Sex Differences in Multiple Sclerosis

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Disclosures and Conflicts of Interest

Disclosures – None with regards to this presentation

Please note that I am not a neurologist or an MS researcher. I am a clinical and laboratory geneticist.

My use of zebras throughout the presentation is due to the way medical students are trained to consider a “differential diagnosis” where the most likely diagnosis is listed first, and the least likely is listed last, the zebra in the herd of diagnoses. When I was a medical student, the genetic diagnosis was the zebra in the list. Many other geneticists also use zebras to designate genetic disorders and genetic/genomic medicine.

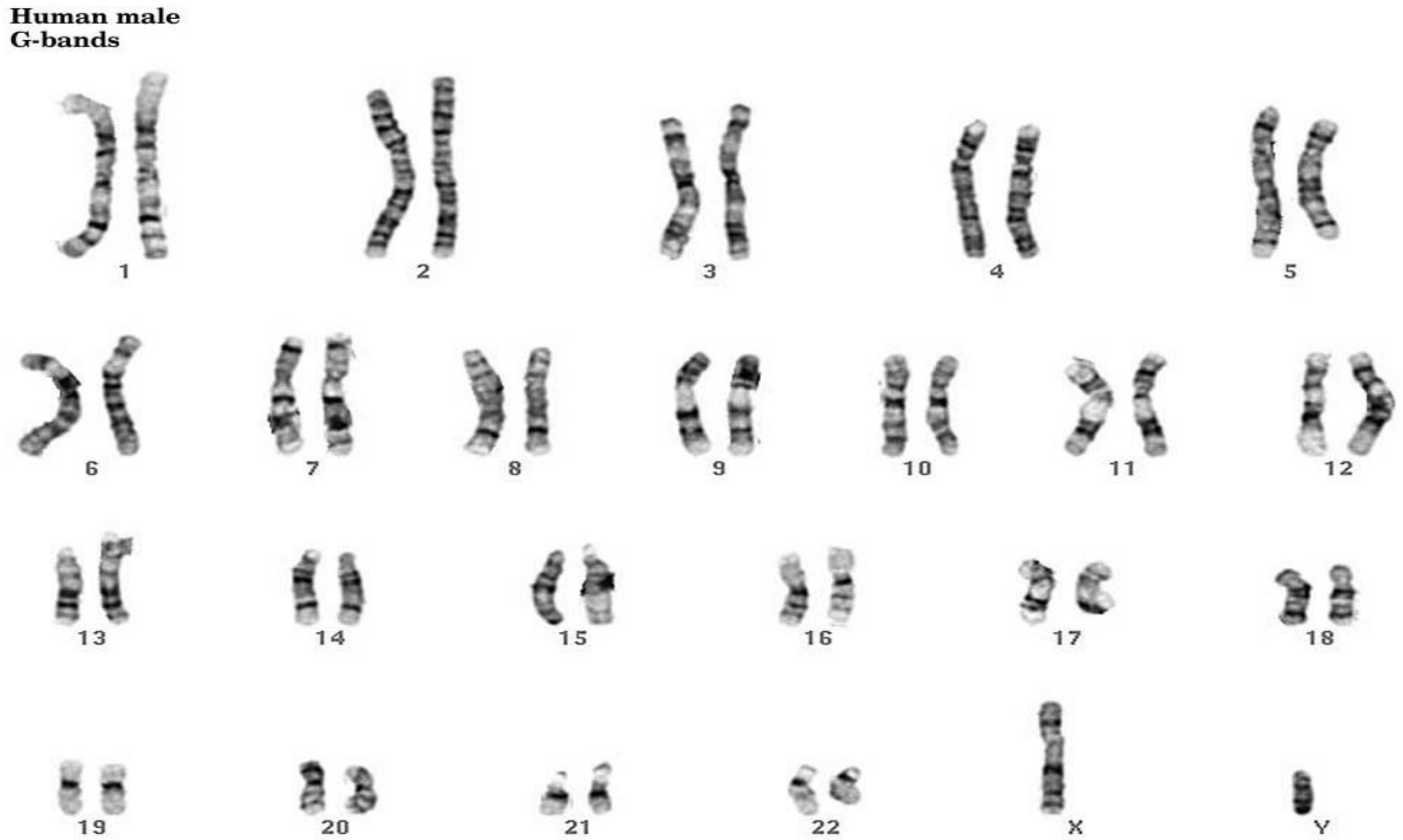
Objectives

- Understand the current ideas about why chromosomal sex influences multiple sclerosis (MS) incidence, course, therapy, and prognosis.
- Learn about the genetic and cellular factors that influence MS.
- Comprehend the types of studies that have been done to uncover why more individuals with female sex chromosomes develop MS.

References to Genetic Sex in This Talk

- Please note that when male or female sex is mentioned in this talk, I 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 female or XY for a male.
- Occasionally an individual has only one X chromosome and is female, or XXY or XYY and is male.
- When there is a difference in the incidence, progression, or prognosis of a disease in females vs males, that is called **sexual dimorphism**.

Normal Male Karyotype



Zebras Amid Their Neighbors

Why is Studying Sex Differences in MS Important?

