to unmeasured or imperfectly measured lifestyle and socioeconomic factors, leading to residual confounding.[4] Case-control studies are also limited by the potential for differential misclassification of the exposure through recall bias. Another limitation of observational studies is reverse causation, which occurs when the disease influences the level of an exposure. This is also of particular concern in MS as disease onset may precede the first clinical manifestations by several years. Indeed, it is not unusual for patients with MS or clinically isolated syndrome (CIS) to initially present with several silent magnetic resonance imaging (MRI) lesions. In addition, health care utilization and fatigue are increased in the years leading to MS diagnosis.[5,6] In an example of possible reverse causation, studies have reported a decreased risk of MS in parous women, suggesting a favorable effect of pregnancy.[7] However, others described a similar association with reproductive history in both men and women, arguing against a biological effect of pregnancy.[8] Restriction of the association to a 5-year period preceding MS diagnosis further raised the possibility of reverse causation wherein subclinical disease may have led to lower procreation.[8]

Largely due to these limitations, associations found in observational studies may fail to be reproduced in randomized controlled trials (RCTs),[9,10] the current gold standard for causal inference. However, certain exposures such as smoking or EBV infection would be unethical to randomize. In addition, long lead time between exposure and disease, as may be the case for several MS risk factors, can make experimental studies

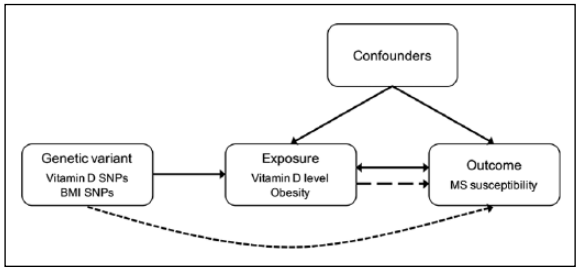

Figure 1. Schematic representation of MR analyses. The causal effect of exposures such as vitamin D level and obesity on the risk of MS (dashed arrow) can be estimated using the genetic variants association with the exposure and the outcome (dotted arrow). The genetic variant is not associated with confounders of the exposure–outcome association and is not susceptible to reverse causation (bidirectional arrow). Note that the dotted arrow does not represent a direct effect of the genetic variants on the outcome (pleiotropy).

impossible. Finally, high costs may be prohibitive, especially when interventions consist of lifestyle modifications or off-patent drugs like vitamin D. These practical considerations for MS prevention trials are magnified by its low incidence rate in the population. For example, an RCT of MS prevention with vitamin D, would likely need to start in the pre-natal period (given the association of immediate post-natal low vitamin D level with risk of MS),[11] continue until at least the mean age of onset of MS and require an unreasonably large number of individuals, given the rarity of the disease. For these reasons, we do not feel that a high-quality MS prevention trial using vitamin D is feasible.

In the absence of high-quality randomized trial data, causal inference can be made using Mendelian randomization (MR). MR uses genetic variants to study the causal effect of a modifiable exposure on an outcome, greatly limiting residual confounding and preventing reverse causation.[9] The basic principle of MR is that if a modifiable exposure is causal in a disease process, then a genetic variant influencing the exposure should also be directly associated with the disease (Figure 1). In other words, single nucleotide polymorphisms (SNPs) conferring a lower level of a certain trait (e.g. vitamin D levels) can be used to measure the effect of that trait on an outcome (e.g. MS risk). The magnitude of the genetic associations with both the exposure and the outcome (which can be derived from different datasets) can then be used to estimate the effect size of the exposure on the outcome, while reducing confounding and reverse causation. This approach offers several advantages. Genetic variants for a given trait are randomly allocated at conception, independent of potentially confounding traits (due to Mendel's second law). Hence, the use of genetic variants as a proxy for a modifiable exposure avoids the spurious association of lifestyle, socioeconomic and other potentially confounding factors that may bias the measure of the relationship between the exposure and outcome.[4] This resembles the conditions of an RCT since the genotype-based groups differ with respect to the exposure, while most other variables are distributed randomly. Furthermore, given that disease processes do not alter genotype (except in cancer and some infections), the direction of effect is known and reverse causation is consequently avoided.

