Alzheimer's pathogenic mechanisms and underlying sex difference

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Abstract:

“Men are from Mars; Women are from Venus.” But both can have Alzheimer’s disease (AD), and it might be sex-specific. AD is a neurodegenerative disease and its prevalence is often reported to be higher for women than men: almost two-thirds of patients with AD are women. One prevailing view is that women live longer than men on average of 4.5 years, plus there are more women aged 85 years or older than men in most global subpopulations; and older age is the greatest risk factor for AD. However, the differences in the actual risk of developing AD for men and women of the same age is difficult to assess, and the findings have been mixed. An increasing body of evidence from preclinical and clinical studies as well as the complications in estimating incidence support the sex-specific biological mechanisms in diverging AD risk as an important adjunct explanation to the epidemiologic perspective. While some of the sex differences in AD prevalence are due to differences in longevity, other distinct biological mechanisms increase the risk and progression of AD in women. These risk factors include 1) deviations in brain structure and biomarkers, 2) psychosocial stress responses, 3) pregnancy, menopause, and sex hormones, 4) genetic background (i.e., APOE), 5) inflammation, gliosis, and immune module (i.e., TREM2), and 6) vascular disorders. More studies focusing on the underlying biological mechanisms for this phenomenon are needed to better understand AD. This review presents the most recent data in sex differences in AD – the gateway to precision medicine, therefore, shaping expert perspectives, inspiring researchers to go

	in new directions, and driving development of future diagnostic tools and treatments for AD in a more customized way.
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Alzheimer's Pathogenic Mechanisms and underlying Sex Difference

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Abstract

"Men are from Mars; Women are from Venus." But both can have Alzheimer's disease (AD), and it might be sex-specific. AD is a neurodegenerative disease and its prevalence is often reported to be higher for women than men: almost two-thirds of patients with AD are women. One prevailing view is that women live longer than men on average of 4.5 years, plus there are more women aged 85 years or older than men in most global subpopulations; and older age is the greatest risk factor for AD. However, the differences in the actual risk of developing AD for men and women of the same age is difficult to assess, and the findings have been mixed. An increasing body of evidence from preclinical and clinical studies as well as the complications in estimating incidence support the sex-specific biological mechanisms in diverging AD risk as an important adjunct explanation to the epidemiologic perspective. While some of the sex differences in AD prevalence are due to differences in longevity, other distinct biological mechanisms increase the risk and progression of AD in women. These risk factors include 1) deviations in brain structure and biomarkers, 2) psychosocial stress responses, 3) pregnancy, menopause, and sex hormones, 4) genetic background, 5) inflammation, gliosis, and immune module, and 6) vascular disorders. More studies focusing on the underlying biological mechanisms for this phenomenon are needed to better understand AD. This review presents the most recent data in sex differences in AD – the gateway to precision medicine, therefore, shaping expert perspectives, inspiring researchers to go in new directions, and driving development of future diagnostic tools and treatments for AD in a more customized way.

Keywords: aging; dementia; gender difference; cognition; hormones; estrogen; menopause

Contents

1. Introduction

Alzheimer's disease is a neurodegenerative disease and the most common cause of dementia (60-80% of cases) [1]. Clinical symptoms include memory loss, apathy, and depression in early stages, impaired communication, disorientation, confusion, poor judgment, and behavioral changes in later stages, and ultimately difficulty speaking, swallowing and walk [2-4]. Pathological hallmarks include parenchymal amyloid senile plaques, intracellular tau neurofibrillary tangles, chronic gliosis, and brain atrophy. AD is a slowly progressive and irreversible brain disorder that begins decades before symptoms emerge. There are three broad phases on AD continuum: preclinical AD, mild cognitive impairment (MCI), and dementia due to AD which can be further divided into the stages of mild, moderate, and severe (**Fig. 1**) [1].

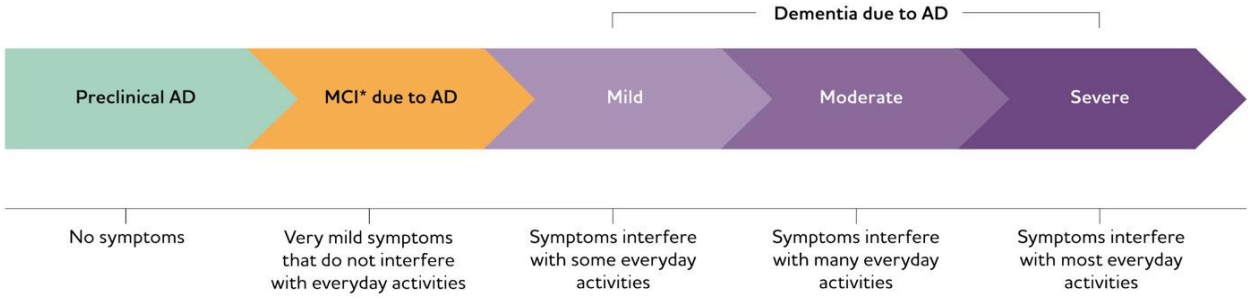

Fig. 1. AD Continuum. (Reprinted/adapted with permission from [1])

1.1 Aging and AD

AD is the sixth-leading cause of death in the US, the fifth-leading cause of death for those age ≥ 65, and a leading cause of disability and morbidity [1]. A person lives through years of morbidity before death as AD progresses. Most AD patients are age 65 or older, so called late-onset Alzheimer's disease (LOAD) except a small portion of early-onset familial AD with genetic mutations. Thus, age is the greatest risk factor for LOAD [2-4]. About 5.8 million Americans age 65 and older have AD in 2020 with 80% are age 75 or older [1]. This means one in 10 persons age ≥ 65 has AD out of the total US population. Moreover, the percentage of people with AD increases with age: 3% of people age 65-74, 17% of people age 75-84, and 32% of

people age ≥ 85 have AD (**Fig. 2**). People younger than 65 can also develop AD, but it is much less common, and prevalence is uncertain.

