Estrogen receptor β in Alzheimer's disease: from mechanisms to therapeutics

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Abstract

Alzheimer's disease (AD) disproportionally affects women and men. The female susceptibility for AD has been largely associated with the loss of ovarian sex hormones during menopause. This review examines current understanding of the role of estrogen receptor β (ERβ) in the regulation of neurological health and its implication in the development and intervention of AD. Since its discovery in 1996, research conducted over the last 15-20 years has documented a great deal of evidence indicating that ERβ plays a pivotal role in a broad spectrum of brain activities from development to aging. ERβ genetic polymorphisms have been associated with cognitive impairment and increased risk for AD predominantly in women. The role of ERβ in the intervention of AD has been demonstrated by the alteration of AD pathology in response to treatment with ERβ-selective modulators in transgenic models that display pronounced plaque and tangle histopathological presentations as well as learning and memory deficits. Future studies that explore the potential interactions between ERβ signaling and the genetic isoforms of human apolipoprotein E (APOE) in brain aging and development of AD-risk phenotype are critically needed. The current trend of lost-in-translation in AD drug development that has primarily been based on early-onset familial AD (FAD) models underscores the urgent need for novel models that recapitulate the etiology of late-onset sporadic AD (SAD), the most common form of AD representing more than 95% of the current human AD population. Combining the use of FAD-related models that generally have excellent face validity with SAD-related models that hold more reliable construct validity would together increase the predictive validity of preclinical findings for successful translation into humans.

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1. Introduction

As the leading cause of dementia and rated as the most feared human disease by the American public, Alzheimer's disease (AD) currently affects 35 million people worldwide, including 5.4 million Americans (Thies and Bleiler, 2013). These numbers are predicted to triple by 2050, with one new case of AD expected to develop every 33 seconds, or nearly a million new cases per year (Thies and Bleiler, 2013). As the number of people affected by AD increases so will costs of health care. In the US alone, it was estimated that $214 billion were spent on AD care in 2014, and the cost is projected to rise to 1.2 trillion in 2050 (Thies and Bleiler, 2013). There is no cure currently available, and no success has been found from over 100 human trials aimed at AD treatment that were conducted over the last decade (McBride, September 14, 2012; Schnabel, July 8, 2013). These unprecedented challenges stress the significance and imperativeness for the development of new strategies targeted for AD prevention and early intervention (Mullard, 2012; Rice, January 25, 2014). It has been estimated that a treatment that delays the onset of AD by just 5 years could reduce the number of people with the disease by nearly 50% in 50 years (Thies and Bleiler, 2013).

2. Sex differences in AD

AD disproportionally affects women and men (Carter et al., 2012; Regitz-Zagrosek and Seeland, 2012). Of the current AD cases, nearly two-thirds are women (Brookmeyer et al., 2011). After age 65, the lifetime risk of AD is 1 in 6 for women (16.7%), whereas 1 in 11 for men (9.1%) (Thies and Bleiler, 2013). In addition, sex is found to influence the development, progression and clinical manifestation of AD. For instance, depression is associated with a 2-fold increased risk for AD in women but not in men; whereas, stroke is associated with a 3-fold increased risk for AD in men but not in women; (Artero et al., 2008). Moreover, AD pathology appears more likely to be clinically expressed as dementia in women than in men (Barnes et al., 2005). AD women tend to exhibit a broader spectrum of dementia-related behavioral symptoms, and experience greater cognitive deterioration than men in the progression of the disease (Chapman et al., 2011; Hall et al., 2012; Irvine et al., 2012a; Schmidt et al., 2008). A recent meta-analysis of neurocognitive data from 15 published studies revealed a consistent male advantage on verbal and visuospatial tasks, and tests of both episodic and semantic memory. It was concluded that women with AD showed worse mental deterioration than men with the disease, even when at the same stage of the condition. In stark contrast, men with AD consistently performed better than women with the disease across the five cognitive areas examined. Most remarkably, the verbal skills of women with AD were worse when compared to men with the disease, which is a striking difference from the profile for the healthy population where females have a distinct advantage (Irvine et al., 2012b).

Sex affects AD at the genetic level as well. First, cognitively normal individuals with a maternal family history of AD were found to express greater phenotypic changes in AD-vulnerable brain regions suggesting a higher risk for developing AD as compared to those with a paternal history or no family history (Berti et al., 2011; Honea et al., 2011; Mosconi et al., 2010). Second, some genetic variants appear to carry a different risk for developing AD in women than in men. As an example, the anti-AD ε2 allele of the apolipoprotein E

gene (APOE2), has been indicated to confer a greater protection against AD in men than in women (Johnson et al., 1998). In contrast, the pro-AD ε4 allele of the APOE gene (APOE4) has been associated with a far more pronounced risk for AD in women than in men (Bretsky et al., 1999; Mortensen and Hogh, 2001; Payami et al., 1996). A meta-analysis found that women with one APOE4 allele had a 4-fold increased risk for AD when compared to women homozygous for the APOE3 allele. However, men with one APOE4 allele had little to no bump in risk (Farrer et al., 1997). The sex-APOE interaction evidenced in those case-control studies was further demonstrated in a recent analysis of the large, longitudinal aging and dementia dataset collected by the National Alzheimer's Coordinating Center (NACC) and from the Alzheimer's Disease Neuroimaging Study (ADNI) involving a total of 5,496 healthy controls and 2,588 mild cognitive impairment (MCI) patients. The analysis found that the risk of clinical conversion associated with APOE4 was significantly greater in women than in men, and such an interaction was present in both the conversion from healthy aging to MCI and in the conversion from MCI to AD (Altmann et al., 2014; Ungar et al., 2014). Moreover, consistent with the findings from the case-control study (Johnson et al., 1998), a significant interaction between APOE2 and sex was also revealed in recent clinical studies in which a protective role of APOE2 was detected in male but not female subjects (Altmann et al., 2014; Ungar et al., 2014).

Furthermore, increasing evidence indicates that sex alone or in combination with APOE genetic status modifies the response effect of AD treatment. A population-based study that examined the effects of FDA-approved medications for AD on clinical progression found a positive association between use of cholinesterase inhibitors and slower progression only in women, particularly in those with an APOE4 allele; in contrast, use of these medications was associated with faster progression in males (Mielke et al., 2012). This sex-specific benefit of AD treatment has also been clinically indicated in treatment effect of intranasal insulin in adults with MCI or AD, and the sex difference was most apparent for APOE4 negative individuals. Specifically, it was found that APOE4 negative men showed cognitive improvement in response to a higher dose of insulin while APOE4 negative women showed worsened performance; however, functional abilities were relatively preserved for women compared with men (Claxton et al., 2013). As reviewed later, another notable area that demonstrates an apparent sex-APOE-treatment interaction is estrogen-containing therapy although the results have been inconsistent. In addition to the interaction with sex, a recent study indicated that APOE also interacts with age to modify the rate of decline in cognitive and brain changes in AD; the presence of an APOE4 allele had a more deleterious effect on the young group of AD patients than the old group of AD patients (Chang et al., 2014). Taken together, these findings underscore the importance of integrating an individual's age, sex and genetic susceptibility and their interaction when examining the clinical efficacy of an AD treatment, although it may present a challenge to the efficiency of the study (Kennedy et al., 2014).