In MR studies, genetic variants assume the role of an "instrumental variable" associated with the outcome only through its association with an intermediate variable of interest. To be a valid instrumental variable, genetic variants must satisfy three assumptions:[9,12] (1) be associated with the exposure of interest; (2) be independent of confounding factors which affect the outcome (independence assumption); and (3) be only associated with the outcome through the exposure of interest (exclusion restriction assumption). The limitations of MR stem from situations where one or more of these assumptions are violated. Pleiotropy is probably the most challenging to address and refers to a situation where a genetic variant influences biological processes that impact the outcome and are independent of the exposure. The presence of pleiotropy can only be assessed indirectly. Nonetheless, robust methods have been developed to detect and correct for its effects,[13] some of which are described below. Other limitations include population stratification (which is confounding by ancestry), canalization, and low statistical power (Table 1).[9,12]

In addition to exploring causal associations between exposures and disease, MR can contribute to the development of new treatments through analysis of genetic variations within drug target loci.[10] For example, an association between a genetic polymorphism of the interleukin-6 receptor (IL-6R) and the risk of coronary heart disease[14] has led to RCTs of tocilizumab, an IL-6R inhibitor, in myocardial infarction.[15]

The emergence of MR as a powerful tool can be attested by its rapidly expanding applications.[16] A PubMed search for the term "Mendelian randomization" or "Mendelian randomisation" identified two papers in 2003 and 213 papers in 2016. Nonetheless, its use in neurology remains rare.[17] Within the field of MS, six original studies based on MR were identified. In the following paragraphs, we detail the contribution of these studies to the understanding of the roles

Table 1. Limitations of MR and how to address them (adapted from Davey Smith and Hemani[12]).

Limitation	Definition	Strategies to avoid/correct for bias
Pleiotropy	Influence of the genetic variant on the outcome through pathways other than the exposure. Can be introduced through linkage disequilibrium with another SNP	Biologic understanding of variant MR-Egger regression Funnel plot of each SNP estimate
Population stratification	Confounding of the genetic–outcome association by subgroups with different disease prevalence and allele frequency	Stratification by ethnicity Maintain homogenous populations Ancestry-informative markers
Canalization	Attenuation of the expected effects of a genetic variant on a phenotype during development through compensatory processes	Unlikely with common variants
Low statistical power	Use of SNPs with small effect on phenotype may result in low power and imprecise effect estimates (wide confidence interval)	Increase sample size Use of multiple variants

MR: Mendelian randomization; SNP: single nucleotide polymorphism.

of vitamin D and obesity in MS pathogenesis. The implications for prevention of MS are substantial as both conditions are highly prevalent, each affecting approximately 40% of the US adult population.[18,19]

Vitamin D levels

Ever since the early description of a latitudinal gradient in MS prevalence, studies have suggested a role for low vitamin D in its etiology. Indeed, large case-control studies identified an association between higher vitamin D levels and a reduced risk of MS.[20,21] This finding was present even when vitamin D levels are measured in the neonatal period.[11] In the Nurses' Health Study cohort, vitamin D supplementation with 400 IU or more decreased MS risk by 40%.[22] Along with this strong body of evidence, results of MR studies from our group as well as others lend strong support for a causal effect of low vitamin D levels in MS etiology.

