The Genetics of Multiple Sclerosis

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Key Points

1. Of multiple sclerosis (MS) genetic heritability, 48% has now been accounted for.
2. The HLA allele *DRB1*15:01* accounts for up to 10.5% of genetic variance underlying MS susceptibility, making it the single largest genetic risk factor; however, this allele has also been found to have epistatic effects on other genetic haplotypes, demonstrating a nuanced and complex impact of gene-gene interactions in MS.
3. Genome-wide association studies (GWAS) have identified more than 200 non-HLA genetic risk factors of MS and have helped to demonstrate that these genetic loci associated with MS are generally related to T-cell activation and proliferation in the immune system.
4. MS has considerable genetic overlap with other autoimmune diseases.
5. Low levels of vitamin D and an increased body mass index (BMI) were found to significantly increase the risk of MS development in Mendelian randomization studies.

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Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by injury to the myelin sheath and subsequent neurodegeneration. The maladaptive autoimmune response in MS results in a myriad of neurological symptoms affecting motor, sensory, coordination, and cognitive functions. MS is a leading cause of disability among young adults and affects females more than males [1, 2]. The identified MS risk factors are varied and likely at interplay with one another. Among them are genetic factors (about 25% of the risk) and environmental factors including living at a high geographic latitude, smoking, obesity, low vitamin D levels, and Epstein-Barr virus (EBV) exposure [3–5].

There has been debate about whether MS is an "outside in" or an "inside out" disease, arising from an abnormal peripheral immune system or from abnormalities within the CNS that trigger an immune response, respectively. As outlined below, the study of the genetics of MS strongly

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implicates that it is primarily a disease of the immune system. MS genetic risk factors are more closely overlapping with those of other autoimmune diseases, including rheumatoid arthritis and type 1 diabetes than with diseases of the CNS [6].

With now more than 230 genetic risk factors identified for MS, leveraging tens of thousands of samples, questions related to gene-gene or gene-environment interactions, genetic influence on phenotype, and pharmacogenetic factors for treatment response have been raised [7]. However, these areas of inquiry have proven to be very challenging, requiring large sample sizes, unified approaches to phenotype, and better understanding of some medications' mechanisms of action. Nonetheless, some gene-environment interactions have been reported, and some genetic factors have been associated with relapse rate and regions of lesion burden. Mendelian randomization experiments have been successfully employed to use genetic drivers of environmental risk factors to support causal associations between these factors and MS risk, eliminating concerns for reverse causation.

The pursuit of genetic risk factors for MS has led to critical insights on the pathophysiology of the disease, and future work will help determine what shapes a person's experience of the disease.

Heritability

Heritability in MS can be defined as the proportion of total variance in risk of disease development that could be explained by genetic factors [8]. Estimation of heritability has largely been based on studies investigating MS risk within families. First-degree relatives (siblings, children, parents) have an increased risk of developing MS (3–5%) [9]. Risk is notably increased to 25–30% in monozygotic twins [9–11]. One of the early twin studies in MS was a longitudinal, population-based study of twins in Canada, which established a 25% risk in monozygotic twins. These twin results have led to estimation that MS genetic susceptibility explains a quarter of the overall risk of the disease. Notably, the Canadian twin study also found that the risk of MS in dizygotic twins is doubled compared to non-twin siblings [11]. These latter results suggest that there may be an influence of the shared intrauterine environment since dizygotic twins and non-twin siblings share the same amount of genetic material, but the former also share very early environmental exposures. Nonetheless, additional studies investigating the MS risk in half-sibling, adoptee, and spouse studies have demonstrated that the primary responsible factor of MS occurrence within families is genetics, more so than the environment of early childhood and adulthood [12, 13]. In sum, there is clustering of MS in genetically related familial groups, and as expected the strongest risk is within monozygotic twins.

In concordant sibling studies, age of onset was determined to be a more important factor than year of onset of MS; this means that siblings who were both diagnosed with MS were more likely to be diagnosed at the same age, rather than in the same calendar year [14, 15]. Genetics was a more powerful predictor than cohabitation [14, 15]. If two siblings had both been diagnosed in the same calendar year at different ages, the environment would be suspected to have played the more important role in MS development. However, being diagnosed at the same chronological age points toward a genetic factor influencing MS predisposition.

As an update to estimations of heritability, a later meta-analysis evaluated eight twin studies from France, United Kingdom (UK), Denmark, North America, Italy, Finland, and Sweden [16]. A biometric multigroup analysis was conducted under a liability threshold model and found meta-analytic estimates for genetic heritability as close to 50% (0.50, 95% CI: 0.39–0.6) [16]. The model also found an estimate of 0.21 (95% CI: 0.11–0.30) for a shared environmental factor of MS and a 0.29 (95% CI: 0.26–0.33) for a unique environmental factor. These results suggest a higher heritability estimate of MS and also a shared very early environmental risk factor. In pediatric MS,

perinatal environmental factors have been associated with risk of disease onset [17].

Sex dimorphism is a predominant feature of MS, with more females affected than males [10, 18, 19]. Sex chromosome contribution to the imbalance in risk has been challenging to study, as X and Y chromosomes are not typically included in genome-wide association studies (GWAS). In a recent large analysis by the International MS Genetics Consortium (IMSGC) of 47,000 cases and 68,000 controls, a locus on the X chromosome was identified with MS risk that reaches genome-wide statistical significance (rs2807267) [7]. The functional consequence of this polymorphism and relation to pathophysiology of MS is not yet fully understood.

