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Sex Differences in Multiple Sclerosis: Risk Factors and Expression*

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Abstract

Multiple sclerosis (MS) is a complex neuroimmune disorder that has been noted to affect more females than males. The chromosomal sex of the affected individual impacts both the incidence as well as the progression of disease. There are now many studies looking into the pathophysiological, genetic, and cellular reasons for the difference in incidence between males and females as well as into the differences in efficacy of treatment. Investigators have identified genetic and chromosomal reasons for this apparent sexual dimorphism, and have delved into the distinction between the immune responses in males and females as well as differences in how the neurons react to the immune stimuli. For example, the number of X chromosomes and the expression of X chromosome-associated genes as well as the parental origin of the expressed X chromosome-associated genes is an important influence in the severity of disease in an animal model of MS. Male patients have more extensive evidence of brain atrophy on MRI scans, corresponding to the more severe progression of MS in males, and the lesions show evidence of increased iron in the outer surface of the lesions. In addition, immune factors are more evident in females than in males in both human disease and in the animal models. This paper will review the data on the sex bias found in MS.

Keyphrases

Multiple sclerosis, sexual dimorphism, sex chromosomes, sex hormones, autoimmunity, genetics, epigenetics, transcriptomics.

Contents

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Introduction

Clinicians caring for patients with multiple sclerosis (MS) have described sex differences in the presentation, progression, and response to therapeutics for many years. There is now a large literature addressing these differences, including the development of animal models for MS. Research has probed the genetics, epigenetics, transcriptomics, cell biology, and imaging characteristics of MS in both humans and animal models. Yet, questions still remain to be addressed. This literature and the unsolved questions will be discussed in this review of sex differences in MS.

Regarding terminology for sex differences and sexual dimorphism, note that when male or female sex is mentioned in this review, we refer only to chromosomal sex, and not gender. The sex chromosomes are called X and Y, and most humans have two sex chromosomes. Typically, chromosomal sex is either XX for a genetic female or XY for a genetic male. Occasionally, an individual has only one X chromosome or three X chromosomes (XXX) and is female, alternatively XXY or XYY and is male. Other possibilities exist, especially when a portion of one X chromosome has been deleted or duplicated. Sexual dimorphism refers to a difference in the incidence, progression, or prognosis of a disease in females vs males. MS is a disease with sexual dimorphism in all aspects of the disorder.

In the past, scientific and medical research in general focused on males based on the erroneous presumption that males and females would have similar incidence, symptoms, progression, and prognosis when the individuals had the same diagnosis. Much of the time, the control group for any clinical study was all male even for disorders that impacted women's health, except for pregnancy. When females had neurological or ill-defined symptoms, the physical indicators were sometimes attributed to hypochondria or hysteria and not to a pathological process. MS symptoms may wax and wane, or may be difficult to observe. However, multiple studies have shown over many years that females are more likely to be diagnosed with, and thus more susceptible to, MS than males. Studies have also shown that males with MS are more likely to have worse disease progression than females (Bove, McHenry, et al. 2016). What factors influence susceptibility and

progression differences in males and females? Examining an animal model of MS has provided some answers.

Animal Model of MS

In experimental autoimmune encephalomyelitis (EAE), encephalomyelitis is induced in an experimental animal (Ryan and Mills 2021; Wiedrick et al. 2021; N. Itoh et al. 2023; Zahaf et al. 2023). Research using several small mammal species has demonstrated that female animals were protected from disease while they were pregnant. This protective nature of pregnancy is also observed in humans. This finding leads to the question of whether hormone levels during pregnancy are protective or whether the immune system functions differently during pregnancy. Only research including both males and females would be able to answer the questions raised by this observation. In EAE experiments, female animals were more susceptible to relapsing-remitting disease, one presentation also seen in humans with MS. Thus, this animal data changed how researchers regarded female subjects compared to male subjects: It was not that the females complained more or had psychological causes of their symptoms.

Golden and R. Voskuhl (2016) discuss the factors which had been implicated in the apparent sexual dimorphism seen in MS. In particular, they review some of the research done on the rodent model. In humans and in mice, there is a gene associated with the development of testes, the SRY or Sry gene, respectively. The authors reviewed the literature in which mouse lines were developed with the Sry gene deleted from the Y chromosome and with the Sry gene inserted into another chromosome. The research demonstrated that the presence of testes alone was not the cause of the sexual dimorphism in EAE mice. There had to be a biological mechanism for the observed differences in the EAE cohorts.

Looking at the mouse EAE model, male mice with chronic EAE had decreased numbers of neurons in cortical layer V of the cerebral cortex when compared to healthy male mice, female mice with chronic EAE, or healthy female mice. Mitochondrial function was most altered in EAE males. Genes were found to be differentially expressed in more significant numbers in male when compared to female mice (Figure 1, panel E, N. Itoh et al. (2023)). The canonical pathways where gene expression was altered was also different in the male mice vs the female mice (Figure 1, panel F, N. Itoh et al. (2023)).

