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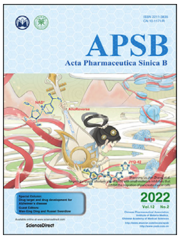

REVIEW

Apolipoprotein E and Alzheimer's disease

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Abstract Genetic variation in apolipoprotein E (*APOE*) influences Alzheimer's disease (AD) risk. *APOE ε4* alleles are the strongest genetic risk factor for late onset sporadic AD. The AD risk is dose dependent, as those carrying one *APOE ε4* allele have a 2–3-fold increased risk, while those carrying two *ε4* alleles have a 10–15-fold increased risk. Individuals carrying *APOE ε2* alleles have lower AD risk and those carrying *APOE ε3* alleles have neutral risk. APOE is a lipoprotein which functions in lipid transport, metabolism, and inflammatory modulation. Isoform specific effects of APOE within the brain include alterations to Aβ, tau, neuroinflammation, and metabolism. Here we review the association of *APOE* with AD, the APOE isoform specific effects within brain and periphery, and potential therapeutics.

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1. Overview

Apolipoprotein E (APOE) is a lipid binding protein and is the predominant cholesterol transport protein in the brain. While the main function of APOE is lipid transport, it also binds and interacts with inflammatory components such as lipopolysaccharides (LPS), amyloid beta (Aβ), beta-glucans, and lipoteichoic acids[1,2]. This function is believed to facilitate clearance of inflammatory and pathogenic molecules suggesting a role for APOE in innate immunity. APOE was discovered in 1975 but its full structure was not resolved until 2011[2–4]. APOE is synthesized and secreted largely in the liver, brain, macrophages, and skin[1,2,5].

In humans, three major isoforms of *APOE* exist. These are known as *ε2*, *ε3*, and *ε4*. These three alleles arose around 7.5 million years ago following the primate–human split[6,7]. Mutations between human and primate *APOE* are non-synonymous and alter protein function. *ε4* was the first allele, with four amino acid changes between human and primates (A18T, T61R, A135V, and V174L)[1,6,7]. Approximately 150,000–220,000 years ago, further amino acid changes to *APOE* resulted in the *ε3* allele (R112C)[1,6,7]. The *ε2* allele originated approximately 80,000 years ago with another amino acid change (R158C)[1,6,7]. *APOE* is on chromosome 19, and the gene is composed of four exons and three introns[1]. The amino acid changes between *APOE* alleles alter the structure and function of the protein[1,6].

The estimated timeline of allele changes in the human population are based on assumption of neutral selectivity[6,8]. Neutral selectivity theory postulates most genetic variation is due to a stochastic process conveying no selection advantage at the molecular level. The *APOE ε3* allele has the highest penetrance in Asia (followed by Europe and Africa). This suggests an Asia origin for the *ε3* allele following human migration[1,6]. Selective pressures on *APOE* alleles are contributed to age-related brain function, resistance to pathogens (such as malaria), climate, fertility, and diet/nutrient sources[6]. Therefore, the assumption of neutral selectivity may render the allele emergence timeline presented above inaccurate[6].

ε3 is the most common isoform of *APOE*, accounting for approximately 80% of alleles in humans. The prevalence of *APOE* alleles varies by continent and latitude. For example, *APOE ε3* alleles constitute 85% of alleles in Asia, 69% in Africa, 82% in North America, 77% in South America, and 79% in Europe[9]. *ε2* and *ε4* are less common, accounting for approximately 5%–10% and 10%–15% of *APOE* alleles found in humans. *APOE ε4* prevalence varies across different populations with 40% penetrance in Central Africa, 37% in Oceania, and 26% in Australia[6]. In Europe, there is a “gradient” of *APOE ε4* allele distribution among populations. With high *ε4* allele prevalence in both northern Europe and Asia at approximately 25% and low allele prevalence in South China and the Mediterranean area at less than 10%[6,10,11]. *ε2* allele distribution and prevalence vary by population as well, with a lack of alleles in African indigenous populations and Australian aborigines. The *APOE ε2* allele is higher than average in Africa with 9.9% penetrance and Oceania populations at 11.1%[6,9]. Variance of *APOE* alleles across climates and populations may reflect selective advantages for specific alleles in specific climates.

