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Neuron

Review

# Mechanisms of sex differences in Alzheimer's disease

Chloe Lopez-Lee,[1,2,3] Eileen Ruth S. Torres,[1,3] Gillian Carling,[1,2] and Li Gan[1,2,*]
[1]Helen and Robert Appel Alzheimer's Disease Institute, Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA
[2]Neuroscience Graduate Program, Weill Cornell Medicine, New York, NY, USA
[3]These authors contributed equally
*Correspondence: lig2033@med.cornell.edu
https://doi.org/10.1016/j.neuron.2024.01.024

## SUMMARY

Alzheimer's disease (AD) and the mechanisms underlying its etiology and progression are complex and multifactorial. The higher AD risk in women may serve as a clue to better understand these complicated processes. In this review, we examine aspects of AD that demonstrate sex-dependent effects and delve into the potential biological mechanisms responsible, compiling findings from advanced technologies such as single-cell RNA sequencing, metabolomics, and multi-omics analyses. We review evidence that sex hormones and sex chromosomes interact with various disease mechanisms during aging, encompassing inflammation, metabolism, and autophagy, leading to unique characteristics in disease progression between men and women.

## INTRODUCTION

First described in a female patient in 1901, Alzheimer's disease (AD) is characterized pathologically by amyloid-β (Aβ) plaques and fibrillar tau tangles. It is the most common form of dementia, and its prevalence continues to rise at an alarming pace.[1] Two-thirds of AD patients are women,[2] and women have a greater lifetime risk of developing AD (1 in 5) compared with men (1 in 10).[1] Although there has been debate over whether this difference is due to the longer lifespan in women, sex has been shown to modulate risk factors and potential disease-causing mechanisms.[3] In addition to biological factors (e.g., chromosomal, epigenetic, or hormonal differences), there are also psychosocial and cultural factors (e.g., access to education, gender differences) potentially contributing to disease risk. In this review, we focus on biological factors to examine sex differences in fundamental disease mechanisms that contribute to neurodegeneration.

Clinical trials are historically biased toward males. Women are frequently excluded for safety reasons due to possible pregnancy, hormonal fluctuations, and contraceptive use.[4,5] Preclinical studies have perpetuated this bias by primarily using male mice only or mixed sex with insufficient power in either sex, often due to increased costs. Exclusion of either sex drastically narrows the applicability of findings to half of the population and hinders the field by making sex-based analyses impossible. Male-biased research has led to inadequate risk calculations and treatment guidelines for women, as seen in drugs for cardiovascular disease.[6] To yield meaningful data and develop efficacious drugs, it is crucial to design experiments with sufficient sample sizes for each sex. This is especially true for mixed sex datasets, which must include a similar representation of male and female samples, with enough power in both sexes independently. Even then, in phenotypes with strong sex differences, one sex can still skew results, reiterating the need for conducting sex-split analyses. Because male-biased research is exceedingly common, the study of sex differences suffers from a relative paucity of available data, especially in complex disease models. This review summarizes sex-stratified findings in AD and related dementias. We believe that understanding the mechanisms underlying sex differences in AD is a crucial step in precision medicine that will lead to more accurate diagnoses and effective treatments for both men and women.

Despite the advancements and growing recognition of sex differences, it is also vital to acknowledge the methodological challenges within the field. For instance, genome-wide association studies (GWAS) have significantly advanced our understanding of genetic anomalies linked to AD. Although numerous X-linked variants are present in the genotyping chips used in GWAS, it is common for studies to discard X chromosome data during the quality control phase. Additionally, when including sex chromosomes in the analysis, the standard autosomal techniques are frequently employed, neglecting the distinct inheritance patterns of the X chromosome.[7] This approach, coupled with the diminished statistical power arising from sex-based stratification in studies with limited sample sizes, makes the identification of sex chromosome single-nucleotide polymorphisms (SNPs) challenging. Consequently, correlations between X chromosome SNPs and AD established in a given GWAS can be difficult to verify in subsequent studies.[8,9] The recently developed software tool, XWAS, provides statistical tests to detect the sex-biased effects of SNPs and higher trait variance due to X inactivation.[10] Embracing tools like XWAS and enhancing sample sizes for greater statistical robustness will significantly aid in detecting X-linked genetic associations in AD.

 

Tremendous strides have been made in understanding the intersecting mechanisms that underpin neurodegenerative diseases, including AD.[11] As highlighted in this review, many of these mechanisms exhibit variations based on sex. We begin by discussing aging, the primary risk factor for AD. Men and women undergo distinct aging processes, marked by different hormonal changes, cellular activities, and age-associated health conditions. Recognizing how these variations in aging influence AD's progression and susceptibility can guide us toward more nuanced interventions. Second, GWAS studies have robustly pinpointed the vital role of innate immunity, especially microglial responses, in AD's development. Consequently, sex-specific immune reactions can shape the way neuroinflammatory actions contribute to AD. Third, the sexes exhibit noted metabolic differences, which have implications for cerebral health. Given the association between metabolic dysfunctions and AD, examining it from a sex-conscious perspective can reveal distinct risk factors and points of intervention. Fourth, proteostasis disturbances, manifested as amyloid β deposition and hyperphosphorylated tau tangles, are central pathological features in AD. Notably, females reportedly show a higher tau burden. We then delve into how sex disparities in protein degradation pathways, especially autophagy, can lead to distinctive disease progression patterns in males and females. Lastly, emerging studies suggest a significant role for the gut-brain axis in neurodegenerative disorders. As the gut microbiome composition varies between males and females, understanding these differences is crucial for unraveling the intricacies of AD's etiology and evolution.

Although this review focuses on AD, it should be noted that sex biases in disease prevalence and progression are seen in many neurodegenerative diseases. As with AD, females are at higher risk of multiple sclerosis,[12,13] although males progress faster through the disease.[14] In contrast, Parkinson's disease is more common in men (relative risk is 1.5 times greater).[15] Women show later onset and milder motor impairment and striatal degeneration than men.[16] Similarly, in sporadic amyotrophic lateral sclerosis, men are more likely to develop the disease, although prognosis and survival time does not differ between sexes.[17] Thus, understanding mechanistic underpinnings of sex differences will be informative beyond the context of AD.

