Age, *APOE* and Sex: Triad of Risk of Alzheimer's Disease

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Abstract

Age, apolipoprotein E ε4 (*APOE*) and chromosomal sex are well-established risk factors for late-onset Alzheimer's disease (LOAD; AD). Over 60% of persons with AD harbor at least one *APOE*-ε4 allele. The sex-based prevalence of AD is well documented with over 60% of persons with AD being female. Evidence indicates that the *APOE*-ε4 risk for AD is greater in women than men, which is particularly evident in heterozygous women carrying one *APOE*-ε4 allele. Paradoxically, men homozygous for *APOE*-ε4 are reported to be at greater risk for mild cognitive impairment and AD. Herein, we discuss the complex interplay between the three greatest risk factors for Alzheimer's disease, age, *APOE*-ε4 genotype and female sex. We propose that the convergence of these three risk factors, and specifically the bioenergetic aging perimenopause to menopause transition unique to the female, creates a risk profile for AD unique to the female. Further, we discuss the unique risk of the APOE4 positive male which appears to emerge early in the aging process. Evidence for impact of the triad of AD risk factors is most evident in the temporal trajectory of AD progression and burden of pathology in relation to *APOE* genotype, age and sex. Collectively, the data indicate complex interactions between age, APOE genotype and gender that belies a one size fits all approach and argues for a precision medicine approach that integrates across the three main risk factors for Alzheimer's disease.

Keywords

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1. Introduction

The greatest risk factors for Alzheimer's disease are age(1–3), the ApoE4 allele(4–6) and female sex (1, 7–10). Prevalence of AD is greater in women (1) whereas the incidence for AD is reported to be comparable in women and men (11) until later age when the incidence is greater in women (12) (Figure 1). Typically, the greater risk of AD in females is attributed to their greater longevity of, on average, 4.5 years.

Herein, we review the three greatest risk factors for AD and propose that it is the interaction between these risk factors that either increase or decrease risk of Alzheimer's. The complexity of the interaction between this triad of risk factors belies simple on factor causality but instead more closely approaches the complexity of factors leading to late onset Alzheimer's disease (LOAD). While this approach takes into consideration three factors that have broad systems biology impact, it still does not fully capture the complexity of the processes leading to risk of LOAD. However considering the interaction between the three greatest risk factors for AD is a step in the right direction.

1.1. Aging

Age remains *the* greatest risk factor for Alzheimer's and is thus a fundamental driver for development of the disease(13). Aging at the global population level is unprecedented and without parallel in the history of humanity(14). During the 20th century the proportion of older persons continued to rise and this trend has continued into the twenty-first century(14). By 2050, the number of older persons in the world will exceed the number of young for the first time in history(14). Between 2015 and 2030, the number of people in the world aged 60 years or over is projected to grow by 56% and by 2050 the global aged population of is projected to more than double(15). Globally, the number of people aged 80 years or over, the "oldest-old" persons, is growing the fastest. People 80 or older currently constitute more than 3% of the population of Northern America and nearly 3% of the population of Europe. Population ageing is a pervasive global phenomenon that will endure for the foreseeable future. By 2050, one in every five people will be aged 60 years or over(15).

The female prevalence of AD is well documented and is generally attributed to the greater life span of women relative to men(1). Globally, women outlive men by an average of 4.5 years(15). However, survival of males is projected to be comparable to females with near equality in longevity between females and males(15). Thus, the greatest risk factor for AD will be equally distributed between the sexes in the near future. Data from the multiple studies show that the incidence of Alzheimer's disease rises exponentially after the sixth decade of life (16).

Aging is a complex progressive process involving every organ and cell system in the body and is the result of coordinated systems biology events that can span decades(17–27). Further, aging is typified by a coordinated series of sequential steps involving specific pathways at each phase in the aging process (17, 28). Systems either driving brain aging are contributors to risk of AD and include glucose hypometabolism and mitochondria dysfunction, innate immune and inflammatory reactions, beta amyloid processing,

dysregulation of cholesterol homeostasis, white matter degeneration and decline in regenerative capacity (16, 17, 19, 21, 22, 24–26, 29, 30)

While aging is typically described and depicted as a linear process, in reality the program of senescence is nonlinear and better represented as step functions between transition states. Aging is typified by transition states and because aging is occurring throughout multiple organ systems the process of aging is highly complex(17, 19–21, 24). Each phase of the aging process can be considered a transition state that can be modified to either accelerate or delay progression to the next phase of aging (17, 22, 25).

It is becoming increasingly clear that complex systems have critical thresholds, often referred to as tipping points, when a system shifts from one state to the other(17). Analyses of transition state dynamics predict three health states, healthy, pre-disease and disease(17). The pre-disease transition state is typically defined as the limit of the normal state that precedes the tipping point into disease. Importantly, the pre-disease state is unstable and is thus potentially reversible. However, the duration of reversibility is limited. The bifurcation point between pre-disease and disease is characterized by a *critical slowing* of the system, in which it becomes increasingly slow to recover from small perturbations to equilibrium(17).

Transition states are inherently unstable and in the case of neurological transition states, indicators of dysfunction at the limits of normal can be signals of instability and tipping points. The presence, variability, intensity and duration of neurological symptoms provide potential advance warning signs of impending risk of later health risks, particularly neurodegenerative diseases. Multiple conditions that emerge during aging, such as metabolic dysregulation, cholesterol dyshomeostasis, insomnia, depression, subjective memory complaints and cognitive decline are associated with increased risk of neurodegenerative diseases later in life such as Alzheimer's(17, 31). Alzheimer's, like multiple neurodegenerative diseases, is characterized by a long prodromal period, 20 years, during which the disease progresses to clinically diagnosed dysfunction. Identifying persons that are at risk for AD while still in a modifiable transition states is critical for reversing or delaying development of Alzheimer's (31).

Individual genetics form the foundation for and can modify the complex multidimensional trajectory of aging (32). Considered below are the two major genetic risk factors of AD, APOE genotype and chromosomal sex.

2. Overview of *APOE*

2.1. Biological Role and Evolution of *APOE*

Apolipoprotein E (ApoE) is a 34-kDa lipid binding protein that functions in the transport of triglycerides and cholesterol in multiple tissues, including brain, by interacting with lipoprotein receptors on target cells (33, 34) (1–4). ApoE is a cholesterol transporter and functions as a key regulator to coordinate the mobilization of cholesterol between cells and to redistribute cholesterol within cells. These functions are particularly critical for the nervous system where ApoE transport of cholesterol is critical for maintenance of myelin and neuronal membranes both in the central and peripheral nervous systems (35). In the

CNS, ApoE functions in conjunction with APOJ and APOC1, which together deliver cholesterol necessary for membrane remodeling, required for synaptic turnover and dendritic reorganization (35). ApoE is particularly critical to the brain as other cholesterol transporters abundant in the plasma, such as ApoA1 and ApoB, are virtually absent in brain thus making the brain particularly reliant upon ApoE for cholesterol transport (35).

APOE, a 3.6 kb long gene, is located on chromosome 19 and (36) encodes for apolipoprotein E (apoE), a 299 amino acid long lipoprotein. Three ApoE isoforms exist in humans: ApoE2, ApoE3, and ApoE4, which differ from one another by single amino acid substitutions at positions 112 and 158, ApoE2 (Cys-112, Cys-158), ApoE3 (Cys-112, Arg-158), and ApoE4 (Arg-112, Arg-158) (5). Substitution of cysteine at position 158 in ApoE2 results in hypocholesterolemia caused by low levels of low-density lipoprotein (LDL), cholesterol(5). In contrast, substitution of cysteine with arginine at position 112 in ApoE4 results in elevation of plasma cholesterol and LDL levels and predisposes the carrier to cardiovascular disease and neurodegenerative disorders, including Alzheimer's disease (AD) (5).