3. Female susceptibility for AD

It is generally conceived that the female susceptibility for AD is due to their longer life expectancy and hence the higher age-associated risk for AD, which, however, is true to a minimal extent. Statistics show that the age difference between females and males is not as

large as traditionally thought. Currently, the worldwide life expectancy for all people is 64.3 years, 62.7 years for males and 66 years for females, a difference of approximately three years, while the average duration of AD can last 8-12 years from the diagnosis. The age difference between sexes appears to be even smaller in rodents; in C57BL/6 mice, female mean life span was 789 ± 42 days, and male was 801 ± 39 days (Selman et al., 2009). Furthermore, results from a meta-analysis of seven sex-specific studies concluded that women were 1.5 times more likely to develop AD than age-matched men (Gao et al., 1998), which is consistent with data derived from the Cache County study that showed a clear increase in the incidence of AD in the female gender (Zandi et al., 2002b). These findings suggest that age does not account, at least not solely, for the sex differences in the prevalence of AD; hence there must be other factors that play a greater role in predisposing females at a higher level of risk for the development of AD.

Research conducted over the last 20 plus years has documented a great deal of evidence that supports the notion that the female vulnerability to AD is largely associated with loss of ovarian hormones during menopause (Zhao and Brinton, 2009; Zhao et al., 2005). Numerous studies have demonstrated that estrogen regulates a wide range of activities throughout the brain including neural development and survival (McEwen, 2002; Simpkins et al., 2005). Estrogen-containing therapy (ET) has been associated with a significantly reduced risk for the development of AD in women when initiated soon after menopause; however, such a benefit disappears and the therapy may even impose an adverse impact if started many years later after menopause during which the hypoestrogenic state might have caused neuronal damage that cannot be reversed by ET (Brinton, 2005, 2008). In support of this "healthy cell bias" of estrogen action, the Cache County Study and the Research Into Memory, Brain Function and Estrogen Replacement (REMEMBER) pilot study found that women who had received ET at the time of menopause and continued for 10 years had a 3-fold lower risk of developing AD (MacLennan et al., 2006; Zandi et al., 2002a). However, as found in the recent Women's Health Initiative Memory Study (WHIMS) that involved high average age of women, when started 10 years after menopause, ET was either to be of no benefit (estrogen-alone) or to afford a negative impact (estrogen plus progestin) on global cognition (Espeland et al., 2004; Rapp et al., 2003; Shumaker et al., 2004; Shumaker et al., 2003). Further follow-up analyses found that among the WHIMS study participants, women who reported using any form of ET before reaching 65 years had a 50% reduction in risk for developing AD or other types of dementia than women who did not use the therapy by that age (Neurology, 2007). In contrast, women who began estrogen-alone therapy after the age of 65 years had an approximately 50% increased risk of developing dementia; and the risk was nearly double among women using the combined estrogen plus progestin therapy (Neurology, 2007). Together, these findings indicate that the timing of initiation in relation to menopause could be a significant regulator of the health impact of ET in postmenopausal women (Manson et al., 2007; Neurology, 2007; Rossouw et al., 2007).

To understand the molecular bases underlying the greater risk associated with the female sex for the development of AD, we recently conducted a pilot study designed to elucidate sex differences in hippocampal aging as demonstrated by the expression profile of a focused set of approximately 180 genes involved in mitochondrial bioenergetics and amyloid metabolism in light of their accentuated roles in preclinical development of AD (Brinton,

2009; Mosconi et al., 2006; Selfridge et al., 2013; Swerdlow et al., 2013; Yao et al., 2009). The results revealed significant disparities in the trajectory of changes with age between female and male brains. The female brain appeared to express a more reproductive aging related profile, whereas the male brain exhibited an aging profile that more closely followed chronological patterns. In the female brain, 44.2% of genes significantly changed during a window of time likely representing the reproductive transition from premenopause to perimenopause. The changes that occurred during this transition were indicative of decreased bioenergetic capacity and increased amyloid dyshomeostasis. In contrast, in the male brain, only 5.4% of genes were altered during this time period. Subsequent changes in the female brain were relatively small; however, in the male brain, most changes occurred at a much older age. Bioinformatics gene network analysis revealed that insulin-like growth factor 1 (IGF1) appeared to serve as a central driver leading to the overall reduced energy metabolism associated with early aging in the female brain. Together, these findings indicate that, first, female brain ages markedly different from male brain. Second, female brain starts to exhibit age-related changes much earlier than male brain, and these changes appear to be closely related to the reproductive stage. Third, the transition from premenopause to perimenopause and the resulting perturbed estrogen signaling may serve as a critical change point that potentially switches a female brain from a metabolically active and healthy status to a hypometabolic and oxidative state, which could be further exacerbated by other genetic and environmental stressors, for example, APOE4 and depression, eventually leading to the onset of AD. Therefore, from the therapeutic perspective, in order to prevent or halt the biological transformations occurring in a female brain that could result in phenotypes at risk for the development of AD pathology, an intervention must be initiated prior to or at the onset of perimenopause, which is much earlier than traditionally thought. Lastly, these findings offer new perspectives for further in-depth studies aimed at understanding and modulating the impact of age-related endocrine changes along with genetic and environmental challenges on the adaptation of the brain and its defense against neurodegeneration. The insights gained from these mechanistic investigations will potentially lead to effective strategies for the prevention, risk reduction, or at least delaying the onset of AD, particularly in the high-risk population of older women.

4. ERβ in the brain: from expression to function

Estrogen receptors exist in two major subtypes, ERα and ERβ. ERβ, first discovered in rat prostate and ovary in 1996, is encoded by a gene located on chromosome 14 (Kuiper et al., 1996), which is separate from the ERα gene on chromosome 6 (Green et al., 1986). ERα and ERβ are widely distributed in a tissue and cell type-specific pattern in both rodents and humans, thus providing an explanation for their distinct regulatory patterns of estrogen action. ERα is expressed at high levels in the "classical" estrogen target tissues such as the uterus, mammary gland, placenta, liver, bone, and cardiovascular system. ERβ, on the other hand, is mainly expressed in "non-classical" target tissues, such as the brain, prostate, ovary, lung, muscle, and urinary tract (Weihua et al., 2003).