In Alzheimer’s disease (AD) populations, *ε4* alleles are overrepresented[12–14]. Approximately 60%–80% of subjects afflicted with AD carry a *ε4* allele[1,12,15,16]. The AD risk conferred by *APOE ε4* is dose dependent[15]. Those carrying one *ε4* allele have a 2–3-fold increased AD risk, while those carrying two *ε4* alleles have a 10–15-fold increased AD risk[1,15,16]. Conversely, *ε2* allele carriers may have a reduced AD risk[1,15,16].

The protein product of *APOE* is a 317 amino acid peptide. The first 18 amino acids are cleaved co-translationally to form a mature 299 amino acid protein[1]. Mouse APOE is considered APOE3-like due to it carrying a threonine at amino acid position 61, but it only shares approximately 70% homology with human APOE[6]. Mouse APOE is six amino acids shorter. The lack of homology with human APOE brings to light possible issues with prior mouse studies focusing on single amino acid mutations in mouse APOE and their relevance to human APOE function[6].

APOE isoforms exhibit differential post-translational modifications. APOE is sialylated which changes isoelectric points of the isoforms. APOE ε4 has a +2 charge, ε3 a +1 charge, and ε2 a neutral charge[1]. Various levels of sialylation alter these charges for each isoform. Variable isoelectric points are due to the arginine–cysteine changes among the isoforms and sialylation differences are due to *O*-linked glycosylation at threonine 194[16,17]. Non-sialylated forms lack neutral sugars[16–18]. Sialylation increases the APOE variation observed in circulation[16,17].

Within APOE amino acids 136–150 are critical for receptor and heparin binding[1,19–22]. The C-terminus is rich in α-helices and functions in lipid binding. Binding of lipids to these residues allows receptor access to residues 136–150. Residues 244–272 interact directly with lipid particles. APOE ε3 and ε4 bind to low density lipoprotein (LDL) receptors with similar affinities, while ε2 has a slightly lower affinity (2% or 50 times weaker)[23]. APOE ε2 and ε3 both bind to smaller phospholipid-enriched high-density lipoproteins (HDL) but ε4 preferentially binds to triglyceride rich/larger very low-density lipoproteins (VLDL). These binding preferences are accounted for with differences in protein domain interactions between the N and C termini of the isoforms. The structure/function relationship of APOE isoforms have been reviewed elsewhere[1,2,4,6,20–22]. Here we will review the role of APOE in lipid metabolism in the periphery and its contributions to AD risk within the brain.

2. APOE and lipid metabolism

APOE is secreted from the liver with VLDL particles. In the small intestine chylomicrons (or ultra-low-density lipoproteins) are synthesized and combine with APOE secreted by the liver[24–26]. Within the central nervous system, ATP binding cassette subfamily A member 1 (ABCA1) and ATP binding cassette subfamily G member 1 (ABCG1) are critical for the transfer of cholesterol and phospholipids to APOE to form lipoproteins[27]. During circulation, VLDL and chylomicrons become enriched with APOE[1,24–26]. Upon interaction with endothelial cells, lipoprotein lipase hydrolyzes the triglycerides from the APOE containing lipoproteins, releasing fatty acids for energy metabolism. APOE functions to transport lipids in endocrine (from liver to distant tissues) and paracrine (within the same tissue type) mechanisms. APOE can redistribute lipids across tissue and cell types[1].

APOE influences lipid metabolism by acting as a ligand for receptors. APOE lipoproteins interact with LDL receptors to regulate levels of VLDL and LDL[1,24,25,28,29]. A second receptor pathway for APOE lipoproteins is the heparan sulfate proteoglycan (HSPG)/low density lipoprotein receptor-related protein (LRP) pathway[1,25,30]. The LRP pathway functions mainly in the liver and is reviewed elsewhere[1,25,28–30]. APOE regulates both catabolic and anabolic lipid metabolism. Increased APOE synthesis, plasma levels, or liver secretion is associated with increased VLDL synthesis and secretion[1]. APOE accumulation on lipoproteins can reduce the lipolysis of lipoprotein triglycerides[1]. This indicates a self-feedback inhibition mechanism for APOE function. APOE induces influx and efflux of cholesterol from cells[2,31]. As a lipid transport protein, APOE is integrated into lipid metabolism across diverse tissue and cell types as shown in Fig. 1.