APOE evolved from the common hominid ancestor of humans and the great apes [9]. While there are three main isoforms of *APOE* in humans (i.e. ε2, ε3, and ε4), all great apes carry the *APOE*-ε4 allele(37). The ε3 allele is the most common in humans, especially in regions with a long-established agricultural economy. However, the ancestral ε4 allele remains high in regions where an economy of foraging still exists or where food-supply is often scarce(38). Although generally more common among African populations, the risk in cognitive decline conferred by carrying the ε4 allele is greater among individuals of European descent, particularly amongst northern European regions (39).

2.2. APOE Protein Structure: *Lipoprotein and Beta Amyloid Binding Motifs*

As depicted in Figure 2, the ApoE protein has an N-terminal receptor-binding region and C-terminal hydrophobic lipid-binding region located at amino acids 244–272. It is the N-terminal region, which contains the two polymorphic positions (i.e. positions 112 and 158) that distinguish the differing isoforms in many ways, such as differing antioxidant profiles (where ε2 ε3> ε4) (40, 41). In *APOE*-ε4, Arginine-61 interactions with Arginine-112 lead to a conformational change causing Arginine-61 to interact with Glutamine-255 in the aqueous environment, resulting in N- and C-terminal domain interactions that do not exist in the ε2 or ε3 isoforms, due to the less hydrophobic Cysteine-112 (42). These interactions are particularly evident when apoE is delipidated, preventing lipoprotein receptor docking and internalization of unlipidated apoE. *APOE*-ε4 also has an arginine-112 to glutamic acid-109 salt bridge, which causes the arginine-61 side chain to extend away from the 4-helix bundle in its structure. In turn, the arginine-61 domain interacts with glutamic acid-255 causing the structure of ε4 to become more compact compared to ε3. Structural differences lead to regions in ε4 that are less protected and less stable than the same regions in ε3 (43). This difference also leads to a binding profile that favors triglyceride-rich very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) over small phospholipid-rich high-density lipoproteins (HDL) particles. Compared to the structure of ε3, ε2 also causes changes in lipoprotein preference, due to impaired lipolytic processing of VLDL, creating increases in VLDL and decreases in LDL cholesterol (44). These differences in the ε2 and

ε4 structures lead to increases in pro-atherogenic lipoproteins, an accelerated trajectory towards atherogenesis compared to ε3, and therefore an increased representation in patients with hyperlipidemia and cardiovascular disease (45). However, despite this high prevalence and the strength of its association with both cardiovascular and cognitive risk, *APOE* testing is still used most exclusively for research purposes only.

2.3 ApoE and Cholesterol Transport

The *APOE* gene is expressed most highly in the liver and brain (34, 35, 46). Over 75% of plasma apoE protein is derived from hepatic synthesis (46). Second to the liver, is brain synthesis of apoE which predominantly occurs in astrocytes, followed by microglia and neurons (47). Given this high production, apoE is the most prevalent brain lipoprotein. The level of *APOE* expression varies by genotype, with ε2 typically having the greatest and ε4 having the lowest expression (48, 49). *APOE* isoform effects on plasma apoE concentration accounts for 20% of inter-individual variability of apoE concentration across all ages, with other factors including BMI, waist-to-hip ratio, oral contraceptives, and sex, also playing prominent role (50).

APOE isoforms differ in their rates of catabolism such that ε2 is catabolized more slowly than ε3 or ε4 (51). The increased rate of catabolism of apoE 4 leads to reduced availability of apoE to serve as a clearance protein, required for the clearance of cholesterol and toxic amyloid-beta (Aβ) oligomers (52).

Excess plasma apoE stimulates hepatic VLDL production and impairs lipolysis whereas a low level of apoE impairs plasma clearance of triglyceride-rich lipoproteins. Accordingly, there is about a 3-fold increase in AD-risk associated with the lowest tertile of apoE plasma concentrations, independent of *APOE* genotype (53). Notably, apoE plasma levels are lowest in MCI patients that progress to AD. Given that ε2 carriers generally show the highest protein concentration, there is some level of reduction in AD-risk in these individuals, while ε4 carriers have an increased risk. Studies of cultured astrocytes show that apoE-ε3 containing astrocytes are able to release more cholesterol to supply neurons than apoE-ε4 containing astrocytes (54).

ApoE-ε4 protein is also less efficient at delivering essential fatty acids such as docosahexaenoic acid (DHA) to neurons, thereby altering the function of glucose transporters and possibly playing a role in the development of insulin resistance, a state commonly attributed to an increased risk of AD (55). Given this alteration, it has been suggested that eiscosapentaenoic acid (EPA)-rich oils may be more suitable in ε4 individuals, especially for use as a hypotriglyceridaemic agent, showing a greater hypercholesterolaemic response to DHA than EPA (56). Indeed, there is a significant interaction between *APOE* genotype and plasma lipid response to both diet and pharmacological therapies (57). Since a ketogenic diet has shown to increase HDL and decrease LDL cholesterol in both obese and normal weight individuals, it has been proposed that ε4 carriers would benefit more from a ketogenic diet than non-carriers, particularly in regards to protection from AD (58–60). Furthermore, diets higher in saturated fats or diets rich in olive oil and high in monounsaturated fats both show more favorable outcomes in

LDL-particle count in ε4 individuals than ε3 individuals (61–64); LDL-particle count is seen as a better measure of atherogenicity than LDL cholesterol itself (65, 66).

Due to the altered protein structure, *APOE*-ε4 is poorly lipidated by ABCA1 and ABCG1, causing it to be more rapidly catabolized than the other isoforms. Furthermore, *APOE* polymorphisms show a differential impact on competition between fasting triglyceride-rich lipoproteins and LDL's for the LDL receptor (LDL-R) (67), ultimately leading to downregulation of LDL-R in states of high VLDL production, as seen in carriers of the ε2 and ε4 allele. These LDL-R interactions are necessary for remnant clearance of the partially metabolized atherogenic chylomicrons and VLDL particles. Interestingly, *APOE*-ε4 in non-human primates does not contain the domain interactions that gives human ε4 its characteristic salt-bridge, thus it behaves more like ε3 in humans in that it does not show preferential binding to triglyceride-rich lipoproteins like the human variant (68). In line with this, it has been proposed that gene targeting to replace arginine-61 with threonine-61 would likely correct the lipid binding preference that leads to the toxic effects of ε4 (69).

The cholesterol efflux regulatory protein (CERP), also known as ABCA1 (ATP-binding cassette transporter), is a major regulator of cholesterol and phospholipid transport and homeostasis. ABCA1 is promotes lipidation of apoE, which allows it to efficiently bind Aβ and facilitate Aβ uptake through LRP1. Studies of APP mice with an ABCA1 deficiency have increased amyloid deposition in the brain, paralleled by decreased levels of apoE. Microdialysis assays indicate that ABCA1 deficiency significantly decreases the clearance of Aβ in apoE-ε4-expressing mice, while there are no significant effects of ABCA1 on Aβ clearance in apoE-ε3-expressing mice (6).

Liver X receptor (LXR) is a nuclear receptor transcription factor that plays an essential role in regulation of cholesterol, triglycerides, fatty acids, and glucose homeostasis. Bexarotene, which activates LXR/retinoid X receptor (RXR) heterodimers, increases the expression of apoE, reduces amyloid plaques, and improves memory performance in an AD mouse model harboring the ε4 allele (70). Likewise, treatment with natural LXR agonists, such as the algae derived Fucosterol has shown to induce transcriptional activation of apoE, and the cholesterol efflux regulator and HDL mediator, ABCA1, without increasing cellular accumulation of triglycerides, a side-effect of other LXR agonists (59).