Disease Complexity of MS

Since MS is an autoimmune disease of the nervous system, it involves both the immune system and the nervous system. T lymphocytes, a type of white blood cell or leukocyte, are activated, making them able to migrate across the blood-brain barrier. Cytokines or chemical messengers released by these T lymphocytes cause cascading activation of microglia and astrocytes and recruitment of macrophages and other lymphocyte. This process leads to demyelination and neurodegeneration. There are three typical presentations of MS: relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS). In addition, there are other more rare autoimmune presentations and MS-like encephalitis. Researchers have looked at the differences in the type of MS in females and males, and demonstrated that although males are less likely to develop MS than females (Figure 2 from Gold et al. (2018)), males with MS often have more severe disease progression.

Several excellent review articles have been published by eminent researchers in the MS field. The following two papers review much of the data that had been published before 2015. The first part of a review Dunn, Lee, et al. (2015) published in the Current Topics of Behavioral Neuroscience 2015 discusses the differences in MS disease incidence in males and females, including the autoimmune mechanisms and the hormonal and genetic factors found in autoimmunity in the central nervous system. They review the rodent model of MS to discuss how autoimmunity in the brain and spinal cord may develop. In the second part of the review Dunn, Gunde, et al. (2015) published in the Current Topics of Behavioral Neuroscience 2015, the authors focus on the rising numbers of females with MS and the more severe outcome in males. They review in depth the data that has been accumulated over years that suggests that there may be differences in the sex-specific interaction with the environment that allows for development of autoimmune disorders. They again discuss the rodent models for MS and other factors that may explain the increased severity and more rapid progression of MS in males.

Lopez-Lee et al. (2022) review the sex differences in several neurodegenerative diseases including MS with emphasis on the immune system. They identify significant differences in male to female ratios of the incidence of MS, higher ratio of HLA-DRB1*15-positive genotype female patients, higher histopathological burden and brain atrophy in males, a higher rate of relapse in RRMS females, shorter time to disability and faster progression in males, a higher rate of conversion from RRMS to SPMS in males, higher CD4+ T-cell counts and CD4+:CD8+ T-cell ratios in females, and higher levels of inflammatory cytokines IL-1beta and TNF in lesions plus a higher percentage of TNF-alpha producing T-cells in males. They used these findings to suggest pathways for treatment based on the immune mechanisms. In Ortona et al. (2016) , the authors look at sexual dimorphism in autoimmune disorders, including MS. The review showed the same factors as the other reviews included as references.

A series of review articles by Voskuhl and colleagues highlight the specific advances in research into the sexual dimorphism found in MS. R. R. Voskuhl and Gold (2012) review the data on the sex differences in MS susceptibility and progression. They discuss the fact that more women have MS than men, while men have a higher rate of progression. They review the information on the effect of pregnancy on MS, noting that pregnancy protects the patient from relapse while the post-partum period has higher rates of relapse. The complexity of the disease and the sexual dimorphism indicate that there are many factors, including genetics, sex chromosomes, hormones, epigenetic effects, and environmental influences. The ability to use laboratory methods to elucidate the mechanisms underlying the disease may lead to more successful treatments.

Identified in R. R. Voskuhl, Sawalha, et al. (2018), one of the sources of the sexual dimorphism in MS is the contribution of sex chromosomes. The researchers used transgenic mice that had sex chromosome manipulation to examine X chromosome imprinting as well as gene expression. They looked at T lymphocytes and at bone marrow chimeric mice to find expression patterns that indicate specific X chromosome gene expression in neurons may lead to differences in autoimmunity and in neurodegeneration. R. R. Voskuhl (2020) discusses a short review of MS disease risk and progression in females and males. R. Voskuhl and Y. Itoh (2022) reviews the data that have been generated about the influence of the X chromosome on neurodegeneration in general and specifically in MS.

Prevalence and Risk Factors for MS

The female to male ratio of MS incidence varies by geographic region, however, it is approximately 2.5:1 to 3.5:1 now. The ratio has only increased over time, and was previously reported as 2:1 to 3:1. Research has demonstrated that the number of male patients with MS has remained steady, while the number of females with MS has increased. Potential mechanisms include gene-environment factors, epigenetic factors, or the decrease in the number of pregnancies across the globe. Could there be other factors that are causing the increase in females diagnosed with MS? Has there been an ascertainment bias? Has MS been underdiagnosed in females who were thought to have psychologically-caused symptoms? These are questions that many researchers are intent at answering.

To date, many factors have been shown to influence MS prevalence, progression, type, and response to treatment with respect to the disease in general and with respect to the sex bias found in MS studies. These factors overlap when investigators talk about prevalence, severity, clinical features, biomarkers that can be used to track disease progression, and response to treatment. In Figure 3 from Bianco et al. (2023), the size of the font used for each factor represents the amount of influence on the aspects of the disease (in black font on the outer rim of the circle) in which there are differences in males and females with MS. Genetics, age, and hormones have the largest influence, while nutritional habits, lifestyle, environment, and epigenetics have the smallest influence.

