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# Integrating Genetics and Environment to Find Causal Mechanisms for Multiple Sclerosis

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## ABSTRACT

Genome-wide association studies (GWAS) have identified hundreds of risk loci for multiple sclerosis (MS), but we have limited knowledge of the mechanisms through which genetic variants mediate risk. Similarly, epidemiological studies implicate numerous environmental risk factors in MS risk, but these cannot identify specific causal mechanisms. We review our current knowledge of genetic mechanisms in MS, including the critical role of expression quantitative trait locus (eQTL) mapping in translating genetic risk loci into causal mechanisms. Molecular and functional context has emerged as an important missing component of these studies, and we discuss how environmental risk factors can be modelled in a quantitative genetic context to identify disease mechanisms. In parallel, we highlight recent advances in which quantitative genetic methods establish a causal role for low vitamin D and obesity in MS, and to dissect the mechanisms through which these operate. As genetic, transcriptional, and epigenetic studies continue to expand, further mechanistic insights for MS are likely to come from the integration of genetic and environmental data.

## 1 | Introduction

Multiple sclerosis (MS) is a chronic progressive disorder in which the immune system targets and destroys central nervous system (CNS) myelin. Approximately 2.8 million people live with MS worldwide [1], making it one of the most common causes of neurological disability in young adults [2]. Females are predominantly affected, at a ratio >3:1 [3]. As MS generally presents between 20 and 30 years of age—during prime working and reproductive years—the social and economic implications of MS are substantial [4].

In the most common form of the disease, termed relapsing-remitting MS (RRMS), recurrent focal inflammation produces episodic neurological symptoms such as vision loss, weakness, numbness, incoordination, bladder dysfunction, and mobility impairment [5]. Most people with MS (pwMS) have numerous relapses, which impair social and occupational function. As recovery from relapses is frequently incomplete, disability accumulates over time.

In addition to relapses, many pwMS experience a slow, insidious progression of disability that leads to impaired ambulation, cognitive dysfunction, and loss of independence [6]. Progressive MS phenotypes are conventionally defined [7, 8] based on when gradual worsening is noted. About 50% of people with RRMS will develop progressive symptoms after an initial relapsing course, a phenotype called secondary progressive MS (SPMS). A minority (~15%) of pwMS present with primary progressive MS (PPMS), where symptoms are progressive from the outset.

Progression in MS occurs in at least two distinct ways. When disability accumulates in a stepwise manner following incomplete relapse recovery, we term this relapse-associated worsening (RAW). Insidious progression that occurs without temporal relation to relapse activity is called progression independent

of relapse activity (PIRA). While the phenotypic classification [7] suggests a neat dichotomization into "relapsing" and "progressive" phenotypes, a retrospective study of 27,000 pwMS involved in clinical trials showed that PIRA occurs throughout the disease course [9]. PIRA can be detected after the initial demyelinating event [10] and in the earliest phases of RRMS [11]. In fact, brain and thalamic volumes are reduced in subjects with pediatric-onset MS compared with age-matched healthy controls [12], suggesting impaired brain growth or neurodegenerative processes at the earliest stages of MS. PIRA events frequently persist, but they may also regress or fluctuate [13]. Specific factors that determine outcome are not well understood. As PIRA is now recognized as the primary driver of disability accumulation in MS [14–17], there is an urgent need to find its underlying immunopathological mechanisms. The emerging concept of MS as a continuous trait [18] rather than a dichotomy has produced calls for a revision of the phenotypic classification [19].

Numerous disease-modifying therapies (DMTs) are available for MS. While DMTs can prevent relapses [20], they have minimal efficacy against progressive symptoms. Current DMTs also have numerous toxicities, including immune impairment, cytopenias, and organ dysfunction. There is a substantial unmet need for more effective therapies that can prevent disability with fewer side effects. By targeting the mechanisms that cause MS or sustain inflammatory demyelination, we may develop treatment and prevention strategies that are likely to succeed in clinical trials [21, 22].

Numerous genetic, environmental, and lifestyle factors contribute to MS risk and pathogenesis [23]. In this review, we explore genetic and environmental factors that predispose to MS, with emphasis on risk factors that are demonstrated to be causal. We discuss an emerging paradigm in which genetic and environmental risk factors, analyzed together, can uncover causal biology, which in turn may lead to novel treatment or prevention strategies for MS.