### Sex chromosomes and sex hormones

The canonical framework for investigating biological sex differences involves two primary sources of sex differences: gonadal hormones and sex chromosomes. Gonadal hormones are primarily produced by ovaries or testes and, as a result, are accessible to manipulation by techniques such as gonadectomy and exogeneous hormone application. The most commonly studied gonadal hormones include estrogen, testosterone, and progesterone. Studying sex chromosomes generally involves comparing XX and XY genotypes. The four core genotype model,[18,19] which generates four genotypes with varying gonad-sex chromosome combinations (i.e. XX with testes, XY with ovaries, XX with ovaries, XY with testes), has been used to disentangle effects of sex chromosomes from gonadal effects. In this model, an effect persisting in ovaries/testes regardless of sex chromosome background represents a gonad effect whereas persistence in XX/XY genotypes regardless of gonadal background is a sex chromosome effect. Trisomy models, which contain three sex chromosomes, enable study of the effects of X versus Y chromosomal dominance. Application of these models thus far has provided insight into the fundamental functions of sex chromosomes and gonadal hormones, as well as how these functions are perturbed in disease-associated processes.

## SEX HORMONES

During aging, women uniquely undergo menopause, a process that occurs over approximately 14 years. During perimenopause, the 1–4 years immediately before menopause, estrogen levels become highly variable. This fluctuation initiates estrogen-related dysfunction in metabolic, inflammatory, and sensory-processing-related molecular pathways.[20,21] Mounting evidence suggests that loss of ovarian hormones during perimenopause contributes to female vulnerability to AD.[22,23] In contrast, men do not appear to undergo a perimenopause equivalent, and instead undergo an overall lesser and more gradual age-dependent decline in the primary male gonadal hormone testosterone.[24] This slower transition may mitigate age-related dysfunction in male hormonal pathways compared with those of females. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are other gonadal hormones affected by the menopausal process in women. Post-menopausal women have up to 10-fold more LH than men.[25,26] LH and FSH both trigger androgen/estrogen production and are often regulated in the same direction. FSH has been shown to contribute to AD pathological burden and downstream cognitive impairment.[27]

Estrogen receptors (ERs) are found throughout the brain and regulate various physiological processes. In cell-based and animal models, estrogen appears to be protective against AD pathology. Specifically, studies suggest that estrogen may reduce levels of Aβ by stimulating the generation of amyloid precursor protein (APP)-containing vesicles from the Golgi-network, thereby promoting APP delivery to cell surfaces.[28,29] In rodent tau models, administering estradiol decreases tau hyperphosphorylation and increases dephosphorylated tau.[30] Hormone replacement therapy (HRT) during menopause and post-menopause has long been considered as a strategy to combat cognitive decline. Early studies suggested that oral estrogen could reduce dementia risk by 34%.[31,32] However, clinical trial results have been inconsistent, with some HRT combinations even increasing dementia risk.[33] Meta analyses have revealed that the negative impact of HRT on global cognition was mainly observed in those over 60 years old, whereas the fewer studies involving younger participants had more mixed results.[34,35] This indicates that a "window of opportunity" may play a role in the efficacy of HRT on AD progression. One theory holds that a "healthy cell bias" in younger patients closer to menopause may enable HRT to be beneficial compared with cells already impaired by neurodegeneration.[36] In terms of intervention to remove hormones rather than replace them, excision of both ovaries and fallopian tubes before menopause worsened age-related atrophy of the entorhinal cortex and amygdala along with increasing dementia risk and risk of other diseases.[37–39]

Encouragingly, a recent study using data from the European Prevention of Alzheimer's Dementia cohort showed that, in

patients carrying apolipoprotein E4 (*APOE4*), HRT improved delayed memory and was associated with higher entorhinal and amygdala volumes than non-users and non-APOE4 carriers.[40] Earlier HRT introduction in APOE4 carriers was also associated with larger hippocampal volumes. This study supports the hypothesis that estrogen modifies AD progression in females in an *APOE4*-specific manner.

## SEX AND NON-SEX CHROMOSOMES

Females typically carry two X chromosomes, one of which undergoes inactivation. However, at least 23% of X-linked genes on the silenced chromosome escape X inactivation in women, resulting in elevated female-specific expression.[41] Of the escapee genes, female:male expression spans 0.50–2.25 but typically falls at ∼1.33, indicating 33% higher female gene expression compared with males.[41] This sex-biased expression is especially important because the functions of the >1,000 genes on the X chromosome span many functions, including immunity and development.[42] The Y chromosome appears to contain relatively few genes and primarily consists of pseudogenes.[43] Approximately 54 genes are homologous between the X and Y chromosomes, including *IL9R*, *TMSB4X/Y*, and *CSF2RA*.[42] Males also have biological mechanisms to enhance expression of X-linked genes, known as dosage compensation. For example, the epigenetic MSL3 complex increases expression of genes on the male X chromosome.[44] A study using the four core genotype (FCG) model showed that, regardless of gonad, T lymphocytes with XY chromosomes express higher levels of the X-linked genes *Msl3*, *Prps2*, *Hccs*, *Tmsb4x*, and *Tlr7* than XX cells.[45] It is worth noting that a recent study of the current FCG model revealed an aberrant translocation of 9 X-linked genes (i.e. *Hccs*, *Amelx*, *Arhgap6*, *Msl3*, *Frmpd4*, *Prps2*, *Tlr7*, *Tlr8*, *Tmsb4x*) in the X chromosome of XY mice, resulting in an artificially higher expression in XY than in XX genotypes.[46] Expression of autosomal genes does not appear to be affected. The elevated expression appeared to be cell-type dependent, and neither expression in brain cell types nor translation to protein level was examined. Nevertheless, appropriate controls would be needed when reporting the chromosomal effects using the current FCG model.

X-linked genes have both protective and detrimental roles in neurodegenerative diseases. The X-linked gene *Kdm6a* is associated with reduced mortality and protection against cognitive deficits in a human APP mouse model.[47] Several X-linked genes, including *IL2RG*, *RAB9A*, and *EMD*, are also associated with female-specific cognitive decline or tau pathology in human AD and aging.[48] The X-linked escapee gene *Usp11* may contribute to increased female vulnerability to tau by increasing levels of tau acetylation, which impedes tau degradation.[49,50] Interestingly, global knockout of *Usp11* reduced acetylated tau to a lesser extent in male tau mice than in female equivalents.[49] *Usp11* is also engaged in a feedback loop with estrogen,[51] and age modifies its extent of X inactivation.[52]

Aging markedly affects the prevalence of mosaic loss of the Y chromosome, which has been linked to AD and other age-related disorders.[53–56] AD brain and plasma transcriptomics indicate that downregulation of the Y chromosome across brain regions is associated with higher age-related risk of developing AD.[57] The specific cell types exhibiting loss of Y can influence downstream disease phenotypes, as AD is associated with more frequent loss of Y in natural killer cells.[53]