Figure 4 from Angeloni et al. (2021) shows a different depiction of the environmental and genetic factors that influence the dysimmunity found in MS patients. For some time, researchers focused on some environmental factors that may influence the development and progression of MS Sadovnick et al. (2021). These include vitamin D Eikelenboom et al. (2009) and sun exposure, smoking, diet, and history of Epstein-Barr virus (EBV) infection. However, the development of dysimmunity and thus MS is different in males and females, and may be due to genetic hyperstimulation in females and genetic suppression in males.

Sex Differences and the Immune System

Figure 5 from Ramien et al. (2016). In the following figure from Ramien et al. (2016), immune cells from males and females are diagramed. In female and male immune cells, receptors for hormones differ significantly. Female cells have estrogen receptors that can be stimulated by either low or high levels of estriol (E2) or high levels of estradiol (E3). Low-level stimulation may cause pro-inflammatory mediators to be induced while the higher levels of stimulation may cause the release of factors that may increase inflammation. In addition, some factors that are possibly released through E2 stimulation may suppress inflammation, although that pathway is not fully understood. In males, the hormone testosterone stimulates immune cells through the androgen receptor. The action of testosterone may dampen adaptive immunity and stimulate innate immunity, or may have other actions within the cell. In females, there are usually two X chromosomes with 1100 genes, while males have one X with a single copy of the X-chromosome genes and a Y chromosome with only 100 genes that mostly are involved with male sexual development. Females are known to be more likely to have increased immunocompetence, with increased immune responses and a higher risk of autoimmunity. Males are more susceptible to infectious diseases.

Figure 6 from Ramien et al. (2016). At the top of figure 6 (Ramien et al. 2016), four mechanisms are listed as influencing pathogenesis, activity and progression of MS: environmental insults, epigenetics, sex chromosomes, and sex hormones. Avila et al. (2018) provides a short review that discusses how hormonal differences may influence inflammation and demyelination. Their premise is that remyelination is augmented by pregnancy, during which the oligodendrocyte precursor cells proliferate and thus increase the ability to remyelinate the damaged areas of the nervous system.

In Giatti et al. (2020), the rodent model of MS and sex steroid hormones are discussed. The authors measured hormone levels, and gene expression in spinal fluid and plasma from EAE animals. They found decreased levels in the spinal fluid from male animals. They suggest that neuroactive steroids may be designed as a treatment modality for MS. These factors target the central nervous system (CNS), including neurons oligodendrocytes, microglia, and astrocytes, and the immune system, comprised of a variety of leukocytes and dendritic cells (DC). Males are more susceptible to the CNS effects of these factors, while in females, there are more inflammatory effects on the immune system. There are also sex-based differences in the rate of neurodegeneration, where male patients are reported to be more susceptible to neurodegeneration. Sexual dimorphism is noted in MS risks, including evident differences in parent-of-origin effects, and inflammation is higher in female patients, causing sex bias in incidence and progression of MS.

Sexual dimorphisms have been found in imaging studies (Koenig et al. 2013; R. R. Voskuhl, Patel, et al. 2020; Chaves et al. 2021). Response to treatment may also differ in men when compared to women (Bove, McHenry, et al. 2016). It is also known that pregnancy reduces the number of MS relapses in many female pre-menopausal patients McCombe (2022).

Transcriptomics and Multi-Omics Analysis

Figure 7 from Català-Senent et al. (2023). Genetic and genomic research has evolved over the past 20 years to included more than the DNA sequence found in the genome. Some researchers have analyzed the transcripts, that is the RNAs found in a population of cells or in an individual, to see what is being transcribed from the genome. This data set is different from a gene expression analysis, which usually focuses only on the transcripts that are turned into proteins, and also different from proteomics, which involves the study of the proteins produced by the genome. Another method used is to look at epigenetics, or how the gene expression is controlled at the DNA level. Genes may be turned off or on through epigenetics. Other -omics methods look at metabolomics, or the metabolic and endocrine products of the genome, or the microbiome, or the bacteria and viruses that naturally reside in an individual. In this flowchart (Català-Senent et al. 2023), the process of a meta-analysis of MS papers to assess sex differences in MS is outlined. This study, in which the subjects and controls included both male and female individuals, revealed additional information about the sex differences in MS.

-Omics includes genomics, epigenomics, transcriptomics and expression studies, proteomics, metabolomics, microbiomics, and more. Transcriptomics and other -omics analyses are complex both in the methodology used to generate the data as well as in the bioinformatics platforms used to analyze the data. Existing databases of genome, transcriptome and other -omics-generated data are often freely available to other researchers. The individuals who perform this type of meta-analysis first have to assess the trustworthiness of the data in the studies that may be analyzed. If a meta-analysis of multiple data sets unintentionally uses flawed data, the result will be distorted, and

the flaw may not be readily identified. If a new data set is created, for example the data presented by Itoh et al. N. Itoh et al. (2023), how well does it mesh with prior work? A data set that contradicts other research may be of high quality and may detect flaws in other investigations, or may find new aspects to investigate that prior research may not have identified. Data sets that mesh well with prior data may add to the confidence that researchers have in existing data. Finding the right gene or data or study is hard!