In the human brain, it was found that, although both ERs were predominantly expressed in limbic-related areas, the highest expression of ERα mRNA was restricted to areas such as the amygdala and the hypothalamus, whereas ERβ mRNA expression was abundant in areas

such as the hippocampal formation, cerebral cortex, and thalamus (Osterlund et al., 2000a; Osterlund et al., 2000b). It should be noted, however, that the findings pertaining to immunolocalization of ERs in the human brain are variable across laboratories using different antibody preparations (Savaskan et al., 2001; Taylor and Al-Azzawi, 2000). In addition to their primary nuclear localization, both ERs are expressed in extranuclear sites as well. For example, both ERα and ERβ were detected in the cytoplasm and neuronal processes of rat hippocampal neurons (Adams et al., 2002; Milner et al., 2001). ERβ was also detected in the mitochondria of rat hippocampal neurons (Yang et al., 2004), as well as in the synaptosomal and synaptic membrane of mouse hippocampal neurons (Nishio et al., 2004). Moreover, the cellular expression and subcellular distribution of ERs can be modified by sex, age and health status. Studies in both rodent and human brains indicate that the expression of ERβ is more subject to a decrease with age; in contrast, the level of ERα is relatively immune to the effect of age (Wilson et al., 2002; Yamaguchi and Yuri, 2012). Nevertheless, aging has been associated with translocation of ERα from the nucleus to the cytoplasm in female but not male brains, and the occurrence of AD neuropathology was accompanied by a high level of nuclear ERα in both female and male brains compared to the level in control subjects (Hestiantoro and Swaab, 2004; Kalesnykas et al., 2005).

Many studies, including our own, demonstrate that both ERα and ERβ contribute to estrogen-induced increased neuronal survival against neurodegenerative insults and the underlying mechanisms, including promotion of mitochondrial viability via regulation of calcium signaling and antiapoptotic protein-mediated signaling cascades (Nilsen and Brinton, 2004; Simpkins and Dykens, 2008; Zhao and Brinton, 2007a; Zhao et al., 2004). However, ERβ appears to play a greater role than ERα in mediating some of the estrogen-exerted neuroprotective actions in the brain. For instance, insulin-degrading enzyme (IDE), a major mechanism involved in β-amyloid degradation in the brain that is decreased in both APOE4 and AD brain, was upregulated by 17β-estradiol treatment mediated by ERβ and the downstream PI3K-AKT pathway, while activation of ERα did not yield a significant result (Zhao et al., 2011b). Moreover, ERβ appears to have a broader involvement in mediating the effects of estrogen on brain development and neural plasticity. The crucial role of ERβ in brain development was first evidenced by the morphological abnormalities, including regional neuronal hypocellularity, especially in the cerebral cortex, and neuronal deficits in the brains of adult ERβ knockout mice. These abnormalities occurred as early as 2 months of age in these mice and progressed with age (Wang et al., 2001). ERβ plays a pivotal role in late embryonic development of the brain as well. In addition to the smaller size of the brains and their fewer neurons in ERβ knockout mice at embryonic day 18.5, compared to those of their wild type littermates, fewer migrating neurons in cortical layers and elevated number of apoptotic cells in the ventricular zones of cerebral cortex were observed in ERβ knockout mice, indicating that ERβ may promote brain development by enhancing neuronal migration and neuronal survival (Wang et al., 2003).

One particular area that demonstrates the role of ERβ in the neural plasticity is its regulation of brain-derived neurotrophic factor (BDNF). As a prototypic neurotrophin, BDNF is structurally related to nerve growth factor but appears to have a greater expression and wider distribution in the CNS, with the greatest concentration found in the hippocampal formation (Murer et al., 2001). A large body of evidence indicates that BDNF plays an essential role in

promoting neuronal survival and differentiation in developing brain (Binder and Scharfman, 2004). In mature brain, BDNF regulates synaptogenesis, synapse maturation and plasticity, and solidifies memory formation and storage (Lu, 2003; Tapia-Arancibia et al., 2008; Yoshii and Constantine-Paton, 2010). In the forebrain, colocalization of ERs, BDNF, and its high affinity membrane receptor, tyrosine receptor kinase B (TrkB), as well as the fact that the BDNF gene contains an estrogen-sensitive response element suggests potential crosstalk between ERs and BDNF-TrkB signaling (Sohrabji et al., 1995; Toran-Allerand et al., 1992). Our recent analyses found that both BDNF and TrkB protein levels were significantly reduced in the hippocampus of ERβ but not ERα knockout adult mice. Additional studies demonstrated that ERβ was necessary for estrogen-mediated upregulation of BDNF in both neuronal cells and brain tissues, and activation of ERβ induced a robust increase in BDNF protein level in experimentally-induced menopausal mouse brains (Aguirre et al., 2010; Aguirre and Baudry, 2009; Zhao et al., 2011a).

The role of ERβ in neural plasticity is paralleled by its modulation of learning and memory. In a Morris Water Maze model, Rissman and colleagues reported that ERβ knockout mice, following 17β-estradiol treatment, exhibited delayed learning acquisition or failed to learn the task, whereas wild type animals displayed significant learning, suggesting that ERβ mediates the estrogen-induced enhancement of learning and memory (Rissman et al., 2002). Similarly, in a hippocampus-mediated fear-conditioning paradigm, ERβ deficiency was associated with profound synaptic deficits and memory impairment compared to wild type controls (Day et al., 2005). ERβ activation increased synaptic protein expression, enhanced long-term potentiation, and improved performance in hippocampus-dependent memory tasks; however, these effects disappeared when ERβ was absent or only ERα was activated (Jacome et al., 2010; Liu et al., 2008; Walf et al., 2008). Further studies indicated that ERα appeared to be more involved in aggressive and sexual behavior, whereas ERβ appeared to be more involved in the regulation of emotional, including anxiety and depression, and cognitive behavior (Gustafsson, 2003; Rhodes and Frye, 2006; Walf and Frye, 2007).