LXR agonists have also been shown to increase the activities of mitochondrial mediated antioxidant enzymes, such as SOD and glutathione (71).

Polymorphisms in the Translocase of the Outer Mitochondrial Membrane (*TOMM40*) gene, which is in linkage disequilibrium with *APOE*, are associated with late-onset AD risk and age of disease onset (72). The expression of both apolipoprotein E and *TOMM40* are significantly increased with AD. *APOE* expression and *TOMM40* expression mRNA levels in the temporal and occipital cortexes are reportedly higher in carriers of the very long TOMM40 variant (523 poly-T), compared to those with the short variant, both in normal individuals and those with AD. This increased TOMM40 expression leads to an increased risk of AD in ε3/ε4 carriers and not ε3/ε3 carriers (72). However, it is unclear what this means in terms of TOMM40 protein levels. For instance, although both show alteration

associated with AD pathology, mRNA of *APOE* expression does not always correlate with apoE protein levels (73). An additional variant at the *TOMM40/APOE* gene cluster (rs2075650) has been significantly associated with LDL and C-reactive protein (CRP) levels, with a trend toward association with HDL and triglyceride levels, proteins that have all been associated with modulation of risk for heart disease and AD (73). Beyond *TOMM40*, most genes involved in the *APOE* gene cluster (i.e. *APOC1*, *APOC2*, and *APOC4*) have been associated with risk for AD. For instance, apoC2, which serves as a measure of triglyceride rich lipoproteins, has been found to co-localize with Aβ (74, 75). Similar findings of increased triglycerides with increased apoC1 have been found, along with strong associations with risk of dementia and insulin resistance (76, 77)

Taken together these findings indicate that there are structural and widespread metabolic differences between the *APOE* isoforms. These widespread differences play a prominent role in conferring risk for Alzheimer's.

2.4. ApoE, Beta Amyloid and Tau

APOE is the best-characterized amyloid-β (Aβ) chaperone, with apoE containing an Aβ-binding motif around region 230–243 (see Figure 1) (34, 78). Functional *APOE* can induce intracellular Aβ degradation through trafficking of amyloid to lysosomes. However, the three isoforms differ in their binding affinities. Specifically, the direct interaction between Aβ and apoE facilitates Aβ oligomerization in rank order ε4> ε3> ε2(79, 80).

As lipidated forms of *APOE*-ε4 bind poorly to soluble Aβ, it is suggested that lipoproteins may contribute in other ways to the dynamics of Aβ clearance along the cerebral vasculature(81). Indeed, *APOE* enables cerebrovascular integrity through the cyclophilin A-NFκB-MMP pathway. Astrocytes in ε3 mice suppress the cyclophilin A-NFκB-MMP pathway, while in ε4 mice they do not, indicating the ε3 isoform may have greater vascular protective capabilities (82). Further, *APOE* knockout mice experience progressive blood-brain-barrier (BBB) breakdown (83).

APOE-ε4 has been shown to increase Aβ deposition and Aβ oligomer formation. Additionally, studies of PiB binding, a technique used to image amyloid, have shown that ε4 increases brain amyloid burden in a dose-dependent manner in cognitively normal individuals and individuals in the prodromal stages of AD (84, 85). Furthermore, ε4 is associated with greater membrane disruption and lysosomal leakage in the presence of Aβ compared to ε3. Interestingly, reductions in glucose metabolism have been shown to correlate better with *APOE* genotype than amyloid load, showing that genotype contributes to reduced glucose metabolism in aging independently of Aβ. Indeed, *APOE*-ε4 individuals show less variation in CSF Aβ levels from a cognitively normal status to mild cognitive impairment (MCI), believed to be a precursor to AD, than do non-carriers (86).

Mice with an ε4 allele insertion show increased tau accumulation, the main component of neurofibrillary tangles seen within somatodendritic and intra-axonal compartments in those diagnosed with AD (87). Moreover, apoE-ε3 is able to bind tau to prevent tau phosphorylation and assembly into paired-helical filaments, while ε4 is not (88). Additionally, ε4 carriers consistently show higher tau, p-tau, and tau/Aβ-42 ratios (89).

Although these associations are present in both sexes, females show a greater association with CSF tau, pointing to possible sex differences along the disease trajectory (10). Future studies involving the use of the lipid-probe theta-toxin, which binds cholesterol, in 3xTgAD and App-NL-F mice, may help further elucidate the sex interactions between apoE and AD pathogenesis (90).

Under conditions of oxidative and excitotoxic stress or neuronal damage, apoE is produced by neurons (47). Due to the domain interaction of ε4, when apoE is synthesized in this state it undergoes proteolytic cleavage to a much greater extent than ε3 (91), with resulting truncated C-terminal fragments entering the cytosol where they become neurotoxic (92) as well as toxic to mitochondria (93). Importantly, during oxidative phosphorylation, mitochondria are one of the primary sources of oxidative stress [92–93]. In line with this, post-mortem microarray analysis of brains of middle-aged cognitively normal *APOE*-ε4 and *APOE*-ε3 carriers without pathological evidence of AD have revealed differences in gene transcripts that are associated with both mitochondrial function and AD, including complex-I gene NADH dehydrogenase, as well as genes associated with insulin signaling, such as CTNNB1 (94). Interestingly, no differences in transcripts directly involved in amyloid processing were found between the groups. Support for the early role of mitochondrial bioenergetics in the progression of AD is strengthened by evidence that young ε4 carriers show reductions in cytochrome-c oxidase (COX) activity, a measure of oxidative metabolism, in brain tissue from the posterior cingulate, even in the absence of fibrillar amyloid (95). Likewise, AD patients with the ε4 allele show higher levels of hydroxyl radicals than AD patients without an E4 allele and reduced levels of glutathione peroxidase, an enzyme with antioxidant capacity that is produced in mitochondria (71, 96). These differences may partially be explained in that mass spectrometry analysis have revealed the methionine-108 region of apoE is considerably more reactive toward free radical labeling in ε4 (97). *APOE*-ε4 carriers also have higher levels of isoprostanes, a marker of oxidative stress, than non-ε4 carriers when matched for cholesterol levels (98). Finally, decreases in mitochondrial respiratory complexes are seen in neurons of ε4 mice and not ε3. In fact, treatment of these cells with GIND25, a small molecule that disrupts the detrimental ε4 structural domain interaction has shown to restore mitochondrial respiratory complex IV levels to a level similar to ε3 neurons (94). A similar compound, IAH, is currently being studied to elucidate its therapeutic potential in AD patients (99). These findings support the early role of energy metabolism in the progression of AD, and suggest that compromises in mitochondrial dysfunction may precede plaque formation in ε4 carriers. It is likely that amyloid fibrils later amplify this mitochondrial dysfunction.

Taken together, these findings indicate that the structural differences in apoE between the isoforms result in a cascade of physiological effects. These differences result in both Aβ-dependent and Aβ-independent changes that serve to effect overall risk for Alzheimer's. In the ε4 isoform, these changes include an increased avidity to bind pro-atherogenic lipoproteins, alterations in the bioenergetic profile, reduced ability for apoE to clear Aβ, and increased accumulation of tau. Furthermore, the apoE ε4 isoform leads to an increased production of reactive oxygen species (ROS) and therefore increased neurotoxic and toxic mitochondrial environments under conditions of oxidative stress.