Figure 8. As with this Where's Waldo? drawing Handford (2019), there are many data points in a meta-analysis, and the researchers engaged in this type of analysis will screen the existing papers to choose those data sets that are most likely to be useful. The question of whether the right data are used is fundamental to the quality of the meta-analysis. In the meta-analysis of research into the deep transcriptome in patients with MS (Català-Senent et al. 2023), data published in 122 papers were screened, and nine separate studies were selected for the meta-analysis. These investigations included both males and females, and had control subjects who were both male and female. Five of these were studies performed with peripheral blood and four with brain tissue. Data were from 474 samples: 189 females with MS, 109 healthy females, 82 males with MS, 94 healthy males. Several genes showed differences in transcription between the affected females and males:

- *KIR2DL3* from blood samples,
- *ARL17B, CECR7, CEP78, IFFO2, LOC401127, NUDT18, RNF10, SLC16A5, STMP1, TRAF31P2-AS1, UBXN2B, ZNF117,* and *ZNF488* from brain tissue.

Brain data used for computational functional analysis showed different altered immune patterns in males and females.

Figure 9 from Bianco et al. (2023). Possible biologic factors that can be used to assess differing progression rates in males and females include those that influence the rate of neurological progression and cognitive decline, which are more rapid in males. There have been multiple imaging investigations into MS. Ontaneda et al. (2023) Rojas et al. (2016) Both MRI studies and post-mortem exams show persistent inflammation and neurodegeneration in males Bove, Musallam, et al. (2016) Chaves et al. (2021).

In R. R. Voskuhl, Patel, et al. (2020), the original findings in brain MRIs in a cohort of male and female MS patients and a matched control group are presented. All scans were done on the same scanner in one facility. The researchers were able to assess specific areas of atrophy to compare the affected females to the affected males. Another part of the assessment included a 9-hole peg test. Both groups showed atrophy in the thalamus. The males with MS had additional atrophy in the putamen and localized cortical regions and showed worse performance on the on the 9-hole peg test. The authors equated these results with the observation that males with MS have worse progression of their disease.

Imaging studies in pediatric patients with MS have also identified sex differences in the enlargement of the choroid plexus Margoni et al. (2022). Cellular mechanisms may contribute to increased damage to axons in MS. Male T cells may increase inflammation more than female T cells in both human MS and EAE. There are sex differences in how humans react to EBV infections and B cells. There is some evidence that prior EBV infection may increase the risk of MS, however, this connection has been contested by other researchers. Looking at cellular factors, females with MS have increased numbers of CD56+ NK leukocytes in the blood. These cells may increase the risk of MS or may be secondary to MS. There is also a difference in microglial reactivity with age when males and females are compared. Astrocytes may be more reactive in males with MS and EAE. More iron accumulates at the rim (edge) of active MS lesions and in the deep gray matter in males with MS Tolaymat et al. (2020). This may affect the rate of decline in male MS patients or may be secondary to the rate of decline. There is also a difference in the susceptibility to demyelination and repair when comparing males and females. Pelfrey et al. (2002) In 11 females with MS, 11 males with MS, and 22 health controls, the researchers measured immunological responses to myelin peptides. The female patients had strong reactions to IFNgamma and no response to IL-5, while the male patients had higher IL-5 responses with much lower IFNgamma responses. The controls had minimal to no responses to either IFNgamma or IL-5. The authors suggest there is a sex bias towards T-helper 1 cell responses in MS.

Tomassini and Pozzilli (2006) This paper reviews the data regarding sex hormones, sexual dimorphism, and MS. The suggestion is made that perhaps the use of female hormones may prove a beneficial treatment for MS.

Neurons may be more vulnerable to circulating levels of neurotoxic molecules in males over females. There are differences in the intestinal microbiome when males and females are compared. A mouse study in which germ-free mice were inoculated with the gut microbiome from discordant human MS twin pairs showed increase in EAE after inoculation with the microbiome from the MS twin. Figure 10 from Alvarez-Sanchez and Dunn (2023) tries to put much of this information together to explain the interrelationship of the factors influencing the sexual dimorphism found in MS and in EAE. This figure shows the interplay of many cellular mechanisms for the sex differencecs found in MS.

Sex Differences and Treatment Opportunities

Systematic reviews of the differences in treatment of MS have been published (Li et al. 2017; Bove, McHenry, et al. 2016). These studies may review efficacy or utility of different treatment regimens. For example, in Austria, there is a nation-wide registry of MS patients. This cohort is more geoethnically similar than subjects in studies performed in more diverse areas. Hegen et al. (2024) analyzed data from 4840 individuals with MS. More females stopped both highly effective and moderately effective therapies early. For the moderately effective treatment, most of these individuals were younger and the rationale for stopping medications was the desire for pregnancy or for nursing a child. There was no difference in female and male de-escalation frequency. Females who stopped the high-efficiency therapies more frequently also had disease progression, adverse events, and other medical issues. Other research has documented differences in the quality of life of MS patients based on their sex (Neto et al. 2018).