In the aged brain, the retention of estrogen-sensitive ERβ actions may offer an important route for maintaining steroid homeostasis and altering plasticity, suggesting that ERβ may be a major target for estrogen therapy in female aging brain (Waters et al., 2011; Zhao et al., 2011a). The role of ERβ in the development and intervention of AD has been supported by several recent studies. Overexpression of ERβ in a rat AD model reduced Aβ deposition in the hippocampus and improved learning and memory of AD rats (Tian et al., 2013). Long et al reported that the frontal cortices of female human AD brains exhibited significantly reduced ERβ, particularly in neuronal mitochondria, which was accompanied with reduced mitochondrial cytochrome C oxidase activity and increased protein carbonylation, suggesting that ERβ deficiency may play an important role in AD pathogenesis in females by contributing to mitochondrial dysfunction (Long et al., 2012). Moreover, our recent study demonstrated that early intervention with an ERβ agonist prolonged the survival, improved the spatial recognition memory, and slowed progression of amyloid pathology in a female AD mouse model (Zhao et al., 2013). Additional evidence that supports the potential of an ERβ-targeted therapy for AD include a recent report which showed that treatment with an ERβ agonist decreased cognitive deficits and β-amyloid levels in a mouse model of AD (George et al., 2013). Together, these results provide the proof-of-concept support that ERβ

could serve as a viable therapeutic target for delaying the aging process of the brain, reducing the risk for the development and delaying the progression of early pathology of AD particularly in females, although it could also benefit male brains (George et al., 2013).

5. ERβ polymorphisms in AD

Since the first study published in 2001, there have been a total of 13 reports that examined the association between ERβ polymorphism and AD risk over the last 15 years (Table 1). Though conflicting evidence exists, the majority of the studies indicate that genetic variation in ERβ increases the risk of AD and this association may be age and sex-dependent with a greater impact in females. For instance, a 4-year prospective cohort study carried out in 249 women with Down syndrome who were non-demented at baseline, showed a 2-fold increase in the risk for AD in women carrying 1 or 2 copies of the minor allele at 3 single nucleotide polymorphisms (SNPs) in introns 6 (rs4365213 and rs12435857) and 7 (rs17766755), and one SNP in intron 8 (rs4986938) of ERβ. Furthermore, this study showed that the association of these four SNPs with an increased risk for the development of AD was observed solely in postmenopausal women (Zhao et al., 2011c). Additionally, a separate case-control study involving 246 Jewish women aged above 80 years old demonstrated that women who were carriers of the minor allele of the ERβ variant, rs4986938, had a 1.7 fold increased risk for developing vascular dementia. This association was specific to the ERβ variant and no association with ERα genotypes or haplotypes was found (Dresner-Pollak et al., 2009). Likewise, a study conducted in 387 subjects with clinically diagnosed probable AD and 467 cognitively normal individuals derived from eastern Finland found that female, but not male, subjects carrying two copies of the ERβ variants, rs1271573 or rs1256043, exhibited a nearly 2-fold increase in the risk of developing AD; a risk which remained significant after adjustment for the APOE genotype and age (Pirskanen et al., 2005). However, contrary to data from previously discussed studies (Dresner-Pollak et al., 2009; Zhao et al., 2011c), the data from this study showed no independent association of the ERβ variant, rs4986938, with the risk of developing AD in either men or women (Pirskanen et al., 2005). The gender differences were further demonstrated in a large cohort study, the Health, Aging and Body Composition (Health ABC) study that involved 1184 male and 1343 female elders living in the US. The study found that one ERβ SNP, rs1256030, was associated with cognitive impairment in both genders, however, two other SNPs exhibited a gender-specific impact, with an increased risk associated with rs1256065 in women and rs1255998 in men (Yaffe et al., 2009). Moreover, a study conducted in Swedish population that focused on a CA repeat in intron 5 of the ERβ gene found that the allele 5 was associated with a decreased risk of developing AD in men but not in women (Forsell et al., 2001).

In addition to gender, the impact of ERβ polymorphism on risk of AD appears to be related to ethnicity as well. A recent study conducted in a multiethnic female cohort involving a total of 1,686 women enrolled in the Washington Heights Inwood Columbia Aging Project (WHICAP), reported that increased risk for AD was associated with four ERβ SNPs (rs944045, rs1256062, rs10144225, and rs2274705) in women of predominantly Caucasian AIMS-defined ancestry. Additionally, when vascular risk factors are taken into consideration, a separate SNP (rs1256059) was associated with increased risk for AD in

women of admixed/Hispanic ancestry. Interestingly, the data reveal a single SNP (rs10137185) that was associated with decreased risk for AD in women who identified themselves as Black. The authors speculate that these findings suggest that the varied effects of risk alleles could be due to different linkage disequilibrium patterns or differences in comorbid risk factors mediating SNP effects on risk for AD by group (Janicki et al., 2014). Moreover, a study carried out in 126 AD subjects of German and Austrian descent and 111 healthy controls indicated that the susceptibility for AD was encoded by the ERβ SNPs rs4986938 and rs1255953 but not rs1255998 despite strong linkage disequilibrium (Luckhaus et al., 2006).

The association between ERβ polymorphism and AD predisposition is further supported by the findings from the multicenter longitudinal Three City (3C) study carried out in a population of 3799 non-demented elderly French women. The data from this study indicate that ERα polymorphisms were not significantly associated with the risk of decline on any of the cognitive tasks. However, significant associations with the ERβ polymorphism, rs1256049, were identified including an increased risk of substantial decline in visual memory, psychomotor speed, and on the incidence of MCI (Ryan et al., 2013). Further analysis indicated that, in support of the initial finding of a significant interaction between ERα and ERβ polymorphisms and the risk for AD (Lambert et al., 2001), the association between ERβ polymorphism and AD appeared to be modified by ERα polymorphism (Ryan et al., 2014). Interestingly, the same study showed a slight association between the minor allele of the ERβ SNP, rs4986938, and a decreased risk of decline in psychomotor speed (Ryan et al., 2013).

The aforementioned studies indicate an association between ERβ SNPs and increased susceptibility to developing AD, however, these studies have been contradicted by others. A cohort study performed in 79 amnestic mild cognitive impairment (MCIa) patients and 144 healthy controls examined the association between the ERβ SNP rs4986938 and the development of MCIa as well as the interaction between rs4986938 and APOE4 in the progression of AD. The investigators reported that the presence of the APOE4 allele, and not the alleles of the ERβ SNP, is a risk factor for the conversion of MCIa to AD (Elcoroaristizabal Martin et al., 2011). Later, the same investigators conducted a case-control study that examined the association between rs4986938 and the risk of MCIa or AD and no association was found (Fernandez-Martinez et al., 2013). Consistent with these two reports, a recent French case-control study that examined 5 different ERβ SNPs (rs4986938, rs867443, rs10144225, rs7154455 and rs1952586) in a population of 1007 AD cases and 647 controls failed to detect a significant association between any ERβ SNPs with AD risk (Goumidi et al., 2011).