Sex effects in MS also include higher transmission of risk through mothers as opposed to fathers, suggesting a genetic or epigenetic factor influencing this gender disparity [20]. Several studies have implicated mitochondrial DNA (mtDNA), which is transmitted to a child strictly from the mother, in MS susceptibility [21, 22]. Mitochondrial dysfunction has been linked to the pathophysiology of chronic neurodegenerative disorders [21, 22]. In regard to demyelinating disorders, a mouse model was established using mtDNA with double-strand breaks that were introduced into myelinating oligodendrocytes [21]. This introduction led to impairment of locomotor function, demyelination, glial activation, and axonal degradation in both male and female mice [21]. These findings suggest that mtDNA damage can cause chronic demyelination and axonal loss in mice [21]. A human haplotype analysis in which mtDNA was sequenced showed a trend toward an overrepresentation of superhaplogroup U in MS participants [23]. Tranah et al. observed in a pooled analysis from seven clinical sites a 1.15-fold higher risk of MS in JT haplotype carriers [24]. These results support the hypothesis that mtDNA variation contributes to MS susceptibility, with potential to explain maternal inheritance of risk.

With the efforts of the IMSGC and generous participation of more than 100,000 individuals, 48% of heritability of MS has now been explained [7]. The specific susceptibility factors are described below and include more than 30 alleles within the human leukocyte antigen region (HLA) and more than 200 non-HLA alleles.

HLA Risk Factors

The human major histocompatibility complex (MHC) is located on chromosome 6 (6021.3) and contains a cluster of more than 200 genes, many of which encode proteins essential to a variety of immune responses [25, 26]. These include the human leukocyte antigen (HLA) class I and class II genes, which encode heterodimeric cell surface glycoproteins that bind and present peptide antigens to T-cell receptors (TCR) [26]. This process of antigen presentation is central to initiation of an adaptive immune response against pathogens and plays a major role in autoimmune disease pathogenesis [25, 26]. Humans have three class I genes, *HLA-A, HLA-B,* and *HLA-C,* which are highly polymorphic and encode the α(alpha) chain of the HLA-A, HLA-B, and HLA-C molecules, respectively [26]. There are three class II gene sets, HLA-DR, HLA-DP, and HLA-DQ, which encode the α(alpha) and β(beta) chains of their respective molecules and are also highly polymorphic [26]. The combination of HLA alleles inherited from each parent is termed the HLA haplotype. The expression of each HLA allele at a locus is codominant [26]. Due to the extensive diversity of HLA alleles, the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System has established a system of nomenclature for HLA genes based on locus, type, subtype, synonymous DNA substitutions within the coding region, differences in the noncoding region, and expression status [27]. Specific HLA alleles have been associated as risk factors for many diseases, including MS [25]. The association between HLA allelic variants and MS susceptibility and progression was first established in 1972 [28, 29]. Since then, many studies have been conducted to better understand how HLA genetic risk factors contribute to MS (Table 11.1) [23, 25–28, 30–44].

Table 11.1 HLA class I and II alleles implicated in MS, referenced in this chapter

	Gene sets	Allele	Associations	Population studied/effected	References
HLA Class II	HLA-DR	DRB1*15:01	Increased risk for MS development; accounts for up to 10.5% of genetic variance underlying MS risk; associated with earlier age of onset in females, oligoclonal band presence, and high IgG levels in CSF	European; African, Asian	[23, 25–28]
		DRB1*13:03	Increased risk of MS development; associated with disease frequency in a population	Ashkenazi Jews	[30, 31]
		DRB1*03:01	Increased risk of MS development	European	[32–34]
		DRB1*15:03	Susceptibility to MS development	African	[35]
		DRB5*01:01	Risk of MS development; attenuates MS severity (DRB5*null patients at an increased risk of developing secondary progressive MS)	African	[35]
		DRB1*14:01	Associated with protective effects against MS development; dominant allele that outweighs the risk of carrying DRB1*15:01	European	[36, 37]
		DRB1*10:01	Negatively correlated with MS occurrence	Middle Eastern	[38]
		DRB1*04:05	Associated with risk of opticospinal MS; severity of MS progression	Asian	[39–41]
	HLA-DQ	DQB1*03	Carriers of homozygous genotype for DRB1*15 and DQB1*03 reached a higher rate of disability significantly faster	European	[42]
		DQB1*15:15	Less frequently found in patients with slowly progressing MS	European	[42]
		DQB1*03:03	Associated with slower MS disease progression	European	[42]
HLA Class I	HLA-A	A*02	Protective effects against MS development, as long as not carrying DRB1*15:01; independently reduces susceptibility to MS	European	[43, 44]
	HLA-B	B*44	Protective factors against MS; also associated with better radiologic outcomes when the disease was present	European	[44]

Abbreviations: HLA human leukocyte antigen, *MS* multiple sclerosis, *IgG* immunoglobulin G, *CSF* cerebrospinal fluid

HLA Factors of Increased Risk

Investigations into the potential genetic heritability of MS began in the 1970s by multiple research groups that used small-sized candidate gene studies to begin to analyze genetic factors related to MS [29, 45]. Among these early studies was investigation of HLA risk factors. The strongest genetic risk factor found to correlate with MS is the *HLA-DRB1*15:01* allele [32]. Carrying a single copy of this allele confers a threefold increase in MS risk in European populations [33]. *HLA-DRB1*15:01* is generally inherited with other HLA alleles as part of an extended *DRB1*1501-DQB1*0602* haplotype [32]. A Human Genome Epidemiology (HuGE) review examined 72 papers investigating the impact of these specific genetic loci published between 1993 and 2004; the review found that in all but a limited few articles, the frequency of *DRB1*15:01* was significantly increased in cases compared to control subjects [30]. The only studies that did not find this association were carried out in non-European populations, suggesting a differential genetic susceptibility among ancestral groups [30]. The large impact of *DRB1*15:01* was also demonstrated in a GWAS, which found that this allele in the class II region of the MHC accounts for up to 10.5% of the genetic variance underlying the risk of developing MS [31]. This is the largest effect of any single factor, which we know of, that contributes to MS risk.