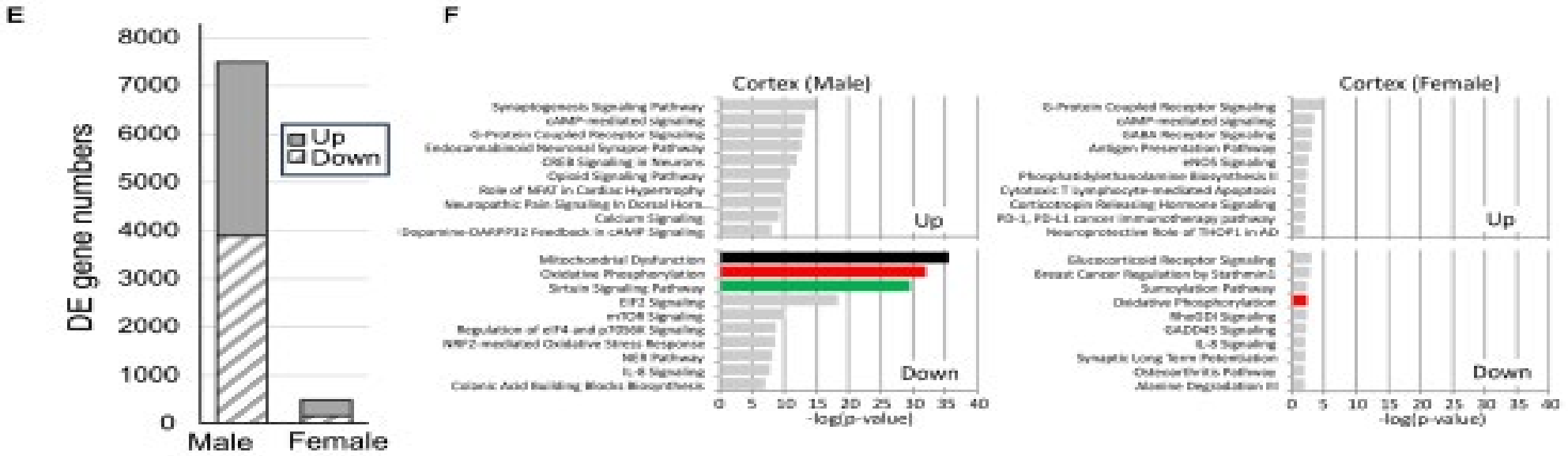
- In the past, scientific and medical research focused on males, with the thinking that males and females would have similar incidence, symptoms, progression, and prognosis when the subjects had the same diagnosis.
- Much of the time, the control group were males even for disorders that impacted women's health (except for pregnancy).
- When females had neurological or ill-defined symptoms, they were sometimes attributed to hypochondria or hysteria.
- However, multiple studies have shown over many years that females are more likely to be diagnosed with (more susceptible to) MS than males.
- Studies have also shown that males with MS are more likely to have worse disease progression than females.
- What factors influence susceptibility and progression differences in males and females?

Animal Model of MS: Experimental Autoimmune Encephalomyelitis (EAE)

- In EAE, encephalomyelitis is induced in the experimental animal. Several small mammal species have demonstrated that females were protected from disease while they were pregnant. This protective nature of pregnancy is seen in humans.
- Does that mean that hormone levels during pregnancy are protective or is the immune system different during pregnancy?
- Only research including both males and females could answer the questions raised by this observation.
- In EAE experiments, female animals were more susceptible to relapsing-remitting disease.
- Thus, this animal data changed how researchers regarded female subjects compared to male subjects: It wasn't that the females complained more! There had to be a biological mechanism for the observed differences.

Findings from 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.