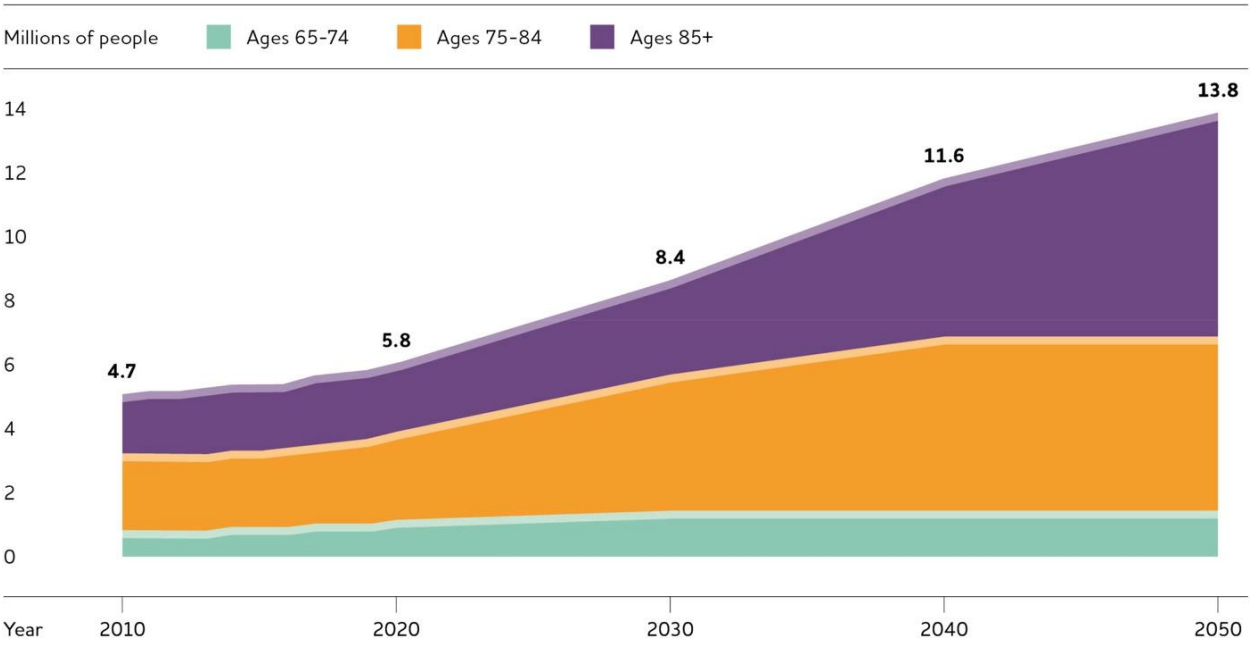

Fig. 2. Projected Number of People Age 65 and Older (Total and by Age) in the US Population with AD (2010 to 2050). (Reprinted/adapted with permission from [1])

1.2 Epidemiologic evidence for sex differences in AD

The prevalence of AD is often reported to be higher for women than men [5-13]. Almost two-thirds of Americans with AD are women. One prevailing reason that has been stated is that women live longer than men on average of 4.5 years, and there are more women aged 85 years or older than men in most global subpopulations, while older age is the greatest risk factor for AD. However, the differences in the actual risk of developing AD for men and women of the same age is difficult to assess, and the findings have been mixed [6-32]. Many studies reported higher age-adjusted numbers for women and/or accelerated progression in women while other studies failed to find an association between incidence, progression, and sex, or remained inconclusive.

There are many factors could attribute to the inconsistent findings among those different studies, including 1) differences in inclusion/exclusion criteria, 2) sample size and statistical power, 3) study design and type – retrospective cohort, prospective cohort, or cross-sectional analysis, 4) cultural differences affecting diet, exercise, and stress response strategies, and 5) the different classification and diagnoses methods on AD cases [13, 19, 29]. The classification bias as an issue in determining the real incidence of AD in men and women is particularly important. It is true that many different criteria have been used across studies. A definitive diagnosis of AD can only be made postmortem while probable AD is routinely diagnosed clinically through the presentation of certain symptoms (e.g., memory loss) in most current AD studies, and other conditions such as vascular dementia, other neuropsychiatric diseases, other neurodegenerative diseases, or traumatic injury or stroke-induced dementia. Some studies use autopsies to verify the

diagnoses of AD while many others use a clinical diagnosis which may categorize other dementia presentations incorrectly as being AD [19, 33]. Moreover, the pre-AD diagnosis of MCI can complicate the timing of the later diagnosis of AD which makes the process of defining the age-of-onset for AD more variable. Therefore, it is not surprising that big discrepancies in the incidence of AD between men and women across studies still remain due to the factors as forementioned [19].