2.5. *APOE-ε4* Specific Risks of Alzheimer's Disease

Among persons diagnosed with AD, up to 60% carry at least one ε4 allele (8). *APOE* ε4 exerts its maximal effect on AD-risk by the early 70's (100), with a reduction in risk after age 85 in both sexes (8). Similarly, the accelerating effect of ε4 on rates of decline diminishes with advancing disease stages, such that *APOE* genotype has no significant effect on overall disease duration. The *APOE*-ε4 allele accounts for as much as 50% of the genetic attributable AD risk (8, 101). In persons diagnosed with AD, *APOE*-ε4 homozygotes carriers are diagnosed at a mean age 68 years whereas in ε4 heterozygotes mean age is 76 years, and 84 years in ε4 noncarriers (6). The *APOE* ε4 gene dose effect on risk and age of AD onset indicates that *APOE* ε4 dramatically increases risk of AD and an earlier age of onset.

In women, carrying one *APOE* ε4 allele shifts the AD risk curve five years earlier, while two alleles shifts the curve to 10 years earlier in both women and men (102) (Table 1). In addition, the risk odds ratio for AD in women with one allele is 3.5–4, while it is 12–15 in *APOE* ε4 homozygote women and men (103, 104).

APOE may serve as an interesting example of antagonistic pleiotropy – a gene that confers advantage in one period of life but later presents as disadvantage. Specifically, young individuals with the ε4 allele show greater neural efficiency on tasks of episodic memory as measured through a more rapid decline in blood-oxygen-level dependent (BOLD) response over learning trials and therefore more efficient use of memory resources (105). They also show better performance in speed of processing, attention and verbal fluency (106). This cognitive benefit extends into middle-age (i.e. 45–55) on measures of attention and prospective memory (107). However, this benefit does not extend to verbal memory at this age. In fact, a study comparing individuals 45–57, and 58–68, and age-matched controls found that the 45–57 apoE-ε4 group performed better on verbal memory tests than the control group, while the 58–68 group showed impairments. At this later age, ε4 older adults also show increased fMRI activation in the association cortex during memory challenge and during tasks involving verbal fluency tests compared to non-ε4 age-matched controls (108, 109), as well as altered connectivity in the default mode network. Further, stressful life events have greater impact on cognition of ε4 individuals of Caucasian-American descent compared to African Americans (110).

Despite the potential early benefits, older ε4 individuals show evidence of early reduction in entorhinal cortex volume (111), greater rate of hippocampal volume loss (112), and early rise in rate of myelin breakdown(113) (Table 1). These individuals also show characteristic patterns of parietal, cingulate, and temporal hypometabolism, consistent with an AD-like pattern in brain glucose utilization (114–116). When stratifying by *APOE* genotype, positron emission tomography (PET) imaging is predictive of AD conversion with 100% sensitivity and 90% specificity (117). Conversely, *APOE* ε2 carriers show evidence of protective mechanisms in the brain, particularly in regards to white matter compared to ε3 carriers (Table 1). Specifically, they exhibit higher fractional anisotropy, a measure of fiber integrity, and lower radial diffusivity, a measure of myelin integrity, in the posterior cingulate and anterior corpus callosum, regions relevant to the progression of AD (118, 119).

3. Sex Differences in *APOE* Genotype and Risk of Alzheimer's

3.1. Evidence for Worse Pathology and Accelerated Degeneration Rates in Females relative to Males: Impact of *APOE-ε4*

Postmenopausal women constitute >60% of the affected Alzheimer population and are those who will bear the greatest burden of the disease (1, 2, 120). Twenty years, 2 decades, ago Farrer and colleagues reported a sex difference in the lifetime risk of AD in women (8). In his seminal report, women with a single copy of the ApoE4 allele was sufficient to increase disease risk associated with two copies of the ApoE4 gene in men (8). This finding was confirmed a year later in a subsequent report by Payami, Schellenberg and colleagues (7) who found that ApoE4 heterozygote men had lower risk than ApoE4 homozygotes; there was not a significant difference between epsilon4 heterozygote males and those without epsilon4 (Table 1). In contrast, epsilon 4 heterozygote women had the same significant twofold increased risk as homozygote men (7). Multiple studies indicate that the *APOE-ε4* risk for AD is greater in women, especially in heterozygous individuals carrying one *APOE-ε4* allele (7, 8, 10, 121). Women with one copy of the *APOE-ε4* allele have a 4-fold increase in the risk of AD, (7, 8, 121) whereas women and men with two copies of the ApoE4 allele exhibit a 15-fold increase in risk (7, 8, 121) and a significantly lower age of onset compared with AD patients carrying ApoE2 or 3 alleles. Consistent with greater AD risk, Barnes, Bennet and colleagues found a even greater sex difference in the impact of pathology and risk of AD [6]. Each additional unit of AD pathology was associated with a nearly 3-fold increase in the odds of clinical AD in men compared with a more than 22-fold increase in the odds of clinical AD in women (122).

Women diagnosed with mild cognitive impairment progressed at faster rates of cognitive decline than men (123). Further, women had an acceleration rate of cognitive decline relative to men. The gender effect was greatest in ApoE4 female carriers. Overall, women ε3/ε4 carriers often show faster age-related decline and greater deterioration of cognition than elderly ε3/ε4 men (8). Indeed, as measured by rates of atrophy and clinical presentation, MCI women decline more rapidly (109) while at the same time experiencing a longer survival rate following diagnosis in ε3/ε4 and ε4/ε4 women (124).

In a study of 131 participants, decreased connectivity in the default mode network in healthy older *APOE-ε4* carriers was identified, with a significant sex interaction in the precuneus, a major hub for the default mode. Specifically, female ε4 carriers show significantly reduced connectivity compared to female ε3 carriers and male ε4 carriers in the anterior cingulate cortex. Additional studies have revealed that cognitively normal female ε4 carriers show greater reduction of functional connectivity in the precuneus compared to ε4 males. This region is structurally connected to the medial temporal lobe, the initial site of tau pathology in AD. This region also shows reduced glucose metabolism in early AD and asymptomatic ε4 carriers (125). Furthermore, MCI female ε4 carriers show more prominent phenotypic features than their male counterparts, such as reduced hippocampal volumes and worse cognitive scores (126). Similar to the differences in cognitively normal ε4 carriers, female ε4 MCI patients show decreased precuneus activity compared to non-ε4 women and ε4 men. In MCI, this decreased connectivity extends to the posterior cingulate (125).

By the age of 40, 15% of ε4 homozygous cognitively normal individuals are amyloid positive, a frequency which occurs 10–15 years earlier than for ε2/ε4 or ε3/ε4 individuals. By 90, more than 80% of ε4 are amyloid positive, although no sex differences are present (127). Given the lack of sex differences in amyloid by ε4, this supports the role of tau in mediating sex differences. In line with this, female ε4 carriers with MCI show greater CSF tau and tau/Aβ ratios compared to ε4 males with MCI (7). Similarly, ε4 women with mild AD are at a higher risk of having both neurofibrillary tangles and Aβ plaques than ε4 men with mild AD (128). These findings support the idea that women ε4 carriers have a greater extent of AD pathology as measured by CSF and pathology analysis at autopsy. While other studies have failed to find a sex difference in AD risk by *APOE* genotype, this can be explained in that there appears to be a differential effect on risk by carrying one ε4 allele compared to individuals who are homozygous for ε4, such that women with one allele are at a greater risk compared to ε3/ε3 women, while men have no increased risk with just one ε4 compared to ε3/ε3 men.