## 2 | Genetic Factors in MS

MS has long been known to cluster within families [24]. While familial aggregation is seen in genetic disorders, such clustering can also reflect shared environmental risk. A genetic basis for MS is inferred by examining how MS prevalence varies among relatives of people with MS. MS concordance in monozygotic (MZ) twins varies substantially, from 5.9% in a population-based French study [25] to 25.3% in a population-based Canadian study [26]. Differences in concordance reflect differences in population prevalence [26], local environmental factors, and/or differences in diagnostic methods. In the higher prevalence Canadian population, MZ twin concordance is higher than dizygotic (DZ) twin concordance (5.4%). Concordance in DZ twins is, in turn, similar to nontwin-sibling concordance (2.9%) in this population [26, 27]. As MZ and DZ twins share a similar familial environment but differ in genetic relatedness (100% for MZ twins, 50% for DZ), the difference in concordance reflects genetic risk for MS. Risk declines progressively in more distant relatives such as half siblings [28], cousins [29], and avuncular pairs [30], further confirming the genetic basis of MS. A meta-analysis of available twin studies estimates that ~50% of MS risk is heritable [31], with the remainder reflecting shared environment (21%) and unshared environment (29%). The absence of a clear inheritance pattern in affected families suggested a complex genetic inheritance driven by numerous independent factors.

The major histocompatibility (MHC) region contains many genetic factors that drive MS risk. Associations with HLA class I alleles HLA-A*07 [32], HLA-A*03 [33], and HLA-B*07 [34], along with class II alleles HLA-DR2 and HLA-Dw2 [35, 36] were among the first noted. At least 32 independent genetic effects are now recognized within the MHC region [37–39]. The DR2 haplotype, containing *HLA-DRB1*1501*, *HLA-DQA1*0102*, and *HLA-DQB1*0602*, has the largest single effect on MS risk, with an odds ratio (OR) of ~3. Epistatic interactions, where the risk conveyed by a particular HLA allele depends on alleles at another HLA locus, are important determinants of MS risk [37, 38, 40].

In addition to the MHC, genome-wide association studies (GWAS) have found hundreds of genetic risk loci for MS [38, 39, 41]. GWAS compare the frequency of common genetic variants (generally those with minor allele frequency >1%) between pwMS and healthy controls. A variant that exists at a higher frequency in pwMS versus healthy controls is said to be associated with MS. More than 200 independent autosomal loci drive MS risk, along with a single variant on the X chromosome [39]. Together, the MHC and validated variants account for ~39% of overall MS heritability [39]. Remaining genetic risk likely arises from a combination of rare variants and gene-environment interactions that have not yet been quantified. A single study that examined rare variation systematically identified five rare variants that increase MS risk [42].

Apart from the MHC, MS genetic risk loci have small individual effect sizes, and thus limited individual utility for disease prediction. The median OR for lead variants of non-MHC autosomal risk loci in a recent MS GWAS was ~ 1.1 (median absolute deviation 0.036) [39]. Polygenic risk scores (PRS) [43] combine multiple risk variants into a weighted average, permitting genetic risk to be quantified for individuals. A recent study showed that inclusion of PRS in a disease prediction model improved discrimination of MS patients from healthy controls even when family history was included in the baseline model [44].

Beyond MS susceptibility, a single variant is now associated with long-term outcomes in MS [45], suggesting a genetic basis for clinical course in addition to disease susceptibility. This number will certainly increase as cohorts with clinical outcome data grow over time.

### 2.1 | Identifying Genetic Mechanisms for MS

GWAS have elucidated much of the genetic basis for MS, but we still do not know the specific molecular mechanisms through which most genetic factors cause MS. This reflects in part a fundamental limitation in GWAS resolution: as neighboring variants are correlated with one another, it is difficult to differentiate their effects statistically. GWAS have identified many loci that predispose to disease, but it is rare that GWAS can identify a single "causal variant" within a given locus. Even where causal variants are known, we have a limited understanding of how