Data from the Framingham Heart Study found that the estimated lifetime risk for AD at age 45 was about one in five (20%) for women and one in 10 (10%) for men, and the risks for both sexes were slightly higher at age 65 (**Fig. 3**) [1, 17]. A recent meta-analysis including 22 studies on sex differences reported all estimates of incidence and prevalence were higher for women than for men despite that the differences were not statistically different [34]. Other studies such as the Cache County Memory Study also confirmed a higher incidence among women, and longevity remains an important reason for more female with AD than male [25, 35, 36]. Taken together, the increasing body of evidence from preclinical and clinical studies as well as the complications in estimating incidence support the sex-specific biological mechanisms in diverging AD risk as an important adjunct explanation to the epidemiologic perspective, which deserves more careful and closer investigations in future studies [19].

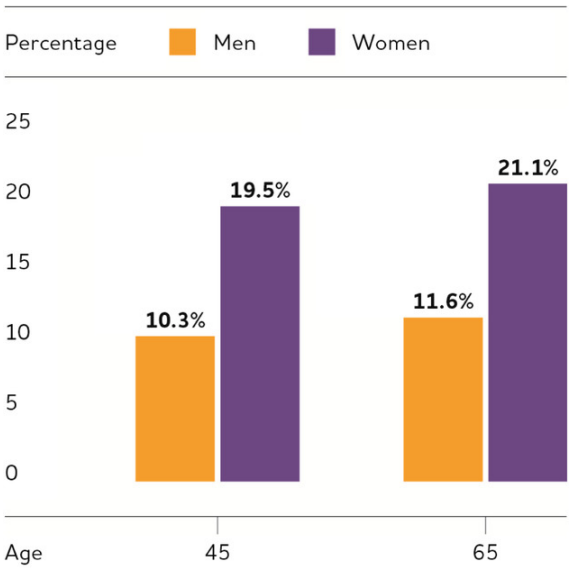

Fig. 3. Estimated Lifetime Risk for AD by Sex at Ages 45 and 65. (Reprinted/adapted with permission from [1])

1.3 Mechanisms attributing to sex differences in AD

If there is a difference in the risk of AD between male and female, there are a number of potential social and biological explanations. One possible reason is that the lower educational attainment in women than in men born in the first half of the 20th century could account for some of the elevated risk, as limited formal education is a risk factor for dementia [37-40]. Interestingly, some investigations using Framingham Heart Study data suggested that men

appear to have a lower risk for dementia due to "survival bias" [17]. So, the men included in the study were the ones with a healthier cardiovascular risk profile and survived beyond age 65 (men have a higher rate of death from cardiovascular disease in middle age than women) and thus a lower risk for dementia [41, 42]. Certainly, more studies are needed to support these interpretations.

While some of the sex differences in AD prevalence are due to differences in longevity, it is very plausible that other socio-economic risk factors as well as distinct biological mechanisms increase the risk and progression of AD in women [43]. The socio-economic risk factors are often referred as gender differences in AD [7]. Women usually have a lower income and lower education than men in most cultures, and they are the primary informal caregivers in their families. The caregiving burden is associated with higher rates of unemployment and an increased psychological risk factors in AD including depression and sleep disorders. In fact, 75% of unpaid caregivers for people with chronic debilitating disease like AD are females [44, 45].

The sexual dimorphism in AD can also be explained by many distinct biological risk factors, including: 1) deviations in brain structure, 2) depression, sleep disorders, and psychosocial stress responses, 3) pregnancy, menopause, and sex hormones, 4) genetic background (i.e., *APOE*), 5) inflammation, gliosis, and immune module (i.e., *TREM2*), and 6) vascular risk factors [7, 13, 19].

Brain structure. It is possible that the sex differences in AD risk arise from the divergent changes in brain structures that male and female have exposed to risk factors. Men usually have a larger brain volume on average, and thus are less sensitive to pathological agents for AD and suffer less or slower structural loss as compared to their women counterparts [2]. Numerous brain imaging studies showed that annual atrophy rates were slower in male MCI and AD patients [30, 46]. Moreover, men with MCI had less atrophy in numerous brain regions and a slower decline in certain cognitive tasks compared to women, as well as less atrophy in numerous regions once an AD diagnosis had been made [47, 48]. Quantitative proteomics studies further revealed more alterations in the white matter and mitochondrial proteomes, redox proteins, ATP synthase and cytochrome oxidase in women [49]. These pathophysiological increases in women suggest women are subjected to more rapid neurodegeneration than men once it starts. In addition, women could be more sensitive to those AD pathologic biomarkers than men. This is in consistent with a recent study, reporting that women had higher CSF total tau and Aβ42 levels, more rapid cognitive decline and hippocampal atrophy, indicative of worse pathologic changes than men [9]. Although evidence showed divergent responses in structural integrity to pathologic insults are sex-specific in AD, the underlying molecular mechanism remain elusive.

Depression. For people with degression, women have higher risk of MCI or AD than men. Interestingly, moderate/severe depressive symptoms were associated with a two-fold higher risk of an MCI in women but not in men though mild symptoms were associated with a two-fold higher risk of an MCI in men but not in women [50, 51]. The fact that depression is more prevalent and severe among females warrants a more careful investigation in the future.