The expression and splicing of genes on non-sex chromosomes also exhibit sex differences.[58] Bulk transcriptomic profiling of 59 human brains showed that autosomal genes exhibit sex-biased exon usage. Using exon-specific probes, 145 genes were identified with sex differences in exon usage, including TREM2 receptors *TYROBP*, *KDM5C*, and *CD44*.[59] Baseline sex differences in autosomal gene expression also occur in specific cell types, such as microglia.[60]

Epigenetic mechanisms contribute to sex differences in autosomal and sex chromosome expression. Estrogen acts as an epigenetic factor by directly altering histone methylation and deacetylation, as well as altering expression by recruiting ERα to gene promoters.[61] In mice, estradiol appears to maintain active chromatin states in both male and female neurons, whereas testosterone primarily promotes chromatin activation and repression in male neurons only.[62] However, the top expression quantitative trait loci in sex-biased autosomal gene transcripts reside in enhancer regions, not in estrogen nor androgen receptor binding motifs.[63] In terms of sex chromosomes, X inactivation is governed by the long non-coding RNA, Xist. However, other lncRNAs, such as Tsix, regulate Xist in mice, although it is unclear whether this function is shared in humans.[64] MicroRNAs (miRNAs) were also recently identified as regulators of sex-specific responses in a mouse model of tau pathology.[65] At baseline, male microglia exhibit higher expression of 61 miRNAs, and removing the miRNA assembler *Dicer* exacerbates tau pathology and reactive microglia in males but not females.[65] Thus, sex differences in neurodegeneration result in part from interactions of sex hormones as well as sex-biased gene expression from autosomes and sex chromosomes.

### Sex differences in aging

Aging is the predominant risk factor for AD and other neurodegenerative diseases. Men and women age differently, and sex differences in aging occur across tissues and are not restricted to the brain.[41,66] Although women live longer than men in general, women often perform worse in physical function examinations at the end of life.[66,67] Men and women's aging differences are often attributed to sex chromosomal and hormonal-driven differences in biology.

Aging is a complex biological process characterized by progressive deterioration of an organism's physiological functions over time, including mitochondrial dysfunction, genomic instability/telomere attrition, epigenetic alterations, cellular senescence, loss of proteostasis, and immune aging. Two interconnected schools of thought seek to explain molecular aging: senescent and programmed theories[66] (Figure 1). Senescent theories focus on damage accumulation, including disposable somas, reactive oxidative species (ROS), and mutation accumulation. Over time, these cells experience wear and tear, ultimately reaching a state of senescence in which they can no longer divide, contributing to the aging process. On the other hand,

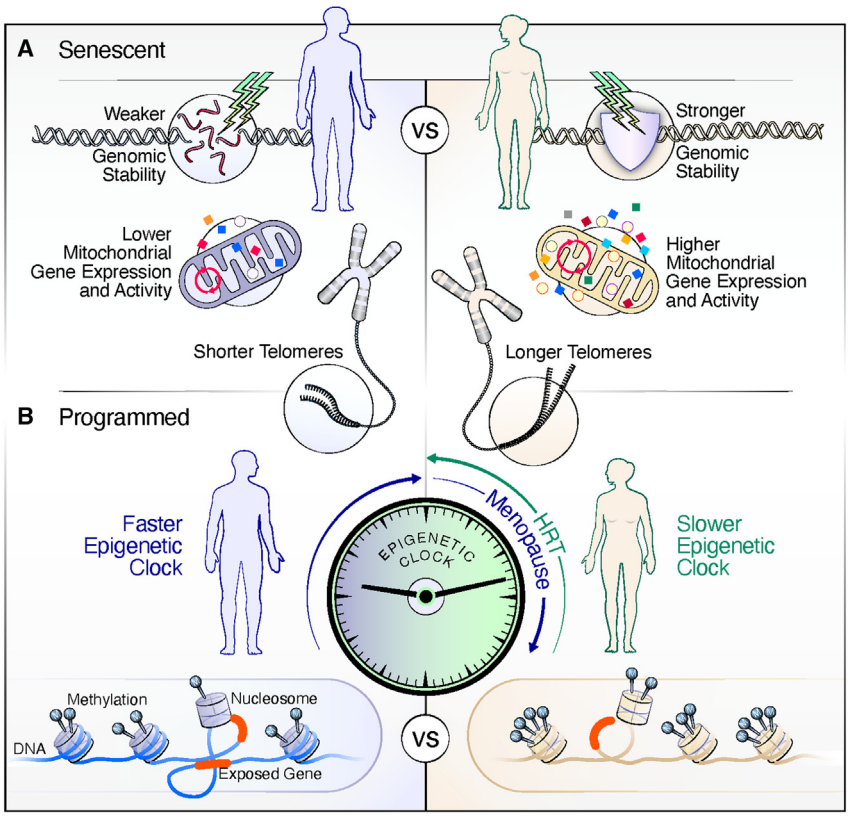


**Figure 1. Sex differences in senescent and programmed theories of aging**

(A) Senescent aging is characterized by accumulation of cellular damage. Higher levels of estrogen in women protect against genomic instability and correspond to higher mitochondrial gene expression and activity.

(B) Programmed aging implicates a predetermined process controlled by genetics and epigenetics. Men and women have sex-dependent differences in DNA methylation across the genome. Epigenetic clocks show a higher "epigenetic age" in men; however, menopause in women speeds up the epigenetic clock, but that action appears to be reduced with HRT. Telomere shortening contributes to both of these theories as cells may only undergo a set number of divisions; thus, telomere length acts as another biological clock.

the programmed aging theory posits that aging is a predetermined process controlled by our genetic makeup. This theory implies that both mitotic and post-mitotic cells are programmed to function for a certain period of time before they start to deteriorate.

Cellular senescence is a state of irreversible cell-cycle arrest that occurs as a response to various stressors, such as genomic instability/DNA damage, oncogene activation, telomere shortening, and mitochondrial dysfunction (Figure 1). Genomic instability is a hallmark of biological aging and DNA damage accumulates throughout life as repair mechanisms become less efficient.[68] Hormonal differences, such as the elevated presence of estrogen in females, protect against cellular senescence via reduction of oxidative stress and ROS.[69] Moreover, the *Tert* gene, which programs telomerase, contains an estrogen receptor element, enabling estradiol to protect against telomere shortening.[70,71] Intriguingly, the expression of senescence markers, such as *p16Ink4a* and *p21Cip1*, appears to accelerate in male mice during aging, but the rate of senescence in female mice is greater near the end of life.[72] Additional studies are needed to explain how sex-biased senescence at specific stages of aging contributes to differences in disease onset, progression, and severity in various neurodegenerative diseases between male and female mice as well as how this translates to men and women.