Patients with MS who carry the *DRB1*15:01* haplotype are more likely to be female and have an earlier age of onset; additionally, this haplotype has also been associated with oligoclonal band presence and high immunoglobulin G (IgG) levels in the cerebrospinal fluid (CSF), both of which are markers of increased CNS autoimmune activity [36, 37]. A recent next-generation sequencing study investigated the *HLA-DQB1*, *HLA-DQA1*, and *HLA-DRB1* alleles in 1403 unrelated European-American MS patients and 1425 healthy controls. This study additionally confirmed that the *HLA-DRB1*15:01* allele occurred at a significantly higher frequency in patients compared to controls [37]. The *HLA-DRB1*15:01*, *HLA-DRB5*01:01:01*, and *HLA-DRB5*01:01:01vl_STR1* alleles were all present in significantly higher frequencies in MS patients compared with controls [37]. Finally, the study also determined that when the *HLA-DRB1*15:01* haplotypes were excluded, the *HLA-DQB1*03:02:01~HLA-DQA1*03:01:01~HHLA-DRB1*04:01:01SG~HLA-DRB4*01:03:01:01* haplotypes occurred at a higher frequency in MS patients compared to controls and were all significantly associated with MS susceptibility [37]. These results illustrate that while the *HLA-DRB1*15:01* allele has a strong impact on MS susceptibility, there are other important genetic loci in the HLA region that influence MS development.

The risk of MS is further modified by other HLA factors. The *DRB1*03:01* and **13:03* alleles have been associated with an increased risk of MS. The relationship between the occurrence of **03:01* and MS was first observed in a study using patients from Sardinia; patients who carried the **13:03* allele were identified to be at a higher risk of MS development [38, 46]. A 2012 Spanish study genotyped *HLA-B, HLA-DRB1*, and *HLA-DQA1* haplotypes in a population of 1069 MS patients and 624 ethnically matched healthy control patients; the results demonstrated that *DRB1*03:01* presence within a haplotype contributed to MS susceptibility [47]. In populations with an increased frequency of MS, the *DRB1*13:03* allele is also considered to be a risk factor for MS development. The **13:03* allele is most frequently seen in Ashkenazi Jewish patients; a study focusing on this population was the first to identify the association of *DRB1*13:03* with MS with a significant effect size [48]. Building from these findings, a study investigating Israeli families impacted by MS found that there was an association of the **13:03* allele with disease frequency [49]. While the effect sizes of the **03:01* and the **13:03* HLA alleles are not as large as the **15:01* genetic loci, they demonstrate important effects on disease susceptibility in certain populations and ethnicities.

The *HLA-DRB1*15:03* allele has also been implicated in MS susceptibility and especially in those of African descent [50]. African-Americans have greater HLA haplotype diversity, as well as

unique patterns of linkage disequilibrium that separates their genetic predisposition to MS from Caucasian populations [51]. A 2008 study found that while *HLA-DRB1*15:01* was still found to be an important factor in MS development in African-American populations, *HLA-DRB1*15:03* was also significantly associated with susceptibility [50]. The *HLA-DRB5*01:01* haplotype is often linked to MS risk in patients of African descent. The *HLA-DRB5* allele also has been found to attenuate MS severity; this is supported by the evidence that *HLA-DRB5*null* subjects are at an increased risk of developing secondary progressive MS [50]. This finding is supported by evidence that *HLA-DRB5* acts as a modifier in experimental autoimmune encephalomyelitis [35]. Additionally, patients who present with *HLA-DRB5*null* carrier allele status are also always in the *DRB1*15:03* haplotypes; *the HLA-DRB1*15:03* haplotype is not observed in Caucasian populations [50].

The strong impact of the *HLA-DRB1*15:01* allele has been shown to interact with other genetic risk alleles within the HLA. The *DRB1*15:01* homozygous genotype was observed to have an epistatic effect on the *DRB1*08:01* allele; the presence of the *DRB1*15:01/*08:01* heterozygous genotype had an increased risk of MS development when compared to the other heterozygous **15:01* genotypes investigated [52]. An important finding from this study was that the *DRB1*08:01* genotype was not predisposing on its own; it only contributed significantly to risk of disease onset when the **15:01* allele was present in the same genotype [52]. These findings add nuance to the understanding of the **15:01* risk alleles and susceptible genotypes for MS and demonstrate the complexity behind genetic predisposition.

HLA Protective Factors

While the aforementioned HLA alleles are associated with an increased risk for MS development, other HLA alleles have been associated with protective effects. These associations tend to be more challenging to identify than risk factors and seem to vary more widely between different ethnic groups. In European populations, an allele commonly associated with protective effects against MS development is *DRB1*14:01* [52, 53]. A UK study observed that protection stemming from this allele is dominant, and carrying this allele outweighed the increased risk from carrying *DRB1*15:01* [53]. In a study of 4347 MS patients, investigating the genetic complexity of MHC haplotypes, the *DRB1*14:01* allele was confirmed as a protective factor, along with *DRB1*01*, and suggested a common mechanism underlying the function of both [52].