Conclusion

MS is a complex disorder and thus sex effects in MS may be categorized in a number of ways (Golden and R. Voskuhl 2016): Incidence, Increasing sex bias over time, Rate of progression, Parent-of-origin differences in inheritance of MS-associated alleles, Protective effects of pregnancy on MS, Differences in immunological function in males and females. None of these categories of sex effects is straight-forward, leaving many avenues for further research.

Significant sexual dimorphisms are found in patients with MS. Some

of these are related to young women's opportunity for having children as well as the protective nature of pregnancy. Many studies have been performed looking at all aspects of MS: ratio of females to males, hormones, immune markers, rates of progression, rates of neurodegeneration, differences in gene expression and cellular processes. Many questions remain! What is it about pregnancy that allows for the tolerance of a fetus that has a distinct genetic make-up when compared to a mother, and does that help slow the relapse rate of MS in pregnant women? What other genes on the X chromosome show differential expression in males and females and also influence the immune responses in women and men susceptible to MS? How can these discoveries lead to new and better treatments of MS? Additional investigation is needed into these and other remaining questions.

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Figure 1: Depiction of the EAE mouse model data with respect to female and male differences in gene expression (panel E) and top 10 canonical pathways for upregulated or downregulated genes, comparing the EAE mice with control mice separately in male and female mice (panel F). differentially expressed (DE); reprinted per CC BY 4.0 license from N. Itoh et al. (2023).

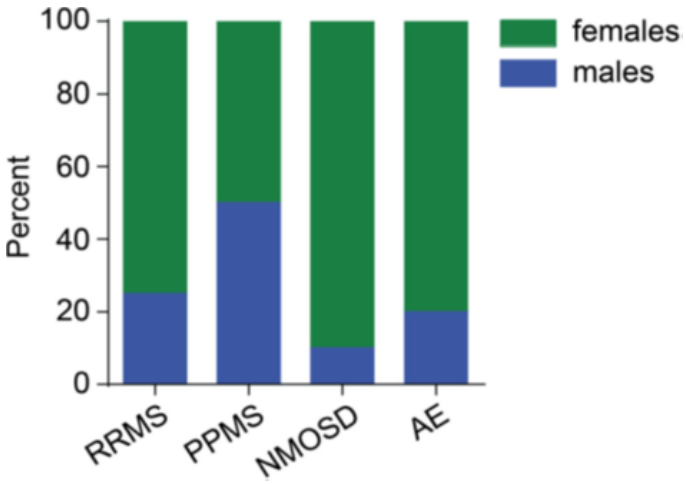

Figure 2: Differences in prevalence for females and males in MS subtypes and related neuroimmune disorders; relapsing-remitting MS (RRMS), primary progressive MS (PPMS), neuromyelitis optica spectrum disorder (NMOSD), antibody-mediated encephalitis (AE); reprinted per CC BY 4.0 license from Gold et al. (2018).

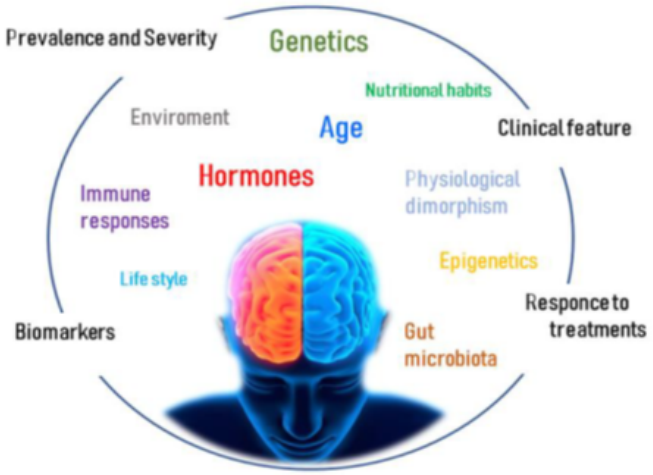

Figure 3: Interplay of multiple factors influencing gender dimorphism in MS; reprinted per CC BY 4.0 license license from Bianco et al. (2023).

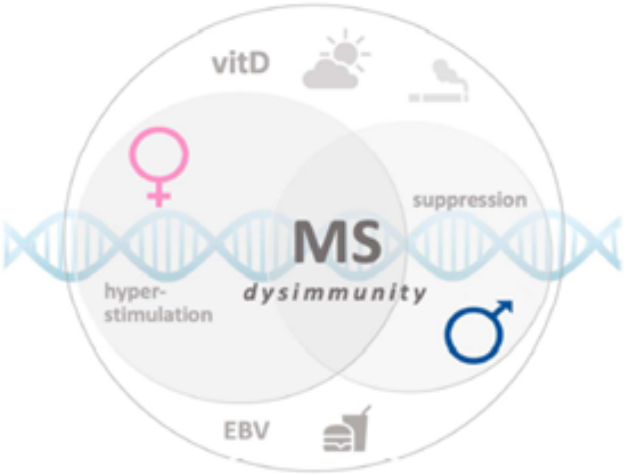

Figure 4: Dysimmunity in MS arises through differing paths in females and males based on environmental factors as well as genetics; reprinted per CC BY 4.0 license license from Angeloni et al. (2021).