Cellular senescence is often associated with mitochondrial dysfunction, and sex differences in mitochondrial function have been observed, with women generally showing greater mitochondrial gene expression and activity,[73] in part due to mitochondrial estrogen receptors.[74,75] Estrogens influence mitochondrial functions, such as reserve capacity and glycolysis, in a protective manner, which may explain why women experience delayed mitochondrial aging compared with men.[76,77] Sex chromosomes have been shown to regulate mitochondrial genes, such as *Pdk4* and *Hk2*, and regulate metabolic phenotypes associated with aging, such as weight gain.[78] Sex differences in metabolism likely affect mitochondrial dysfunction-dependent senescence, indicating the importance of better understanding sex-specific metabolic contribution to senescence.

In support of programmed theories in aging, several epigenetic clocks have been described based on patterns of DNA methylation, which plays a crucial role in gene regulation. The epigenetic age in males often exhibits higher predicted biological age than in females (Figure 1), and this difference is seen across various tissues and contributes to the mortality risk disparity between the sexes.[79] In women, earlier menopause is linked to increased epigenetic age, whereas HRT is associated with lower epigenetic age[80,81] (Figure 1). Genome-wide DNA methylation differences between sexes show significant disparities, particularly on the X chromosome.[82,83] Using the AD neuroimaging initiative database, a study examined the association between epigenetic age acceleration, cognitive impairment, sex, and biomarkers of AD risk. Although sex, cognitive impairment diagnosis, and *APOE ε4* alleles were not associated with epigenetic age acceleration, females exhibit a slightly more accelerated epigenetic aging using the skin and blood clock in the transition from normal cognition to cognitive impairment than males.[84] A recent meta-analysis study found age-related sex differences in DNA methylation patterns, with differentially methylated sites being enriched in imprinted genes but not in sex-hormone-related genes.[85] Thus, it is important to consider sex differences when using epigenetic aging clocks for research or clinical purposes, as they can impact the accuracy of age predictions and the identification of individuals at higher risk for age-related diseases.

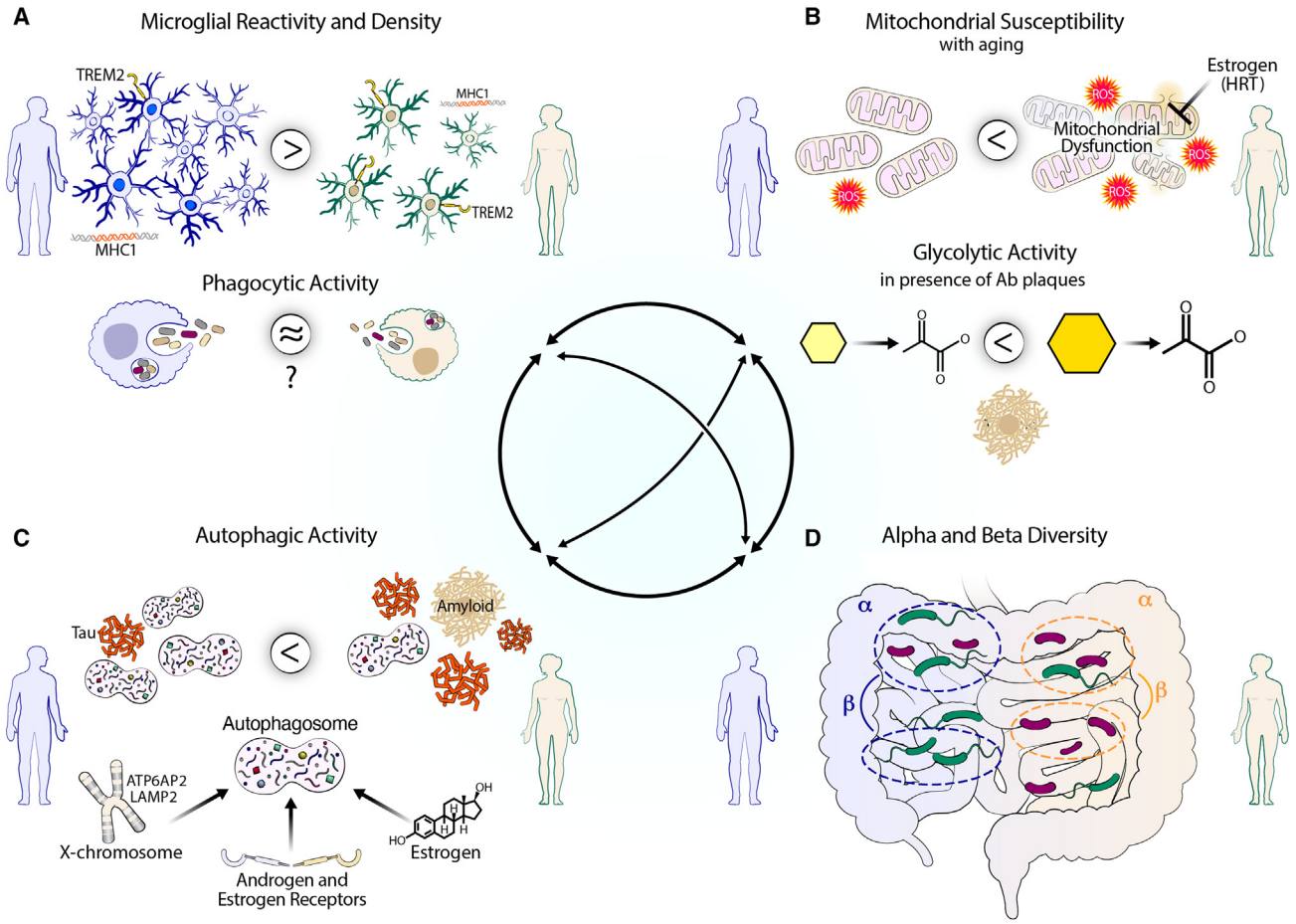


**Figure 2. Sex differences in mechanisms associated with neurodegeneration**

(A) Sex differences in microglia number, response, and phagocytosis.

(B) Sex differences in metabolism during aging. Estrogen protects mitochondrial health in females; reduced estrogen during menopause may lead to metabolic dysregulation and increase female vulnerability to cellular stress and disease.