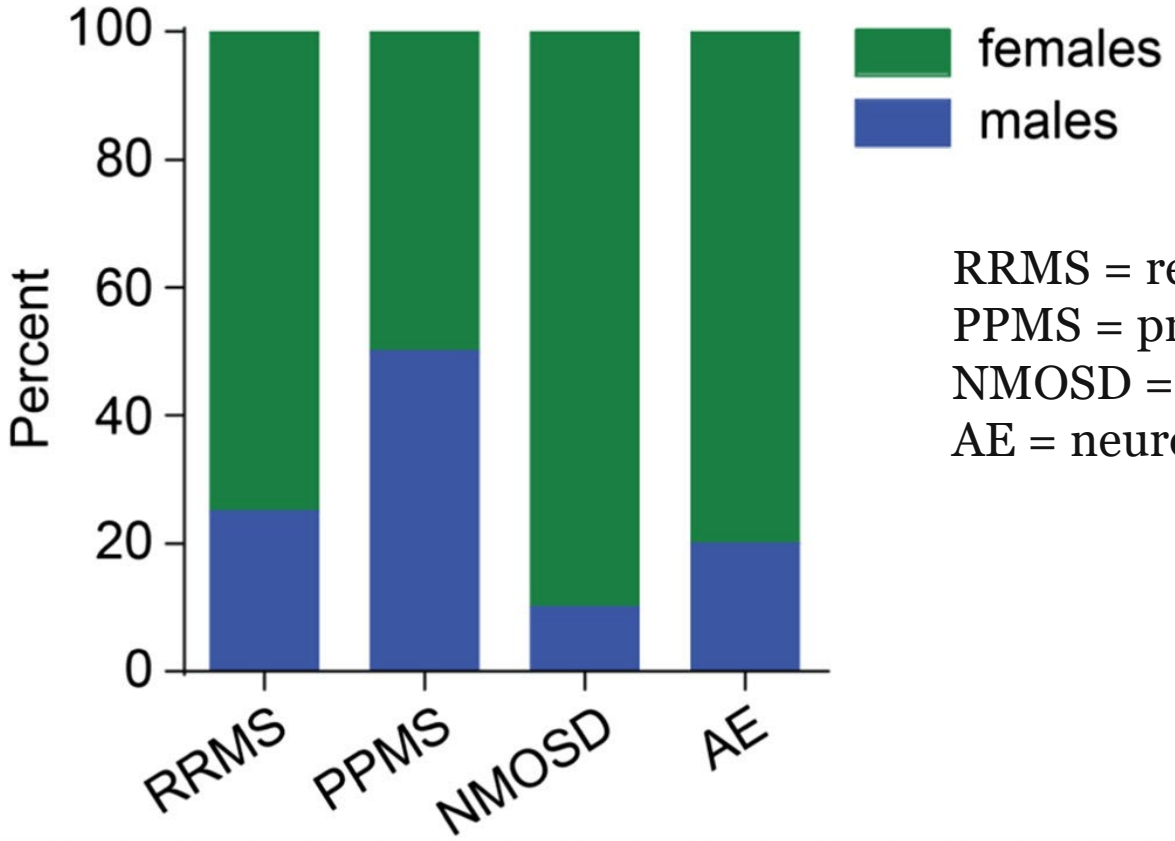
DE = differentially expressed

Itoh et al. 2023

MS Disease Complexity

- MS is an autoimmune disease of the nervous system. Thus, it involves both the immune system and the nervous system.
- T lymphocytes are activated, then able to migrate across the blood-brain barrier.
- Cytokines 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 different presentations of MS:
  - Relapsing-remitting (RRMS)
  - Secondary progressive (SPMS)
  - Primary progressive (PPMS)
  - Other more rare autoimmune presentations, and MS-like encephalitis

Female to Male Ratio of MS Subtypes



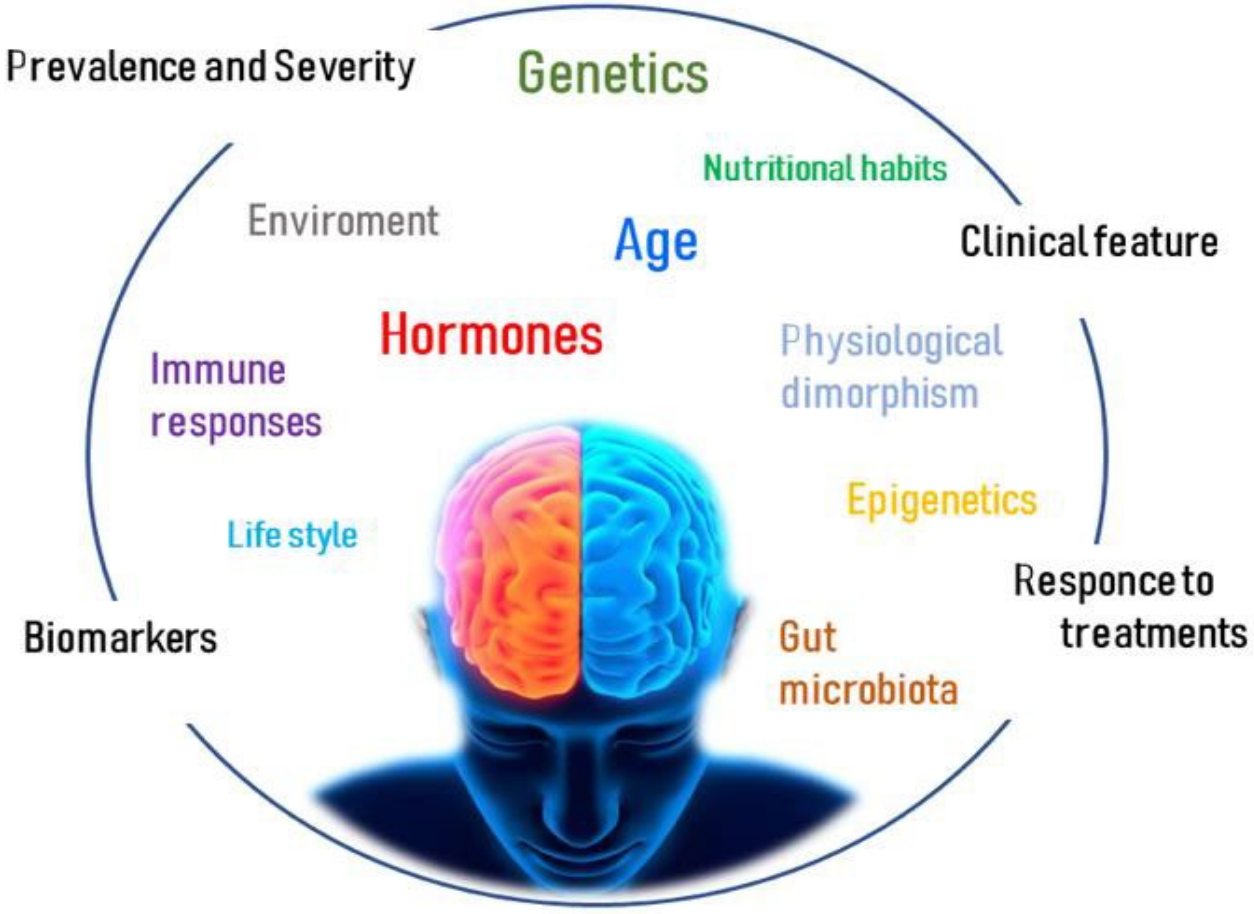
RRMS = relapsing-remitting MS
PPMS = primary progressive MS
NMOSD = neuromyelitis optica spectrum disorder
AE = neuronal antibody-mediated encephalitis MS

Gold et al 2019

Increasing Numbers of Females with MS Over Time: Why?