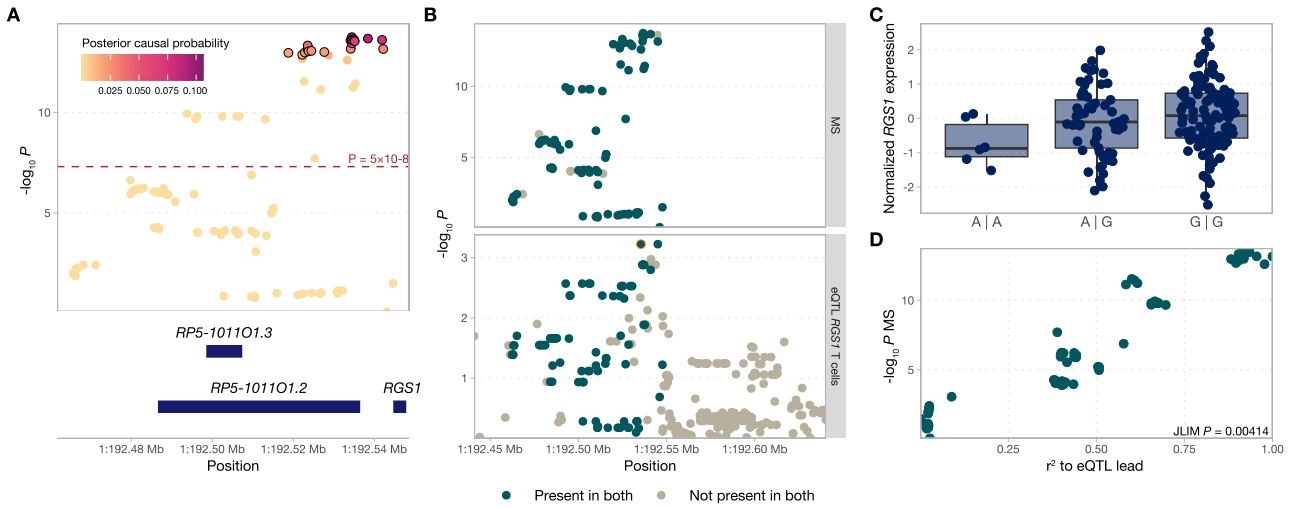


**FIGURE 1** | Disease mechanisms are uncovered by eQTL mapping. (A) GWAS highlights a region of chromosome 1 containing numerous variants associated with MS. Thirty-five SNPs reach $p < 5 \times 10^{-8}$, the threshold for genome-wide significance (red dashed line). There is likely to be only a single causal variant in this region; strong correlations among the associated variants make it difficult to identify the causal variant. We used a fine-mapping technique to calculate the probability that each SNP is the causal variant (fill color). A total of 19 SNPs (circled in black) are contained in the 95% credible set; these SNPs collectively account for 95% of the association signal. (B) The MS association (top facet) overlaps with an eQTL signal for *RGS1* in T cells (bottom facet). (C) *RGS1* varies with the genotype at the strongest eQTL SNP (outlined in orange in panel B): the GG genotype predicts higher expression than AA or AG. (D) The MS risk signal declines as a function of correlation to the lead eQTL variant, indicating a shared causal variant underlies both signals.

these variants perturb molecular and cellular function to cause MS.

As a rule, the variants most strongly associated with MS are noncoding, meaning they do not change protein sequence or structure. MS risk variants are enriched in chromatin regions that are open to transcription factor (TF) binding [46]. Open chromatin regions that contain MS risk variants tend to be active in immune cells [47]. These findings suggest that MS risk variants exert their effects by altering gene expression in immune cells. To find the specific causal mechanisms through which MS risk loci cause disease, we need to identify the individual gene(s) that are dysregulated and the specific cell type(s) in which this occurs.

Expression quantitative trait locus (eQTL) mapping is the most important technique to link MS risk loci to downstream mechanisms [48] (Figure 1). In eQTL mapping, gene expression is measured in a cell type of interest and tested for association with genetic variants nearby (typically those variants within 1 Mb of the gene of interest, that is, *cis*-eQTLs). Where the same variant drives both MS risk and expression of a given gene, we infer that the variant causes disease by altering expression of the gene in the cell type under study. eQTL studies are typically done in healthy donors to avoid the confounding effects of disease or its treatment; causal inference is possible in part because MS does not alter the genetic sequence.

Expression QTL studies have identified downstream mechanisms for many MS risk loci, but the majority remain unexplained. Applying a rigorous colocalization technique to eQTL data from CD4 T cells, monocytes, and neutrophils, Chun et al. identify downstream gene targets for ~1/3 of autoimmune disease risk loci [49]; the remaining 2/3 are unexplained. There are several reasons for this mixed success. First, as mentioned above, GWAS resolution is limited: while many risk loci are known, specific causal variants are usually unknown. Second, eQTL studies are typically much smaller than disease susceptibility GWAS, and early efforts [50–52] were likely underpowered.

In addition to power and resolution, there are several biological reasons for the incomplete success of eQTL studies. First, we must consider the cell types in which MS mechanisms occur. While early studies examined lymphoblastoid cell lines [53, 54], whole blood [55, 56], or small collections of predefined, flow-sorted immune cells in aggregate [57], we now know that expression QTLs are frequently cell type-specific [50]. Early studies could not capture disease mechanisms that are restricted to particular cell classes. More recent studies have applied eQTL methods to diverse collections of peripheral immune cells [58, 59] and other disease-relevant cell types such as microglia [60]. With single-cell transcriptomics technology, we can now perform eQTL analysis at single-cell resolution, assessing the precise immune cell subsets in which disease biology occurs [61, 62]. While the single-cell approach promises to identify additional disease mechanisms, a recent single-cell eQTL study found causal eQTLs for only 39 of 90 examined MS GWAS loci [62].

In addition to particular cell types, transcriptional disease mechanisms may operate in specific environmental and molecular contexts. In a recent study, autoimmune disease risk variants were found to alter chromatin accessibility in peripheral blood mononuclear cells (PBMCs) without influencing gene expression [63]; this suggests that many autoimmune risk loci regulate gene expression in a context-specific manner (e.g., only when a particular transcription factor is expressed) and that previous eQTL studies did not capture these functional contexts. Response

eQTLs (reQTLs) are eQTLs that are specific to, or vary with, particular environmental conditions (e.g., T cell activation).