In summary, 8 out of the total of 13 reports have shown that genetic polymorphism in ERβ is a component of AD susceptibility; however, 5 reports did not find such an association (Table 1). One major factor that may have contributed to the disparities among studies could be the composition of the study population. One common feature shared in 4 of the 5 reports that failed to find an association is that those studies were all based on a mixed population of both genders (Elcoroaristizabal Martin et al., 2011; Fernandez-Martinez et al., 2013; Ryan et al., 2014). In contrast, of the 8 studies that showed a positive result, 7 studies were either

conducted only in women (Dresner-Pollak et al., 2009; Janicki et al., 2014; Luckhaus et al., 2006; Ryan et al., 2013; Zhao et al., 2011c) or stratified by gender (Pirskanen et al., 2005; Yaffe et al., 2009), and all showed a significant association between ERβ polymorphisms and AD susceptibility in women. Taken together, despite the differences among studies, it is clearly indicated that ERβ polymorphisms increase the risk for cognitive impairment and/or AD in women, providing further support of a role for ERβ signaling in the maintenance of neurological health and the modulation of AD predisposition predominantly in the female population. With respect to a possible role of ERβ in male brain, three studies reported mixed results showing that certain genetic variations of ERβ increased (Yaffe et al., 2009) or decreased (Forsell et al., 2001) or had no effect (Pirskanen et al., 2005) on the likelihood for developing cognitive impairment or susceptibility for AD in men. Therefore, further investigations are needed in order to reach a clear conclusion.

6. ERβ and human APOE isoforms

APOE mediates the redistribution of lipids among cells and is expressed at highest levels in brain and liver. Human APOE gene exists in three major isoforms coded by three distinct alleles, ε2, ε3, and ε4. APOE2 is relatively rare, with only 5% incidence, and it is recognized as a protective variant against AD (Kim et al., 2009; Liu et al., 2013). APOE3 is the most common isoform present in 75% of the population, and it is considered to be risk-neutral in AD. APOE4 occurs in only 20% of the population, however, it accounts for approximately 65% of AD cases (Kim et al., 2009; Liu et al., 2013). As the greatest genetic risk factor for AD, APOE4 increases the risk of preclinical cognitive decline, lowers the age of onset of AD, and decreases the response to AD treatments (Corder et al., 1993; Poirier et al., 1993; Ward et al., 2012; Yaffe et al., 1997).

As reviewed earlier, increasing evidence indicates that the link between APOE4 and AD is far more prominent in women, suggesting that female sex hormones play a role in modulating the effect of APOE4 in the development of AD. This concept is supported by several lines of research findings. APOE expression is modified by the genetic variants of APOE; APOE4 is associated with a lower level of APOE in the brain compared to both APOE2 and APOE3 brains. APOE expression is regulated by a genomic mechanism involving ER as a result of the presence of an estrogen response element (ERE) on the promoter of the APOE gene (Lambert et al., 2004). 17β-estradiol increases the expression of APOE in brain regions responsible for learning and memory, including hippocampus and cortex (Levin-Allerhand et al., 2001). APOE expression in the brain is also affected by the estrous cycle in a brain region specific manner, and regional variability in APOE protein level appears to vary as a function of the ER subtype (Struble et al., 2003). Differential regulation of APOE protein expression by ERα and ERβ is further demonstrated in both cultured neurons and brain tissues (Wang et al., 2006; Zhao et al., 2011a).

Interaction between female sex hormones and APOE genotype has also been demonstrated by studies that investigated APOE modulation of the effects of estrogen in the brain under both normal and pathological conditions (Struble et al., 2008; Yaffe et al., 2000). 17β-estradiol increased the extent of neurite outgrowth in cultured adult mouse cortical neurons that expressed the human APOE2 or APOE3 genes, but had no effect on neurons from non-

expressing mice or in those supplied with exogenous APOE4 protein (Nathan et al., 2004). Similarly, in a familial AD mouse model expressing human APOE gene isoforms, treatment with 17β-estradiol decreased amyloid deposition in the brains of APOE2 and APOE3-bearing mice, whereas amyloid deposition was increased in the brains of APOE4-bearing mice (Kunzler et al., 2014). Consistent with the findings in animal models, a study conducted in 2,716 elderly women found that ET use was associated with less cognitive decline in APOE4-negative but not APOE4-positive individuals (Yaffe et al., 2000). Similarly, in a cross-sectional study, women who were non-APOE4 carriers exhibited the highest level of learning and memory performance after ET, while women who were APOE4 carriers performed no better after ET than did APOE4 carriers who did not receive ET (Burkhardt et al., 2004). ET appears to interact with APOE genetic variants in AD brain as well. As demonstrated by a 12-month randomized and placebo-controlled AD study, women patients without APOE4 exhibited better mood and cognition with ET treatment (Valen-Sendstad et al., 2010). In addition to the nullification of the benefits of ET, the APOE4 status could also transform ET into a toxin, as found in the Nurses' Health Study in which ET was associated with a worse rate of cognitive decline among APOE4 carriers (Kang and Grodstein, 2012).

Together, these findings indicate that estrogen may have a dual effect in the brain modulated by APOE genotype, and it tends to exert a positive outcome when APOE4 is absent while an opposite outcome could happen when APOE4 is present. This conclusion, however, is contradicted by studies that found that estrogen use was associated with a beneficial effect in APOE4 carriers (Jacobs et al., 2013; Ryan et al., 2009; Yue et al., 2007). Current HT was associated with a decreased risk of dementia associated with APOE4 (Ryan et al., 2009), and no effect was found when APOE4 is absent (Jacobs et al., 2013). In line with these findings, a study conducted in a Chinese population found that long-term, low-dose ET was beneficial in women APOE4 carriers, and no significant difference was found between HT treatment and control groups among women APOE3 carriers (Yue et al., 2007). The discrepancy could be caused by potential confounding factors, which warrants further investigations that can lead to a clear understanding of this very important phenomenon.

Interestingly, despite the increasing number of studies that demonstrated an interaction between estrogen and APOE isoforms, there have been nearly no studies that examined the involvement of the ER subtype. Our recent analyses demonstrated, for the first time, that activation of ERβ differentially regulated insulin/IGF1 signaling pathways in mouse brains expressing human APOE2, APOE3 or APOE4. Activation of ERβ increased the metabolic activity in both APOE3 and APOE4 brains, whereas the impact was substantially lower in APOE2 brain. These data provide preliminary support for the role of the potential interaction of ERβ signaling with APOE genotype in modulation of susceptibility to AD. ERβ signaling deficiency resulted from age, menopause, or genetic polymorphisms contribute to the increased risk of AD in elderly women, and the extent of the risk could be modified by the APOE genetic status. Future studies of the interactive effects, at the molecular level, of APOE genotype with sex, female sex hormones, and ERβ signaling, is critically needed. These investigations will very likely lead to sex and APOE genotype-specific targets for AD prevention and risk reduction particularly in high-risk population of female APOE4 carriers.