APOE isoforms differentially affect blood lipid profiles in humans[1,32]. *APOE ε2* is associated with increased levels of APOE/triglycerides and decreased levels of APOB/cholesterol[1,32]. *APOE ε4* is associated with decreased levels of APOE/triglycerides and with increased levels of APOB/cholesterol[1,32]. *APOE ε3* is considered of neutral consequence to blood lipid profiles. These alterations of circulating lipid profiles influence the risk of atherosclerosis based on *APOE* isoform expression.

While *APOE ε2* may decrease the risk of AD, it increases the risk of type III hyperlipoproteinemia (HLP) or dysbetalipoproteinemia, a disease which increases the incidence of atherosclerosis[1,33,34]. Patients with type III HLP have high cholesterol, triglycerides, and abnormal accumulation of β-VLDL[1,33]. While all patients with type III HLP are homozygous for *APOE ε2*, a large portion of *APOE ε2* homozygotes (~90%) do not develop this disease. There are likely other contributing factors beyond *APOE* genotype which contribute to the onset of type III HLP[1,33].

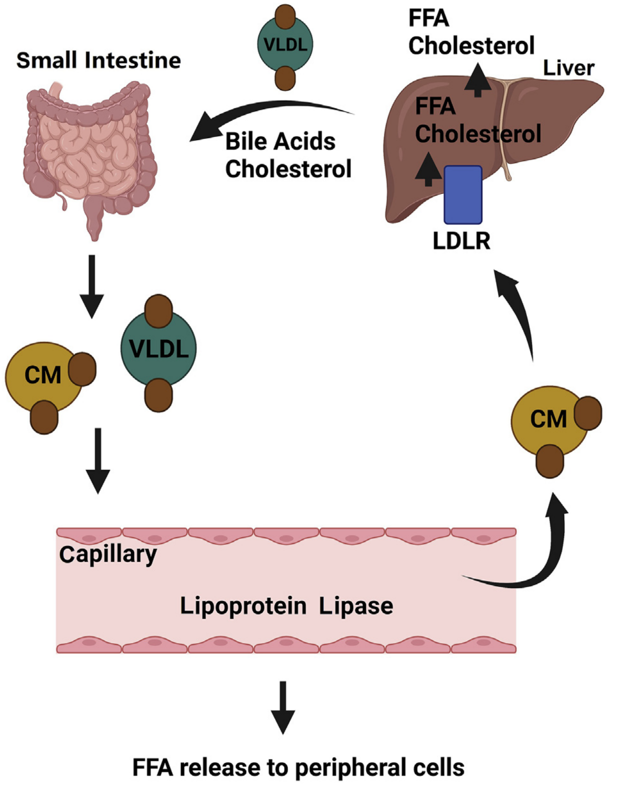

Figure 1 APOE lipid transport across diverse tissues. APOE is secreted from the liver with VLDL, cholesterol, and bile acids. In the small intestine, APOE is combined with chylomicrons (CM) and released with VLDL/APOE particles. In capillaries, lipoprotein lipase releases APOE from free fatty acids (FFA) for distribution to peripheral cells. CM/APOE particles are transported to the liver where LDLR releases FFA and cholesterol into the liver. Figure created with Biorender.com.

APOE ε4 genotype places individuals at an increased risk of heart disease[20,32,35–38]. This is likely due to the association of *APOE ε4* with elevated LDL and APOB[1]. APOE ε4 binding to VLDL decreases its lipolytic processing which contributes to changes in lipid profiles[1]. APOE does play a role in atherosclerosis as *ApoE* knockout mice have severe atherosclerotic lesions with high lipid levels (including β-VLDL)[39,40]. Macrophages likely play a large role in atherosclerosis. Mice lacking *ApoE* in macrophages are more susceptible, while mice expressing *ApoE* only in macrophages are protected from atherosclerosis[41,42].