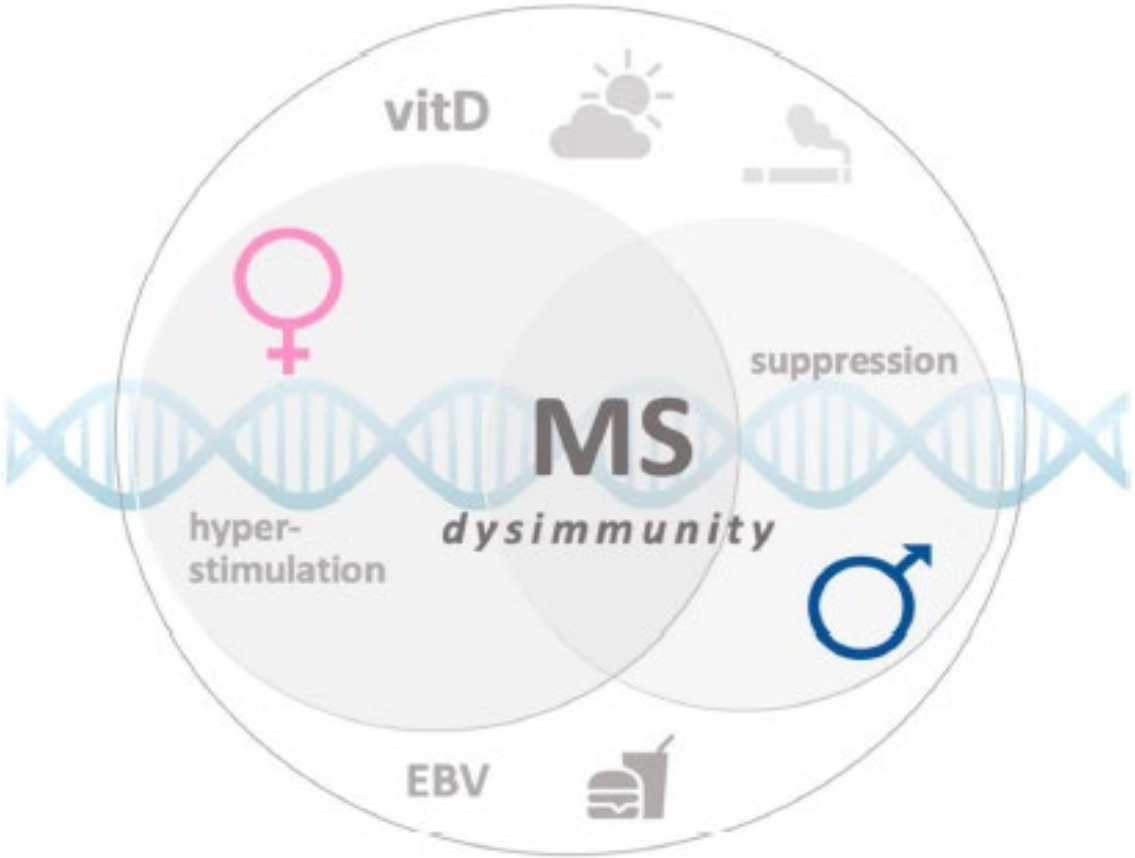
- The female to male ratio of MS incidence varies by geographic region. It is approximately 2.5:1 to 3.5:1 now.
- The ratio has only increased over time.
- Research has demonstrated that the number of male patients with MS has remained steady, while the number of females with MS has increased.
- Potential 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?

