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Exploring Sex-Based Mechanisms of Inflammation and Neurodegeneration in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by both immune-mediated inflammation and progressive neurodegeneration. While both processes occur in all individuals with MS, females more often present with heightened inflammatory activity, whereas males tend toward accelerated neurodegeneration and disability progression. This review synthesizes current evidence on biological, hormonal, genetic, epigenetic, and environmental mechanisms underlying sex-based differences in relapsing-remitting MS (RRMS), the most common MS subtype. We integrate findings from human studies, animal models, and molecular research to examine the contribution of genetics and epigenetic regulation including sex chromosomes, single nucleotide polymorphisms (SNPs), and microRNAs (miRNAs) to MS pathophysiology. We discuss the influence of sex hormones, including estrogen, progesterone, and testosterone, and environmental risk factors such as Epstein-Barr virus infection and vitamin D3 deficiency. Evidence suggests that X-linked immune-related genes, hormone - immune interactions, and sex-specific epigenetic regulation shape differential immune responses and neuronal vulnerability in males and females with MS. miRNAs emerge as critical molecular bridges linking genetic susceptibility, hormonal milieu, and environmental exposures to downstream inflammatory and neurodegenerative pathways. Understanding the mechanisms driving sex differences in MS may enable the development of targeted interventions addressing both neuroinflammation and neurodegeneration. Integrated miRNA - mRNA analyses, explicitly powered to assess sex as a biological variable, hold promise for identifying novel biomarkers and therapeutic targets. Such approaches could inform more personalized treatment strategies and improve long-term outcomes for all people with MS.

Keywords

multiple sclerosis, sex differences, neuroinflammation, neurodegeneration, epigenetics, MicroRNA

Introduction

Multiple sclerosis (MS) is an acquired demyelinating disease of the central nervous system (CNS) marked by immune-mediated inflammation and chronic neurodegeneration, processes that together drive disease activity and disability progression (Figure 1) (Harbo et al., 2013; Lublin et al., 2014). Affecting over two million people worldwide, MS is the leading cause of non-traumatic neurological disability in young adults (Bove & Chitnis, 2013; Harbo et al., 2013). Although a specific causative etiology for MS remains unknown, it is thought to result from unique combinations of environmental, genetic, epigenetic, biological, and sex specific factors. However, we have an incomplete understanding of how each of these specific risk factors contribute to the varied clinical course in everyone with MS, and this gap in our knowledge remains a barrier to precision medicine in MS.

Relapsing-Remitting MS (RRMS), the most common subtype of MS, accounts for ~85% of new diagnoses (Lublin et al., 2014) and is the subtype on which this review is focused. Notably, RRMS disproportionately affects females, with a female-to-male ratio of approximately 3:1, a disparity that appears to be increasing globally (Bove & Chitnis, 2013; Harbo et al., 2013; Lublin et al., 2014). Indeed, sex is one of

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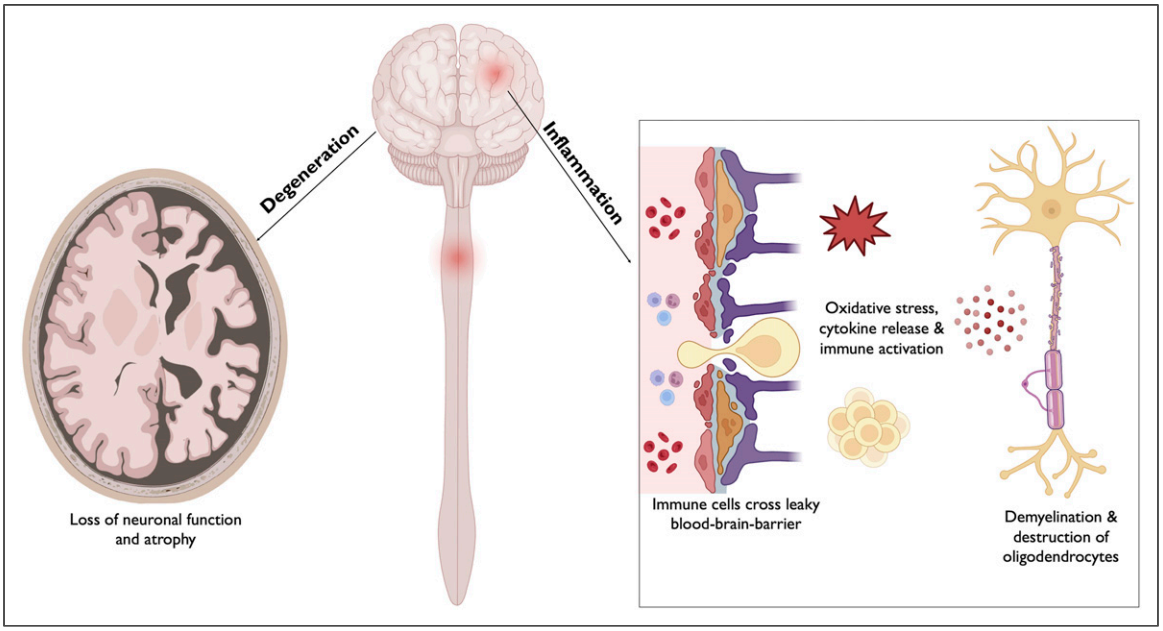

Figure 1. MS is an Autoimmune Disease Characterized by Both Neurodegeneration and Neuroinflammation (Image Created in BioRender.com)

the most well-established risk factors for RRMS (Bove & Chitnis, 2013; Harbo et al., 2013; Lublin et al., 2014). While both sexes experience inflammation and neurodegeneration, clinical patterns often differ: females tend to exhibit heightened inflammatory disease activity during their reproductive years. Males, though less likely to develop MS, often experience more severe neurodegeneration and greater disability (Figure 2) (Bove & Chitnis, 2013; Lublin et al., 2014). Understanding the molecular underpinnings behind these sex-based differences is critical to advancing treatment.