Sleep disorders. The development and progression of AD pathology has been linked to sleep disorders. The production and clearance of Aβ are correlated with the wake/sleep cycles as Aβ production mainly occurs when awake while its clearance occurs mainly during the sleep stage [52, 53]. Older people tend to have poor-quality sleep which leads to reduced Aβ clearance and increased Aβ accumulation in the brain as well as the increased risks of developing AD [54]. Sleep may also selectively attenuate synaptic strength between neurons, decreases cellular stress, and restores brain energy reserves [55]. Sex differences in risks of developing sleep disorders are well-established with women having more sleep problem and reaching a peak during menopause [13]. Although women are generally more prone to present sleep disorders, further study is needed to understand the association between sleep and the sex-specific risk of developing AD.

Stress. Stress and increased stress hormone (e.g., cortisol) levels have been associated with cognitive impairment and AD [56]. Changes in stress hormone signaling have been found in AD patients. In AD patients, the levels of corticotrophin releasing factor 1 (CRF1) were found much higher in hippocampal brain regions, which may alter the hypothalamus-pituitary-adrenal axis signaling, resulting in hippocampal atrophy [57, 58]. In general, women are more vulnerable to stress-related disorders, and levels of cortisol in women with mild-to-moderate AD were found much higher than in men. Preclinical animal studies also confirmed that high level of CRF1 signaling is linked to increased AD pathologies including elevated amyloidosis and tauopathy [57, 58]. More investigations are required to fully understand the stress-related sex dimorphism in AD pathogenesis.

There are certainly other possible biological risk factor attributing to the sex dimorphism in AD, including but not limited to obesity, diabetes mellitus, metabolic syndrome, and thyroid dysfunction which have been reviewed elsewhere. Next, we put more emphases on sex hormones, APOE, TREM2, and vascular risk factors in the following sections.

2. Sex hormone in AD

Endocrinological factors especially sex steroid hormones have been found associated with onset and progression of AD, and their changes during lifetime are risk for AD [24, 27, 59]. The normal age-related depletion of estrogens in women and androgens in men, resulting in a loss of neuroprotective hormone effects, is viewed as the primary reason for an increased risk of AD. It is also known as the sex hormone "activational effects" which are the more transient actions of sex hormones in the adult [27, 60]. In addition, emerging evidence suggests that developmental effects of sex hormones responsible for sexual differentiation of the brain may yield a female brain that is inherently more vulnerable to AD pathogenesis. This is also known as "organizational effects" which are the long-lasting or permanent roles of hormones in sexual development and differentiation [27, 60]. Sex steroid hormones including estrogens, progestogens, and androgens exert a variety of activational effects in the brain that increase neural health and resistance to MCI and AD through regulations of amyloidosis, tauopathy, and gliosis [27, 61, 62]. Moreover, sex hormones can act more generally to increase brain function and resilience.

Estrogens. Sex differences in AD are often significantly linked to the estrogen in women, which has attracted the most attentions in preclinical and clinical studies [63-66]. Estrogen is mainly produced in the ovaries, but a significant amount is also synthesized in the brain, especially the

primary female hormone estrogen 17β-estradiol (E2). Evidence showed that E2 can regulate synaptic plasticity, promote neural survival, and mediate sex-specific behaviors [67]. Therefore, the depletion in endogenous estrogen levels during menopause was proposed as a trigger for the development of AD in women. In agreement, some but not all investigations showed that estrogen reductions in adulthood are linked to higher risk of AD in women [63-65]. Lifetime estrogen exposure suggest that dementia risk is associated with reduced estrogen. Childbearing women showed higher risk of cognitive impairment and dementia than nulliparous women as pregnancies lead to an overall decrease in women's lifetime exposure to estrogen [27, 68]. On the other hand, one study suggested that longer lifetime estrogen exposure may increase risk of dementia [69]. The correlative relationship between low estrogen and AD is also supported by surgical menopause-induced estrogen depletion [70-72]. Surgical menopause conducted prior to natural menopause, not after, disrupted the normal cyclic production of sex steroid hormones, resulted in faster cognitive decline and more tauopathy [71]. Preclinical studies also showed increased soluble Aβ levels in the brains of wild-type mice after ovariectomy (OVX) [61, 73]. In agreement, OVX in various AD transgenic female mice led to significant acceleration of Aβ pathology and worsening of behavioral performance compared with gonadally intact controls, and estrogen replacement therapy blocked this effect [61, 63, 66, 74]. However, in some other AD transgenic strains, OVX or estrogen therapy did not change the brain Aβ level significantly, suggesting that differences in transgenes or strains with different neurosteroidogenesis might mediate the relative impact of estrogen in these models [75, 76].