Factors that Influence Multiple Sclerosis and Other Neurodegenerative Diseases



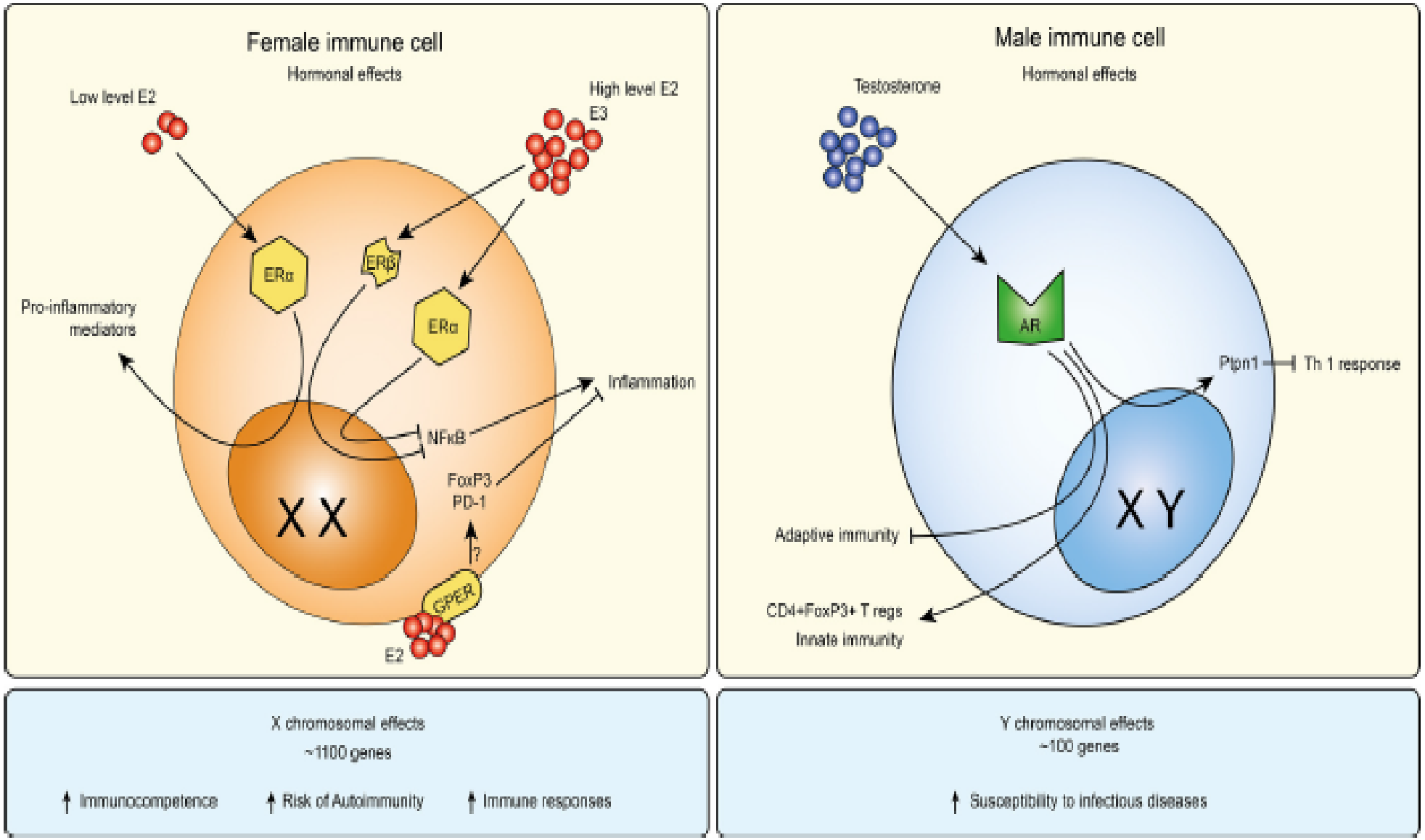
Bianco et al 2023

Risk Environmental and Genetic Factors in MS Sex Bias



Angeloni et al 2021

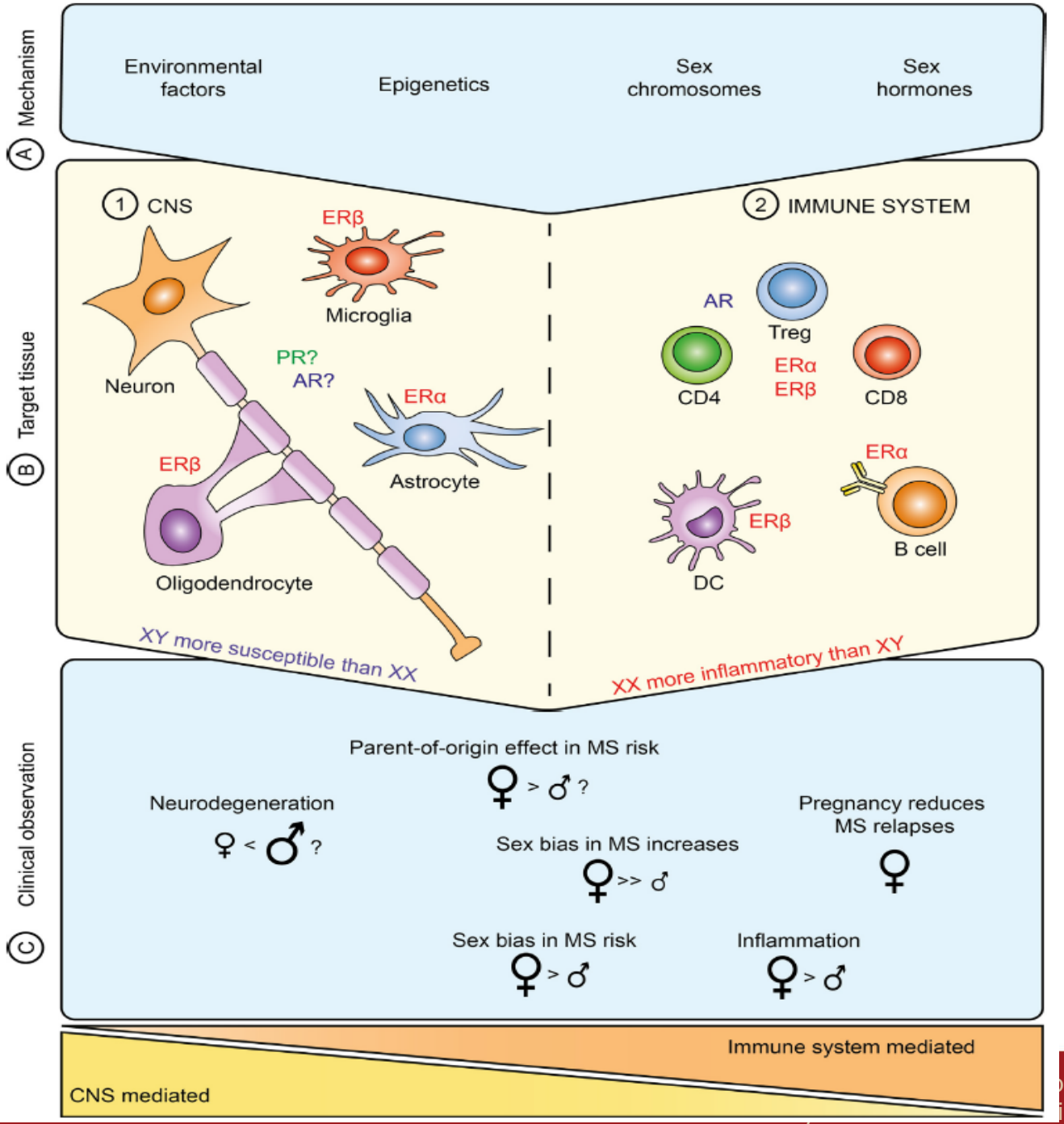
Effects of Hormonal and Genetic Sex Differences on the Immune System