This narrative review offers a focused synthesis of select biological mechanisms that may underlie the observed sex-differences in inflammation and neurodegeneration between males and females with RRMS. We begin by examining sex chromosomes and hormones, which are well-established modulators of immune and neurological processes. We then explore environmental exposures, such as Epstein-Barr Virus and vitamin D, and introduce the concept of epigenetics as a potential bridge between genetic, environmental, and other contributing risk factors. The overarching goal is to highlight mechanistic pathways with the greatest biological plausibility supported by current evidence, with an emphasis on those most likely to inform future epigenomic research and ultimately, more personalized treatment strategies in MS.

Methods

Search Method

This narrative review was informed by targeted searches of PubMed/MEDLINE, Web of Science, CINAHL, Google Scholar, and the Cochrane Library. Searches emphasized 2010–2025 to capture mechanistic pathways that may contribute to sex-based differences in MS, while retaining earlier seminal studies when relevant. Core search strings combined terms for multiple sclerosis with sex/sex-based concepts and key mechanistic domains, for example:

- ("multiple sclerosis" OR MS) AND (sex OR "sex-based" OR gender OR male OR female) AND:
  - (neuroinflammation OR neurodegeneration OR atrophy)
  - (hormone* OR estrogen OR estradiol OR progesterone OR testosterone)
  - (epigenetic* OR microRNA OR miRNA)
  - (genetic* OR "X chromosome" OR SNP OR eQTL)
  - (environment* OR "Epstein–Barr" OR EBV OR "vitamin D" OR microbiome OR obesity OR tobacco)

Relevant articles were also identified via backward citation searching (scanning reference lists of included papers) and forward citation tracking (locating newer articles that cited key sources).

Criteria

Articles were included if they: (1) were published in English; (2) addressed multiple sclerosis with relevance to sex differences (biological/physiological mechanisms or outcomes), with a focus on RRMS but not limited to it; and (3) contributed mechanistic insight into inflammation and/or

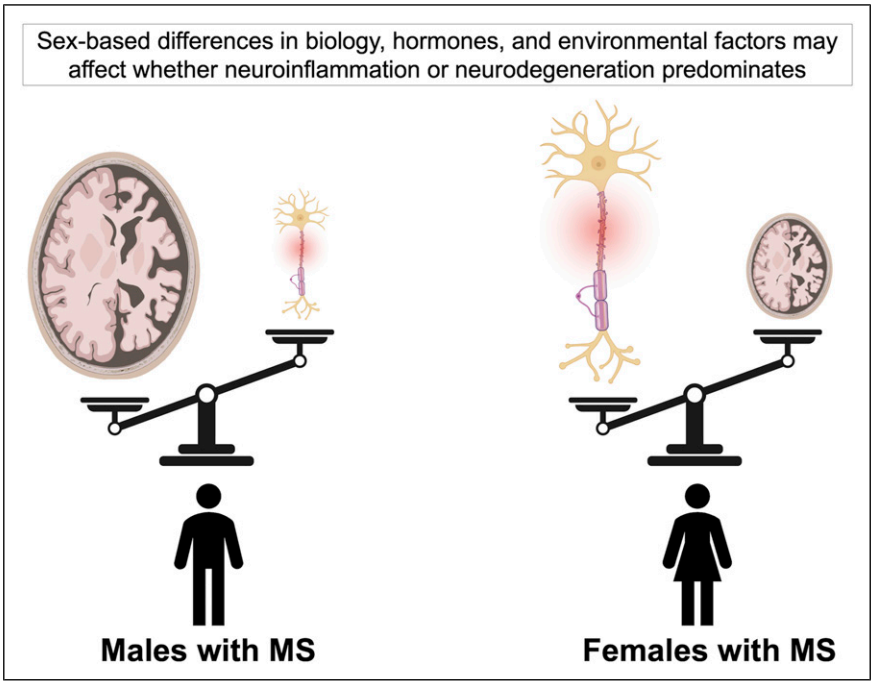

Figure 2. Conceptual Framework (Image Created in BioRender.com)

neurodegeneration (e.g., genetics/eQTL/SNPs, epigenetics/miRNA, sex hormones, immune pathways, EBV, vitamin D, microbiome, obesity, tobacco). Eligible study types included human clinical/translational studies, animal or cell preclinical studies, reviews/meta-analyses synthesizing biological mechanisms, and consensus/guideline papers when mechanistically informative.

Studies were excluded if they: (1) did not pertain to MS or to sex/sex-based mechanisms; (2) focused exclusively on psychosocial/health-services topics without biological or mechanistic relevance; (3) were non–peer reviewed (e.g., editorials, opinion pieces, preprints) or conference abstracts without full data; or (4) were single-patient case reports unless uniquely illustrative of a mechanistic point.