(C) Basal autophagy is lower in women than men throughout life and may contribute to more tau and amyloid accumulation. Chromosomal and hormonal factors may also contribute to this.

(D) Men and women have distinct alpha and beta gut microbiome diversities. The gut microbiome and neuroinflammation, metabolism, and autophagy are all linked to each other, increasing the complexity of sex differences in neurodegeneration.

Telomere shortening is important in both the senescence and programmed aging theories. In the senescence theory, after a number of divisions, cells hit the so-called Hayflick limit, mainly due to telomere shortening, and this leads to aging. On the other hand, the programmed aging theory considers telomere length as a biological clock that determines cell lifespan and, thus, influences aging. Women generally have longer telomeres than men do, a distinction noticeable from birth[86] and also evident in mice[87] (Figure 1). This variance aligns with the observed difference in average lifespan between the sexes. Notably, the *DKC1* gene, encoding dyskerin, affects telomerase activity in embryonic stem cells and is expressed from both X alleles in female embryonic cells, potentially leading to elongated telomeres prior to embryo implantation.[88] This phenomenon could be instrumental in establishing sex disparities in telomere length and lifespan. Further studies are warranted to clarify the relationship between embryonic telomerase levels and sex-based differences in telomere length and longevity.

As the largest risk factor for multiple neurodegenerative diseases, including AD, aging significantly affects several physiological and molecular processes, such as inflammation, autophagy, metabolism, and the microbiome. These age-related changes can contribute to the onset and progression of AD. Interestingly, sex differences modulate these processes, which may explain the observed sex disparities in AD. The upcoming sections will delve into each of these mechanisms to explore how they are affected by aging and sex, and the resulting implications for AD (Figure 2).

## Sex differences in maladaptive innate immune responses

Large-scale human genetic and transcriptomic studies, combined with experimental evidence from animal models, suggest that innate immune mechanisms are a driving force in the pathogenesis of neurodegenerative diseases. In AD, large-scale GWAS revealed that many risk genes are specifically expressed

or enriched in microglia and myeloid cells in the periphery.[89] Whole-genome sequencing studies led to the discovery of rare variants of TREM2, a transmembrane receptor highly expressed in microglia and myeloid cells, that increase AD risk 2- to 3-fold.[90,91] In a co-expression network analysis, the immune and microglia module—of which TREM2's adaptor TYROBP is a key component—was most relevant for late-onset sporadic AD pathology.

Microglia exhibit sex differences in several innate immune responses at baseline. Sex differences in microglial number are both age- and region-dependent, with a male increase in microglial number across the cortex, hippocampus, and amygdala by 13 weeks of age.[92] Male microglia had higher *MHC-I* gene expression, potentially indicating a favorability for antigen presentation compared with females.[92] When presented with fluorescent beads, male and female microglia display similar phagocytic ability; however, this is likely context-dependent, as female microglia exhibit greater phagocytic ability than male equivalents when incubated with palmitic acid.[93] Whether sex differences in phagocytosis emerge in the presence of endogenous stimuli seen in neurodegeneration, such as myelin debris or Aβ plaques, remains unknown.

Importantly, microglial sex differences also persist in neuroinflammatory phenotypes. Young male and female microglia display sex-specific transcriptomic profiles in which male microglia upregulate pro-inflammatory genes, including *NF-κB*[60] (Figure 2). Aging may induce a switch in directionality of sex differences in inflammation, as microglia from aged females demonstrate stronger activation of inflammatory processes than those from aged males.[94] Notably, several immune genes linked to inflammation are present on the X chromosome, such as *Il1rapl1*, *Il2rg*, *Tlr7*, and *Ikbkg*. However, these genes are subject to complex relationships with estrogen[95] and aging[96] and thus do not necessarily translate to higher global inflammation in women. Furthermore, macrophages with an intact Y chromosome exhibited higher expression of pro-inflammatory *Il1b* and *Ccr2*.[97] The male skew toward inflammatory responses has been evidenced by higher numbers of activated microglia. Male and female microglia also respond differently to acute inflammatory stimuli.[98] Administration of lipopolysaccharide *in vitro* affects the microglia transcriptomes of neonate males and females differently: male microglia upregulate pro-inflammatory IL-1B and TLR4. *In vivo*, administering estradiol with lipopolysaccharides worsened microglial IL-1B upregulation in female hippocampi but not male equivalents, reiterating the divergent responses elicited by estrogen depending on sex.[99]

Sex differences also occur in AD rodent models. In AppNL-G-F knockin mice, where the *APP* gene harbors the Swedish (*NL*), Beyreuther/Iberian (*G*), and Arctic (*F*) familial AD mutations, amyloid deposition accelerated the transformation of homeostatic microglia to activated response microglia (ARMs). Compared with male mice, microglia in female AppNL-G-F mice progress faster into ARMs.[100] In another AD mouse model, which used APP/presenilin 1 (PS1) mice hemizygous for the *APP* Swedish mutations (*KM670/671N1*) and *PS1 ΔE9* mutation, females had a greater plaque load than males, accompanied by worse spatial learning impairment.[101,102] Female mice had greater levels of *Trem2*, *Tyrobp*, *Clsd*, and *Ccl6* than genotype-matched males, mirrored by increases in activated microglial morphology.[103] Interestingly, the *R47H* mutation of the prominent innate immune risk gene *TREM2* only exacerbated tauopathy-induced spatial memory deficits associated with pro-inflammatory transcriptomic changes in female mice, not in male mice.[104] Further exploration of the nuances of sex-specific neuroinflammation is required to determine whether such inflammation plays a formative role in downstream neurodegeneration and the underlying mechanisms.

Microglial ApoE may play a critical role in tau-mediated neurodegeneration.[105] Women are more susceptible to APOE4-associated risk, but the driving mechanism underlying this effect is currently unclear. In a transgenic amyloid model (5XFAD) on either a humanized *APOE3* or *APOE4* background, microglial plaque coverage, or ability of microglia to surround a plaque, was highest in male *APOE3* mice, with reductions in plaque coverage induced by both *APOE4* genotype and female sex. Similarly, increased microglial *Trem2* expression was also associated with higher plaque coverage. Interestingly, the pattern of amyloid burden was inversely related to microglial plaque coverage, with *APOE4* genotype and female sex showing the highest amyloid levels. These results suggest sex-biased efficiency in plaque coverage as a possible contributor to the increased AD risk associated with the *APOE4* genotype and female sex.[106]

Human AD studies investigating sex differences remain sparse. In post-mortem tissue from AD patients, microglia appeared uniformly ramified in male brains, whereas female brains exhibited highly diverse microglial morphology.[103] Men also displayed increased microglial density compared to women. In the parietal cortex, the plaque area was greater in women than men but, interestingly, men demonstrated greater amyloid staining in their vasculature.[103] In human AD brains, single-nuclei RNA sequencing (RNA-seq) analyses revealed striking sex-specific transcriptomic alterations associated with the *R47H TREM2* mutation, especially in microglia.[104] However, due to variabilities in human disease conditions and in disease-modifying SNPs, much larger datasets are needed to provide a more comprehensive understanding of sex-specific microglial responses in AD.