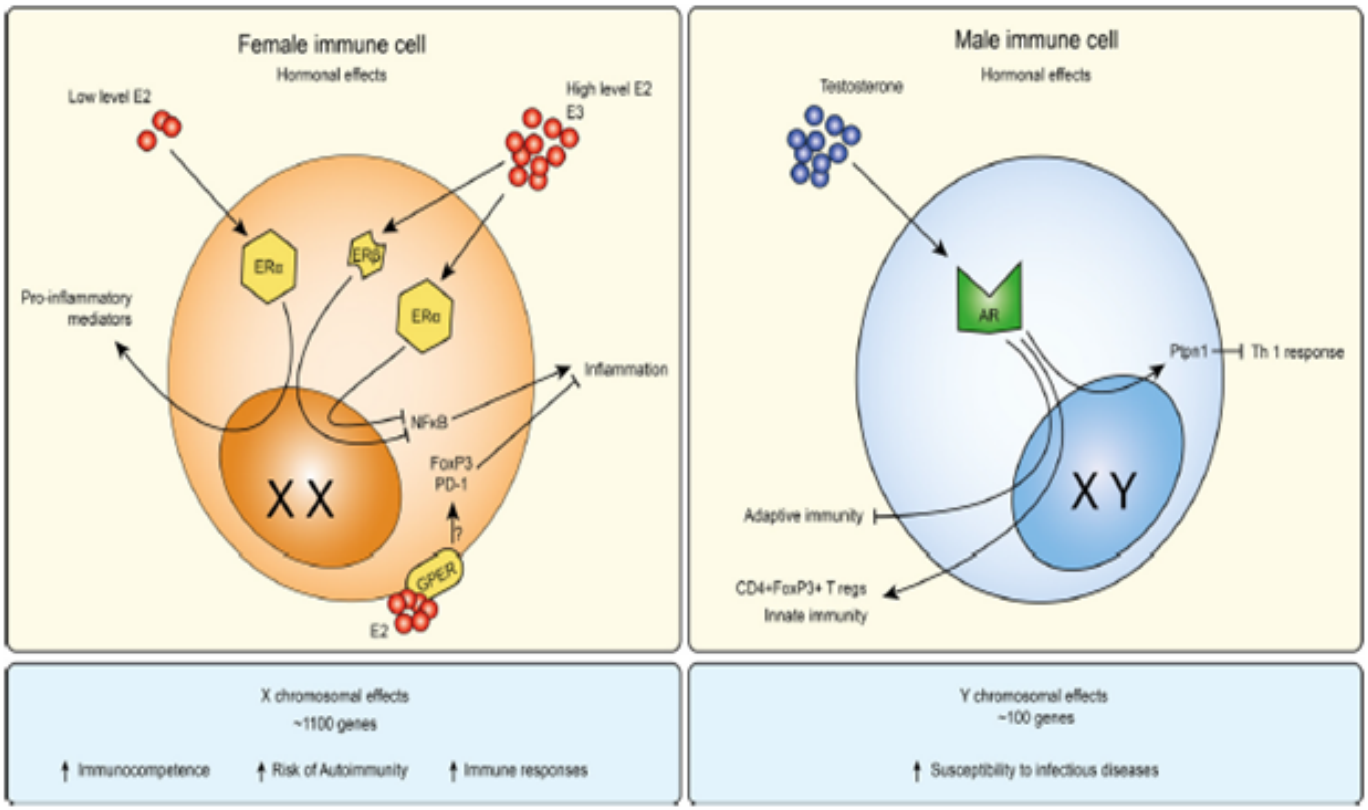

Figure 5: Comparison of the cellular pathways in females (in orange) and males (in blue) in the development of MS (top panels); comparison of the numbers of genes on the X and Y chromosomes (bottom panels); estriol (E2), estradiol (E3); reprinted per CC BY 4.0 license from Ramien et al. (2016). Differences in the cellular pathways cause females to have increased immunocompetence, increased risk of autoimmunity, and increased immune responses, while males have increased susceptibility to infectious diseases.