Information Extraction

From each included source we extracted: MS population/phenotype (and sex distribution when available); study design and model (human/animal/cell; inclusion of sex chromosome complement or hormone manipulation); mechanistic domain(s) (i.e., immune pathways, hormonal signaling, epigenetic/miRNA regulation, genetic variants/eQTLs, environmental modifiers such as EBV or vitamin D); primary findings related to sex differences in neuroinflammation and/or neurodegeneration; key effect modifiers (e.g., pregnancy, menopause/andropause); and major limitations. For review articles, we noted scope and consensus/conclusions. Two reviewers independently screened titles/abstracts and discussed full-text eligibility and extracted elements to consensus; discrepancies were resolved through discussion. Because the intent was to synthesize mechanistic themes rather than to catalog all studies exhaustively, PRISMA procedures were not applied.

Sex Differences in MS Disease Course

MS is characterized by both inflammation and neurodegeneration (Figure 1), though not always in equal measure (Figure 2). An MS relapse is an acute inflammatory CNS attack, where new or worsening symptoms arise due to infiltration of immune cells targeting myelin, the protective covering of nerve fibers. While acute CNS inflammation, or relapses, cause sudden symptom worsening, neurodegeneration of MS is a more gradual process involving irreversible nerve cell and axon loss that contributes to long-term disability, even in the absence of active CNS infiltrative inflammation.

Evidence suggests that females tend to have a more inflammatory disease course than males. A retrospective population study of 3,028 male and 6,619 female Danish MS patients found females had a 16% higher relapse rate characterized by acute inflammatory CNS attacks (Magyari & Koch-Henriksen, 2022). While such findings highlight group-level trends, they do not imply that males are protected from inflammation or that females are spared from neurodegeneration—both processes occur in both sexes (Figure 2).

While males are less likely to develop MS, they often experience a disease course characterized by accelerated

neurodegeneration and greater physical disability (Bove & Chitnis, 2013; Lublin et al., 2014). Compared to females, males with MS tend to accumulate disability at a faster rate, experience more pronounced cognitive decline, and exhibit more severe brain atrophy (Magyari & Koch-Henriksen, 2022). Additionally, males face an increased risk of mortality associated with MS, underscoring the potential consequences of unchecked neurodegenerative processes (Magyari & Koch-Henriksen, 2022).

All current FDA-approved disease-modifying therapies (DMT) for RRMS effectively reduce the frequency of CNS inflammatory events (i.e., clinical relapses) but are less effective at slowing neurodegeneration. This limitation is clinically important for all people with MS, but is particularly concerning for males, who on average exhibit more rapid neurodegenerative change. Importantly, females comprise approximately 70% of the study samples used in pivotal DMT trials for MS, making males an underrepresented population in MS research (ORWH, 2022). While none of these trials have reported significant sex differences in treatment response, they were not specifically designed or powered to detect such differences, leaving critical questions about the efficacy of DMTs in males versus females unanswered.

Our conceptual framework (Figure 2) illustrates that while both sexes with MS experience inflammation and neurodegeneration, females show a relative predominance of inflammation and males of neurodegeneration. We hypothesize that investigating these patterns may reveal mechanisms driving MS phenotypes. The framework acknowledges overlap between groups but emphasizes relative differences that may hold mechanistic significance. Clinical observations of these sex-specific disease courses underscore the need to explore the biological and molecular drivers—such as genetic susceptibility, epigenetic regulation, immune dynamics, sex hormones, and environmental exposures. Clarifying these mechanisms is critical for developing personalized strategies that target inflammation, degeneration, or both, rather than relying on a one-size-fits-all approach.

Sex Chromosomes and Genetic Variants

Sex Chromosomes

Sexual dimorphism in MS partly stems from differences in sex chromosomes. Females inherit two X chromosomes, while males inherit one X and one Y chromosome. Whereas the Y chromosome contains around 100 genes, mostly related to male reproduction, the much larger X chromosome carries approximately 2,000 genes, many involved in immune regulation. Thus, the X chromosome encodes a number of genes potentially influencing the immune-mediated CNS inflammation implicated in MS. Sex-based differences in X-chromosome gene expression may arise from several mechanisms including male X chromosome hemizygosity, epigenetic modifications of the maternal or paternal X chromosome and differential X chromosome gene dosage in females, potentially influencing both inflammation and neurodegeneration (Du et al., 2014; Golden et al., 2019).

The X chromosome genes involved in immune function include *FOXP3*, *TLR7*, *TLR8* and *CD40.* The *FOXP3* gene encoding forkhead box P3 (FOXP3) is of relevance to MS given that it functions as the lineage specification factor for regulatory T cells (Tregs), which are immune suppressor cells critical for the maintenance of peripheral immune tolerance (Sakaguchi et al., 2008). Impaired Treg function has been associated with MS (Viglietta et al., 2004) and linked to reduced FOXP3 expression in individuals with MS (Huan et al., 2005). Tregs were more likely to be impaired in individuals with RRMS than progressive MS (Venken et al., 2006). Therefore, altered FOXP3 expression and Treg function are implicated in the loss of CNS immune tolerance in RRMS. Animal studies have shown a mechanism whereby sex-differences in Foxp3 expression may occur (Voskuhl et al., 2018). Using a transgenic mouse system to dissociate sex chromosome effects from that of sex hormones, Voskuhl and colleagues showed that the maternally inherited X chromosome had relative hypomethylation at the Treg-specific demethylated region of the *Foxp3* gene, compared to the paternally inherited X chromosome. The relative hypomethylation would predict higher *Foxp3* expression from the maternally inherited X chromosome. Because males inherit the maternal X chromosome and because of male X chromosome hemizygosity, males could have higher Foxp3 expression, more Tregs and resistance to autoimmunity because of parent-of-origin difference in DNA methylation at the X chromosome. To our knowledge, it remains unknown whether such sex differences exist in persons with MS, but the study illustrates the complex mechanism whereby the epigenetic change (i.e., hypomethylation at the Treg-specific demethylated region) combined with the biology of sex chromosomes (i.e., male X chromosome hemizygosity) can lead to sex differences in gene expression.