3.2. *APOE* Genotype and Response to Estrogen

On average, ApoE ε4 negative women have better cognitive performance than ApoE ε4 positive women(129). Further, ApoE ε4 positive women were at greater risk for cognitive impairment (129). The impact of estrogen therapy in postmenopausal women is complex. ApoE ε4 negative women receiving estrogen or hormone therapy had the highest level of cognitive performance, whereas women positive for ApoE ε4 receiving estrogen or hormone therapy performed worse than ApoE ε4 carriers not receiving therapy (129). Notably, treatment with tamoxifen, a selective estrogen receptor modulator and estrogen receptor antagonist, ameliorated cognitive deficits observed during the menopausal transition, especially in ApoE ε4 women (130).

The contradictory effects of estrogenic agonists and an estrogen receptor antagonist in ApoE ε4 carriers suggest that the female ApoE ε4 brain is different that the ApoE 3 carriers. We propose that one fundamental difference between the ApoE ε4 brain and Apoe 2 and 3 brains is in reliance of the ApoE ε4 brain on ketone bodies as a bioenergetic fuel. Reliance of the ApoE ε4 brain on ketone bodies as a bioenergetic fuel puts that brain at risk for utilization of its own white matter as fuel (29). In the female brain, estrogen activates the system biology of glucose metabolism while simultaneously suppressing the ketogenic system in brain thereby promoting brain reliance on glucose as its primary fuel to generate ATP (17, 28, 131–138). If the ApoE ε4 brain is a dual fuel dependent brain, being dependent upon glucose and ketone bodies, then suppression of the ketogenic system in the ApoE ε4 brain would result in reduction in a critical fuel to generate ATP. In the case ApoE 2 and 3 carriers, estrogenic activation of the glucose metabolism system and suppression of the ketogenic system is beneficial as estrogen is promoting and sustaining brain utilization of the primary fuel system glucose. In contrast, in a dual fuel dependent brain, one dependent upon both glucose and ketone bodies, suppression of the ketogenic pathway would put that brain at metabolic risk. Reliance of the ApoE ε4 brain on ketone bodies as a bioenergetic fuel is consistent wtih evidence from multiple laboratories demonstrating that ApoE ε4 carriers are glucose hypometabolic prior to decline in cognitive function (117, 139, 140).

Estrogen receptors play a key and contrasting role in regulation of ApoE gene and protein expression (33) and risk of AD(141). ERα up-regulated ApoE mRNA and protein expression whereas in contrast, ERβ down-regulated ApoE mRNA and protein expression(33). These data suggest that use of ER-selective ligands could provide therapeutic benefit to reduce the risk of AD by increasing ApoE expression in ApoE 2 or 3 allele carriers and decreasing ApoE expression in ApoE4 allele carriers. Polymorphisms in both estrogen receptor alpha (rs4986938) and estrogen receptor β (rs2234693) have been associated with increased risk of dementia and AD (141).

Estrogen therapy may prove beneficial in *APOE-ε4* women who show a favorable response by increasing ABCA1 production. In fact, estrogen has been shown to increase ABCA1 mRNA expression in mice as well as postmenopausal women receiving hormone therapy (142, 143). Competitive interactions between estrogen receptor β and LXRβ for transcriptional coactivator RAP250 likely modulate estrogens effects on ABCA1 levels (143).

Postmenopausal APOE-ε4 positive women age 49–69 who discontinued their hormone therapy regimen exhibited telomere shortening, an index of biological aging, to a greater extent than women who do not carry an ε4 allele or ε4 women not receiving hormone therapy (144).

Estrogen exposure of mitochondria is implicated in regulation of mitochondrial functioning and activates manganese superoxide dismutase (MnSOD) antioxidant activity (145). Conversely, APP and Aβ disrupt mitochondrial function and this is partially mediated by Tom40 interactions (146). The Tom40 protein is an active channel for protein sorting, and is crucial to healthy mitochondria (146). As previously mentioned, the *TOMM40* gene is in linkage disequilibrium with *APOE* (72). Notably, estrogen treatment has been shown to increase both apoE and Tom40 levels through activation of estrogen receptors (147). Estrogen treatment also modulates the apoE receptor LDL related receptor protein (LRP1) (148). Estrogen treatment in ovariectomized mice also causes increases in LRP, an apoE binding protein, in the hippocampus and neocortex (149). Along these lines, apoE synthesis is required for estrogen-induced neuroprotection and neurite outgrowth (148, 150, 151) and is lost in the presence of ε4 (151).

Collectively, these data indicate a complex interaction between the estrogenic and ApoE systems that deserve greater investigation at the systems biology level of analysis and particularly at the interaction between these two systems during nature aging.

3.6. Lack of Sex Differences in Young May Support a Role of Hormone Loss During Menopause

Infants harboring the *APOE-ε4* allele have lower gray matter volume and lower white matter myelin water fractions, indicating reduced myelin integrity in AD-relevant regions compared to non-carriers. These include the precuneus, posterior cingulate, lateral temporal, and occipitotemporal regions (152). These infants also present with greater water fraction and gray matter volume in frontal regions, with no observable sex differences. Interestingly, they have greater myelin water fraction in regions that myelinate later and reduced water fraction

in regions that myelinate early, potentially indicating a condensed white matter development trajectory. As AD is first associated with myelin loss in regions that myelinate late in development, a reduction in white matter development would put *APOE-ε4* carriers at greater risk. In line with this, differences in *APOE* genotypes are associated with a steeper rate of myelin breakdown in late-myelinating frontal regions, and *APOE* has shown to mediate myelin maintenance and repair mechanisms (153). While there are observable differences in brains of *APOE-ε4* carriers quite early in development, the impact of sex on *APOE* related changes in white matter or gray matter are not always found in studies looking at children or adolescents (111, 152). This evidence suggests that sex differences conferring greater risk of AD in ε4 women could be synergistic with events that occur during mid-life or later.

Interestingly, apoE concentration shows sex-specific alterations consistent with the time-scale of puberty, as well as menopause in women (**see** Figure 3). Specifically, females show higher apoE concentrations than males until around age 17, the age of puberty completion in women (154, 155). These differences are then reversed around the average age of menopause (48, 156). Importantly, this perimenopausal transition is marked by a bioenergetic shift in the brain to utilizing ketone bodies as fuel (17, 60, 157) with estrogen loss post-menopause resulting in decreased metabolic function in the brain (158–160).

3.7. Evidence for Protection of ε2/ε2 and ε3/ε3 Genotypes in Women

Over 87% of centenarians are ε2/ε3 or ε3/ε3 among the majority of populations studied, including France, Japan, Spain, Italy, and Finland (161–164). While the ε3 allele is the most common, the prevalence among this age group is greater than the general population. Notably, interactions with ACE polymorphisms, shown to impact cardiovascular risk, likely impact the role of *APOE* in promoting longevity (165). Although ε2 is generally believed to be protective against AD, evidence exists to suggest this is true in ε2/ε3 individuals of both sexes, but only in ε2 homozygous females [110; 134]. In line with the role of ε2 in promoting longevity, one study of Swedish individuals found that ε2 was associated with a reduced risk of AD only in females until age 85, when the allele stops being protective (166). Additionally, there is a decreased mortality rate in ε2 women, while this is not always observed in men (167). However, it is important to note that carriage of the ε2 allele is associated with a greater risk for hyperinsulinemia and diabetes in both sexes, both of which increase the risk for Alzheimer's (168, 169).

The *APOC1* gene, which is contained in the *APOE* gene cluster, has also been associated with longevity. Specifically, one SNP in this gene (rs4420638) has been identified as protecting against AD and promoting resilience. Interestingly, this SNP is in linkage disequilibrium with *APOE* and shows sex specific differences (170). In fact, *APOC1* allele and genotype frequencies have been identified as significantly different in elderly women age 84 and older compared to younger women (171).