APOE functions in lipid metabolism across many tissue and cell types while also influencing disease risk for atherosclerosis, heart disease, type III HLP, and AD. Evidence is emerging that systemic effects of APOE may affect AD risk[43–45]. For example, heart disease and atherosclerosis are risk factors for AD and cognitive decline in aging. However, APOE mediates direct effects within the brain, and these are discussed below.

3. APOE and the brain

APOE is expressed in astrocytes, choroid plexus cells, microglia, and vascular mural cells[5]. Numerous studies cite expression of APOE in stressed neurons[46–51]. APOE binds neuronal low density lipoprotein receptors (LDLRs) including LRP1 to transfer lipids into neurons from surrounding cells. APOE lipid transport between neurons and astrocytes is illustrated in Fig. 2. Peripheral APOE impacts brain health. *ApoE* null mice have synaptic

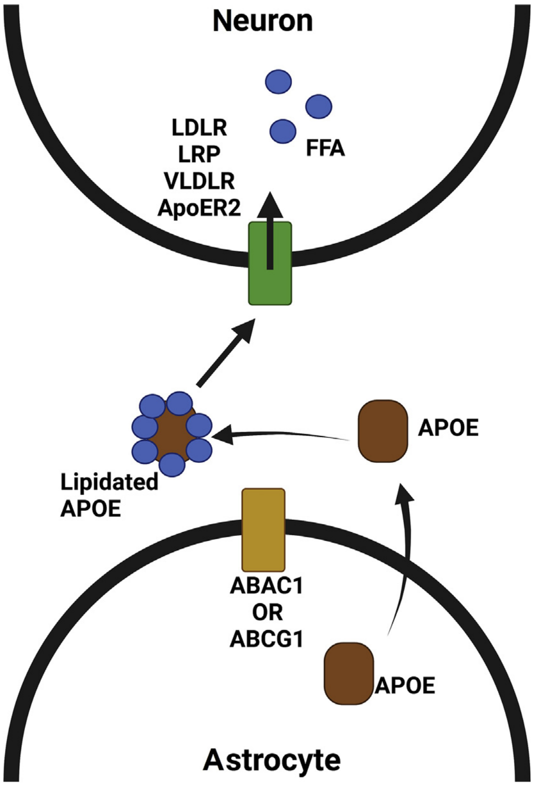

Figure 2 APOE lipid transport between neurons and astrocytes. APOE is secreted from astrocytes and then lipidated by ABCA1 or ABCG1. Lipidated APOE is carried to neurons where receptors (LDLR, VLDR, LRP, or ApoER2) remove APOE from the lipids to release FFA into neurons. Figure created with Biorender.com.

dysfunction, and this can be improved by restoring peripheral *ApoE* expression[52].

In healthy individuals which are *APOE ε3/ε4* carriers, the ratio of APOE ε4 to APOE ε3 in plasma correlates with loss of gray matter volume and abnormal glucose metabolism. APOE ε4 is associated with increased cortical atrophy and decreased gray matter volume in those with AD[53–55]. Overall, APOE isoforms affect lipid transport, glucose metabolism, mitochondrial function, synaptic plasticity, Aβ, tau, and cerebrovascular function within the brain.

APOE ε4 is the greatest genetic risk factor for late onset AD (LOAD)[1,20]. *APOE ε4* also influences risk and outcomes for stroke, traumatic brain injury (TBI), multiple sclerosis (MS), Parkinson's disease (PD), and frontotemporal dementia (FTD) in some studies[55–67]. AD is defined by cognitive decline, memory loss, and postmortem extracellular Aβ plaques and intracellular neurofibrillary tangles (NFTs). AD subjects show decreased cerebral glucose metabolism, mitochondrial dysfunction, neuroinflammation, loss of proteostasis, vascular changes, and insulin resistance[68–71]. These findings are not unique to the brain in AD subjects as they are also observed in the periphery[72]. APOE influences these facets of AD pathology as discussed below and highlighted in Fig. 3.