Response eQTL studies have uncovered context-dependent genetic mechanisms in numerous cell types under diverse stimuli. Fairfax et al. performed eQTL analysis after stimulating $CD14^+$ monocytes with interferon-gamma or lipopolysaccharide [64]. They identified dynamic and context-dependent eQTLs that overlapped with disease-risk loci. Compared with eQTLs present in baseline conditions, context-dependent eQTLs were located further from the pertinent TSS. Kim et al. [65] report similar findings in LPS-stimulated monocytes. Response eQTLs have also been identified in monocyte-derived DCs [66, 67], primary monocytes [59, 65], and activated CD4+ T cells [58, 68, 69].

Alasoo et al. identified response eQTLs in human macrophages after stimulation with interferon-$\gamma$ or Salmonella [70]. They showed that 60% of stimulus-specific eQTLs also influenced chromatin accessibility, and that the variants' effects on chromatin accessibility could frequently be detected before stimulation. This suggests that response eQTLs may perturb enhancer priming.

Several recent studies have identified response eQTLs in immune cells at single-cell resolution. Schmiedel et al. examined 19 distinct T cell subsets after stimulation with anti-CD3 and anti-CD28 [61]. They showed that 4308 genes were genetically regulated (e-genes) after stimulation; nearly half of these (1946) showed no genetic control in unstimulated conditions. In total, 261 e-genes colocalized with autoimmune disease risk loci; 26 e-genes overlapped with MS risk loci. This study shows that immune activation reveals regulatory effects of risk variants that remain hidden in the basal state, underscoring the importance of functional context. While most reQTL studies to date have employed crude functional stimuli, these methods can also be used to model specific environmental stimuli that are relevant for a particular disease. Future reQTL studies are likely to uncover MS disease mechanisms by modelling the environmental risk factors that drive MS risk.

Finally, most eQTL studies conducted to date focus on variants within a narrow window around each gene, typically ±1 Mb of the transcription start site (i.e., *cis*-eQTLs). This is done to increase power, because *cis*-eQTLs tend to have larger effect sizes than *trans*-eQTL [71–73]. Early studies with small sample sizes were powered to detect them. This *cis*-centric framework neglects distant regulatory mechanisms, including *trans*-eQTLs, in which a variant influences the expression of genes elsewhere in the genome through 3D chromatin interactions or regulatory cascades and gene networks. Though *trans*-eQTLs are understudied, we know that they are common in both model organisms [74, 75] and humans [50, 71, 73, 76, 77] and that they are relevant to human disease [56, 72, 78, 79]. In fact, early studies using linkage analysis in bulk cell populations showed that *trans*-eQTLs are more common than *cis*-eQTLs [76]. Though the GTEx study found many more *cis*- than *trans*-eQTLs [80], this study examined whole tissue gene expression. We now know that *trans*-eQTLs are frequently cell type- [50, 62] and stimulus-specific [50, 56, 64, 65], thus may not be detected in complex admixtures. Indeed, resolution of precise cell types has proven an effective strategy to detect *trans*-eQTLs [50, 62, 65, 79]. In addition to cell-type specificity, our efforts to find *trans*-acting transcriptional mechanisms for MS risk loci have been limited by the enormity of the search space, particularly as the number of known genetic variants has increased. It is likely that future *trans*-eQTL studies, employing larger cohorts and more focused search strategies, will uncover distal regulatory mechanisms through which MS risk variants predispose to MS.

## 3 | Environmental Factors in MS

Observational studies implicate numerous environmental factors in MS risk. Notable examples that increase risk include exposure to cigarette smoke [81] and organic solvents [82], Epstein–Barr virus infection [83], adolescent obesity [84], lack of sun exposure, and insufficient vitamin D [85]. Several factors have been associated with reduced MS risk, including the use of oral tobacco [86, 87], high coffee consumption (more than 900 mL/day) [88], and cytomegalovirus infection [89]. In this section, we describe the evidence for vitamin D insufficiency and adolescent obesity—two factors that are now established as causal risk factors for MS—and microbiota, an emerging environmental risk factor of interest.

### 3.1 | Vitamin D Insufficiency

The first suggestion that vitamin D may play a role in MS pathogenesis came from observational studies in North America that found higher MS prevalence at higher latitudes [90–92]. Similar gradients are also noted in Japan [93], China [94], and Spain [95]. An inverse, south-to-north gradient is also found in New Zealand [96].

While several potential explanations were considered initially, Acheson [97] was the first to hypothesize that latitude gradients reflect ultraviolet exposure. A northeast-to-southwest gradient was described in France [98]; the observed gradient aligned with mean monthly UV exposure [99]. Another ecological study in France examined 2667 MS cases registered in the national agricultural health system and found a strong inverse correlation between annual and wintertime (December–March) UVB exposure and MS prevalence by region, with the effect being more pronounced in women [100]. A meta-analysis of global prevalence data showed that MS prevalence increases by 20% for every 10° of latitude north of the equator, with a turning point around the tropics (~23° latitude). Again, exposure to UVB radiation emerged as a strong environmental predictor of MS risk [101].