Another mechanism contributing to sex differences in X-linked gene expression is incomplete X-chromosome inactivation. In females, inactivation normally silences one X chromosome through DNA methylation to prevent gene overexpression, yet ~15% of genes escape this process, leading to higher expression of some X-linked genes. (Voskuhl, 2020). This escape can lead to higher expression of X-chromosome encoded genes in females. Interestingly, males with Klinefelter's syndrome (XXY) exhibit increased susceptibility to MS and other autoimmune diseases, underscoring the role of X chromosome dosage in immune-mediated disease risk (Seminog et al., 2015). Whether incomplete X-inactivation contributes to MS risk or disease activity remains unknown.

Mouse models of MS have shown that animals with an XY chromosome complement experience greater neurodegeneration and disability than those with an XX complement, even when sex hormone effects are controlled (Du et al.,

2014). These findings suggest that sex chromosome complement may contribute to the more severe disease course often observed in males, independent of hormonal influences. In humans, the extent to which XY chromosome–linked mechanisms drive neurodegeneration in MS remains unclear, but these results highlight an important knowledge gap. Identifying the epigenetic and molecular pathways by which sex chromosome complement influences neuronal vulnerability could provide insight into sex-based disparities in disease course. Future research should consider the interplay of sex chromosome biology—including gene variants, incomplete X-chromosome inactivation, male X chromosome hemizygosity, and parent-of-origin epigenetic modifications—when investigating the mechanisms underlying sex differences in MS.

Genetic Variants: Single Nucleotide Polymorphisms (SNPs) and MS Pathogenesis

Genome wide association studies (GWAS) identified over 200 SNPs associated with MS, many of which are linked to genes involved in immune cell function and inflammatory pathways (Han et al., 2020; Huang et al., 2017; Patsopoulos et al., 2019). SNPs are variations in a single DNA base pair that can influence gene expression and play a critical role in disease susceptibility and progression (Patsopoulos et al., 2019). There are approximately 10 million SNPs in the human genome that occur in both coding (i.e., exons) and non-coding (i.e., introns) gene regions (Wu & Jiang, 2013). While at least a third of SNPs in exons alter protein function, the 93% of SNPs that occur in non-coding regions, such as promoters, enhancers, and 3′ UTRs, are increasingly being recognized for their roles in altering gene expression by affecting RNA splicing, transcription factor binding, and miRNA expression (Orozco et al., 2022). Other "disease-associated SNPs" may not directly impact gene expression themselves but can be inherited together with nearby functional variants due to linkage disequilibrium—a phenomenon where genetic variants that are physically close on a chromosome are statistically correlated and tend to be co-inherited (Sved & Hill, 2018). In such cases, the identified SNP acts as a marker that points to the true causal locus driving changes in gene expression and cell function (Sved & Hill, 2018).

A genetic variant in the form of a SNP has been identified in the X chromosome that confers a small but significant risk of developing MS (Patsopoulos et al., 2019). This SNP (rs2807267) lies downstream of the genes *VGLL1* (vestigial family-like member 1), a transcriptional cofactor, and *RNU6-320P (RNA U6 small nuclear 320 pseudogene)*. The mechanism whereby this genetic variant confers risk of MS remains unknown and needs further research. Using UK Biobank data, Borziak and Finkelstein (2022) also identified 20 X-linked SNPs in genes related to myelination and immune function. Remarkably, remyelination-related SNP variants were absent in men, while women carrying both immune- and remyelination-related SNPs exhibited a 20-fold increase in MS risk, suggesting that disruptions in these X-linked pathways may underlie higher MS susceptibility in females. Additionally, some MS risk-associated SNPs may undergo epigenetic modifications by environmental factors, such as the Epstein-Barr Virus (discussed in detail later in this review), in a sex-specific manner, potentially driving differential gene expression between males and females and contributing to disparities in MS progression and severity (Keane et al., 2021).

Many of the SNPs associated with MS act as expression quantitative trait loci (eQTLs), regulating gene expression and potentially interacting with epigenetic factors to influence inflammation and neurodegeneration in MS (Han et al., 2020). For example, the SNP rs1414273 within the intron of the CD58 gene locus is an MS-associated eQTL: carriers of the risk allele exhibit lower CD58 mRNA levels and higher miR-548ac levels, suggesting a genotype-dependent effect on RNA processing that impacts immune regulation (Hecker et al., 2019). These eQTLs demonstrate how genetic susceptibility to MS may be shaped by the epigenome, influencing immune response and neurodegenerative pathways. We hypothesize that certain immune-related eQTLs could be more highly expressed in females due to estrogen-mediated epigenetic modifications, intensifying inflammation. In males, other eQTLs might interact with the Y chromosome or respond differently to environmental triggers, contributing to faster neurodegeneration. By examining these potential interactions, we can better understand how genetic risk factors may manifest differently in each sex, offering insights into more targeted, personalized treatment strategies.

Potential Mechanisms Underlying Sex Differences in MS

As described above, GWAS studies have identified several risk alleles for MS. However, the genetic variants associated with MS each individually confer only a small risk of developing MS (Parnell & Booth, 2017). Hormones and environmental exposures also play critical roles in shaping immune responses and neurodegeneration and may interact with genetic factors to influence MS susceptibility, progression, and clinical outcomes. In this section, we first examine hormones, then environmental factors, and finally other elements that may modulate MS.