3.1. Aβ

Aβ is generated from sequential proteolysis of amyloid precursor protein (APP). APP is cleaved through two pathways, nonamyloidogenic and amyloidogenic. In the nonamyloidogenic pathway, APP is cleaved by α-secretase into a C-terminal fragment of 83 amino acid length (C83) and soluble APP α (sAPPα). The C83 fragment is trimmed by γ-secretase to form the APP intracellular domain (AICD) and p3 fragments. In the amyloidogenic pathway, APP is cleaved by β-secretase into a C-terminal fragment of 99 amino acid length (C99). The C99 fragment is trimmed by γ-secretase to form the APP intracellular domain (AICD) and Aβ fragments. These pathways have been reviewed elsewhere[73]. The process of Aβ generation is understood but the underlying reason for the shift to favor amyloidogenic APP processing in AD is not understood[74].

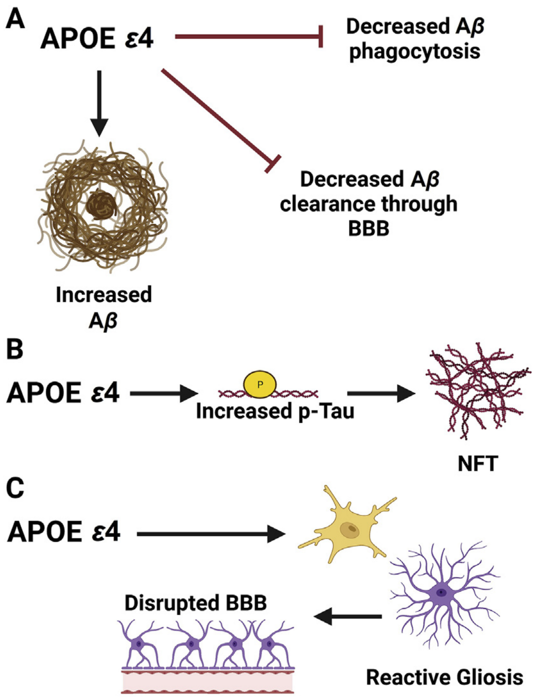

Figure 3 APOE effects on Aβ, tau, and neuroinflammation. (A) APOE ε4 increases Aβ and plaque formation, reduces Aβ clearance through the blood−brain barrier (BBB), and reduces Aβ phagocytosis by glia. (B) APOE ε4 increases tau phosphorylation (p-Tau) which increases neurofibrillary tangle (NFT) formation. (C) APOE ε4 increases reactive gliosis and neuroinflammation which can lead to a disrupted BBB. Figure created with Biorender.com.

APOE ε4 is associated with increased brain Aβ pathology in a gene dosage dependent manner. Homozygous *APOE ε4* carriers have the highest Aβ pathology burden compared to heterozygous carriers[62,75–77]. APOE ε4 influences Aβ pathology through increasing its accumulation within brain, development of oligomeric species, and plaque formation[78]. APOE binds Aβ directly, and APOE ε4 binds more efficiently than APOE ε3[79]. Despite disease status carrying an *APOE ε4* allele is associated with increased brain fibrillar Aβ levels[80]. Deposition of brain Aβ over time appears to be higher in those that carry an *APOE ε4* allele[81,82]. Those who are heterozygous *ε2/ε4* do not gain protection from the *ε2* allele, as when compared to *ε3/ε4* individuals, there were no differences in brain Aβ burden[83]. These studies suggest a strong relationship between *APOE ε4* isoforms and Aβ burden.

Decreased Aβ clearance could be an underlying mechanism for increased brain Aβ accumulation in *APOE ε4* carriers[84–88]. APOE ε4 has been shown to reduce Aβ clearance[87,89]. Aβ is cleared from the brain through interstitial fluid, the blood−brain barrier (BBB), or cellular/enzymatic degradation[90]. *In vivo* and *in vitro* evidence show that APOE mediates Aβ transport through the BBB. APOE ε4 binds preferentially to VLDL (see above) and the VLDL receptor has a slower clearance rate of APOE/Aβ complexes compared to other receptors (LRP1) bound by other APOE isoforms[85]. Further studies have examined the specific portions of APOE responsible for Aβ clearance. A truncated APOE ε4 protein missing amino acids 166−299 showed reduced Aβ clearance, suggesting this is the critical region facilitating Aβ clearance[91].