A first study by Mokry et al.[23] selected four SNPs associated with the level of 25-hydroxyvitamin D (25OHD) in the SUNLIGHT GWAS, which included 33,996 individuals of European descent. The effect of these SNPs on the risk of MS was assessed in the International Multiple Sclerosis Genetics Consortium (IMSGC) Immunochip study, the largest in MS (14,498 MS cases and 24,091 controls).[3] Two SNPs not ascertained in the Immunochip genotyping platform were subsequently extracted from the second largest study, the IMSGC/WTCCC2 GWAS (9772 cases and 6332 controls).[24] MR analysis of the effect across the four variants revealed that a standard deviation decrease in natural log-transformed 25OHD levels doubled the risk of MS (odds ratio (OR) = 2.02; 95% confidence interval (CI) = 1.65–2.46; $p = 7.72 \times 10^{-12}$). In vitamin D insufficient individuals (25–50 nmol/L), one standard deviation corresponds to variations of ~35–75 nmol/L. Furthermore, as each SNP was in proximity to genes implicated in vitamin D synthesis (*DHCR7* and *CYP2R1*), transport (*GC*), or metabolism (*CYP24A1*), the effect on MS risk is more likely to act through vitamin D rather than other pathways (i.e. pleiotropy). Individual estimates for each SNP on risk of MS were concordant, further decreasing the likelihood of pleiotropy.

Another group replicated evidence of low vitamin D's causative effects on MS by conducting MR analyses in two different populations, the Kaiser Permanente Medical Care Plan in Northern California (KPNC) and the Swedish case-control studies Epidemiological Investigation of Multiple Sclerosis (EIMS) and Genes and Environment in Multiple Sclerosis (GEMS).[25] The combined populations included 7391 MS cases and 14,777 controls. The authors calculated a genetic score for each individual based on the number of 25OHD increasing alleles at three different loci (*GC*, *DHCR7*, and *CYP2R1*), weighted by their effect on 25OHD level. The meta-analysis of both populations showed that an increase in the genetic score, reflecting higher 25OHD levels, was protective against MS (OR = 0.85, 95% CI = 0.76–0.94; $p = 0.003$). The association persisted after controlling for sex, year of birth, ancestry, smoking, HLA-DRB1*15:01, and 110 non-HLA MS risk variants. Similarly, an increase in the same genetic score was also associated with a decreased risk of pediatric-onset MS (OR = 0.72; 95% CI = 0.55–0.94; $p = 0.02$) in a study of 569 cases and 16,251 controls.[26] Given that these associations were not reported in measured or transformed 25OHD levels, their magnitude cannot be directly compared to Mokry et al.'s study.[23]

Given that large-scale RCTs of vitamin D to prevent MS are not presently feasible, the MR studies presented above may provide the strongest evidence yet for a causative role of lowered vitamin D levels in the development of MS. Of note, these studies explore the effect of lifelong exposure and do not address the possibility of a critical time window, nor do they address the role of vitamin D on disease activity in patients with established MS.

Obesity

A growing body of evidence over the past decade suggests a role for obesity in the development of MS. A body mass index (BMI) $\geqslant$ 30 kg/m^2 during late adolescence or early adulthood has been consistently associated with a two-fold increase in the risk of MS compared to normal weight.[27] Childhood obesity has also been related to pediatric[28] and later-onset MS,[29] although other studies dispute this finding in later-onset MS after controlling for late adolescence obesity.[27,30] Recent MR studies have confirmed the etiologic role of obesity in MS risk.

A study by our group[31] identified 70 independent SNPs that were genome-wide significant in GIANT,[32] the largest GWAS for BMI (n = 322,105). Similar to the vitamin D studies, effect estimates of the BMI-associated SNPs on risk of MS were obtained from either the IMSGC Immunochip study or IMSGC/WTCCC2. MR analysis showed that a standard deviation increase in BMI, which corresponds approximately to a category shift from overweight to obese, raised MS risk by 40% (OR = 1.41, 95% CI = 1.20–1.66, $p = 2.72 \times 10^{-5}$). Several sensitivity analyses were conducted to ensure that MR assumptions were satisfied. One such analysis is the MR-Egger regression, a method to detect and potentially control for presence of pleiotropy adapted from the Egger regression for small study bias in meta-analysis studies.[13] In this study, the MR-Egger intercept was centered at the origin and the slope coefficient was concordant with the standard analysis, decreasing the likelihood of pleiotropy. Using the separate populations from KNPC and EIMS/GEMS, Gianfrancesco et al.[33] independently confirmed the presence of causal effect of BMI on MS. Based on a genetic risk score comprising 97 variants from GIANT, a higher BMI increased the risk of MS (OR = 1.10; 95% CI = 1.05–1.15) after adjustments for birth year, sex, education, smoking status, ancestry, and genetic predictors of MS.