## Sex differences in metabolism

Metabolic dysregulation is a key converging process in aging and neurodegeneration, and differs between aging males and females. Alterations in metabolism are detectable at early stages of AD, and impaired energy metabolism precedes cognitive impairment, indicating mitochondrial dysfunction may be a causal factor in the disease.[107,108] AD patients display abnormally low positron emission tomography (PET) measurements of glucose metabolism in several brain regions correlating with disease severity,[109–111] and glucose hypometabolism can be used to predict conversion of mild cognitive impairment to AD.[112] Women appear to have early protection against metabolic dysfunction. Using advanced machine-learning techniques, a study evaluated a comprehensive dataset of brain PET images collected from a wide age range (20–82 years) of cognitively healthy men and women. Aged female brains produced a younger metabolic brain age than male counterparts, according to an algorithm based on regional glucose levels,

oxygen consumption, and cerebral blood flow.[113] Intriguingly, higher female brain metabolism may confer early disease resistance. Women with mild-to-moderate AD appear to exhibit cognitive advantages, which disappear when adjusted for metabolic rate or as disease pathology becomes more severe.[114] Similarly, sex differences are observed in oxidative stress.[115] Women of reproductive age generate higher levels of antioxidant enzymes and lower levels of hydrogen peroxide, NADPH oxidase, and homocysteine than men, showing a protective effect of female sex on metabolic processes.[116,117] The decrease in estrogen levels after menopause removes this sex difference, reducing antioxidant capabilities and increasing ROS production and homocysteine to levels similar to those of men.[117]

These early protective effects of the female sex on brain metabolism may arise from sex differences in the mitochondria. In healthy individuals, peripheral mononuclear blood cells from adult women show significantly higher mitochondrial complexes I, I+II, and IV, as well as increased uncoupled respiration, electron transport chain (ETC) capacity, citrate synthase activity, and ATP levels, compared with men.[73] Similarly, mitochondria isolated from female rodent brains show greater ETC activity, ATP production, and NADPH-linked respiration than males,[118] supporting higher mitochondrial function in females.

Alterations in metabolism are detected at early stages of AD, and impaired energy metabolism precedes cognitive impairment, indicating that mitochondrial dysfunction may be a formative factor in the disease.[107,108] Patients with AD display abnormally low glucose metabolism in the brain that correlates with disease severity,[109–111] and glucose hypometabolism can be used to predict conversion of mild cognitive impairment to AD.[112,119] In animal models of AD, age-induced metabolic remodeling appears to occur earlier in females than in males.[120] In one study of wild-type mice, expression of 44% of genes changed in female mice between 6 and 9 months of age in contrast to only 5% of genes in equivalent males. Of the 44% altered in females, most genes were downregulated, and half of the downregulated genes were involved in energy metabolism. Network analysis of the differential genes in females identified the apolipoprotein clusterin (*Clu*), an AD risk factor, as a central node, connecting decreased bioenergetic metabolism with increased amyloid dyshomeostasis. The decline in energy metabolism in females included functional domains, such as glycolysis, pyruvate dehydrogenase/tricarboxylic acid cycle, and electron transport chain/oxidative phosphorylation.[120] Microglia likely play a key role in enforcing sex differences in metabolic activity in the context of AD, as female microglia from APP/PS1 mice shift to glycolysis in the presence of amyloid plaques, whereas male microglia do not.[103] Although comparisons between wild-type males and females were not the main focus of this study, there also appear to be baseline sex differences in glycolysis in the absence of Aβ plaques.

Unsurprisingly, sex differences in mitochondrial function are regulated by estrogen. Estrogen induces mitochondrial biogenesis and increases mitochondrial respiration in neurons and glia,[121,122] likely due to estrogen-induced increases in PGC1α expression, a transcription factor controlling energy metabolism and mitochondrial dynamics.[118] In the 3xTg mouse model of AD that exhibits both amyloid deposition and tau inclusions, ovariectomy resulted in decreased mitochondrial respiration and increased mitochondrial Aβ levels. These mitochondrial Aβ levels are reversed with estrogen treatment,[123] illustrating the protective role of estrogen on mitochondrial function. Estrogen may exert protective effects through other mechanisms, including increased transcription of mitofusins[123] and mtDNA.[118] Ovariectomy reduces mitofusin levels in rats and increases mitochondrial fission in both wild-type and 3xTg AD mouse brains.[123] Pre-menopausal women have increased mtDNA abundance with age, but after menopause, female mtDNA decreases at a similar rate as in aging men, implicating estrogen in the regulation of mtDNA abundance.[124] Overall, women had more mtDNA in their blood than males at any age.[124] The specific mechanisms by which estrogen affects mitochondrial dynamics through the transcription of PGC1α, mitofusins, and mtDNA should be further explored in the future. Additionally, while ample research connects estrogen regulation to metabolism, there is limited information on the regulatory contribution of sex chromosomes, a critical area to examine in future studies.

Overall, evidence indicates that estrogen exerts beneficial effects on mitochondrial function and oxidative stress, which may provide women with early resilience against disease. Reduction in estrogen levels during menopause may counter these protective benefits, leading to metabolic dysregulation and rendering women more vulnerable to dysfunction. A neuroimaging study found that post-menopausal women show glucose hypometabolism in parieto-temporal cortices reminiscent of early AD pathology,[125] supporting the idea that reduced estrogen contributes to metabolic dysregulation. However, cerebral blood flow and ATP production are also increased in post-menopausal women, and this ATP production positively correlated with global cognitive performance,[125] suggesting a potential compensatory mechanism to adapt to post-menopausal metabolic changes. Future studies should investigate metabolic remodeling in the female brain during menopause to better understand how the brain compensates for loss of estrogenic regulation and to highlight areas of female metabolic vulnerability during the aging process.