Ramien et al 2016

E2 = Estriol; E3 = Estradiol

Clinical Evidence and Potential Mechanisms of Sex Differences in the Pathogenesis, Activity, and Progression of Multiple Sclerosis

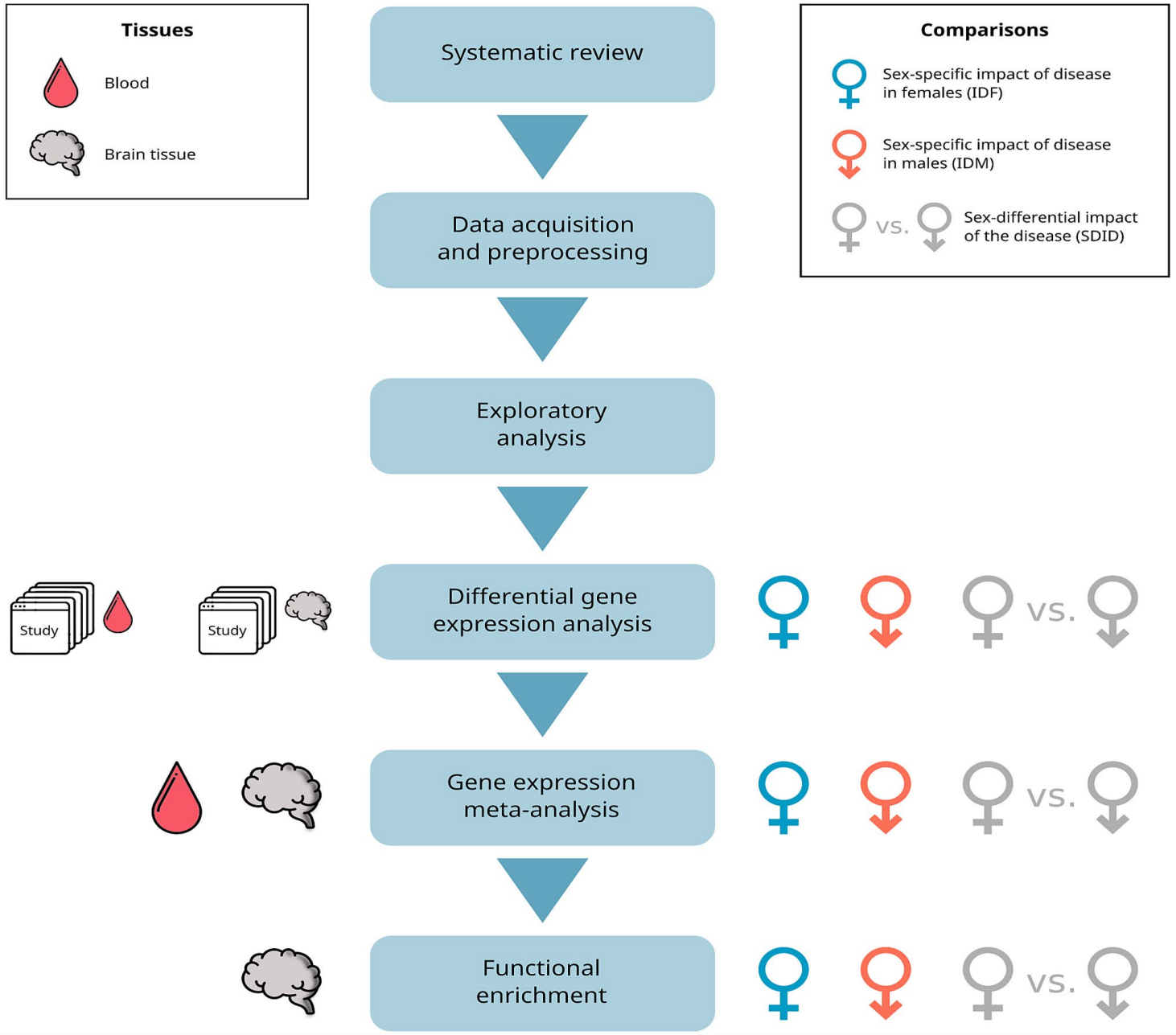


Ramien et al 2016

Analyzing the Transcriptome (or Other –Omics) May Help Reveal Mechanisms

- 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 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 methods are looking at metabolomics, or the metabolic and endocrine products of the genome, or the microbiome.