Using the same adult BMI genetic risk score, a recent MR study reported a similar association in pediatric-onset MS (OR = 1.17; 95% CI = 1.05–1.30; $p = 0.01$).[26] In addition, the authors assessed the effect of 28 SNPs associated with childhood BMI derived from the literature. Surprisingly, these did not significantly alter the risk of MS (OR = 1.02; 95% CI = 0.79–1.33; $p = 0.88$). Given that the mean age at onset in the study was between 14 and 15 years, childhood obesity would more appropriately test the effect of obesity on MS risk, rather than adult obesity. While 28 variants contributed to the childhood BMI genetic score, only 15 were genome-wide significant in the largest childhood BMI GWAS meta-analysis (n = 35,668).[34] The score was also unweighted, likely introducing a potential limitation as alleles are unlikely to have similar effect sizes.[35] Finally, the instrument was not specific to childhood BMI as 11 of 28 variants either overlapped or highly correlated with adult variants. This is relevant as BMI-related genetic variants have complex age-specific effects.[36]

In addition to identifying environmental risk factors, MR can be used to investigate underlying biological mechanisms between exposure and disease. Lower vitamin D bioavailability in obese individuals has been suggested as an explanatory mechanism for the effect of obesity on MS.[37] Indeed, MR showed that a unit (kg/m^2) increase in BMI reduced 25OHD concentrations by 1.15% ($p = 6.52 \times 10^{-27}$).[38] While this potentially confounding variable may bias the association between BMI and risk of MS in classic epidemiologic studies, the MR study on pediatric-onset MS demonstrated that vitamin D and BMI genetic variants independently contributed to the disease risk.[26] Alternatively, adiponectin, an anti-inflammatory adipocyte-derived cytokine inversely correlated with BMI, has been proposed as a mediator in the association between obesity and MS. Indeed, levels differed between MS cases and controls,[39] and the hormone was protective in the experimental autoimmune encephalomyelitis (EAE) model.[40] However, an MR study showed large effects of adiponectin level on MS to be unlikely, as a lifetime exposure to sizable differences of genetically determined adiponectin levels did not influence MS susceptibility (OR = 0.93; 95% CI = 0.66–1.33; $p = 0.61$, per two standard deviations change in adiponectin level).[41] Although the underpinnings of the relationship between obesity and MS remain unclear, studies have revealed an interaction between BMI and HLA risk genes in the development of the disease.[42]

Conclusion

Massive investments in large-scale GWAS over the past decade have established reliable genetic variants for a multitude of modifiable environmental risk

factors, providing unprecedented opportunities for genetic epidemiology and, specifically, MR. Summarized data from publicly available, large sample size meta-analyses from genetic consortia further increase the power of these studies. MR can overcome many of the limitations of classic epidemiology and thus constitutes a powerful tool to assess causal effects of environmental risk factors in MS without reliance upon animal models. This methodology has provided strong evidence in support of a pathogenic role for lowered vitamin D level and obesity in MS, including pediatric-onset MS. The implications for prevention are considerable given the high prevalence of the exposures and the fact that they can be safely corrected. Furthermore, the contributions of MR in other fields of medicine have gone beyond exposure–outcome associations to generate new treatment targets and inform drug development. However, limited availability of genetic studies in non-Caucasians and the need to maintain population homogeneity to avoid bias in MR studies has largely excluded individuals of non-European ancestry, limiting the findings' generalisability. In summary, MR can provide important insights into the etiology of MS in humans, a disease particularly difficult to study through observational epidemiological methods, given its unknown timing of onset.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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