7. ERβ-targeted therapeutics for AD and beyond

One of the major problems inherent in the traditional ET formulations is that they act non-selectively as agonists in all tissues that contain ERs, which leads to both desirable and unwanted outcomes (Zhao and Brinton, 2006b, 2007b). On one hand, ET has been established as the most effective therapy to treat menopausal symptoms such as hot flashes. Moreover, ET has been well demonstrated for its important role in maintaining bone health, for example, prevention of hip fracture as found in WHI studies. On the other hand, this non-selective agonistic nature increases the risk of breast and endometrial cancer and thromboembolism. In the search for a non-feminizing and safe estrogen alternative therapy for long-term use to sustain neurological health, two venues of approach have been pursued: one is the development of and assessment of tissue-selective ER modulators and the other is the development and evaluation of ERβ-selective agonists, as exemplified by our own work (Zhao and Brinton, 2005, 2006a, 2009; Zhao et al., 2007; Zhao et al., 2009; Zhao et al., 2013; Zhao et al., 2011a; Zhao et al., 2005; Zhao et al., 2006).

Therapeutically, an ERβ-targeted approach presents several major advantages. First, selective activation of ERβ reduces potential antagonistic interactions between the two ERs. ERα and ERβ have been shown to work in a complex manner, both complementary and sometimes antagonistic, in a number of biological systems. Our earlier study showed that activation of both ERα and ERβ by different agonists led to decreased neural responses indicating an antagonistic interaction (Zhao and Brinton, 2007a; Zhao et al., 2004). Second, selectively targeting ERβ minimizes undesirable events mediated by ERα in reproductive tissues. In comparison with their impact with a large degree of overlaps in the brain, ERα and ERβ play a more differential role in reproductive systems. ERα has been shown to serve as the primary mediator of sexual development and modulation, and promote cell proliferation in response to estrogens; whereas ERβ has a much smaller impact on these processes and has been shown to be anti-proliferative in breast and uterine tissues. These differential regulatory patterns present an optimal opportunity for an ERβ-selective agonist to reap the health benefits associated with ERβ without activating untoward effects mediated by ERα (Zhao and Brinton, 2006a). Moreover, the lack of uterotrophic activity associated with an ERβ agonist eliminates the need to combine with a progestogen in the treatment, which has been proven as a compounding factor for the effectiveness of ET, for example, the presence of a progestogen and a different treatment schedule could exert an inhibitory effect against estrogen-mediated neural responses (Aguirre et al., 2010; Nilsen et al., 2005; Zhao et al., 2012). Together, these therapeutic advantages make ERβ a promising and safe target for the development of therapeutic agents that could benefit various indications in which ERβ plays a role.

The therapeutic potential of an ERβ-based intervention for AD has been directly demonstrated in both female and male AD mouse models as reported in two recent articles. One came from a recent study from our group, in which we demonstrated that in a female triple transgenic AD (3×Tg-AD) mouse model, when initiated prior to the appearance of AD pathology, a 9-month dietary supplementation with an ERβ-selective phytoestrogenic (phyto-β-SERM) formulation promoted physical health, prolonged survival, improved spatial recognition memory, and attenuated Aβ deposition and plaque formation in the brains

of AD mice (Zhao et al., 2013). This formulation is currently being assessed in phase I/IIa human trials designed to evaluate the dosage, safety, pharmacokinetics, and proof-of-concept efficacy for mitigation of memory complaints in menopausal women. The other report demonstrated the efficacy of an ERβ agonist in male AD animals. It was found that chronic co-administration of a selective androgen receptor (AR) modulator (SARM) and a selective ERβ agonist in gonadectomized male 3×Tg-AD mice improved long-term memory, reduced anxiety-like behavior, increased the expression of Aβ-degrading enzymes, and reduced soluble Aβ levels (George et al., 2013). In addition, the combination treatment increased the level of AR in the hippocampus of male AD mice likely via ERβ since treatment with the SARM alone did not yield a significant effect (George et al., 2013). These compelling data warrant further investigations of the therapeutic potential of an ERβ-based approach for AD stratified by disease status, sex/gender, ApoE genotype, and ERβ polymorphisms, to address the important questions including: 1) Would there be a "critical window of opportunity" for an ERβ-targeted therapy to exert a neuroprotective effect? In other words, would an ERβ-targeted therapy be potentially effective in preventing or delaying the onset of AD, slowing the progression of early-stage AD, or would it also provide some benefits in treatment of mid-to-late stage AD? 2) Would an ERβ-targeted therapy be potentially effective in female brain only, or would it also exert some benefits in male brain as suggested by the study discussed above (George et al., 2013)? 3) Would the therapeutic effects of an ERβ-targeted therapy be potentially modulated by ApoE genetic status? 4) Would there be a potential interaction between an ERβ-targeted therapy and ERβ risk alleles? Ultimately, in order to increase the possibility of translational success, preclinical findings need to be further validated in non-familial AD (FAD) models that are more in line with the pathophysiological conditions of the majority of human AD cases, i.e., the late-onset sporadic AD (SAD), the most common form of AD representing over 95% of current human AD population.

To date, several dozen AD models that mimic the genetic cause of AD have been developed, including the widely used models such as PDAPP (hAPP^{V717F}) (Games et al., 1995), Tg2576 (hAPP^{K670N,M671L}) (Hsiao et al., 1996), J9 (hAPP^{K670N,M671L,V717F}) (Hsia et al., 1999), 5×FAD (hAPP^{K670N,M671L,I716V,V717I} + hPS1^{M146L,L286V}) (Oakley et al., 2006), 3×Tg-AD (hAPP^{K670N,M671L} + hPS1^{M146V} + hTau^{P301L}) (Oddo et al., 2003), and the recently developed human neural stem cell derived three-dimensional culture system ((hAPP^{K670N,M671L,V717I,} PS1-dE9) (Choi et al., 2014). A common feature of these models is that they all carry some mutant form of human genes related to FAD that lead to overproduction of neurotoxic Aβ peptides and amyloid plaques as well as neurofibrillary tangles in some models. These models have proven valuable in understanding the mechanisms underlying certain aspects of AD pathology and preclinical evaluation of the therapeutic effects of AD candidate drugs, for instance, the effects on amyloid pathology and cognitive function. Overall, since these FAD-related models accurately simulate the histopathological hallmarks of AD, they generally hold excellent face validity; however, they have shown poor predictive validity that could be closely associated with their unreliable construct validity. First, since human SAD is not caused by genetic mutations related to amyloid or tau production, it is very likely that the findings derived from FAD-related animal models are not replicated in human SAD patients. Second, aging is the

greatest risk factor for human SAD, however, in FAD-related animal models, overexpression of human mutant genes accelerate the onset of AD-like pathology, with amyloid plaques appearing even when the animals are 2-months old (Lee and Han, 2013), which compromises the role of the age factor in the pathogenesis and intervention of AD. It has been proposed that the current trend of deficits in AD translational research could in part be attributed to the discord between preclinical studies that were mostly conducted in FAD-related animal models and clinical studies that were carried out in patients mostly with late-onset SAD (Franco and Cedazo-Minguez, 2014; Laurijssens et al., 2013). Therefore, there is an urgent need for novel models that more adequately reflect the underlying etiology of human SAD, which would increase the construct and thus predictive validity of the models. Such models will be crucial for successfully translating preclinical findings to humans in the future.