## Autophagy

Autophagy dysregulation has been implicated in multiple age-related disorders, including neurodegeneration.[126] The three major types of autophagy are macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA).[127] Macroautophagy involves the formation of a double-membraned phagophore, which turns into an autophagosome and eventually fuses with a lysosome, whereas in microautophagy, cargo is directly taken up through invagination of the lysosomal membrane. In CMA, chaperones transport individual unfolded proteins to the lysosomal membrane, where HSC70 recognizes these substrates and binds LAMP2A for translocation into the lysosome.[127] Both macroautophagy and CMA decline with age, facilitating the buildup of toxic protein aggregates,[127] and the expression of autophagy-related proteins is decreased in the AD brain.[128]

Sex differences in autophagy may contribute to early protein aggregation and disease vulnerability in women. Females have lower basal autophagy than males do throughout their

lifetimes,[126,128] which may allow for higher protein aggregation in women (Figure 2). Reduced autophagic induction or flux results in a failure to clear Aβ and tau protein aggregates, and this aggregation further inhibits autophagy, resulting in self-sustaining pathology.[128] Accordingly, women have higher levels of pathological tau than men do, particularly in individuals with high Aβ pathology or the *APOE4* allele.[129]

Studies point to sex hormones as major regulators of autophagy (Figure 2). Both androgen and estrogen receptors transcriptionally regulate autophagic genes involved in the induction, expansion, and maturation of phagophores.[126] In fact, potential androgen or estrogen receptor binding sites have been identified in the promoter regions of two-thirds of autophagy proteins, and 84% of core autophagy proteins can be transcriptionally regulated by sex hormone receptors.[130] Unlike its effect on mitochondrial regulators, estrogen acts to reduce autophagic gene expression. The presence of estrogen or ERs is associated with suppression of autophagy, and ovariectomized animals or animals lacking ERs have higher basal autophagy in several cell types.[128]

Sex chromosomes may influence autophagic regulation as well. Many genes regulating autophagy are found on the X chromosome, including *ATP6AP2* involved in lysosomal acidification, CMA regulator *LAMP2*, and members of the Rab protein family involved in macroautophagy.[126,127] LAMP2 protein levels are a rate-limiting factor for CMA, and reduction of CMA with aging is attributed to reduced LAMP2A stability on the lysosomal membrane.[127] Thus, sex differences in *LAMP2* expression and function should be further investigated to determine whether they have a role in the described sexual dimorphisms in autophagy.

A recent study exploring the genetic regulation of autophagy, based on sex, demonstrated that phosphatase and tensin homolog (PTEN), which enhances autophagy by inhibiting the protein kinase B (AKT) pathway, is primarily expressed in female mice cortices, whereas Klotho, serving a similar function, is prevalent in male mice cortices.[131] Despite these differences, levels of phosphorylated AKT remained consistent across both sexes, suggesting complementary roles of PTEN and Klotho. Given the association of diminished PTEN and Klotho levels with age-related diseases, analyzing how their balances shift due to aging, pathology, and menopause could yield insights into sex-specific autophagy alterations in disease states.

In sum, lower basal autophagy in females may contribute to early accumulation of protein aggregates, such as the increased tau burden in women,[129,132] creating disease vulnerability. After menopause, loss of estrogen may disrupt the protective estrogenic effects on mitochondrial metabolism, thereby increasing female susceptibility to cognitive decline.

## Gut microbiome

Investigation of the interaction between the gut microbiome and the brain is a relatively new field. On a molecular level, the microbiome is necessary for sex-specific rhythms of gene expression as well as metabolic functions and fat distribution.[133,134] The microbiome also appears to affect processes that have exhibited sex biases and are central to neurodegenerative disease, such as inflammation and phagocytosis[135] (Figure 2). In a mouse model of AD, striking sex differences were observed in the effects of microbiome dysregulation on amyloid plaques.[136] Administering antibiotics acutely during early-life mitigates Aβ pathology and reactive microglial morphology in male but not female APP/PS1 mice.[136] In both baseline and antibiotic-treated states, males and females show sex-specific microbiota profiles. However, antibiotic treatment alters expression of 940 genes in male APP mice but only one gene in equivalent females. Genes with lowered expression from baseline to antibiotic-treated in males were concentrated in immune ontologies, including microglial response pathways. Importantly, Aβ pathology is not ameliorated in microglia-depleted male mice treated with antibiotics, supporting cross-talk between microglia and microbiome in modifying plaque pathology.[136]

Progress has been made in identifying specific microorganisms affected during AD. One study applied machine learning to unveil 19 predictive microbes correlated with levels of Aβ and p-tau in the cerebrospinal fluid (CSF) of humans.[137] Microbes are often clustered based on genomic similarity, called operational taxonomic units (OTUs). In AD patients, richness of the microbiome was reduced via estimates of OTU coverage and number of species compared with controls.[138] Alpha diversity, indicating the richness and abundance of OTUs, was also decreased in AD patients. Beta diversity, the comparison of microorganism composition between samples, was significantly different between AD patients and controls. When monitoring microrganisms by genera and families, 13 genera were significantly different between AD and control along with nine families; 7/13 genera were correlated with Aβ, and 6/13 with p-tau in CSF.[138] Of note, this cohort was 72% female, so some of the observed differences may be sex specific. Few studies have been performed monitoring the microbiome in the simultaneous contexts of AD and sex.

Whether the influence of sex on the microbiome is primarily hormonal, sex chromosomal, or comprises interactions of the two has not yet been defined. The microbiome can influence processing of sex hormones, such as androgen reuptake and excretion.[135] Moreover, gonadectomy and supplementation with the androgen dihydrotestosterone affect microbiota composition.[139] The influence of gonadal hormones on the gut microbiome and peripheral diseases has been reviewed.[140] However, the effect of sex chromosomes on the gut microbiome has yet to be fully explored. Knocking out *Fmr1*, the gene with a causal variant for fragile X syndrome, has been shown to alter several bacterial species.[141] However, a limiting step in exploring the gut-brain axis remains methods to analyze microbiota data in the context of genetic variations. A method was recently developed to analyze X chromosomal associations with microbiome data and applied to a human dataset to uncover multiple X-linked associations.[142] Expansion of GWAS to include the X chromosome here would also be immensely helpful, as autosomal GWAS have been used to elucidate genetic variants associated with microbiome phenotypes.[143] Although much remains to be understood, dysregulation of the microbiome appears to exhibit sex bias and may ultimately contribute to the larger sex differences seen in AD risk and progression.