CSF analysis of MCI patients revealed higher levels of total tau in female ε4 carriers, and lowest levels of phosphorylated tau in ε3 females over ε3 males, potentially pointing to a protective factor in ε3 females over males (121). Additionally, cognitively normal ε3 homozygote females had higher CSF amyloid than ε3 homozygous males (121). Further,

apoE serum concentrations continue to rise with age in ε3 women to a greater extent than ε4 women or ε3 men (48) (Figure 3). Given the role of *APOE* in Aβ sequestration and clearance, this increase likely provides for protection in ε3 women (121). Unfortunately, most studies looking at *APOE* genotype effects on the brain and CSF levels group all non-ε4 carriers together, making it difficult to more adequately assess whether or not the ε3 allele is protective in homozygous women.

In the latest study of over 8,000 individuals by Altman and colleagues comparing the risk of ε4 status by sex, an increased relative risk for female ε4 carriers (both heterozygous and homozygous) was identified over female ε3 homozygotes in progressing from normal cognition to MCI, or from MCI to AD. The absolute risk values were higher for male ε4 carriers with 22.4% of ε4 heterozygous male carriers progressing from normal cognition to MCI, whereas 19.8% of female ε4 heterozygous carriers did (121). Rather than indicating female ε4 carriers are at a greater risk, what this might indicate is that the impact of having an ε4 is greater in females, likely because there is some protective mechanism for the ε3 females. Indeed, the ε3 females showed the lowest risk of all groups. Specifically, only 13.9% of ε3 homozygous females progress from normal cognition to MCI, while 20.7% of males in this same category progress; additionally, 24.2% of females progress from MCI to AD, while 28% of males progress, indicating protection in the female ε3 carriers (121). Likewise, there is a higher proportion of male *APOE* ε3 homozygotes than females that have progressed to AD in the NACC Uniform Data Set, a database which involves data collected from 34 AD centers across the US (N=11,654) (172, 173).

3.8. Sex Differences in Cardiovascular Risk by *APOE* Genotype and Effects on Vascular Dementia

In a Framingham Heart Study of 3413 participants, age-adjusted period prevalence of cardiovascular disease (CVD) was related to *APOE* genotype with a higher rate for men than women (18.6% in the ε4 group for men and 9.9% for ε4 women) (64). Notably, while the ε2 allele was protective for women (4.9%), this protection was not seen in males with the ε2 allele (18.2%), indicating that the presence of either the ε2 or ε4 alleles in men is associated with greater CVD risk. Furthermore, the overall odds of CVD for men within the ε2 group was 1.94-fold greater than that for men within the ε3 group, while ε2 women were at a .91 reduced risk compared to the ε3 women (64). In a separate Framingham Heart Study of 7901 participants, mortality due to cardiovascular disease that was 6 times higher in men than women at ages 45–54 (174). The increased period prevalence in risk for heart disease for men was still evident when comparing the lowest risk group of males (i.e. ε3/ε3) to the highest risk group of women (i.e. ε4/ε4). However, after the age of 65, the sex difference in risk between males and females was less than 2-fold.

The incidence for vascular dementia has been shown to be higher in men than women (12). In fact, results from a recent study looking at sex differences by *APOE* genotype, cannot rule out that more vascular dementia pathologies could have been present in the male participants. While female ε4 carriers showed greater tau levels than ε4 males with MCI, the differences are in line with a potential vascular dementia in the males, as vascular dementia presents with similar CSF Aβ but lower total tau and phosphorylated-tau levels than AD

(12). Importantly, participants presenting with potential vascular disease are often not included in studies of AD. While this is beneficial in that it limits potential confounds, there is also a growing understanding in the connection between AD and cerebrovascular health. Given that men are at a greater risk for heart disease and other cardiovascular events, especially towards the young-old age spectrum (e.g. 65–74), understanding this group would shed light on the sex differences in ε4 carriers.

Although cardiovascular deaths in late mid-life cannot explain all differences in dementia rate by sex, a substantial part of the difference in dementia risk between females and males could derive from incidence of cardiovascular disease before the age of 65. At mid-life lifetime risk of AD, is not different for men and women whereas after midlife for women and men and subsequent to menopause in women, from 65 years of age onward, women have a 2 fold greater lifetime risk of AD then men. While one could speculate that lower risk of AD in males over the age of 65 is due to survivor cohort. This hypothesis will be testable at the population level as males gain longevity comparable to females in the near future(15).

Men with ε2/ε3 alleles exhibit higher insulin levels compared with controls, indicative of a state of insulin resistance that has been associated with impaired clearance of atherogenic triglycerides. In women, estrogen therapy has been shown to improve insulin sensitivity, potentially providing protection prior to menopause (64). Men also show greater levels of SREBP2 expression, a protein that interacts with apoE to further regulate lipid homeostasis (175). Moreover, estrogen can upregulate apoE gene expression by increasing apoE mRNA (147). In fact, female rats also show higher expression levels than males (176). Given the impact of estrogen on apoE levels, this higher expression would allow for greater protection in terms of both cognition and heart health in females until menopause, when estrogen levels decline dramatically. This increase likely proves protective in ε2 women against CVD, while detrimental in ε4 women towards the risk of AD due to the pathological structure and function of ε4.

3.9. Evidence for Worse Effects in Men or No Sex Difference

Most intervention studies that include a separate analysis of possible therapeutic benefit in ε4 individuals, fail to consider that carrying an ε4 allele is associated with an earlier age of diagnosis and accelerated pathology. Therefore, age-matched studies often yield negative results in ε4 individuals. Similarly, AD shows an altered trajectory in time towards diagnosis between the sexes (see Figure 2), (177) likely blurring the sex interactions in ε4 carriers. For instance, the association between *APOE* genotype and cognitive decline is significant only in women over the age of 70 (9). Additionally, ε2 participants are often excluded from analysis, though studies that have included them indicate that having the ε2/ε4 allele is associated with an increased risk for AD compared to ε3/ε3 [19; 59; 134]. Given the differential impact this genotype has on women and men for cardiovascular health, it is likely that surviving men would exhibit an increased risk for AD with this genotype compared to females (178). However, there is likely to be a selection pressure on men ε4 carriers, such that those that overcome the prominent impact of this allele on cardiovascular risk likely possess other factors that compensate for the presence of an otherwise 'frail' genotype.

Low plasma apoE is associated with a decrease in hippocampal volume, and shows less of a sex difference in concentration levels in ε4 individuals than between the other genotypes (179). However, normal ε4 men show greater AD-pathology and worse memory performance than normal ε4 women, pointing to an earlier incidence of vulnerability (127). Notably, years of life lost in individuals with at least one ε4 allele are 2.5-fold greater for men than for ε4 women (167). Furthermore, there is some evidence of a sex by *APOE* gene dose interaction, such that the significant effects of ε4 on risk for decline in episodic memory are generally less dependent of zygosity in females than males (180). Likewise, ε4/ε4 men are at higher risk of AD than ε4/ε4 women (8). For example, in a recent study, a greater proportion of ε4/ε4 men progressed from normal cognition to a state of MCI than did ε4/ε4 women. Similar trends were seen for individuals starting as MCI at baseline and progressing to AD (121). Additionally, episodic memory as measured through the Kendrick Object Learning Test has been reportedly worse in normal ε4 homozygous men than women (180). Furthermore, studies on the effect of *APOE*-ε4 homozygosity in men with MCI have shown that ε4 men have smaller hippocampal volume than ε4 homozygous females (126).