Alleles that have been further linked to protective effects against MS development are found in the class I HLA loci, including HLA A and HLA B. The *HLA-A*02* allele was linked to protection against MS in a study in Portuguese MS patients [54]. The results of this study found that the *HLA-A*02* allele decreased the risk of developing MS and that this effect was independent from the impact of *HLA-DRB1*15:01* [54]. A later study found that the *HLA-A*02* allele independently reduced susceptibility to developing MS [39]. In a Scandinavian cohort study of more than 3000 patients and healthy controls, it was observed that all *HLA-A*02*-bearing haplotypes were protective against MS development, as long as they did not carry the *HLA-DRB1*15:01* haplotype as well [40]. This study also identified a single class I haplotype, which carried *A*02-C*05-B*12*, which negated the risk of carrying *HLA-DRB1*15:01*, demonstrating a complex role of class I alleles in MS risk and development [40].

The same study that demonstrated supporting evidence for the protective role of *HLA-A*02* also identified the *HLA-B*44* allele as a protective factor and found that it was associated with better radiologic outcomes when the disease was present [39]. These brain-related outcomes include better brain parenchymal function and decreased T2 hyperintense lesion volume [39]. Both the *HLA-A*02* and *HLA-B*44* alleles were assessed after accounting for the effect of *HLA-DRB1*15:01*.

A study that investigated *HLA-DRB1* and *HLA-DQB1* alleles in 120 Iranian MS patients found that the *DRB1*10:01* allele was negatively

correlated with MS occurrence, suggesting a potential protective effect against the disease, but the sample size was very modest [55]. While a proportion of the healthy controls carried this gene, none of the MS patients did [55]. A separate case-control study in the Slovak population identified the *DRB1*07, DRB1*13*, and *DRB1*03* alleles to be protective against MS development, along with the *DRB1*13-DQB1*06* and *DRB1*11-DQB1*03* haplotypes [56]. Part of these results was replicated in a study using a Finnish cohort, which found the *DRB1*13~DQB1*06* haplotype to have protective properties against MS [57]. Two independent investigations from Brazil and Canada found protective effects of the *DRB1*11* allele, signifying an important multicultural correlation for this specific loci [58, 59]. The results of multiple studies investigating protective HLA factors against MS development have demonstrated that increased, region-specific research is needed in order to fully identify different factors.

In total, 32 loci within the MHC region have been confirmed to have association with increased or decreased risk to have MS [7]. While these loci represent a substantial proportion of MS heritability, the rest of the currently identified factors reside within the autosomal non-MHC genome.

Genome-Wide Association Studies: Non-HLA Risk Factors

Although early studies of non-HLA genetic risk factors identified a few alleles of interest, the results were not initially reliably replicated, largely due to modest sample sizes. In 2007, a study by Fisher et al. demonstrated that MS is likely characterized by a diverse spectrum of risk allele frequencies underlying susceptibility—a theory termed the polygenic model of MS [60]. The risk in evidential support for the polygenic model of MS genetic heritability gave the theoretical support necessary to begin intensive genome-wide studies that utilized large datasets to effectively screen thousands of single nucleotide polymorphisms (SNPs) [61]. These GWAS were made possible through technological advancements and the development of novel methodological approaches such as the SNP array and have provided the foundation for later advances in our understanding of the genetic heritability of MS. GWAS are conducted by comparing the allele frequency at each position in the genome between case and control subjects, assuming an additive model of genetic effects, and a significant difference between the two highlights a genetic association to MS risk [62]. GWAS research investigations are agnostic, or hypothesis-free, and target the entire genome by tagging linkage disequilibrium (LD) blocks; this means that they require large sample sizes of participants, but the sample is not restricted to family-based relationships. Case-control samples can be used. Over time, statistical thresholds have been adopted in order to account for multiple comparisons and to ensure reproducibility in independent studies [63]. The current standard for genome-wide significance level is $P < 5*10^{-8}$, which is equivalent to $P < 0.05$ after the Bonferroni correction for the number of independent tests in the entire genome, given LD between known variants [63].

The first GWAS targeted non-HLA elements of MS and was completed by the International Multiple Sclerosis Genetics Consortium (IMSGC) in 2007 [64]. Using 1540 parent-affected offspring trios, this study was able to identify two genetic loci outside of the HLA genes that had previously not been implicated in MS: the interleukin-7 receptor (IL-7R) and the interleukin-2 receptor (Il-2R) [64]. Both of these genetic loci are linked to cytokine receptors, and replication studies have provided evidence for a causal link between these two receptors and MS pathogenesis [65–68]. The gene product of IL-7R, the interleukin-y receptor alpha chain, forms a complex with a gamma chain cytokine receptor that is critical for the proliferation and survival of T and B lymphocytes in the immune system [66, 69]. Genetic aberrations in this complex have been demonstrated to lead to immune deficiency syndromes [70]. An mRNA assay study using peripheral blood mononuclear cells (PBMCs) in MS patients found that there was an increased expression of the IL7 signaling path-

way in the CSF of patients compared to controls [66]. This suggested that the disruption of this signaling pathway in the CNS could partially account for the onset of MS. Polymorphism in the IL-7R gene was classified as a significant risk factor for MS, as identified by four independent case-control and family-based datasets [65]. Additionally, the IL-2R gene, which was also implicated in MS through the preliminary GWAS, encodes a subunit of a receptor for the pro-inflammatory cytokine interleukin 2 (IL2); this cytokine has been associated with multiple autoimmune diseases in addition to MS, including rheumatoid arthritis and type 1 diabetes [64, 71, 72]. IL-2R helps to maintain suppressive functions of a T-cell subtype, which in turn facilitates effector and memory T-cell differentiation and plays an important role in immune system function [73]. Furthermore, the IL-2R gene is regulated by vitamin D in CD4+ T-cells, implicating a known environmental factor in MS development in genetics [71].