Sex Hormones in MS

Sex hormones play a significant role in shaping immune responses, contributing to the disparities observed in autoimmune diseases such as MS.

Estrogen

There are three main types of estrogen, each predominant at different life stages in females: estrone (E1) during menopause, estradiol (E2) during reproductive years, and estriol (E3) during late pregnancy (Avila et al., 2018; Deckx et al., 2013). Estrogens exert their effects through estrogen receptor (ER) α and β, expressed on various cells including immune cells thought to be central to MS pathogenesis, such as T and B lymphocytes (Deckx et al., 2013; Ysrraelit & Correale, 2019).

Estrogens play a well-known role in modulating the adaptive immune system, and estrogen fluctuations are especially critical for immune regulation in females during their reproductive years. There are no notable sex differences in MS susceptibility before puberty or after menopause, underscoring estrogen's influence on MS risk during childbearing years (Alcina et al., 2012; Jiang et al., 2018). This is further demonstrated by the reduction in MS disease activity during pregnancy, when high estrogen levels promote the expansion of Tregs to protect the fetus, followed by a heightened risk of relapse postpartum as estrogen levels drop (Avila et al., 2018; Harbo et al., 2013).

Estradiol (E2), the predominant estrogen during the reproductive years, is of particular interest in RRMS, exhibiting both anti- and pro-inflammatory effects that appear to be dose dependent. At lower concentrations, E2 is associated with increased inflammatory activity, including the induction of T helper (Th) 1 cytokines such as IFN-γ and activation of natural killer (NK) cells, which may drive autoimmune responses (Deckx et al., 2013; Maglione et al., 2019; Ysrraelit & Correale, 2019). In contrast, higher levels of E2, such as those observed during pregnancy, are associated with reduced MS disease activity. This effect may be mediated in part by E2's ability to enhance the expression of *FOXP3*, a key transcription factor for Tregs, through the action of ERα. E2 promotes the differentiation of Tregs, which suppress inflammatory responses and maintain peripheral immune tolerance, including in the CNS (Adurthi et al., 2017; Garnier et al., 2018; Polanczyk et al., 2004). Studies in mice have demonstrated that E2 treatment increases Foxp3 expression in CD25-CD4 + T cells in an ERα-dependent fashion (Polanczyk et al., 2004), likely through its transcription factor activity of ERα (Adurthi et al., 2017). In addition, estrogen may act to control immune responses by modulating T helper 17 responses (Garnier et al., 2018). Clinical studies have shown that oral administration of estriol (E3) can reduce inflammation in MS patients, highlighting the potential of estrogen-based therapies. However, translating these findings into practice requires further research to determine the optimal route, timing, and dosage, and to clarify which patients are most likely to benefit (Avila et al., 2018; Deckx et al., 2013; Ysrraelit & Correale, 2019).

Beyond immunomodulation, estrogens also promote remyelination and reduce axonal loss, potentially slowing the progression of neurodegeneration in MS (Rojas et al., 2013). MRI studies have demonstrated that cortical atrophy is more extensive in males compared to females with MS, particularly when females are limited to those with pre-menopausal disease onset (Rojas et al., 2013, 2016). This finding underscores the potential protective effects of ovarian hormones against neurodegeneration.

Notably, estrogen appears to have contrasting effects on lesion pathogenesis in males versus females with MS. In males, estradiol levels are positively correlated with lesion burden on MRI (Harbo et al., 2013; Tomassini et al., 2005). Luchetti et al. (2014), investigated steroid synthesis and signaling in MS lesions and normal-appearing white matter and identified sex-specific patterns in hormone signaling pathways. In males with MS, there was local upregulation of aromatase, the enzyme responsible for estrogen biosynthesis, and ERβ, along with increased tumor necrosis factor (TNF) mRNA expression in lesions. These findings suggest that inflammatory signals stimulate estrogen synthesis and signaling in males, as TNF was found to upregulate ERα expression in primary cultures of human microglia and astrocytes. In contrast, females showed upregulation of 3β-hydroxysteroid-dehydrogenase, a key progesterone biosynthetic enzyme, and progesterone receptor expression within lesions, pointing to a shift toward progesterone signaling. Together, these findings highlight the complexity of hormonal regulation in MS lesions and suggest that localized hormone production and receptor signaling may shape lesion biology in a sex-specific manner.

Testosterone

In contrast to females, males typically produce lower levels of IFN-γ due to the immune-modulating effects of androgens, such as testosterone (Voskuhl, 2011). Males also generally produce higher levels of interleukin-10 (IL-10), an anti-inflammatory cytokine that regulates immune responses (Voskuhl, 2011). Testosterone has been shown to enhance the regulatory Treg phenotype in males, promoting immune tolerance and reducing autoimmunity (Voskuhl et al., 2018; Walecki et al., 2015). This cytokine balance and enhanced Treg activity may help explain the lower incidence of autoimmune diseases in males, despite their tendency to experience more severe disease manifestations when MS does develop.

In addition to its effects on immune regulation, testosterone exhibits neuroprotective properties that may reduce the severity of MS-related neurodegeneration. Testosterone has been shown to promote oligodendrocyte function and myelination, both of which are crucial for repairing the myelin sheath that is damaged in MS (Voskuhl et al., 2018). Testosterone also helps reduce neuroinflammation by inhibiting the activation of microglia and astrocytes, which are key players in the CNS inflammatory cascade (Tomassini et al., 2005).