4. Conclusion

Collectively, the data indicate a complex interaction between the trial of greatest risk factors for Alzheimer's disease, age, *APOE* genotype and sex. The complexity of this interaction remains to be fully and specifically characterized. While investigating the interaction between three systems across multiple transition states of aging is incredibly challenging at the basic and clinical levels of analysis, it is feasible using a big data bioinformatic approach. Clearly there are sex differences in risk of AD that are modified by APOE genotype. But even at this level there is a fair degree of variability that is not explained by either APOE genotype or by sex suggesting that the systems biology of aging is driving factor.

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Highlights

- The three greatest risk factors for Alzheimer's disease are age, APOE-ε4 genotype and female sex.

- Convergence of these three risk factors, creates unique sex differences risk profiles for Alzheimer's disease.

- The bioenergetic shift of the perimenopause to menopausal transition, unique to female, creates a risk event that likely exacerbates effect of APOE-ε4 positive females to thereby contribute to greater lifetime risk of Alzheimer's disease in women.

- Increased risk of earlier onset of Alzheimer's is evident in APOE-ε4 homozygote males whereas greater risk of later onset AD is evident in females.
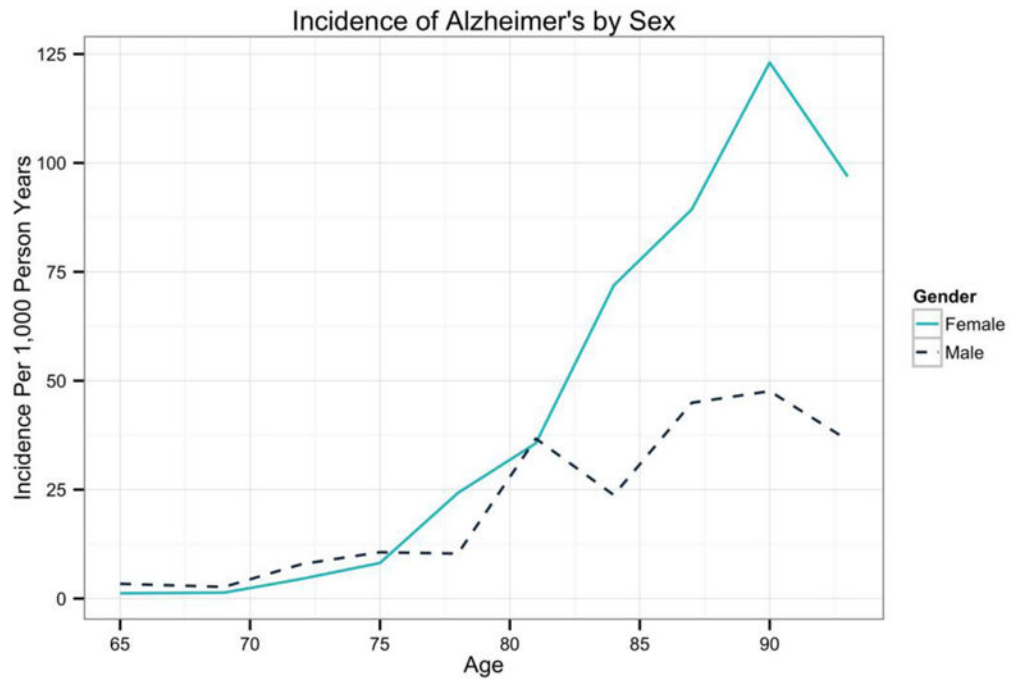
Figure 1.
Sex-specific incidence estimates of Alzheimer's per 1,000 person years. Obtained with data from the Cache County Study [12]. Data reported in Ruitenberg et al., [12] indicate that men are at greater risk than women for developing earlier onset AD. However, this sex difference is reversed by age 75, with women at a 2 fold greater risk for AD, thereafter.
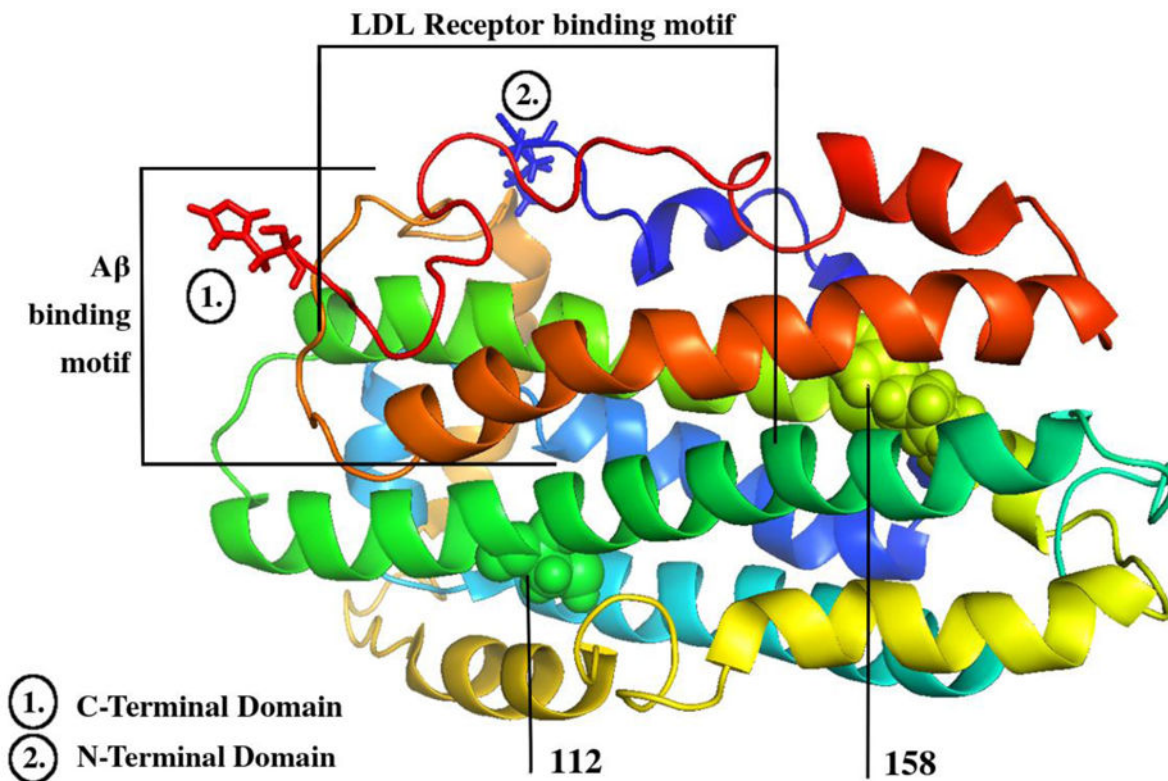
Figure 2.
ApoE NMR protein structure. Created from the RCSB Protein Databank and the PyMol graphics viewer (PDB ID: PO2649) [186]. This figure shows the nuclear magnetic resonance protein structure of apoE ε3. Lipoprotein binding (1) and Aβ-binding (2) motif regions are delineated [34, 78]. Residues 112 and 158, which are altered between (and therefore determine) the differing isoforms, are also labeled [42–43].
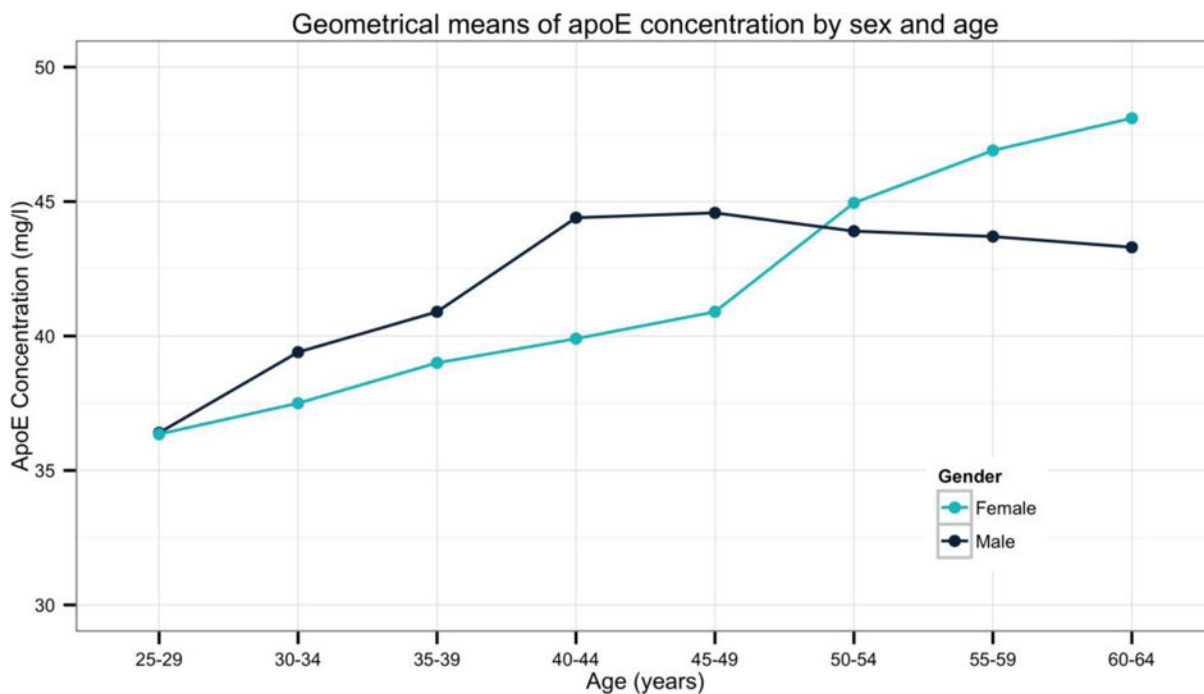
Figure 3.