After the first GWAS was completed and the roles of IL-7R and IL-2R in MS were identified, the results were quickly replicated by independent studies; the buildup of investigation surrounding GWAS and heritability in MS led to a marked increase in genetic studies and sample sizes [65, 66]. In order to create a larger subject pool and achieve greater statistical power, the IMSGC expanded to include approximately 23 research groups from 15 countries. By 2010, a large series of GWAS and meta-analyses made possible by this expansion increased the number of confirmed genetic loci associated with MS to 26 [74]. While the study was able to maximize its subject pool by using case-controls instead of family-based, this method created confounding such as population stratification that limits interpretation of results [74]. To control for this, a novel approach called the variance component method was used to adjust for this genomic inflation bias [74]. Another GWAS was conducted by the IMSGC a few years later, which identified 52 loci that were definitely associated with the risk of developing MS, including 29 completely novel loci [75]. Of these identified genes, 23 were previously known to be involved in other autoimmune diseases; this suggested that a common mechanism may underlie several autoimmune disorders [75]. A GWAS in 2013 brought the number of established MS-associated genetic loci up to 110, with 103 of these loci existing outside of the HLA [76]. The most recent GWAS, conducted in 2017 by the IMSGC, included a sample size of more than 115,000 case and control subjects [77]. This study found that the total number of MS risk-associated genes is approximately 233, with 200 of these loci non-HLA related and 32 HLA-related [77]. The speed with which the multiple consecutive GWAS were able to identify increasingly large numbers of genetic loci outside of the HLA that are involved in this multifaceted disease is a testament to this study design in our understanding of MS susceptibility.

Pediatric-onset MS may represent an extreme example of genetic susceptibility given the young age of disease development. To date, many of the adult susceptibility alleles have been confirmed in this population, although some with potentially higher effect sizes than seen in adults [78]. MHC class III variants were also associated with MS risk in children [78]. A caveat of these studies is that sample sizes are limited in this more rare MS population.

A strength of the GWAS was their incredibly large datasets that generated important connections between genetic loci and disease prevalence, but they are not without limitations. LD is the idea that the regions where SNPs exist as indicated by GWAS are not overly specific; these regions can be expansive sections of the genome that include several genes that could potentially be implicated in MS [79]. Additionally, incredibly large sample sizes were required to detect even marginal genetic associations with minor effects, under any inheritance model; this means that the era of GWAS has mostly come to an end, due to the increasing statistical need for datasets of larger sizes. Follow-up experiments have helped to refine our understanding of the conclusions reached by GWAS in the past decade and look to new methodologies.

The conclusion from sustained GWAS investigations into MS has been that the loci associated

with MS are generally related to T-cell activation and proliferation in the immune system [80, 81] and that MS susceptibility genes are enriched in all immune cell types [7]. Transcriptomic and epigenetic enrichment analyses demonstrate that T-cell biology is a major feature of MS but stops short of naming it the key characteristic of the disease; alternatively, it is noted that there are many active components in MS outside of T-cell activity, including adaptive and innate immunity in pathogenesis [10, 80–82]. However, this picture of the genetics of MS is not complete, and statistical analyses have demonstrated that there are potential remaining genetic factors in addition to the environmental factors that remain to be elucidated. Susceptibility studies have demonstrated that while MS has important genetic components, and these implicate the immune system as the origin of the disease, it is not an entirely heritable disease.

Non-Caucasian Populations

The majority of research conducted on the genetics of MS has included participants with European ancestry; ethnic minority populations have been underrepresented in heritability research [83]. While historically MS has been reported to have the highest prevalence in Caucasians with Northern European ancestry, it has also been consistently reported in most ethnic groups [84]. In order to untangle the complex genetic interactions that lead to MS, it is imperative to investigate the genetic differences between populations that lead to the same disease.

When compared to Caucasian populations, people with African ancestry have a statistically smaller risk of MS development; however, if they do develop MS, they may be at risk for faster disease progression and increased disability [85]. These observations implicate genetic factors as potential modifiers of the differences in MS risk and phenotype in different populations, but environmental factors may also notably contribute.

The MHC class II *HLA-DRB1*15* and *HLA-DRB1*15:03* alleles are susceptibility factors in populations of African descent. As mentioned in a previous section, African-Americans have greater HLA haplotype diversity, in addition to patterns of LD that changes their genetic predisposition to MS as compared to Caucasian populations [51]. The *HLA-DRB1*15:03* allele has been significantly associated with MS susceptibility in cohorts with African descent [50]. The class II *HLA-DRB*15* alleles have been observed to indicate severity of disease progression in this cohort [50, 86]. A study investigating differences in MS clinical outcomes in 673 African-American and 717 European American participants demonstrated that African ancestry of HLA correlated with an earlier age of onset and increased disability, as measured by the Multiple Sclerosis Severity Score (MSSS) and cane dependency [87].

In order to investigate the MS susceptibility genetic profile in African-Americans, a replication study investigated allele frequencies of 19 SNPs in 12 MHC genetic loci in 918 patients and 656 controls [88]. The results showed both HLA and non-HLA factors were associated with MS risk; it also demonstrated consistent findings of SNP associations compared to the literature in Caucasian populations, showing common immunological mechanisms for MS development [88]. A follow-up study was conducted a few years later, which used an expanded population size of 1162 cases and 2092 controls to assess the association of MS risk variants in a population with African ancestry [89]. This study, using a significance threshold of $p < 0.01$, found none of the *HLA-DQB1* alleles were significantly associated with MS; conversely, researchers identified eight SNPS associated with MS outside of the MHC [89]. While research has suggested there are not large differences in HLA risk factors among African and Caucasian populations, more modest sample sizes in these studies limit the identification of factors that may be rare in the population or have small effect sizes.