Androgens. In parallel to the association between low estrogen in women and higher risk of AD, reduced testosterone levels may increase the risk of AD in men. This is supported by several lines of evidence [77, 78]. There are low serum levels of testosterone in men with AD, and low serum testosterone and AD risk is apparent at least ten years before dementia diagnosis clinically. Moreover, significant reduction in brain testosterone levels was observed in men with early and late stages of AD neuropathology than age-matched normal controls, and there was a reverse correlation between brain testosterone levels and brain soluble Aβ levels in early AD [79]. Lastly, androgen-deprivation therapy for patients with prostate cancer increased serum Aβ levels and the risk of AD significantly [59]. In line with this, preclinical studies suggested that decrease in brain androgen level was closely associated with increase in soluble Aβ in rats. Endogenous testosterone depletion by orchiectomy (ORX) resulted in increased level of soluble Aβ in brain, and could be reversed by androgen therapy but not estrogen [80]. Similar results were observed in 3×Tg-AD and APP23 male mice that testosterone is a negative regulator of Aβ [81]. Collectively, available evidence is prone to suggests that decreased testosterone in men but not in women increases the risk of AD in men, and more closer investigations are needed to fully illustrate androgen's role in AD risk.

Estrogen replacement therapy (ERT). If it is true that estrogen depletion due to menopause in women plays a critical role in increased risk of MCI and AD compared to age-matched men, then ERT should help reverse or alleviate such sex-specific AD pathology. However, clinical studies resulted in inconclusive data, with some even showing adverse effect on cognition and AD risk [36, 64]. It is surprising to see these clinical results, but it is possible that ERT confers other risks like cardiovascular problems that interact with AD pathogenesis to worsen cognition. It is also not clear whether estrogen plus progesterone or progesterone alone could have different outcomes compared to those postmenopausal women enrolled in ERT. Interestingly, preclinical

studies have demonstrated that ERT can reduce risk of AD during certain "critical windows" – ERT had a higher likelihood of neuroprotective in the perimenopause period but not post-menopause period [19, 27]. This is in consistence with the data from several clinical trials including the Cache County Memory Study [36], the WHIMS-Young study [82] and the Kronos Early Estrogen Prevention Study (KEEPS) [83]. Therefore, the "Window of Opportunity" hypothesis emerged arguing that neuroprotective effect of estrogen depends on the age and stage of menopause, and ERT started during post-menopause when a new hormone-receptor equilibrium is already achieved may disturb the established balance, resulting in adverse effect on cognition and AD. Moreover, there is another "Healthy Cell Bias of Estrogen Action" hypothesis which suggests that the benefits of ERT decline over one's lifetime as cognitive health declines during aging [84-86]. So, the effects of estrogen on cognitive function may progress from beneficial to neutral or deleterious over time. This explains why estrogen administered later in life (post-menopause) had neutral or adverse effects on cognition. In this scenario, estrogen is protective only if neurons are healthy at time of its administration while is negative if neuronal health is compromised since neurons during perimenopause are more likely healthier than at post-menopause stage. Although, there are merits in these two inter-related hypotheses, critical underlying cellular and molecular mechanisms remain elusive.

3. Sex and APOE in AD

ApoE is a lipoprotein responsible for transport of cholesterol and phospholipids, and there are three genetic variants – APOE2, APOE3, and APOE4 [13]. The APOE4 has been established as the strongest genetic risk factor for LOAD and related dementia whereas APOE2 is somewhat neuroprotective [4, 12, 14, 20, 23, 29, 31, 87]. In addition, ApoE has Aβ-binding motif and is the best characterized Aβ chaperone which can facilitate Aβ degradation via trafficking of amyloid to lysosomes [31]. Evidence showed that APOE-ε4 may increase Aβ oligomer formation, Aβ deposition as well as upregulation of β-site APP cleavage enzyme (BACE1) [23]. APOE4 was showed to accelerate BACE1 by indirectly modulating cholesterol quantities and increased APP recycling. Recent studies also demonstrated that ApoE regulates tau pathogenesis independent of Aβ pathology, and ApoE4 leads to a gain of toxic effect on tau pathologies [88].

APOE-ε4 increases one's risk of having AD while ε2 form decreases one's risk compared with having the ε3 form. People with one copy of ε4 may have three-fold higher risk of AD compared to those with two copies of ε3 form, and the risk hikes to (8-12)-fold higher if having two copies of ε4 form. Moreover, people with the APOE-ε4 form are shown to have amyloidosis and dementia at a younger age than those having ε2 or ε3. Among all AD patients in US, up to 65% had at least one copy of APOE-ε4 gene [1, 4, 29].

Although APOE4 is the biggest genetic risk factor for AD development, women with at least one copy of APOE-ε4 often exhibited more risk and faster cognitive decline and deterioration than counterpart men. APOE4-associated risk of AD and MCI peaked earlier in women than in men, giving significant elevation between the ages of 65 and 75 for AD (4.37 times increased risk in women compared to 3.14 in men) and 55 and 70 for MCI (1.43 in women versus 1.07 in men) while the APOE4-associated risk for AD or MCI among men peaked in the 75- to 85-year-old group [89]. In agreement, women with APOE4 have more risk of converting from MCI into AD compared to non-APOE4 carrier women or men with any APOE isoform [90]. The sex dimorphism is somehow most significant among APOE3 and APOE4 heterozygotes and are

most apparent in females with menopause [4, 20, 29, 89]. These findings indicate that estrogen and APOE4 interaction may play a key role in elevated risks of AD development in women compared to men.