Migration studies suggest that the risk associated with higher latitudes is established in early life. Individuals who move from areas with high MS prevalence (such as the UK and northern European countries) to low-risk countries (such as South Africa) before the age of 15 adopt the lower MS risk of their new country [102]. Those who migrate after age 15, however, retain a risk level comparable to their country of origin. Migrants from South Asia to the UK had a higher risk of MS when they migrated before age 15 compared with those who migrated after [103]. A retrospective study of migrants from the UK and Ireland to Australia found that migrants who developed MS in Australia were more likely to have migrated after 15 years of age [104], further supporting the notion of an environmental factor that acts in early life.

An association has also been observed between the month of birth and MS risk in the Northern Hemisphere. Researchers analyzed MS cases in countries such as Canada, the United Kingdom, Denmark, and Sweden, comparing them with population controls and unaffected siblings. They found a 9.1% increase in MS risk for individuals born in May, and an 8.5% risk reduction for those born in November. The authors proposed that maternal vitamin D deficiency during pregnancy, due to reduced sun exposure in winter months, could explain this pattern [105]. Though such seasonal patterns may be confounded by seasonal variation in birth rates [106], seasonal fluctuations in MS births have now been replicated in several independent cohorts [107–109].

Additional research has focused on outdoor activity and MS risk, showing that spending more time outdoors between the ages of 16 and 20 was associated with a 45% reduction in MS risk [110]. Another study explored the effect of sun exposure on MS risk in monozygotic twins [111]. Seventy-nine MS-discordant monozygotic twin pairs were asked about childhood outdoor activities, and a sun exposure index was calculated. The twins that did not develop MS had higher sun-exposure scores, and each sun-exposing activity reduced the risk of MS by 25%. Childhood outdoor activity thus decreased MS risk independent of genetic susceptibility.

#### 3.1.1 | Mendelian Randomization Establishes Causality for Low Vitamin D

Numerous lines of observational evidence support a role for low vitamin D in MS pathogenesis, but these studies may suffer from confounding by unknown variables and reverse causation. Mendelian randomization (MR) [112–114] is a method of causal inference that uses genetic variants as instruments to assess the causal effect of an environmental exposure on a trait of interest. As genetic variants are randomized at meiosis, MR analyzes genetic data in a manner analogous to a randomized clinical trial (RCT) to establish causality for environmental traits subject to a limited set of assumptions [113, 114].

Approximately 29% of population variance in serum 25(OH)D is attributed to genetic factors [115], and several large GWAS have been conducted for vitamin D [116–119]. Using a four-SNP instrument from the SUNLIGHT GWAS [117], Mokry et al. [85]. applied MR to the latest MS GWAS [39] to show that low serum vitamin D is a causal risk factor for MS. Controlling for unmeasured confounders, the risk of MS increased by 2.0-fold for every standard deviation decrease in log-transformed serum 25-hydroxyvitamin D. A causal association with vitamin D was confirmed in an independent study of two epidemiological cohorts [120] using a three-SNP instrument from the same GWAS. Similar findings are also reported for pediatric-onset MS [121]. Jacobs et al. used MR to show a causal effect of vitamin D that is independent of childhood BMI [122]. Intriguingly, the authors showed a small reverse causation effect, with MS predisposing to low vitamin D. Though this is not further discussed in the paper, sun avoidance by MS patients could contribute to such an observation.

As GWAS cohort sizes have increased, so has the number of loci that influence vitamin D. A recent GWAS identified 143 independent loci influencing serum vitamin D level [119]. Using a 20-SNP instrument from this GWAS, Wang et al. [123] confirmed the causal influence of low serum 25-hydroxyvitamin D on MS.

### 3.2 | Obesity

Obesity has emerged as an important risk factor for MS [124]. Initial evidence for obesity came from the Nurses' Health Study (NHS) and NHS II [125]: female participants reported their height and weight at baseline, and the authors calculated BMI at age 18. Over 40 years of follow-up, 241 cases of MS were confirmed in NHS and 352 in NHS II. Obesity (BMI $\geq$30 kg/m$^2$) at age 18 was associated with more than double the risk of developing MS compared with women whose BMI was between 18.5 and 20.9 kg/m$^2$. The association persisted after controlling for smoking behavior, latitude of residence at age 15, and ethnicity. No increased risk of MS was observed after the authors adjusted for reported body size at ages 5, 10, and 20 years. These results suggest that weight in adolescence, more than in childhood or adulthood, is critical in determining MS risk [125].