Clinically, males with MS are more likely to have low testosterone compared to healthy controls, and testosterone insufficiency has been linked to increased gadolinium-enhancing lesions on MRI, reflecting active CNS demyelination (Harbo et al., 2013). This finding is particularly relevant in the context of andropause, the age-related decline in circulating testosterone. MS onset in men often occurs later than in women, aligning with the gradual decrease in testosterone availability that occurs with aging (Ysrraelit & Correale, 2021). Thus, loss of androgenic protection may contribute to the delayed but often more aggressive disease course observed in males (Harbo et al., 2013; Ysrraelit & Correale, 2021). Adding to this complexity, a recent study reported increased MS risk among transgender individuals receiving estrogens and anti-androgens, underscoring the importance of androgen signaling in modulating disease susceptibility (Ysrraelit & Correale, 2021). Together, these observations highlight testosterone's dual immune-regulatory and neuroprotective roles and suggest that reduced androgen activity—whether through aging, primary hypogonadism, or medical suppression—may exacerbate MS risk and progression. These observations have spurred interest in testosterone supplementation trials (clinicaltrials.gov ID NCT03910738), though results to date are preliminary and require larger studies to determine efficacy and safety.

Other Hormones

While estrogen and testosterone have been the primary focus of prior research, other hormones also merit attention despite their roles being less clearly defined.

Progesterone

Progesterone receptors are expressed on key immune cells including T cells, B cells, and dendritic cells. In most immune contexts, progesterone exerts anti-inflammatory effects such as increasing Treg differentiation and reducing IFN-γ production, but its influence on the immune response is complex (Ysrraelit & Correale, 2019). While progesterone has been reported to enhance certain co-stimulatory pathways in antigen-presenting cells, this may occur alongside an overall shift toward immune tolerance, possibly by selectively modulating which T cell subsets are activated (Ysrraelit & Correale, 2019). In the experimental autoimmune encephalomyelitis mouse model of MS, progesterone treatment reduced disease severity, even when administered after disease onset, indicating its therapeutic potential in MS by modulating immune responses and supporting myelin repair (Hughes, 2011). To date, there have been no human clinical trials of progesterone monotherapy in MS, and studies of estrogen–progestin combination therapies, such as in postpartum relapse prevention and as add-on treatment to interferon-β, have not demonstrated robust clinical benefits despite some MRI improvements (Pozzilli et al., 2015; Vukusic et al., 2009).

In addition to its role in immune signaling, progesterone has shown neuroprotective effects by promoting the proliferation and maturation of oligodendrocyte progenitor cells, crucial for myelin repair (Hughes, 2011; Ysrraelit & Correale, 2019). It remains possible that progesterone's effects in MS depend on timing, dosage, or interaction with other hormones.

Prolactin

Prolactin is a hypothalamus-pituitary-gonadotropin axis hormone with a primary role in supporting lactation and is also known to impact immune regulation. Questions remain regarding the role of prolactin in MS. Elevated prolactin levels have been reported in MS patients, particularly during relapses and in those with hypothalamic lesions or optic neuritis (Deckx et al., 2013; Ysrraelit & Correale, 2019). Hyperprolactinemia can promote autoreactive B-cell activity and increase the production of IFN-γ and IL-2 by Th1 cells, suggesting that prolactin may contribute to inflammation in MS (Deckx et al., 2013; Ysrraelit & Correale, 2019). Animal models have suggested that prolactin has proinflammatory effects but may also have a neuroprotective effect in terms of promoting remyelination (Gregg et al., 2007; Riskind et al., 1991). On the other hand, exclusive breastfeeding reduces the risk of postpartum relapse MS relapse (Holz et al., 2022; Langer-Gould et al., 2009), which suggests the possibility that high prolactin levels sustained by breastfeeding may be protective against relapse in MS. Further research is needed to untangle prolactin's potential role in MS.

Environmental Risk Factors in MS

Environmental factors, in addition to genetic and hormonal influences, likely play a pivotal role in MS onset and progression. The rising global prevalence of RRMS among females since the 1980s points to behavioral and environmental shifts rather than genetic causes (Parnell & Booth, 2017). These exposures can interact with genetic predispositions and influence epigenetic mechanisms—heritable changes in gene expression that do not alter DNA sequence but may modify disease risk and severity (Esteller et al., 2024; Jones et al., 2019). Such epigenetic modifications may contribute to the observed disparities in MS risk between males and females.

No single factor alone is sufficient to cause or drive MS. Instead, MS appears to result from the convergence of genetic susceptibility and environmental triggers, with epigenetic regulation potentially acting as a mediator between the two (Gacias & Casaccia, 2014; Han et al., 2020; Huang et al., 2017). Unlike fixed genetic variants, epigenetic modifications (e.g., DNA methylation, histone modification, non-coding RNA activity) are dynamic and reversible, making them attractive therapeutic targets.

Epstein-Barr Virus (EBV)

EBV is a herpesvirus that establishes lifelong infection in host B cells, cycling between latent and lytic phases. During latency, EBV persists in B cells by expressing a limited set of viral genes, classified into latency types (I–III), to evade immune detection while maintaining the viral genome (Keane et al., 2021). Recent studies propose that EBV infection is a necessary precursor for the onset of MS, but infection alone is not sufficient to trigger the disease (Bjornevik et al., 2023). Nevertheless, EBV is the only universal risk factor identified across the MS population, making it a critical environmental exposure to understand, particularly in the context of sex differences. While males and females are equally susceptible to EBV infection, females generally mount a more robust immune response, with higher antibody titers and stronger humoral responses compared to males (Alvarez-Sanchez & Dunn, 2023; Hedström et al., 2020; Keane et al., 2021). This enhanced response is largely influenced by estrogens, which promotes B cell function and antibody production. In contrast, androgens have immunosuppressive effects, leading to a more subdued immune response in males. This sex difference in viral immune response may help explain why females are more prone to immune-mediated diseases-including MS, systemic lupus erythematosus and rheumatoid arthritis-following EBV infection (Keane et al., 2021).