Although estrogen decline in women due to menopause is implicated in cognitive vulnerability in AD, clinical ERT showed that females with APOE4 background receiving estrogen treatment had more cognitive decline than those APOE4 females without ERT [91-94]. Interestingly, ERT led to cognitive improvement for women with APOE2 or APOE3 isoforms [95]. This contradicting result could be explained by the positive loop feedback mediated by estrogen receptors alpha and beta (ERα and ERβ), which are involved in upregulation or downregulation of ApoE protein expression, respectively [96]. Thus, APOE4 carries receiving ERT may suffer an exacerbated ApoE4 overexpression stemming from ERα stimulation by estrogen whereas APOE2/3 carriers with the same treatment may benefit from neuroprotective ApoE2/3 overexpression [96]. Moreover, another explanation is related to the effect of estrogen on metabolic pathways in the brain [97, 98]. Previous studies showed that estrogen promotes glucogenic metabolic pathways but inhibits ketogenic pathways for ATP in female brains. In AD patients with APOE4 form, there is a trend of metabolic shift from glucogenic pathway to a more ketogenic system that relies on utilizing white matter for fuel in the brain. Since APOE4 brains rely on a dual metabolic system of both glucose and keto, estrogen therapy could suppress ketogenic pathway, leading to a compromised ATP production in brain [13]. In contrast, promoting glucogenic pathway and mitigating ketogenic pathway in APOE2/3 carriers by estrogen can lead to a more positive and conducive bioenergetic profile for ATP production because APOE2/3 brains are more tune to using glucose as the primary metabolic pathway [97, 98].

Clearly, APOE plays an important role in AD development and treatment responses with major differences between APOE isoforms and between men and women. More studies are needed to illustrate the underlying mechanisms of this sex-specific impact on AD pathogenesis with different APOE background.

4. Sex and immune module TREM2 in AD

Neuroinflammation and neuro-immune response is another important risk factor for AD development. Several key genetic polymorphisms are indicated in the immune module of AD pathogenesis including TREM2, CD33, and CR1 [13, 19]. Other diseases and environmental factors like viral infection, obesity, TBI, Ca^{2+}/Mg^{2+}-dyshomeostasis may also increase the inflammation. Neuroinflammation involves the activation of reactive glial cells including microglia and astrocytes, leading to higher levels of pro-inflammatory cytokines and Aβ accumulation. Non-steroid anti-inflammatory drugs were found to reduce the risk of AD progression in people over 55 age.

Sex differences in immune systems including neuro-immune modulation of memory and cognitive function also exist. Women usually have stronger immune responses to stimulations than men involving different pathways and immune cells, correlating to higher susceptibility to infections in male whereas higher incidence of autoimmune disorders in females [99]. One possible reason is the differences between sex hormones and sex chromosomes in men and women [99]. Sex differences in dysregulation of glial cell-mediated neuro-immune responses

have been implicated in AD and TBI [100]. However, sex-specific cellular and signaling mechanisms in neuro-immune modulation is still largely unclear.

A recent preclinical study demonstrated that a gene module of "immune response" was significantly upregulated aged mice, and this module was led by the top hub genes including TREM2 and TYROBP [4]. Trem2 is the key regulator of microglial functions in the brain, and the R47H risk variant of the TREM2 gene is a genetic risk factor of AD [101, 102]. Interestingly, in tau mice, female expressing an AD risk variant of R47H-TREM2 exhibited a stepped-up microglial reaction and had worse memory while the same TREM2 mutation did no such thing in male mice [101-103]. The maladaptive microglia are a likely culprit behind the greater memory loss in females. Microglia in both sexes expressed the disease-associated microglia (DAM) signature of genes. However, expression of certain DAM genes shifted only in females with R47H-Trem2 form. The R47H increased the expression of many pro-inflammatory cytokines of the DAM signature and lessened expression of a suite of neuronal genes in female mice while R47H variant exerted no overt change on the DAM signature in male mice [103]. This sexual dimorphism seemed not due to more tau pathology in females, but to an altered microglial response to that burden. Multiple lines of evidence suggest that microglia respond differently between the sexes in some instances, but the driving force, underlying molecular mechanisms, and how they ultimately affect the course of disease, remain elusive.