These findings were replicated in a large case-control study in Sweden [126]. A total of 1571 cases were matched to 3371 controls from the national population register. Participants were asked about their current height and weight, as well as their weight and BMI at age 20. BMI >27 kg/m$^2$ at age 20 associated with a twofold increased risk of MS, and this was statistically significant in both men and women [126]. In the same cohort, this group later showed that the association with BMI at 20 was independent of body size at age 10 [127], providing a critical window in which obesity may influence MS risk. Interaction of obesity with HLA risk alleles is reported in multiple cohorts [127, 128].

In a pediatric demyelinating disease cohort, 75 newly diagnosed pediatric cases of MS or CIS were identified, most of these in girls. Obesity was associated with an increased risk of MS and CIS in girls, but the association was not statistically significant in boys [129]. The authors considered several explanations, including whether obesity in children increases the risk of MS/CIS, but with delayed symptom onset until adulthood. They also proposed that high estrogen exposure combined with inflammatory mediators from adipose tissue may accelerate disease manifestation in adolescent girls.

A prospective, longitudinal study of 302,043 school-age children in Denmark showed that higher BMI at age 7–13 was associated with higher MS risk in girls [130]; girls above the 95th percentile for BMI had a 1.61–1.95 times higher risk of MS compared with girls below the 85th percentile. Effect sizes were smaller and nonsignificant overall among boys, likely reflecting decreased power in males. A large observational study from California used logistic regression models to control for prior infectious mononucleosis and established genetic risk factors. They showed that BMI $\geq$30 kg/m$^2$ in one's twenties increased MS risk twofold in females. There was no such association in males. The authors hypothesized that obesity may increase MS risk by promoting a chronic low-grade inflammatory state, or potentially by interacting with gut microbiota [131].

In the EnvIMS study, participants in Norway and Italy were asked to evaluate their body size retrospectively using standardized body silhouettes at 5-year intervals from age 5 to 30. After adjusting for age, smoking, and outdoor activity, large body size (silhouettes 6–9) increased MS risk in Norway, particularly at age 25, with OR 2.21 in men and 1.43 in women [132]. No significant associations were observed in Italy, possibly reflecting other environmental and genetic factors, or methodological differences between the cohorts. Though consistent with prior studies, these findings should be interpreted with caution as recall bias may influence retrospective self-reports of body size.

#### 3.2.1 | Mendelian Randomization Establishes a Causal Role for Obesity

Numerous lines of observational evidence implicate obesity in establishing MS risk, but these are subject to confounding, recall bias, and reverse causation. We now have large GWAS for body mass index [133, 134], permitting Mendelian randomization to establish causality. Mokry et al. [133] used 70 genetic variants that reached genome-wide significance in the GIANT study as instruments to show that obesity has a causal effect on MS risk; a 1 standard deviation increase in genetically determined BMI increased the odds of MS by 41% [135]. Gianfrancesco et al. used an 87-SNP instrument to show a causal influence of genetically-determined BMI on MS risk in two independent cohorts [136]. In this analysis, they controlled for sex, year of birth, education, ancestry, smoking status, and MS-associated genetic variation. In a sex-stratified analysis, the association remained significant in women but not in men, though with wide confidence intervals suggesting limited power in the smaller male sample [136].

Jacobs et al. use MR to show that genetically determined childhood BMI is a causal risk factor for MS, independent of vitamin D status [122]. Harroud et al. [137] used multivariable MR to show a causal effect of childhood BMI on MS. The odds of developing MS increased by 1.26 for every standard deviation increase in BMI. The effect attenuated after adjusting for adult BMI. In contrast, adult BMI exhibited a causal influence that remained after controlling for childhood BMI. The authors conclude that the causal effect of childhood obesity on MS risk is primarily mediated by the persistence of obesity into early adulthood, with no evidence of a direct causal effect of obesity specifically during childhood. Controlling for pubertal timing showed that the effects of obesity on MS risk are independent of hormonal changes during puberty [137].

## 4 | Microbiota

Recent years have seen increasing interest in a potential role for gut microbiota in modulating MS activity. A bidirectional link has been described—referred to as the gut–brain axis—in which metabolic, endocrine, and immunologic mediators influence the development and differentiation of neurons and glial cells, as well as the integrity of the blood–brain barrier [138, 139].

Intestinal dysbiosis occurs when there is an imbalance in the gut microbiota, and this may influence autoimmunity. Reduced overall microbial diversity has been described in pwMS [140], and bacterial genera associated with immunomodulatory properties appear to be underrepresented. Short-chain fatty acids (SCFAs), produced through bacterial fermentation and known to increase regulatory T ($T_{reg}$) cells and suppress $T_H17$ responses, are decreased in pwMS [139, 141]. Some studies report altered SCFA profiles in pwMS, including lower relative acetate compared with butyrate/propionate, which inversely correlate with inflammatory markers but not with clinical activity, radiological activity, or neurofilament levels; these findings should be interpreted cautiously, as SCFAs have context-dependent pro- and anti-inflammatory effects [142]. A Consistent reduction in SCFA-producing bacteria (such as *Firmicutes*, *Roseburia*, *Coprococcus*, and *Lachnospiraceae*) has been described in pwMS, along with an increase in bacterial genera associated with pro-inflammatory responses (including *Bacteroidetes*, *Akkermansia*, and *Ruminococcus*) [140].