Flowchart Describing a Deep Transcriptome Meta-analysis that Revealed Sex Differences in Multiple Sclerosis

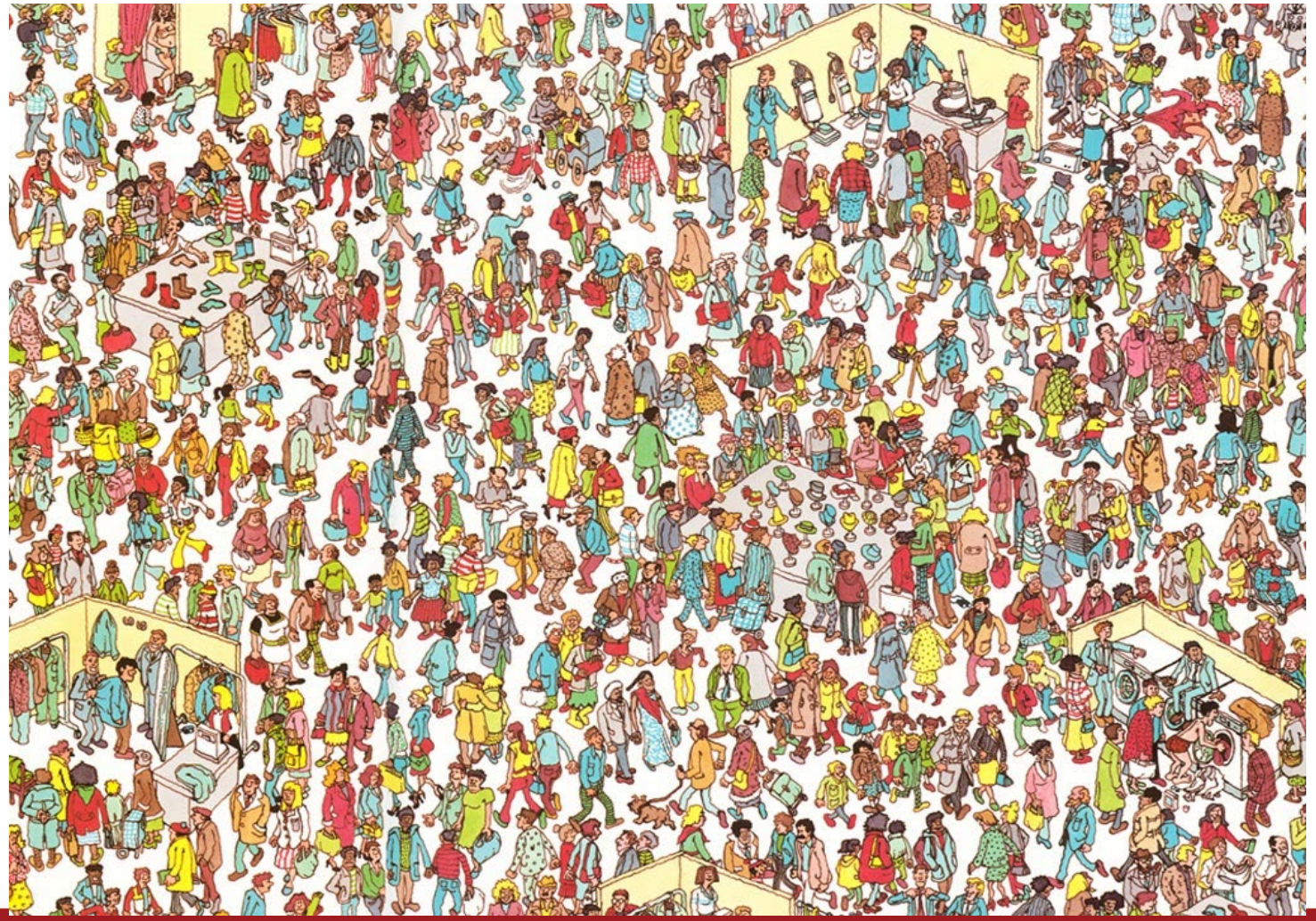


Catala-Senent et al 2023

Transcriptomics and Other –Omics Analysis is Complex!

- -Omics includes genomics, epigenomics, transcriptomics and expression studies, proteomics, metabolomics, microbiomics, and more.
- Databases already exist with transcriptome and other –omics data. The data is often freely available to other researchers.
- How trustworthy is this data?
- If a meta-analysis of multiple data sets unintentionally uses flawed data, the result will be distorted, and the flaw may not be identified easily.
- If a new data set is created (Itoh et al, 2023), how well does it mesh with prior work?

Finding the Right Gene or Data or Study is Hard!



Results of the Deep Transcriptome Meta-analysis

- Data published in 122 papers were screened, and nine separate studies were selected for the meta-analysis. Five 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 “functional” studies showed different altered immune patterns in males and females.