In addition to those with European or African ancestry, there are distinct patterns of MS disease diagnosis and course in those with Asian genetic ancestry. A Japanese study found an association of *DRB1*15* alleless with a more typical course of MS; if a patient had no presence of this allele,

they were more likely to have the clinically distinct opticospinal MS [90]. Opticospinal MS in Asian patients was associated with the risk variant *DRB1*04:05*; this allele was linked to a disease course characterized by an earlier age of onset, reduced severity, and a statistically smaller number of brain lesions compared to similar studies in Caucasian populations [90, 91]. However, these studies predate research surrounding neuromyelitis optica (NMO) and myelin oligodendrocyte (MOG) antibodies. A meta-analysis investigated the association between HLA genes and MS in Chinese populations and found that the *HLA-DR2.DRB1*15* haplotypes were associated with risk of MS in Chinese patients but to a lesser extent than in Western MS population [92]. Additionally, this study identifies *HLA-DR9* alleles as conferring resistance to MS in this population [92]. Wang et al. conducted a whole-exome sequencing study on MS patients from Southern China, in an attempt to identify genetic variants in a Chinese population compared to Caucasian populations [92]. They found 17 variants that were previously unreported in the literature as being related to MS that were shown to have significantly different frequencies between the MS patients and healthy controls, specifically a rare variant located on exon 7 of TRIOBP [92]. While this research has helped to fill in our understanding of the genetics of MS in patients with Asian ancestry, the small sample size (n = 8) means that the study will have to be further supported by continuing investigation.

Multiple Sclerosis Genetic Overlap with Other Autoimmune Diseases

Multiple sclerosis has been characterized as an autoimmune disorder, a definition that has been supported by the implication of HLA genetic loci in its etiopathogenesis. Autoimmune diseases, or AIDs, are known to have some amount of similar implicated biological mechanisms that lead to an overactive immune response (Fig. 11.1). In MS, the largest single risk factor is the presence of the *HLA DRB1*15:01* allele. As described previously, this allele follows what is called the additive model; the more copies of the allele present in the genome, the more genetically susceptible an individual is to developing MS [53, 93]. This phenomenon is also seen in other autoimmune diseases, such as celiac disease, narcolepsy, and type 1 diabetes; in all of these cases, homozygosity for a gene increases risk in comparison to heterozygous genetic groups [41, 42, 94]. The similarity between dosage effects in HLA genes of known autoimmune diseases and MS strongly suggests that MS arises primarily from an abnormal peripheral immune system and provides the theoretical foundation for further parallels between MS and other AIDs.

GWAS have provided researchers with the ability to use large sample sizes in order to unravel some of the genetic components of complex diseases, like MS [46]. The results from these studies demonstrate that MS-related genes overlap notably with genetic loci that have previously been associated with many other AIDs [95]. GWAS demonstrate that just over one-third of the known MS risk loci overlapped with regions that have previously been associated with other AIDs, including celiac disease, type 1 diabetes, and rheumatoid arthritis [72].

Autoimmune diseases, such as MS, are incredibly complex; the polygenic mechanisms underlying each individual pathology remain challenging to fully elucidate as the field is only beginning to reach sample sizes that allow for studies of gene-gene interactions. In addition to genes underlying risk factors present in multiple AIDs, there are also genes that act as protective factors for some AIDs but risk factors for others. Sirota et al. compared genetic profiles of six autoimmune diseases, including MS, and five non-autoimmune diseases, in order to identify SNPs that are both protective and susceptibility factors [96]. The study identified two broad classes of autoimmune diseases, where specific MHC SNPs that make an individual susceptible to one protect against the other. Rheumatoid arthritis and ankylosing spondylitis belong to one class, while MS and autoimmune thyroid diseases belong to the other, and they are differentiated by certain MHC polymorphisms [96]. The