ApoE serum concentrations by sex and age (N=6,934). From data collected in the ApoEurope project, obtained with permissions from [48]. Data indicate that men have greater apoE concentrations than women until age 50–54. While men experience progressive declines in apoE concentrations following this age, apoE concentrations rise in women. As low serum levels of apoE are associated with an increased risk for AD, this tipping point might explain the discordant findings in risk by sex, such that men are at risk due to a reduced production of apoE, while ε4 women produce an overabundance of as isoform with impaired function.

Table 1

Summary of the results outlined in this review. Both protective and risk factors associated with APOE genotype by sex are outlined.

Summary of Sex Differences by APOE Genotype		
Genotype	**Sex**	**Findings**
ε2/ε2	Males	More severe lipidemia and atherosclerosis due to high plasma apoE [64]
	Females	Reduced AD risk in women <85, increased after age 85 [166]
	General	Increased prevalence of hyperlipidemia and cardiovascular disease [45] ApoE concentrations are generally the highest in this group, protecting from AD [48, 53]
ε2/ε3	Males	Increased AD risk due to higher incidence of insulin resistance [64]
	Females	Estrogen and ERT improve insulin sensitivity, compensating for the increased risk in males [64] Reduced cardiovascular risk and AD risk in women <85, increased after age 85 [64, 166]
	General	Associated with increased longevity and protection from AD [161–165]
ε3/ε3	Males	Potentially protective against AD, although at higher risk than genotype-matched females [121]
	Females	MCI patients show lowest levels of p-tau, associated with delayed disease progression [121] More favorable CSF amyloid profiles than ε3/ε3 men [121] Lowest AD risk compared to males and all other APOE genotypes in females [121] Increases in apoE concentration with age provide protection from AD risk [121, 48] Associated with increased longevity and protection from AD [161–165]
	General	Protective against AD [for example: 121, 127, 39, 110, 69, 162, 166]
ε2/ε4	Males	Increased risk of cardiovascular disease compared to ε3/ε3 individuals [64]
	Females	Protection from cardiovascular risk and AD [121, 64, 166]
	General	Greater AD risk in individuals of European descent [39] Evidence of brain amyloid accumulation occurs 10–15 years later than in ε4/ε4 carriers [127]
ε3/ε4	Males	Increased risk for MCI and AD, although slower decline [121]
	Females	Significant effects on risk for decline in episodic memory [180] Higher MCI and AD risk than non-ε4 carriers and male ε3/ε4, comparable to ε4/ε4 men [7, 121] Increased likelihood of pathological levels of CSF tau and tau/Aβ ratio [121] Compared to ε3/ε4 men, faster age-related cognitive decline and longer survival rates [8, 109, 124]
	General	In 70+ normal ε4 carriers have greater amyloid PET than other genotypes [127] Greater AD risk in individuals of European descent [39] Evidence of brain amyloid accumulation occurs 10–15 years later than for ε4/ε4 carriers [127]
ε4/ε4	Males	Higher risk of MCI and AD than ε3/ε4 or ε2/ε4 males or ε3/ε4 and ε4/ε4 females [8, 121, 166]
	Females	Comparable AD risk to ε3/ε4 females [6]; longer survival rates following AD diagnosis [7,8]
	General	Over 30% develop AD by age 75, with greater risk in individuals of European descent [39] ApoE concentrations are generally the lowest in this group, a predictor of AD risk [48, 53] Highest risk for brain Aβ accumulation [84–85]
General ε4 findings	Males	Years of life lost in men ε4 carriers are greater than in genotype-matched women [167]
	Females	Greater prevalence of AD in female ε4 carriers than males [7, 8] Greater alterations in precuneus and anterior cingulate cortex connectivity [10, 125] Associations between APOE and cognitive decline evident later in females [9, 127] More prominent phenotypic features in female MCI ε4 carriers – reduced hippocampal volume and worse cognitive scores [126] CSF tau, p-tau, and tau/Aβ-42 levels highest in MCI ε4 carriers compared to non-carriers with MCI, particularly for females [7, 10, 12, 89] Women ε4 with mild-AD are more likely to have both neurofibrillary tangles and amyloid than ε4 men, indicating greater pathology [128] Postmenopausal ε4 women on ERT exhibit signs of neuroprotection and preservation of telomere length compared to ε4 women not receiving treatment [130, 144, 150]
	General	APOE-ε4 show greater neural efficiency on episodic memory tasks [105] and better performance in speed of processing, attention, and verbal fluency until mid 50's when declines are evident [64, 106–107] Reduced myelin integrity in AD-relevant regions evident in infancy [152]

Summary of Sex Differences by APOE Genotype		
Genotype	**Sex**	**Findings**
		Increased dementia risk (AD, PDD, VaD, and DLB) and cardiovascular risk [12, 64, 121] Carrying one ε4 allele shifts the AD risk curve 5 years earlier (OR=3.5–4) [102–104] Two alleles shifts the AD risk curve by 10 years (OR=12–15) [102–104] *APOE*-ε4 exerts its maximal effects on AD risk by the early 70's [100] Up to 65% of individuals with AD harbor at least one ε4 allele [8] CSF Aβ less predictive of conversion from normal to MCI than for other genotypes [86] By 40, 15% cognitively normal *APOE*-ε4 carriers are amyloid positive [127]

Aβ: Amyloid-beta 1–42; AD: Alzheimer's disease; DLB: Dementia with Lewy Bodies; ERT: Estrogen replacement therapy; MCI: Mild cognitive impairment; OR: Odds ratio; PDD: Parkinson's dementia; P-tau: Phosphorylated tau; VaD: Vascular dementia.