Sex-specific interactions between MS risk genes and EBV latency III infection may further explain the higher susceptibility of females to MS. Keane et al. (2021) found that the expression of the EBV latency III master regulator, EBNA2, is negatively correlated with the estrogen receptor ESR2 in female lymphoblastoid cell lines (LCLs), but not in male LCLs. Additionally, correlations between the EBV latency gene LMP1 and EBV DNA copy number with ESR2 were stronger in females. The study also identified sex-specific expression of MS risk loci, with 26 eQTLs found exclusively in females and 15 in males. These findings support the idea that genetic variation can modify the immune response to EBV in a sex-dependent manner, potentially amplifying inflammatory pathways in females and influencing MS risk.

Vitamin D

Vitamin D is primarily produced through skin exposure to sunlight, and its deficiency is thought to contribute to the higher risk of MS observed in populations living at higher latitudes, where sunlight exposure is reduced (Lublin et al., 2014). Notably, females with vitamin D3 deficiency face a seven-fold increased risk of MS, a trend not observed in males (Nashold et al., 2009). This discrepancy may be explained by a synergistic relationship between vitamin D3 and estrogen in females. Studies in mouse models of MS have shown that female mice with vitamin D3 deficiencies could only raise their levels through supplementation if they had sufficient estrogen (Spanier et al., 2015). Additionally, vitamin D3 and estrogen interactions seem to influence the Foxp3 gene pathway, which is essential for immune regulation. Low vitamin D3 levels may decrease Foxp3 expression by promoting methylation at the Treg-specific demethylated region, resulting in a more inflammatory T-cell phenotype (Sintzel et al., 2018; Walecki et al., 2015). In this way, vitamin D deficiency may have a more pronounced effect on immune dysregulation in females than in males.

Other environmental factors—such as the gut microbiome, obesity, and tobacco use—also contribute to MS pathophysiology, partly through effects on miRNA expression. Females tend to harbor pro-inflammatory gut bacteria, while males show more anti-inflammatory profiles, with microbiota shifts influencing epigenetic pathways (Duarte-Silva et al., 2022). Obesity may worsen MS in females as estrogen amplifies leptin's pro-inflammatory effects (Cordiero et al., 2024; Evangelopoulos et al., 2014). Tobacco use increases MS risk and severity in both sexes, potentially via miRNA regulation, though risk may be greater in men (Bove & Chitnis, 2013; Palacios et al., 2012).

MicroRNAs and Epigenetic Regulation in MS

The central dogma of genetics describes the flow of genetic information from DNA to RNA to protein, a process that is intricately regulated at multiple levels. Double-stranded DNA is transcribed into single-stranded messenger RNA (mRNA) in the nucleus, which is then processed, exported, and translated into proteins by ribosomes in the cytoplasm. Epigenetics refers to somatically heritable changes in gene expression that do not involve changes in DNA sequence (Esteller et al., 2024). Such epigenetic alterations can occur in DNA, RNA, and protein molecules via DNA methylation, non-coding RNAs, and post-translational chromatin protein modifications such as histone methylation and acetylation, respectively (Esteller et al., 2024; Li, 2021; Zhou et al., 2021). Importantly, epigenetic changes can be mediated by environmental and biological factors that ultimately impact changes in gene expression (Zhou et al., 2025).

For example, microRNAs (miRNAs)—small (19–24 nucleotide) single-stranded non-coding RNAs—serve as critical epigenetic regulators by modulating gene expression post-transcriptionally. Following export from the nucleus and maturation, miRNAs bind to target mRNA, typically within the 3′ untranslated region, where they induce mRNA degradation, repress translation or less commonly activate translation. With over 1,800 known miRNAs targeting up to 60% of human genes, these molecules are central to a wide array of biological processes, including immune regulation and neurodevelopment (Sharma & Eghbali, 2014). The X chromosome is notably enriched in miRNAs compared to the Y chromosome and autosomes (Di Palo et al., 2020), underscoring their potential role in the heightened risk of MS and the more inflammatory disease course observed in females.

In MS, miRNAs provide a dynamic and adaptable layer of gene regulation, capable of integrating genetic susceptibility, hormonal signaling, and environmental exposures. This makes them uniquely positioned to help explain the complex sex-based differences in MS pathophysiology—particularly the tendency for females to exhibit greater neuroinflammation and males to experience more neurodegeneration. Importantly, miRNAs are not static; they respond to cues from the body's internal environment (i.e., sex hormone fluctuations) and external exposures (i.e., viral infections, vitamin D levels, smoking). Through these interactions, miRNAs act as molecular bridges linking upstream triggers to downstream immune and neurodegenerative processes.

Emerging research in MS and other autoimmune diseases supports the idea that miRNA dysregulation plays a key role in shaping the disease course, and that these effects are often sex specific. Mouse models of MS and rheumatoid arthritis, for example, have demonstrated that certain miRNAs amplify inflammatory responses in females, while others influence neurodegenerative pathways in males (Dai & Ahmed, 2014; Li et al., 2020). For instance, miR-155, a well-characterized pro-inflammatory miRNA, is frequently upregulated in females with MS and may contribute to heightened disease activity (Dai & Ahmed, 2014; Li et al., 2020). Conversely, miR-1-3p and other neurodegeneration-associated miRNAs may be more active or dysregulated in males, aligning with their greater burden of brain atrophy and disability over time (Dai & Ahmed, 2014; Li et al., 2020).