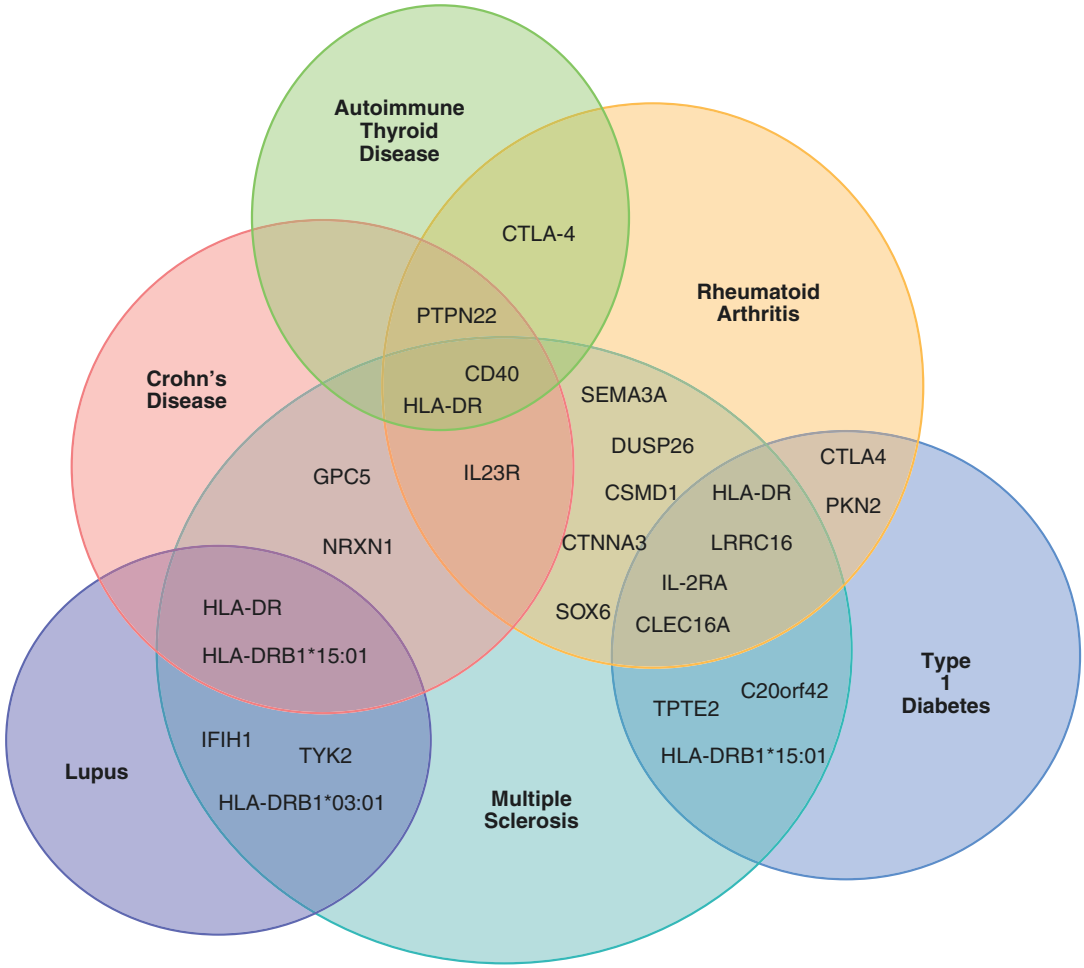

Fig. 11.1 Examples of multiple sclerosis genetic overlap with other autoimmune diseases

study hypothesized that the SNP alleles associated with different autoimmune phenotypes may interact differently with both environmental and other genetic factors, thereby changing the biological context of the SNP in different people [96]. Further investigation is necessary to understand the relationships between these AIDs more clearly.

Susceptibility Factors and MS Disease Course

In a multifaceted disease such as MS, there are a multitude of both environmental and genetic risk factors (as well as interactions among these) that may impact not only disease onset but also phenotype. Preserving function and day-to-day quality of life is an especially important driver of medical standard of care. In order to slow the progression of MS as best as possible, it is important to continue investigating the impact of different susceptibility factors on disease course.

The HLA is one of the most important sites of genetic heritability for MS diagnosis and may have importance also for phenotype. Carrying *HLA-DRB1*15:01* has been associated with earlier age of MS [97, 98]. The HLA alleles *HLA-DRB1*15:01, HLA-DQB1*03:01, HLA-DQB1*03:02, HLA-DQB1*06:02*, and *HLA-DQB1*06:03* have been associated with more severe MS progression, as indicated by

patient magnetic resonance imaging (MRI) scans and evidence of increased inflammation and neurodegeneration [99, 100]. The presence of *HLA-DRB1*15:01* may also be associated with greater lesion burden in the spinal cord [101]. In addition, the *HLA-DRB1*04:05* allele has been shown to influence the severity of MS progression, independent of environmental risk factors such as latitude, in a Japanese cohort study [102]. Patients with this specific allele were shown to have lower MSSS scores, lower frequency of brain lesions meeting the Barkhof criteria, and decreased levels of CSF abnormalities compared to *HLA-DRB1*0405-negative* patients [102]. A similar study used 282 non-related Slovak patients to investigate the impact of the *HLA-DRB1* and *HLA-DQB1* alleles on MS progression [56]. The study demonstrated that carriers of a homozygous genotype for *DRB1*15* and *DQB1*03* reached a higher rate of disability significantly faster than noncarriers; additionally, *DRB1*15:15* was found to be less frequent in patients with slowly progressing MS, and *DQB1*03:03* was found to be associated with a slower disease progression [56]. Better MRI outcomes (T2 burden and atrophy) have also been observed in those carrying *HLA B*44* [39]. Genetic loci within the HLA may also be partially responsible for an individual's response to several disease-modifying therapies (DMTs). Fifteen specific HLA alleles were found to have modifying effects on DMTs, all of which led to a reduction in the MS severity score (MSSS), a type of disability status calculation for the severity of MS [103].

In addition to HLA-related genetic susceptibility factors impacting MS disease progression, non-HLA factors have been associated with disease course [104, 105]. In a set of approximately 800 participants, modest associations were found between some allelic variants outside the HLA and MS phenotype (age at onset, MSSS, brain parenchymal volume, and T2 lesion load), but many of these did not reach genome-wide significance [104]. A genetic risk or burden score of non-HLA susceptibility alleles has been associated with age at onset and the presence of oligoclonal bands [106]. A similar unweighted genetic risk score was not associated with relapses in pediatric-onset MS [107]. The non-HLA MS susceptibility variant within the *AH1* gene is associated with MS relapse hazard in both children and adults [108]. In a genome-wide analysis, variation at a loci associated with the gene *LRP2* (rs12988804) was also associated with relapses in children and adults [105].

Worse clinical outcomes and more severe disease progression were also found to be predicted by the rs12959006 variant in the myelin basic protein (MBP), a major component of the myelin sheath in the CNS, even though this genetic variant is not associated with risk of disease onset [109]. Unexpectedly, a SNP detection study identified genetic variants located in CPXM2, IGSSF9B, and NLRP9 that have the potential to modulate MS disease course; the relationship found was so strong that these genes may be used as disease activity biomarkers to identify MS patients with divergent disease courses [110].