5. Sex and vascular risk factors in AD

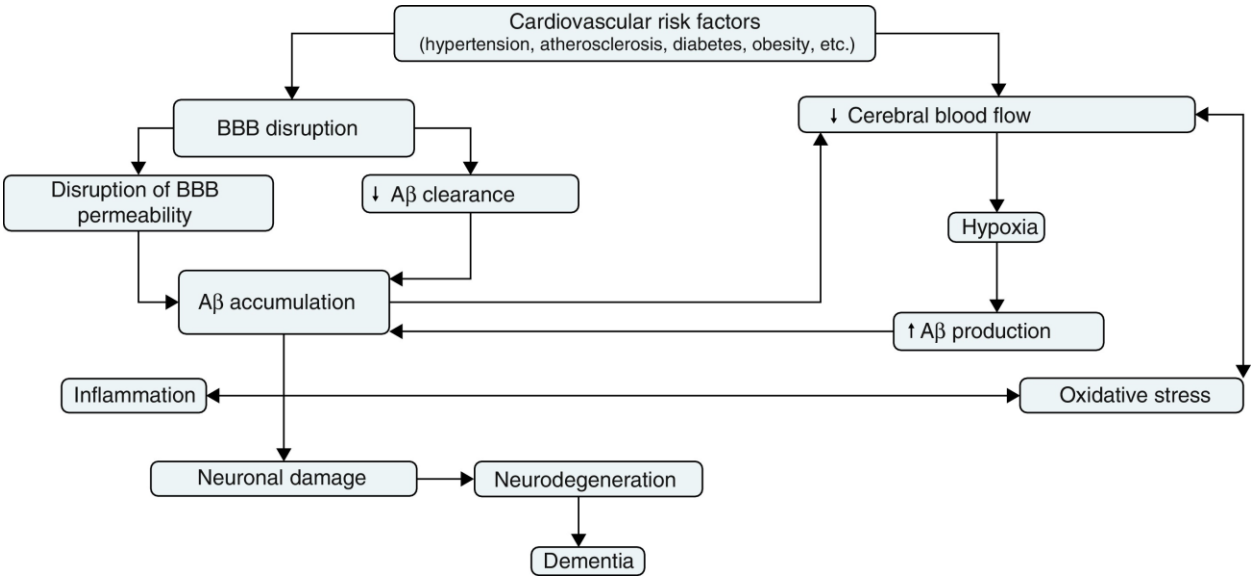

Fig. 4. Vascular hypothesis explaining the link between vascular dysfunction and AD. (Reprinted/adapted with permission from [104])

It is well established that vasculature plays a critical role in the progression of AD as a main comorbidity contributing to AD pathogenesis. More than 50% of all patients with vascular cognitive impairment (VCI) will advance to dementia [105]. The vascular hypothesis as an alternative to amyloid hypothesis argues that cardiovascular risk factors including hypertension, atherosclerosis, diabetes, obesity, and other microvessel pathology lead to reduced cerebral blood

flow, BBB disruption, hypoxia, impaired Aβ clearance, elevated oxidative stress/inflammation, and ultimately neurodegeneration and dementia (**Fig. 4**) [104]. In agreement, a recent study showed that BBB breakdown is an early event in the aging human brain in MCI patients and early clinical stages of AD [106]. Vascular abnormality may act in synergy with brain Aβ levels through a positive feedback loop where vascular risk factors promotes Aβ accumulation in the brain parenchyma, and Aβ accumulation in turn aggravates vascular dysfunctions. Accumulating evidence suggest there is also a link between vascular dysfunction, neuronal damage, and inflammation in AD where cerebral vasculature-involved inflammation may precede Aβ deposition which in turn promotes the inflammatory responses.

APOE4 also plays an important role in acceleration of BBB breakdown and brain capillary pericytes degeneration. Latest evidence from human studies suggest that people with at least one APOE-ε4 are distinguished from those with APOE-ε3 by BBB breakdown in medial temporal lobe and hippocampus, apparent in APOE4 carriers without cognitive impairment and more severe in those with cognitive impairment [107]. Surprisingly, this APOE4-mediated BBB breakdown is not related to amyloid or tau pathology [107]. These findings suggest that APOE4 leads to BBB dysfunction predicting cognitive decline independently of AD pathology.

The sex differences in vascular risk factors for AD development are also emerging. Men have a much higher incidence of coronary artery disease (CAD) than women in all ages, which is linked to cognitive decline with brain microvascular lesions [108]. One possible reason is the protective effects of estrogen against inflammation, oxidative stress, and atherosclerosis in females [109]. While some studies showed that men have a higher incidence of stroke than women and at much earlier ages, other studies found women have a higher risk of stoke due to their longer lifespan [110-112]. Some unique vascular risk factors to women are hypertensive pregnancy disorders, including preeclampsia, eclampsia, and chronic gestational hypertension, which are directly associated with rain lesions and cognitive impairment [113, 114]. Women at age of ~60 with prior history of hypertensive pregnancy disorders had more brain atrophies after pregnancies compared to those with normotensive pregnancies [113].

The effects of APOE genotype on sex differences for vascular risks have also been explored. There was a significantly higher rate of CVD for men APOE4 carriers (18.6%) than counterpart women APOE4 carriers (9.9%) as shown by a Framingham Heart Study with 3413 participants [115]. The APOE-ε2 seemed protective for female (4.9%), but not for male (18.2%), suggesting ε2 or ε4 is associated to higher risk for CVD in male. The mortality due to cardiovascular disease in men was 6-fold higher than in women at ages between 45 to 54 according to another Framingham Heart Study with 7901 participants, and this difference was still evident even comparing the lowest risk group of men (i.e. ε3/ε3) to the highest risk group of women (i.e. ε4/ε4) [17]. Thus, it is speculated that lower risk of AD in men older than 65 years is due to this so called "survivor hypothesis," which is supported by the evidence that there is no differences between male and female for midlife risk of AD while significantly higher risk for female after midlife, and post-menopause from age of 65 years and older [29].

6. Gap between clinical and preclinical studies

Although sex-specific differences are implicated in the heterogeneities of AD prevalence and clinical manifestations by clinical and preclinical studies, there is always a gap regarding

behavior and cognitive performance, disease course and prognosis, and pathology between human and animal studies. For example, a recent study by comparing human brain aging with mouse models revealed major gaps in our understanding of sex differences across species on APOE and sex interactions [116]. Aging male APOE4 carriers had 7-fold more microbleeds than women, while it was opposite to male EFAD mice that had 7-fold fewer. However, the EFAD mice showed modest female excess in prevalence of Aβ-positive vessels or CAA, consistent with some postmortem human studies. This supports the notion that AD is a uniquely human condition.