The interplay between sex hormones and miRNAs adds yet another layer of complexity. Estrogens have been shown to upregulate miRNAs that promote regulatory T cell differentiation and neuroprotection, while testosterone may downregulate miRNAs implicated in inflammation and axonal damage (Dai & Ahmed, 2014; Shansky, 2015). These hormone-miRNA interactions may help explain temporal patterns in MS activity, such as the reduction in relapses during pregnancy, increased risk of relapses during the postpartum period, and the potential acceleration of neurodegeneration after menopause. Additionally, environmental risk factors such as EBV, vitamin D3 deficiency, obesity, and tobacco use may alter miRNA expression profiles in ways that vary by sex, creating distinct molecular cascades that drive MS onset and progression differently in males and females.

Taken together, miRNAs are more than passive markers of disease—they are integrators of the genetic, hormonal, and environmental influences discussed throughout this review. Their ability to mediate these complex, layered interactions holds tremendous promise for clarifying the biological basis of sex differences in MS. Profiling miRNA expression in males and females with RRMS could uncover novel biomarkers of disease activity, treatment response, and prognosis. Furthermore, miRNAs may point to therapeutic targets capable of modulating immune and neurodegenerative pathways in a sex-specific, personalized way. As our understanding of miRNA function expands, so too does the opportunity to develop more tailored and effective interventions that address the unique trajectories of MS.

Conclusion and Future Directions

Sex-based disparities in MS susceptibility and progression are likely driven by a complex interplay among immune system differences, genetic and epigenetic factors, sex hormones, and environmental influences. Both males and females experience inflammation and neurodegeneration, but their relative predominance often differs—females more frequently show heightened neuroinflammatory activity, whereas males tend toward neurodegenerative decline. These patterns are shaped by interactions among X-linked immune-related genes, hormone-mediated immune modulation, and environmental exposures such as EBV and vitamin D3.

Despite advances in our understanding of MS, significant gaps remain in deciphering the precise molecular mechanisms behind these sex-based differences. The relative contributions of genetic predispositions and epigenetic modifications in driving neuroinflammatory versus neurodegenerative pathways are still unclear. miRNAs, as epigenetic regulators, hold great promise in addressing these questions. Investigating miRNA profiles in males and females with MS could illuminate how miRNAs modulate gene expression to drive neuroinflammation in females and neurodegeneration in males, clarifying the ways in which genetic risk factors are either amplified or mitigated through epigenetic changes. Because miRNAs are stable in peripheral blood and other accessible tissues, they could be incorporated into longitudinal monitoring strategies to detect shifts toward more inflammatory or degenerative trajectories—supporting earlier, more tailored intervention. Targeting dysregulated miRNAs offers the potential to modulate inflammatory responses in females and enhance neuronal resilience in males, addressing the unique therapeutic needs of each sex.

The simultaneous interrogation of miRNA and mRNA expression represents a promising approach to bridging these knowledge gaps because it enables researchers to uncover the regulatory interactions that drive sex-based differences in MS. miRNAs act as master regulators, influencing mRNA stability and translation, and together they form intricate networks that determine cellular processes. By studying miRNAs and mRNAs in tandem, future research could identify specific miRNA-mRNA interactions that govern inflammatory and degenerative pathways in males and females. This dual-layer analysis could provide opportunities to pinpoint how epigenetic regulation contributes to disease processes in a sex-specific manner, offering insights that single-layer approaches cannot achieve. Furthermore, this integrated approach can help reveal how genetic and environmental factors interact with epigenetic mechanisms to shape MS progression. Understanding these mechanisms has clear clinical implications. A sex-specific lens can guide targeted prevention strategies, patient education, and individualized risk counseling. For

example, vitamin D monitoring may be particularly important in premenopausal women, while EBV vaccination—should it become available—could have universal benefit but potentially stronger impact in reducing female MS incidence.

Epigenetic changes can be reversed through lifestyle and pharmacotherapeutic interventions (Zheleznyakova et al., 2017). Therefore, such epigenetic studies cannot only identify biomarkers for improved risk assessment but also can identify specific molecular targets for reversal (Garcia-Bloj et al., 2016; González Molina et al., 2025; Stolzenburg et al., 2015). It is hoped that epigenetic biomarker profiling may ultimately inform individualized interventions leading to reduced disability in MS. Current FDA-approved DMTs for RRMS are primarily effective in reducing neuroinflammation and preventing relapses but are less effective in slowing neurodegeneration—a critical gap given that neurodegeneration is a key driver of long-term disability for all individuals with MS. This limitation is especially concerning for males, who may exhibit faster progression and earlier brain atrophy, but it also impacts females as they age or transition to progressive forms of MS. Future research must address this therapeutic blind spot by investigating how DMTs affect both neuroinflammatory and neurodegenerative pathways—and whether their effects differ by sex, disease stage, or underlying biological profile. Understanding how miRNA dysregulation contributes to both inflammation and degeneration could help identify new therapeutic targets that go beyond relapse control. Ultimately, integrating miRNA and mRNA data while explicitly considering sex as a biological variable could help deepen our understanding of MS heterogeneity and support the discovery of novel biomarkers and therapeutic pathways. This approach could help move the field toward more personalized and comprehensive care strategies, ensuring that advances in research translate into better outcomes for all individuals affected by MS.

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