The above research supports the concept that genetic risk factors have some impact on the severity of MS. Further understanding the intricacies of these susceptibility factors could help improve treatment approaches for MS patients in the future.

Mendelian Randomization: Support for Environmental Risk Factors

Mendelian randomization studies offer the ability to study environmental risk factors with minimal concern for reverse causation. This kind of study assesses the impact of genetically determined variation in a biological pathway marker on a disease, which helps scientists to support or reject the pathway's hypothesized causal role in disease etiology [5]. Mendelian randomization studies are ecologically valid and natural studies that attempt to emulate randomized control trials, the gold standard for assessing causality in a relationship. The genetic alleles driving an environmental risk factor (cards dealt at birth before disease onset) can be used as a proxy for the risk factor in the analysis. Being upstream of the final risk factor level and non-mutable by the disease

itself or other confounding environmental factors, these alleles greatly reduce concerns of reverse causation and other confounding.

Vitamin D

In research cohorts across the world, people with MS exhibit lower vitamin D levels than healthy controls [111, 112]. In those with MS, individuals with more disability or higher relapse rates have lower vitamin D levels than those with less severe disease [113]. These observations may in part explain the historical observation that MS prevalence is greater in higher latitude areas with less sunlight [114]. Vitamin D has been demonstrated to have direct effects on the immune system, making it a plausible risk factor, but concerning among many of these studies is the possibility that the observation is explained by reverse causation—that those with MS-related disability venture outside less, causing low vitamin D levels.

In order to understand vitamin D mechanisms more thoroughly and how they relate to MS, Mendelian randomization studies have been undertaken. The study by Mokry et al. used almost 34,000 subjects to demonstrate that alleles of vitamin D metabolism genes caused genetically determined variation in vitamin D status; this means that vitamin D level is not just linked to sun exposure but also to baseline genetics [103]. The study found that individuals with genetically determined vitamin D deficiency had more than a 10x risk of developing MS compared to those with the highest quartile of genetically determined vitamin D levels [103]. A GRS of three variants associated with vitamin D level is associated with increased risk of pediatric-onset MS [115]. These studies demonstrate a causal link between the genetically determined vitamin D levels and the pathology of MS.

Mendelian randomization can also be used to assess associations with phenotype. In a cohort of approximately 200 pediatric participants with MS, a vitamin D genetic risk score was associated with relapse rate, demonstrating that those with highest risk for low vitamin D levels had 2.6-fold higher relapse rates [116]. These results were replicated in an independent cohort of children with MS [116].

Obesity

In addition to vitamin D, the process of Mendelian randomization has also been used in order to determine the causality of the relationship of obesity with MS risk and progression. The justification for using this type of investigation is similar to the reasoning behind vitamin D studies: in order to establish causality and understand the nature of the relationship between obesity and MS in a naturalistic setting with unmeasured confounders.

A growing body of evidence has demonstrated that obesity has some level of impact on MS onset and progression. A body mass index (BMI) of more than 30 kg/m^2 during late adolescence and early adulthood is associated with a twofold increase in MS risk in women compared to those with a healthy weight [117]. Obesity in childhood has also been correlated with the diagnosis of MS in pediatric- and later-onset patients [118, 119].

In order to better understand the relationship of obesity and MS, Mendelian randomization studies were conducted. A 2016 investigation used a two-sample MR approach with summary statistics from both the Genetic Investigation of Anthropometric Traits (GIANT) consortium and the International MS Genetics Consortium (IMSGC); the study weighted the effect of SNPs on MS by their effect on BMI. The large collaborative effort found that genetically elevated BMI was causally associated with risk of MS development [120]. A separate-sample MR study using an international dataset found a causal effect of increased BMI on susceptibility of MS using 19 established variants that predict BMI [43]. This study also identified the fat mass and obesity-associated gene, FTO, to be implicated in MS; specifically, FTO alone increased the risk of MS. FTO has been previously associated with cancers, Alzheimer's disease, dementia, and cognitive decline in healthy adults [44, 121–123].

These results suggest that not only does BMI have an indirect impact on MS through inflammatory pathways, but there are characteristics of a genetically elevated BMI that directly increase disease risk. BMI-related genes are also associated with increased risk of pediatric-onset MS [115].

Obesity has also been shown to play a role in vitamin D levels in the body, thereby demonstrating an indirect impact that an increased BMI may have on MS [124]. A MR study investigated a proposed theory that lower vitamin D bioavailability in obese patients constitutes the biological mechanism through which obesity impacts MS [124, 125]. The study found that for every unit increase in BMI, there was a reduced 25OHD (vitamin D) concentration in the body by 1.15% [124]. A separate study importantly found that both vitamin D and obesity genetic variants have independent impacts on disease risk [115]. The results of these Mendelian randomization studies demonstrate important variables in an individual's risk for developing MS and have shown that BMI has both indirect and direct impacts on MS diagnosis and progression.

Conclusion

The aforementioned research has clearly demonstrated that MS is a partially heritable disease. Genetic factors both within and outside the HLA region are associated with MS risk, the greatest influence of which is from carrying the *HLA-DRB1*15:01* allele. GWAS studies over the last decade have captured the strong genetic similarities of MS with other autoimmune diseases, suggesting MS is an "outside in" disease. More work is needed to understand the relationship of genetic factors with disease course, requiring more consistency across research cohorts in how investigators phenotype the disease. Mendelian randomization studies have grown in prevalence as a methodology with which to analyze the impact of environmental factors leveraging genetic drivers of those factors to minimize bias and reverse causation forms of confounding. With the larger sample sizes now available, it will also become possible to better study epistatic effects among susceptibility factors as well as begin to study critical gene-environment interactions that likely lead to onset of disease.

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