In addition to neurodegenerative diseases like AD, the diverse influence of ERβ in the human body has made it a potential target for a multitude of other conditions as well, including menopausal symptoms, cardiovascular disease, multiple sclerosis, depression, and endocrine-related cancers (Nilsson et al., 2011; Zhao and Brinton, 2006a). MF101 is an oral, botanically derived extract that selectively regulates ERβ, and is demonstrated by both preclinical and clinical data for its therapeutic promise for treating postmenopausal vasomotor symptoms without increasing cancer risks (Grady et al., 2009; Leitman and Christians, 2012). Our own research has provided further support for the therapeutic potential of an ERβ-based therapy for the improvement of both physical and neurological health during menopause (Zhao et al., 2009; Zhao et al., 2011a). Another major application associated with ERβ is its potential utility as a target for the development of cancer therapy (Fox et al., 2008; Hartman et al., 2012). In breast tissue, ERα promotes cell proliferation in response to estrogens, whereas ERβ exerts anti-proliferative effects (Hartman et al., 2009). The presence of ERβ is associated with favorable outcomes in women with breast cancer treated with adjuvant tamoxifen (Younes and Honma, 2011). In males, as the predominant ER in the prostate gland, ERβ regulates prostate growth by down-regulating AR expression (Prins and Korach, 2008). Selective activation of ERβ induced apoptosis in epithelium and stroma of benign prostatic hyperplasia, as well as androgen-independent tumor cells implicated in recurrent prostatic cancer (McPherson et al., 2010). Therefore, it is reasonable to speculate that imbalanced or loss of ERβ expression could play a pivotal role in tumor development and progression (Bardin et al., 2004; Gallo et al., 2012). In support of this hypothesis, overexpression of ERβ has been shown to act as a tumor suppressor by modulating the transcription of genes involved in cell growth control, cell cycle progression and apoptosis, and is associated with longer patient survival (Pinton et al., 2009; Pinton et al., 2010). Again, given these positive findings, the therapeutic potential of ERβ-selective agonists for treating these conditions needs to be further explored in appropriate animal models before moving into human studies.

8. Concluding remarks

Since its discovery in 1996, ERβ has been widely demonstrated for its role in the regulation of a broad spectrum of brain activities from development to aging. ERβ polymorphisms have been associated with accelerated brain aging and increased risk for the development of AD

predominantly in women. Future studies to examine the interactive impact of ERβ signaling deficits and genetic or environmental stressors on the adaptation and defense of the brain against neurodegeneration are critically needed. The current trend of lost-in-translation in AD drug development underscores the urgent need for novel models that recapitulate the etiology of human late-onset SAD, the most common form of AD representing over 95% of all current human AD cases. Combining the use of FAD-related models that generally have excellent face validity and SAD-related models that hold more reliable construct validity would together increase the predictive validity of preclinical findings for successful translation into humans.

Acknowledgments

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Highlights

- AD differentially affects women and men.

- Sex interacts with APOE genetic isoforms to modulate the risk for AD.

- ERβ is involved in a broad spectrum of brain activities from development to aging.

- ERβ polymorphisms are associated with increased risk for cognitive impairment and AD in women.

- ERβ-based approach holds therapeutic promise for AD prevention, risk reduction, and early intervention.

- Human late-onset sporadic AD models are critically needed for successful translation of preclinical findings into humans.

Table 1

ERβ polymorphisms in AD (2001-2014)

Study & Year of Publication	ESR2 Gene Variants	Study Design and Subjects	Clinical Assessments	Diagnoses Criteria	Major Findings
Forsell et al. 2001	14 alleles of CA repeat (intron 5)	Case-control study in Swedish population: AD Cases: n=336,122 men, 214 women, age at disease onset = 66 ± 9, age= 71±9 Controls: n= 110, 37 men, 73 women, age = 68 ± 11	Not provided	AD Diagnoses: DSM-IV criteria Control Criteria: Cognitively healthy and no family history of dementia	Allele 5 with 18 CA repeats was associated with a decreased risk of developing AD in men but not in women.
Lambert et al. 2001	rs4986938 (3′-UTR)	Case-control study in UK Caucasian population: AD Cases: n = 186, 39% males, age at disease onset = 64.6 ± 11.8, age = 67.9 ± 12.5 Controls: n = 405, 47% males, age = 62.6 ± 14.9	Not provided	AD Diagnoses: DSM-III-R & NINCDS-ADRDA criteria for definitive or probable AD Control Criteria: Without dementia criteria and with full integrity of cognitive functions	No association of rs4986938 with the risk of developing AD in either gender was found.
Pirskanen et al. 2005	IVS3-1842A>G rs1271573 rs1256043 rs1256059 rs4986938	Case-control study in Finnish population: AD Cases: n=387, 116 men, 271 women, age at disease onset = 71.8 ± 7.1 Controls: n=467, 185 men, 282 women, age when sampled = 69.5 ± 5.1	A thorough investigation that included a medical history, physical, neurological and neuropsychological examination, routine laboratory tests, and brain imaging.	AD Diagnoses: NINCDS-ADRDA criteria for probable AD and confirmation of AD pathology according to CERAD criteria for AD; MMSE score = 17.2 ± 7.0 Control Criteria: MMSE score = 27.0±1.4, or neurophysiological test yielding no signs of dementia	Variation in the ESR2 gene was associated with an increased risk of AD in women, whereas it did not contribute to disease susceptibility in men. Specifically, the rs1271573 T/T and rs1256043 T/T genotypes were associated with a nearly 2-fold increase in the risk of AD in women, and remained significant after adjustment with the APOE genotype and age.
Luckhaus et al. 2006	rs4986938 rs1255998 rs1255953	Case-control study in German-Austrian population: AD Cases: n = 126, 44 men, 82 women, age = 76.9±9.8 Controls: n=111, 41 men, 70 women, age = 74.1±3.7	An extensive screening that included routine laboratory workup, psychometric testing and cerebral imaging.	AD Diagnoses: NINCDS-ADRDA criteria for probable or possible AD Control Criteria: Confirmed to be free of cognitive impairment by psychometric testing and psychiatric interview	Susceptibility for AD was associated with rs4986938 and rs1255953 but not with rs1255998.
Dresner-Pollak et al. 2009	rs4986938	Case-control study in Female Jewish population from Israel: AD cases: n = 118, age=83±7 VaD cases: n = 60, age=82±6 Controls: n=68, age=81±7	Clinical assessments included Folstein MMSE, Hachinski ischemia scoring and computed tomography.	AD Diagnoses: NINCDS-ADRDA criteria for probable AD VaD Diagnoses: NINDS-AIREN criteria for probable VaD Control Criteria: Cognitively intact women with MMSE score > 25	rs4986938 was associated with susceptibility for VaD in elderly Jewish women.
Yaffe et al. 2009 (Heatlh ABC Study)	rs1255998 rs1256049 rs1256065 rs1256030	Prospective cohort study in community-dwelling elders living in Memphis and Pittsburgh in the US: n = 2527, 1184 men (mean age = 73.7),	The modified mini-mental state examination (3MS) was administered during the baseline visit and repeated at year 3 and 5 follow-up visits.	Cognitive Impairment: Defined as a 3MS decline of 5 or more points at either follow-up visit.	1) 2 SNPs (rs1256065, rs1256030) were associated with likelihood of developing cognitive impairment in women.