Patient Sex-Related Immune Features

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]

= Increased Inflammation, Estrogen Protection, Immune Response

= Increased Cognitive Decline and Neurodegeneration

Bianco et al. 2023

Possible Biologic Factors in Differing Progression Rates

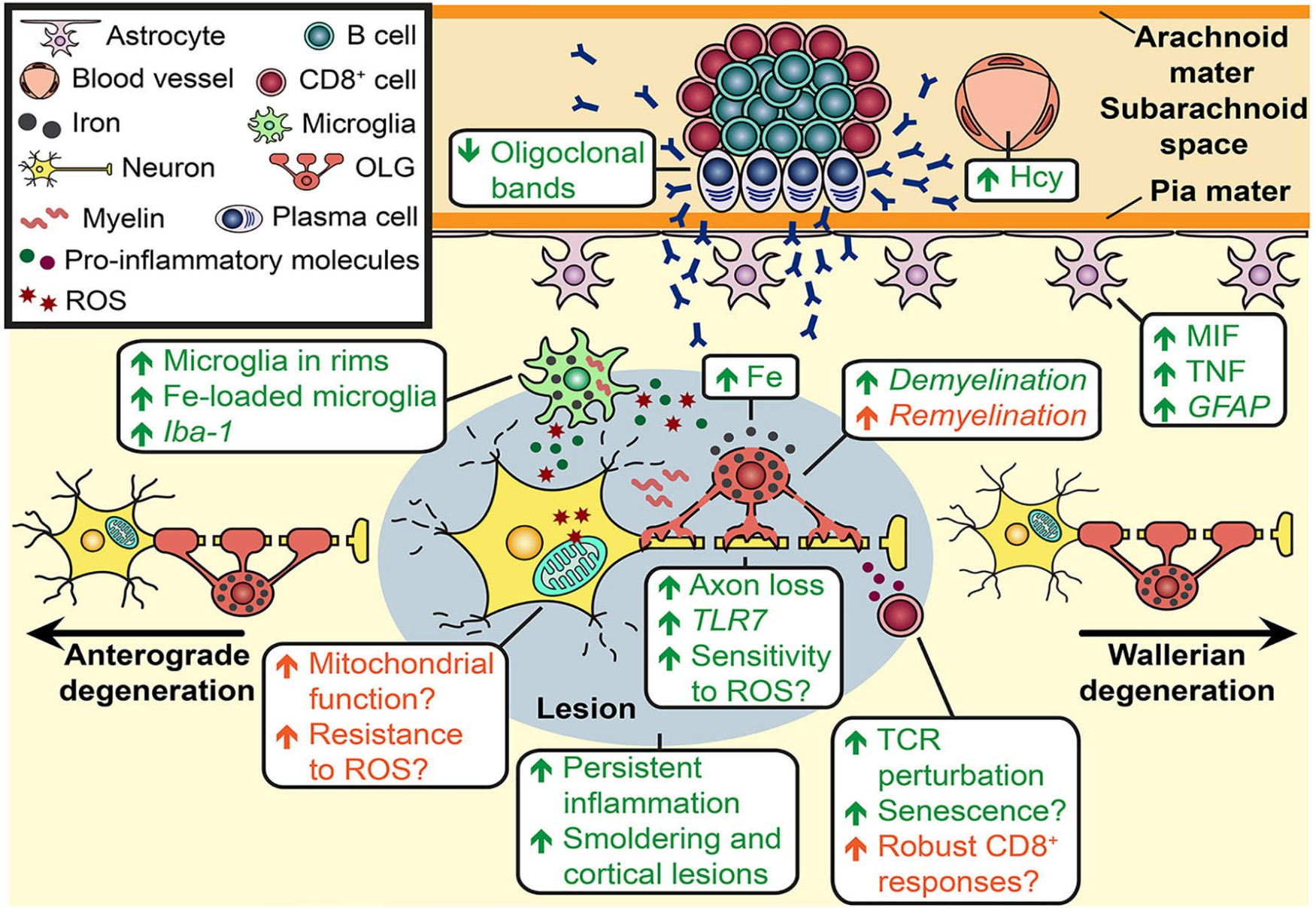
- Neurological progression and cognitive decline are more rapid in males.
- Both MRI studies and post-mortem exams show persistent inflammation and neurodegeneration in males.
- Mechanisms within cells may contribute to increased damage to axons in MS.
- Male T cells may increase inflammation more than female T cells in both human MS and EAE.
- There are sex differences in how humans react to EBV infections and B cells.
- Females with MS have increased numbers of CD56 NK cells in the blood.
- There is a difference in microglial reactivity with age when males and females are compared.
- Astrocytes may be more reactive in males with MS and EAE.

Possible Biologic Factors in Differing Progression Rates (2)

- More iron accumulates at the rim of active MS lesions and in the deep gray matter in males with MS.
- There is also a difference in the susceptibility to demyelination and repair when comparing males and females.
- 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.
- The next figure tries to put much of this together.
- Note: ROS = reactive oxygen species and OL = oligodentrocytes

Cellular Mechanisms that May Be Involved in Sex Differences in MS Progression

Green = male
Orange = female
Italics = EAE model



Alvarez-Sanchez and Dunn 2023

Sex Differences in Treatment Opportunities

- In Austria, there is a nation-wide registry of MS patients. This cohort is more geoethnically similar than studies performed in diverse areas.
- In a study analyzing 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.

Looking for Zebras Among the Herd

Words of Caution

- MS is a complex disorder and thus sex effects in MS may be categorized in a number of ways:
  - 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.

Summary

- Significant sex differences 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!

Thank you!

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- I also thank all of the MS patients, their families, and the clinicians and researchers who contributed to the studies I have referenced.

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