Furthermore, intestinal dysbiosis has been shown to compromise the integrity of the intestinal epithelial barrier, leading to increased permeability. This increased permeability, measured using the lactulose/mannitol ratio as an indicator, has been reported to be greater in pwMS compared with healthy controls [143]. It has been proposed that this may facilitate the translocation of microbial components and microorganisms into the systemic circulation, potentially influencing immune function [143].

Vitamin D has also been shown to influence microbial diversity. Higher vitamin D levels have been associated with an increased abundance of beneficial microbial genera in the gut [144]. Another study found that high-dose vitamin $D_3$ supplementation in healthy adults altered the microbiota of the upper gastrointestinal tract, increasing microbial diversity and reducing opportunistic gammaproteobacterial species; as bacteria do not express vitamin D receptor (VDR), the authors suggested that the influence of vitamin D on the microbiota is likely indirect, mediated through host immune responses [145]. Studies evaluating vitamin D supplementation have shown mixed results. In some analyses, performed on biopsies from the sigmoid colon, ascending colon, and ileum, vitamin D supplementation did not significantly alter microbial diversity [145]. However, in women with vitamin D deficiency, 12 weeks of vitamin D supplementation was associated with an overall increase in gut microbial diversity, including a rise in species considered beneficial [144]. Other studies in pwMS reported that vitamin D supplementation at a dosage of 5000 UI per day increased the prevalence of *Akkermansia* species, which are associated with increased intestinal barrier integrity [146].

Within studies of intestinal permeability, vitamin D is essential for maintaining intestinal epithelial integrity by sustaining the expression of tight junction proteins (claudin, occludin, ZO-1, ZO-2, and vinculin). By promoting the synthesis of tight junction proteins, vitamin D strengthens intercellular connections and protects the intestinal mucosa from infections and toxins [147]. In animal studies, mice lacking vitamin D receptors (VDR) in their intestinal epithelial cells demonstrated reduced levels of claudin-2. It has been observed that VDR transcriptionally regulates the expression of claudin-2 in intestinal epithelial cells [148]. Overall, available data suggest that the gut microbiota and

intestinal barrier integrity play a role in the pathophysiology of MS, likely through the gut–brain axis and its impact on systemic immune responses. Although numerous studies in humans and experimental models have provided important insights into this interaction, a causal relationship between gut dysbiosis, altered permeability, and disease activity or progression has not yet been definitively established.

## 5 | Bringing Genetics and Environment Together to Find Mechanisms for MS

Genetics and observational epidemiology have identified many factors that determine MS risk, but they have been less successful at delineating the molecular mechanisms that lead to demyelination. A growing literature suggests that we can identify novel causal mechanisms for MS by considering genetics and environment together: quantitative genetic methods can bring precision and reproducibility to the study of environmental risk factors, while environmental risk factors can provide essential context for genetic studies.

As discussed above, much of the genetic risk for MS operates through gene regulation, but conventional eQTL studies have found a minority of the mechanisms through which GWAS risk loci operate. It is now clear that context is key: MS risk variants operate in particular cell types and specific environmental conditions. While early response eQTL studies used crude stimuli, a growing number of reQTL studies are modelling specific disease-relevant conditions. Barreiro et al. [66] stimulated primary dendritic cells with Mycobacterium tuberculosis and identified response eQTLs that overlapped with GWAS loci for pulmonary TB infection. Response eQTLs have also been detected in PBMCs after stimulation with influenza A [149], and rhinovirus [150], and in memory T cells after TB infection [151]. While MS-specific conditions have not yet been modelled in an eQTL framework, it is easy to see how causal environmental factors such as low vitamin D and obesity could be used to decipher the transcriptional effects of MS risk variants in a relevant context. Such studies would have the potential to uncover both genetic and environmental risk mechanisms.

Just as environmental factors can provide essential context to delineate genetic mechanisms, a new generation of studies is employing genetic methods to establish causality for environmental factors. As discussed above, Mendelian randomization has established low vitamin D and adolescent obesity as causal risk factors for MS, but we do not know the precise mechanisms through which these predispose to MS. Proposed mechanisms for obesity's effect include reduction in bioavailable vitamin D [152], induction of persistent, low-grade inflammation, and increased leptin and decreased adiponectin levels [153]. To address this question, Harroud et al. performed a Mendelian randomization mediation analysis examining BMI, serum vitamin D, leptin, and adiponectin [154]. They showed independent causal effects for vitamin D and BMI. Considering vitamin D and obesity together, they estimated that only ~5.2% of the obesity signal is explained by vitamin D. Genetically determined leptin and adiponectin levels did not increase MS risk, so the mechanism through which obesity increases MS risk remains unknown. As GWAS cohorts increase in size and MR methods improve, it is likely we will achieve similar mechanistic insights for other environmental factors.