## Limitations and future directions

AD drug studies thus far have lacked adequate data regarding sex-specific effects. Notably, the recently approved anti-Aβ

treatment for AD, lecanemab, demonstrated much less clinical benefit to women compared with men,[144] an alarming result that has been largely ignored.[145] Moreover, this result was also found in the Emergency vs Delayed Coronary Angiogram in Survivors of Out-of-Hospital Cardiac Arrest (EMERGE) trial for aducanemab,[146] highlighting the prevalence of this concern. A significant number of AD therapeutic studies do not present sex-segregated outcomes.[147] Systematic reviews of cholinesterase and memantine treatments reveal scant reporting on differences in tolerability and efficacy between men and women.[148,149] Studies examining the impact of lifestyle on AD indicate that both sexes seem to respond similarly to lifestyle intervention.[150] However, results from the larger, international World Wide Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (WW-FINGER) study have not yet been shown.[151] In contrast, multi-domain interventions in the comparative effectiveness dementia & Alzheimer's registry (CEDAR) study suggest that women may experience greater benefits than men in terms of cardiovascular and lipid risk metrics, though not in cognitive measures.[152] Going forward, clinical studies should more thoroughly evaluate potential sex-related variations in efficacy and side effects to enhance treatment outcomes for both women and men.

Much is still unknown about the underlying causes of sex differences in disease, and many technical limitations contribute to this problem. Menopause studies in mice have remained difficult because female mice do not experience the typical peri- and post-menopausal stages resulting in low estrogen levels.[153] Ovariectomies and hormone replacement have been used to study the influence of loss of circulating estrogen, but ovariectomies lack the fluctuating estrogen levels seen during perimenopause. The accelerated ovarian failure model utilizes the toxin 4-vinylcyclohexene diepoxide to mimic the estrous acyclicity and fluctuation and low estrogen levels after menopause (reviewed in Marongiu[154]). Application of this model in the context of AD pathology may yield fruitful mechanistic indicators of whether HRT can be beneficial against AD if administered within a specific window. One epigenetic study generated a lifetime estrogen exposure model, which identified an epigenetic signature for breast cancer risk.[155] Such models could provide crucial information if applied to AD, not just for disease progression but also for the timing of HRT.

The combination of models probing sex-based effects with models of disease, such as the FCG model crossed with tau transgenic models, would greatly advance the field. Although such studies often require large sample sizes, such a design could potentially uncover innate sex biases in processes such as aging[156] and demyelination. In studies in which this combination is not used, investigating both sexes within a disease model is necessary. We have seen that when these analyses are performed, striking sex differences arise in the effects of AD risk variants such as *R47H*,[104] *APOE4*,[157] and *BIN1*.[158] For full transparency of potential sources of sex differences, it is important to acknowledge when a disease model contains an inherent sex-biased construct such as the *Thy1* promoter, which contains an estrogen receptor element and contributes to higher amyloid beta pathology in female 5XFAD mice.[159]

This review primarily focuses on molecular contributors to sex differences in AD-related processes. However, sex differences in circuitry may also affect AD pathological accumulation and spread. Women with AD demonstrate more global AD pathology, primarily driven by tau tangles, than men with AD. Heightened tau pathology in women was highly significant across ten brain regions, including the entorhinal cortex and hippocampus, whereas amyloid pathology was elevated in women within six brain regions.[160] However, sex differences do not occur in Lewy bodies or TDP-43 load.[160] Tau has been shown to disrupt neuronal circuits, but it is unknown whether this effect is sex-biased.[161] Sex-biased factors can shape neuronal circuitry, such as expression of Estrogen receptor 1, which can regulate aversion by excitatory projections in hypothalamus.[162] Even so, sex-based projections in response to AD pathology remain largely unknown. Sex differences in brain atrophy and cognitive decline during AD have been reviewed elsewhere[107,163] and may serve as indicators of underlying sex differences in neuronal circuitry.

We acknowledge that this review is limited in scope as we only address sex as a biological and physiological factor. Gender (i.e., self-identification with a specific sex or other identity and resulting environmental, societal, and cultural influences) should also be studied to better understand AD.[164] Further, we address sex differences associated specifically with AD; however, comorbid disorders (e.g., diabetes, cardiovascular disease) and psychiatric disorders (e.g., depression) also present with sex-differences[165] and likely interact in a complex manner to influence AD development and progression.

## Conclusions

From the current literature reviewed here, sex differences are a result of both separate and interacting contributions of gonadal hormones and sex chromosomes on neuroinflammation, epigenetics, metabolism, autophagy, and other molecular areas, including the microbiome. Microglia have become apparent as a common denominator in AD risk and progression across many of these fields, suggesting that microglia serve as a crucial modulator in disease risk and progression. Forthcoming studies will further elucidate the role of microglia in AD, and potentially AD's sex bias in risk toward women.

Given the coverage of several fields here, it is difficult to come away with one conclusion regarding sex differences. Rather, sex differences are highly context dependent. Thus far it does not appear that either sex chromosome or gonadal influence predominates over the other, but the current evidence investigating these relationships is limited. We recognize that there are many gaps in the current knowledge of sex differences for the AD mechanisms we have discussed, and there are many other important mechanisms underlying AD that currently lack sex-specific research altogether. However, one fact holds true: further elucidation of the role of sex in disease-associated processes is required to design effective treatments. Given the multi-faceted sex biases in prevalence, pathology, and progression observed in AD patients, it remains highly likely that sex will need to be considered to develop a therapeutic that is effective in AD for both sexes.

Much of the current research assessing sex differences in neurodegeneration is either descriptive or attributes sex differences solely to an association with sex steroid hormones, such as estrogen. However, we believe that careful dissection of these relationships via rodent FCG or 4-vinylcyclohexene diepoxide

models can establish a more concrete understanding of effects related to gonadal hormones versus chromosomes, as well as determining causal relationships. Although the inclusion of sex forces researchers to expand studies and bolster sample sizes, we advocate for the planning of well-powered groups when split by sex to expand our understanding of sex in AD. Better understanding of these differences can only enhance our understanding of disease etiology and lead to more effective disease therapies.

## ACKNOWLEDGMENTS

We would like to thank Gan lab members for their discussion and input. C.L.L. is supported by the Gilliam Fellowship (HHMI); G.C. is supported by F31AG079560 (NIA); L.G. is supported by R01AG072758 (NIH), R01AG074541 (NIH), the Tau Consortium, and the JPB Foundation.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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