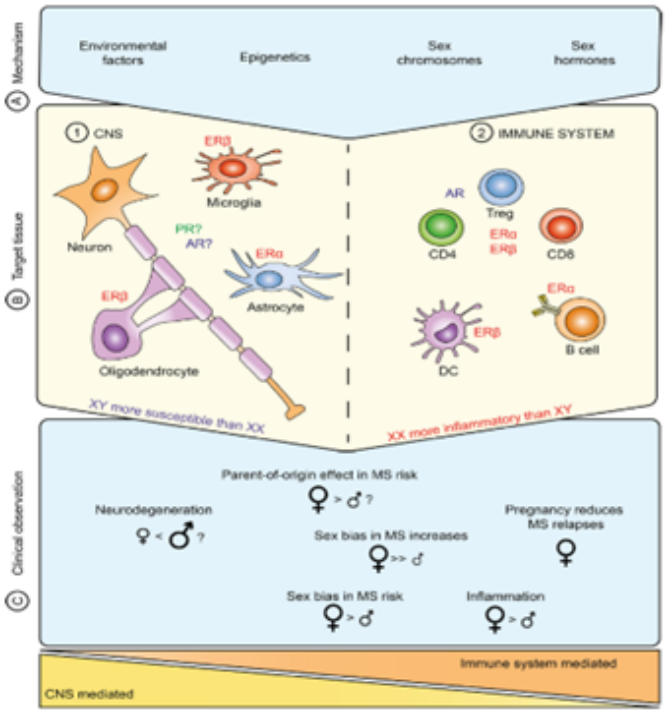

Figure 6: Mechanisms that cause sexual dimorphism in MS (panel A); target tissues and cell types that demonstrate female vs male differences (panel B); clinical observations reveal sexual dimorphism (panel C); symbol size shows the relative influence of each factor; circle on plus sign (female), circle with small arrow (male); left side of diagram shows CNS mediated effects and right side immune system mediated effects; reprinted per CC BY 4.0 license license from Ramien et al. (2016).

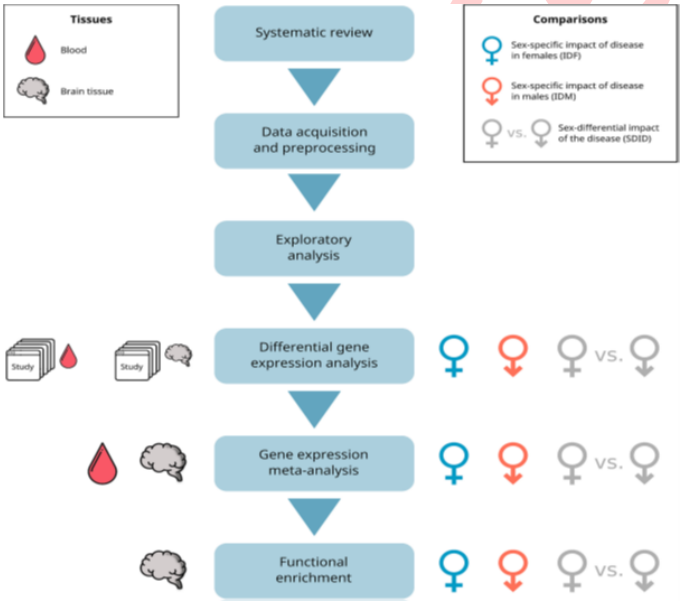

Figure 7: Methodological steps in the data analysis performed by Catala-Senent *et al.*; reprinted per CC BY 4.0 license license from Català-Senent et al. (2023).

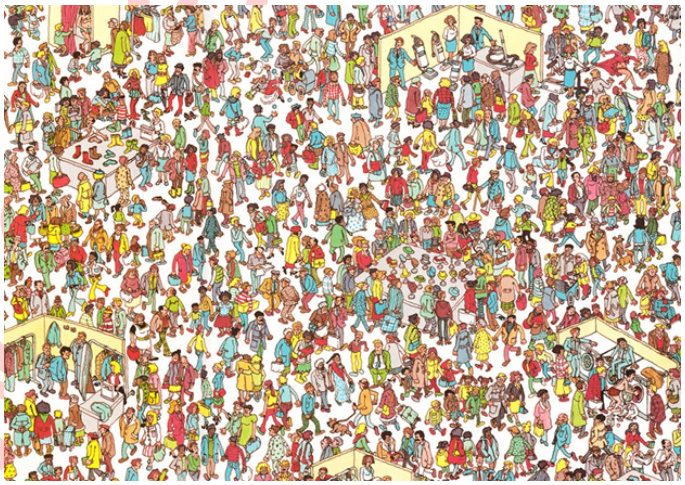

Figure 8: Drawing from "Where's Wally?" by illustrator Martin Handford (Handford 2019), known as "Where's Waldo?" in North America, demonstrating the difficulty in identifying a specific item in a complex collection when there are many similar items. In "Where's Waldo?", there are multiple persons wearing red and white clothing as does Waldo. He is located in the upper left area near the cash register. Figure reprinted per CC BY 4.0 license from Handford (2021).