Beyond vitamin D deficiency and classical adipokines, obesity is associated with chronic low-grade inflammation driven by macrophage infiltration into adipose tissue and an imbalance toward pro-inflammatory adipokines (e.g., leptin, resistin, visfatin) with reduced anti-inflammatory mediators (e.g., adiponectin, apelin) [155]. This promotes $T_H1/T_H17$ polarization, reduces regulatory T cells, and activates pathways such as JAK/STAT and NF-$\kappa$B, potentially enhancing T cell–mediated demyelination in MS [156]. In parallel, saturated fatty acids activate the NLRP3 inflammasome, increasing IL-1$\beta$ and IL-18, while obesity-related gut dysbiosis and increased intestinal permeability further amplify systemic immune activation relevant to MS [155].

As mentioned above, there is strong evidence linking Epstein–Barr virus infection to MS risk. While the effect size is large [83], causality for EBV has not yet been formally shown, and precise causal mechanisms remain unknown. In fact, while a recent preprint used Mendelian randomization to show a causal relationship between EBV viral load and Hodgkin lymphoma risk, no such causal relationship was shown between EBV viral load and MS risk [157]. Similarly, we have strong evidence that smoking behavior may increase MS risk, but causality has not been proven. As GWAS cohorts increase in size, it will become more feasible to establish causality with MR. As discussed above, advanced techniques such as MR mediation analysis will permit the dissection of independent causal pathways.

This review focuses on eQTL and transcriptional mechanisms that cause MS, but QTL methods can be used to study proteins (pQTLs), chromatin accessibility (caQTLs), and other epigenetic features. As larger and more detailed datasets become available, quantitative genetic techniques will permit the construction of detailed causal pathways from MS risk loci and environmental risk factors, to epigenetic regulation, to protein levels, to cellular function.

## 6 | Translating MS Mechanisms into Therapeutics

In this review, we have explored genetic and environmental risk factors for MS and how these can be co-analyzed to uncover causal mechanisms for MS. As our primary goal in identifying causal mechanisms is to translate these to therapeutics or prevention strategies, it is appropriate to take stock and ask how we are doing. Though therapies that arise from GWAS have a higher likelihood of success in clinical trials [21, 22], we are not aware of a single approved MS therapeutic that arose initially out of MS GWAS. While disappointing, it is likely too early to abandon hope. As we discussed above, specific causal mechanisms are known for a minority of loci, and rapid advances are being made with a variety of new eQTL approaches.

More attention has been paid to vitamin D as a potential immunomodulatory therapy for MS. While low vitamin D is a causal risk factor for MS, it is less clear whether vitamin D supplementation can alter the clinical course of MS once it is established. Some observational studies suggest that lower

vitamin D levels may be associated with a higher risk of progression [158], including a greater prevalence of deficiency in SPMS compared with RRMS and associations with increased brain atrophy [159]. Higher vitamin D levels have also been linked to lower disability progression in some studies [160]. RCTs of vitamin D supplementation in established MS have been largely negative [161–176], and a recent systematic review of RCTs involving high-dose supplementation showed no overall effect of vitamin D supplementation on relapse rate, disability progression, or new T2 lesions at 6–24 months [177]. These findings largely echo a previous Cochrane review [178] that rated the quality of evidence as "very low," largely due to the small sizes of available RCTs.

A recent RCT of high-dose vitamin D in clinically isolated syndrome—individuals with a single demyelinating attack but who do not fulfill MS criteria—showed lower radiologic disease activity and decreased time to disease activity, suggesting a potential modulatory effect [179]. Overall, the role of vitamin D supplementation in MS progression remains uncertain and continues to be an area of ongoing research.

From a prevention perspective, epidemiological and genetic evidence suggest that avoiding obesity in childhood and adolescence may reduce the future risk of MS [180]. A 2025 national cohort study showed that both childhood and adolescent obesity are associated with an increased risk of MS in adulthood, with a clear dose–response relationship. However, treatment of pediatric obesity did not modify this risk, highlighting the importance of early prevention of severe obesity [180].

Mechanistically, obesity appears to interact with other environmental factors such as gut dysbiosis and EBV [181, 182]. Excess weight and Western diet promote pro-inflammatory changes in the gut microbiota [182], while a case–control study suggests that overweight and obesity in early adulthood may act synergistically with EBV infection markers (e.g., high anti–EBNA-1 antibodies and history of mononucleosis) to further increase MS risk [181]. To date, there are no interventional trials showing that weight loss in adolescence reduces MS risk, supporting a focus on early prevention and broader lifestyle strategies [180].

### Author Contributions

A.M.L. wrote and edited the manuscript. M.R.L. wrote and edited the manuscript.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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