To explore the role estrogen and menopause in AD, mice models have been constructed to replicate different elements of human menopause including natural aging, ovariectomy (OVX) and hormone replacement [71, 117]. However, they often fall short to recapitulate the human menopause process adequately [86]. The intact aging mice model fails to achieve very low levels of estrogen while the mice OVX model lacks perimenopause stage. To bridge the gap, a better mice model mimicking the human menopause should be used, and the innovative accelerated ovarian failure (AOF) using the chemical 4-vinylcyclohexene diepoxide (VCD) could be used (**Fig. 5**) [86]. It uniquely recapitulates hormonal changes that occur during human menopause, including estrous acyclicity and fluctuation (as in human perimenopause), followed by undetectable, estrogen levels (as in human post-menopause). This model also allows for the dissociation of the effects of aging from the effects of hormone levels in young mice [86, 118].

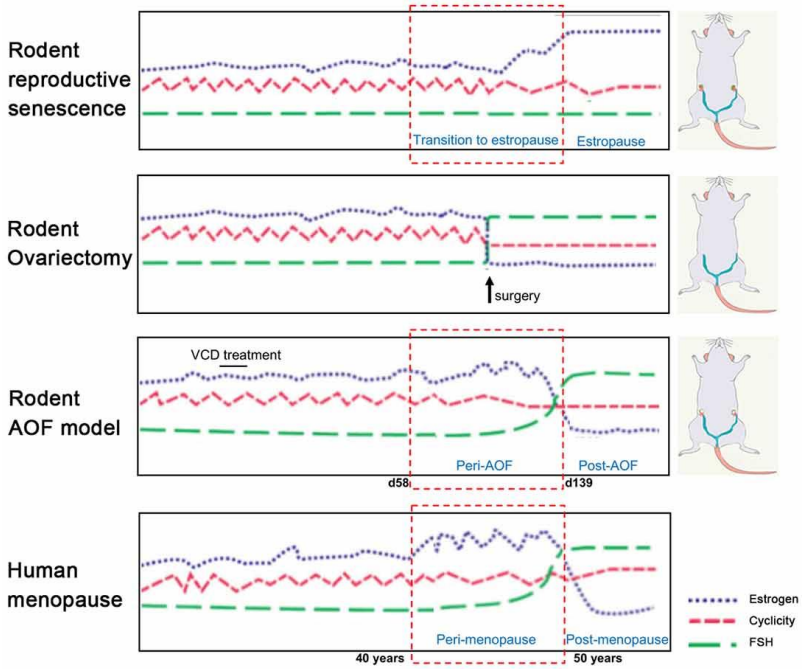

Fig. 5. Models of menopause in rodents and human. (Reprinted/adapted with permission from [86])

Preclinical animal models of AD are critical to gaining a better understanding of pathogenesis and to assess the potential of novel therapeutic approaches. The fact that AD clinical trials so far has very limited success could be, at least partially, related to the premature translation of high successes in animal models that mirror only limited aspects of AD pathology to humans. Animal

models are advantageous in offering the option to conduct preclinical testing *in vivo* [119]. New knock-in mouse models are potentially more representative of physiological models of AD. Non-human primates may provide more genetic similarity to humans and a more physiological relevant pathogenesis of AD, but are limited by availability, costs, time, and inconsistency [119]. To enhance the success of translation from preclinical studies to patients, a better understanding of the strengths and weakness of different preclinical models and the use of more than one model could be helpful.

7. Conclusion

There is mounting evidence to support sex and gender differences on the risk of AD development [6-13, 16, 21, 24, 25, 48, 90, 99, 101, 110]. The underlying mechanisms for the apparent differences remain elusive, but intrinsic biological risk factors are increasing prominent besides the longevity for women. Among them, sexual dimorphism in CNS structures, changes in sex hormone signaling, risk genes and sex interactions, immune responses, and vascular diseases are considered important determinants. Biological sex and common co-morbidities for AD should be considered in both preclinical and clinical studies. Providing that there are currently no effective treatments available for AD, it is critical that we understand how to mitigate risk factors for this devastating disease in both sexes.

To fully answer this question on sex differences in AD, more large-scale comprehensive AD risk assessments, biomarker collection, and genetic stratification are needed [7]. Better disease models therefore could be generated from big data, including individual variability like sex, phenotypic, genetic, epigenetic, biomarker, lifestyle, and psychosocial characteristics. These data-driven large-scale studies will stimulate a paradigm shift towards precision medicine and precision pharmacology in AD, highlighting the need for tailored interventions considering specific biological make-up including sex for each individual.

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January 5, 2021

Dear Dr. Eichmann,

Thank you very much for inviting us to contribute a review on Alzheimer's disease to *Cellular and Molecular Life Sciences (CMLS)*. We are very pleased to submit our manuscript, entitled "*Alzheimer's Pathogenic Mechanisms and underlying Sex Difference*", to be considered as a candidate for *CMLS*.

Enclosed is a manuscript drafted by Dr. Zhu at Stony Brook University, Dr Axel Montagne from Edinberg University and me, with 5 figures. Thank you very much for your time and consideration.

Sincerely,

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