NDs	Female/Male Ratio	Main IMMUNE FEATURES (In Vivo/Vitro)		
		Female	Male	In Common
MS	3:1 [10]	Higher neutrophils/macrophages activity [11] Higher $CD4^+$ T cell, $CD4^+/CD8^+$ ratio [11] APCs are more competent [11] Higher PGR expression in microglia [17] Higher expression of IL-21, IL-27, and IL-18 [18] Notable Treg, TH1/TH2 variability [18,19]. (*Mice*) Higher Th1 cytokine production [20]	Higher NK cells [11] Higher $CD8^+$ Tcell [11] Higher $CD3^+$ and TNFα [21] Higher IL-1β and TNF [17] APCs secrete IL-10 [21] (*Mice*) Higher lymphocyte infiltration [20]	M1 in early MS shifts to M2 in later stages [22] Patients with more severe disease have higher proportions of lesions with foamy microglia/macrophages [17] TNFα is increased by macrophages/microglia during the early development of sclerotic plaques [21]

Figure 9: Immune factors categorized by whether found more in females or males or both in humans and in the mouse model of MS; those in the female column are associated with increased inflammation, estrogen protection, and an immune response, and those in the male column are associated with increased cognitive decline and neurodegeneration; reprinted per CC BY 4.0 license from Bianco et al. (2023).

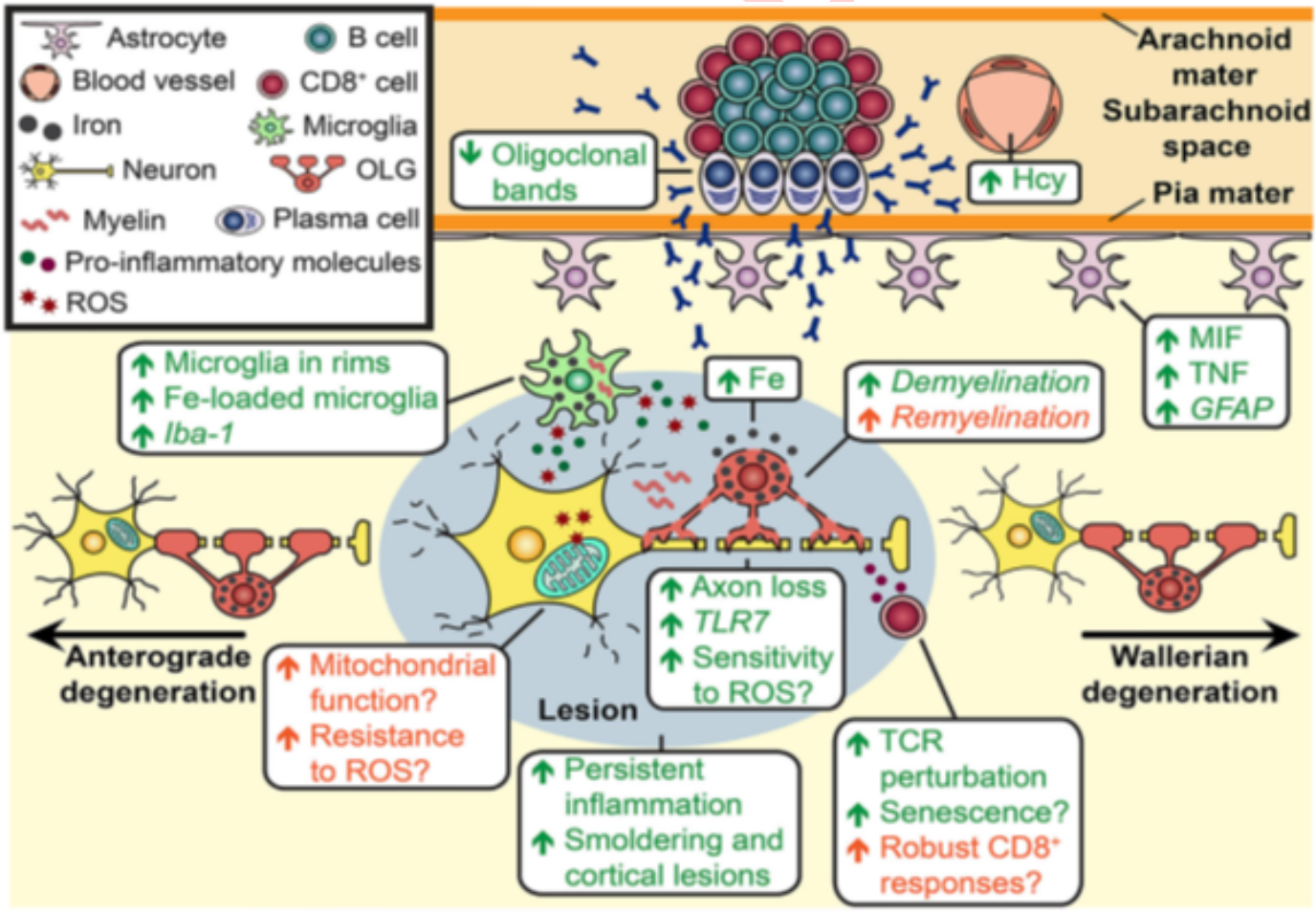

Figure 10: Diagram depicting the comparative brain tissue effects in males and females with MS, including cell types and signaling factors within the tissues and the functional effects of these signaling factors; green = male, orange = female, italics = EAE model, Fe = iron, ROS = reactive oxygen species, Hcy = homocysteine, OLG = oligodentrocytes; genes and proteins indicated by their common symbols; reprinted per CC BY 4.0 license from Alvarez-Sanchez and Dunn (2023).