Study & Year of Publication	ESR2 Gene Variants	Study Design and Subjects	Clinical Assessments	Diagnoses Criteria	Major Findings
		1343 women (mean age = 73.4), non-demented at study onset 1343 women (mean age = 73.4), non-demented at study onset			2) 2 SNPs (rs1255998, rs1256030) were associated with cognitive impairment in men.
Zhao et al. 2011	13 SNPs including: rs17766755 rs4365213 rs12435857 rs4986938	Prospective cohort study in a community-based cohort of women with down syndrome resided in the north eastern region of the US: n = 249, age = 31-70, non-demented at study onset	Evaluations of cognition and functional abilities, behavioral/psychiatric conditions and health status were administered during the baseline visit and repeated at 14-18-month intervals for 4 years. Cognitive function was evaluated with a test battery designed for use with individuals with down syndrome varying widely in their levels of intellectual functioning.	Dementia Diagnoses: Based on the recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability. A substantial and consistent decline over the course of follow-up (of a duration of at least one year). Control Criteria: Without cognitive or functional decline, or some cognitive and/or functional decline that was not of significant magnitude to meet dementia criteria.	Two-fold increase in the risk of AD was found in postmenopausal women carrying 1-2 copies of the minor allele at 3 SNPs: rs17766755, rs4365213, rs12435857, and rs4986938.
Goumidi et al. 2011	rs4986938 rs867443 rs10144225 rs7154455 rs1952586	Case-control study in Caucasians originating from northern France: AD cases: n = 1007, 33% males, age at disease onset = 70.0 ± 8.3, age = 74.8 ± 8.8 Controls: n = 647, 37% males, age = 73.1 ± 8.3	Not provided	AD Diagnoses: DSM-III-R & NINCDS-ADRDA criteria for probable AD Control Criteria: Subjects without DMS-III-R dementia & with intact cognitive function & MMSE scores 25 & no family history of dementia.	No significant association between ESR2 SNPs and AD risk was found.
Elcororaristizabal Martin et al. 2011	rs4986938	Prospective cohort study in Spanish population: MCIa cases: n = 79, 51.9% females, age = 72.15 ± 709 Controls: n = 144, 57.2% females, age = 71.60 ± 8.42	Symptomatic and cognitive assessment was administered during the baseline visit and repeated every 12 months for 39 months.	MCIa Diagnoses: Peterson's criteria AD Diagnoses: DSM-IV & NINCDS-ADRDA criteria for probable and possible AD. Control Criteria: No memory complaints & no severe disease & no previous neurological disease & Clinical Dementia Rating scale score = 0.	No association between rs4986938 and MCIa patients who converted to AD was found.
Fernandez-Martinez et al. 2013	rs4986938	Case-control study in Caucasian participants living in Spain: MCIa cases: n = 204, 61.3% females, age = 70.25±8.6 AD cases: n = 350, 71.1% females, age = 72.17±8.3 Controls: n = 262, 59.5% females, age = 74.00 ± 9.6	Participants were evaluated using a broad battery of neuropsychological tests. For patients with MCIa and AD, evaluations also included routine blood tests.	MCIa Diagnoses: Peterson's criteria AD Diagnoses: DSM-IV & NINCDS-ADRDA criteria for probable and possible AD.	No association between rs4986938 and the risk of MCIa or AD was found.
Ryan et al. 2013 (*Three City Study*)	rs1256049 rs4986938	Prospective cohort study in community-dwelling elderly women from three French cities: n = 3799, age = 69-77, non-demented at study onset	Cognitive assessments (global function, verbal fluency, visual memory, psychomotor speed, and executive function) were performed at baseline and the 2-, 4-, and 7-year follow-ups.	MCI Diagnoses: Defined as an education and age-matched score in the lowest quintile on at least one cognitive test & with a cognitive complaint.	1) rs1256049 was associated with an increased risk of substantial decline in visual memory, psychomotor speed, and on the incidence of MCI 2) rs4986938 was associated with a decreased risk of decline in psychomotor speed.

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Ryan et al. 2014 (*Three City Study*)	rs1271572 rs1256049 rs4986938	Prospective cohort study in community-dwelling elders from three French cities: n=6959, age ≥ 65, non-demented at study onset	Clinical assessments including standardized questionnaires and clinical examinations were performed at baseline and the 2-4-, and 7-year follow-ups.	Dementia/AD Diagnoses: DSM-IV & NINCDS-ADRDA criteria.	No significant association between ESR2 variants and the risk of dementia or AD was found.
Janicki et al. 2014 (*Washington Heights Inwood Columbia Aging Project*)	20 SNPs including: rs944045 rs1256062 rs10144225 rs2274705 rs10137185 rs1256059	Prospective cohort study in a multiethnic community of elderly women residing in northern Manhattan, New York, US: n = 1686, age = 72.0 ± 6.7, non-demented at study onset	All subjects underwent an in-person interview of general health and functional ability followed by a standardized assessment, including medial and medication history, physical and neurological examination, and a neuropsychological battery. Assessments were conducted at 18-24 month intervals over a mean of 6.1 years of follow up.	AD Diagnoses: NINCDS-ADRDA criteria & excluded MCI or low neuropsychological scores in order to obtain the most robust phenotype.	1) Increased risk for AD was associated with four SNPs (rs944045, rs1256062, rs10144225, rs2274705) in women of predominantly Caucasian origin. 2) rs10137185 was associated with a decreased risk for AD in women self-identified as Black. 3) rs1256059 was associated with an increased risk for AD in women of admixed/Hispanic ancestry when vascular risk factors were included.