APOE ε4 also decreases cellular uptake and enzymatic degradation of Aβ. *In vitro* and *in vivo* experiments have shown that astrocytes from *APOE ε4* human carriers (or mice) have impaired uptake of Aβ[92,93]. Other studies suggest APOE does not play a critical role in astrocyte Aβ clearance but LRP1 does. This suggests APOE could exhibit competitive binding of LRP1 with Aβ[87,94]. In microglia, APOE mediated cholesterol efflux mediates delivery of Aβ to lysosomes, and microglia harboring an *APOE ε4* allele have reduced Aβ clearance. Stem cell-derived microglia carrying *APOE ε4* alleles show reduced Aβ phagocytosis when compared to those carrying *APOE ε3* alleles[95]. Overall, APOE ε4 appears to impart effects on Aβ clearance pathways across multiple cell types.

Aβ clearance can also occur through enzymatic degradation by neprilysin and insulin degrading enzyme (IDE). *APOE ε4* carriers show decreased expression of these enzymes. In postmortem human brain samples, *ε4* carriers had decreased expression of neprilysin and IDE[96–98]. APOE ε4 may affect neprilysin mediated Aβ degradation in microglia specifically[95]. Overall, the mechanism and link of APOE isoforms to function of these enzymes requires further research.

A final mechanism for Aβ clearance is through the perivascular system. Mouse studies suggest a role for APOE ε4 in reducing perivascular drainage of Aβ[99]. Other clearance pathways are less studied, including the glymphatic system. APOE is delivered from the glymphatic system into the brain and the rate of this transport is affected by APOE isoforms[100]. Overall, this topic deserves more attention in the AD field.

The evidence that APOE affects Aβ fibril formation/seeding/oligomerization is weak. Most *in vitro* studies are inconsistent[70]. However, studies implicate that APOE ε4 induces Aβ fibrilization more rapidly than APOE ε3[101]. *In vivo* studies suggest that reduction of APOE levels in astrocytes before Aβ fibrils are formed reduces Aβ pathology[102]. However, this mechanism does not have an effect after Aβ fibrils are formed. Expression of APOE ε4 in astrocytes of mice was found to increase Aβ pathology when compared to APOE ε3 isoform expression[90,103].

Aβ biology is also affected by expression levels of APP. *In vitro* studies suggest APOE increases the transcription of APP. Separate studies show that APOE ε4 increases Aβ production in stem cell-derived neurons[92,104]. *In vivo* studies have failed to replicate *in vitro* findings. Mice with humanized *APOE* show no change in Aβ production between APOE isoforms[105]. However, there are concerns regarding the relevance of humanized *APOE* mouse models and the effects of mouse receptors interacting with human proteins.

3.2. *Tau*

Tau pathology is observed in AD and other neurodegenerative diseases (known as tauopathies). In AD, tau forms insoluble intercellular NFTs. Tau hyperphosphorylation has been shown to decrease the solubility of tau and promote tangle formation. Tau biology and pathology have been extensively reviewed elsewhere[106,107]. *APOE ε4* interacts with tau pathology in AD.

Postmortem studies indicated that homozygous *APOE ε4* carriers have higher tau pathology than heterozygotes or non-carriers[77,108−110]. *In vivo* studies show that neuronal expression of APOE ε4 increases tau hyperphosphorylation[111]. This could increase NFT formation. Stem cell-derived cerebral organoids from *APOE ε4* carriers have higher tau phosphorylation when compared to *APOE ε3* carrier-derived models[112]. Humanized *APOE* mice expressing mutant tau (P301S) show increased brain atrophy and neuroinflammation with APOE ε4 expression[113]. Other studies have shown increased tau pathology in mice harboring human *APOE ε2* alleles when compared to other isoforms[114].

Fragments of APOE have effects on tau biology and pathology. C-terminal truncated forms of APOE ε3 and ε4, bearing amino acids 1−271 were shown to interact with phosphorylated tau species[115]. Truncated APOE ε4 was more likely to interact and induce NFT formation than truncated APOE ε3. APOE residues 245−260 are critical for the tau interaction and NFT formation[116]. In transgenic mice expressing neuron specific APOE ε3 or ε4, C-terminal truncated APOE products were observed[46]. These C-terminal APOE products were more prominent with APOE ε4 expression and with age, the APOE truncation patterns were like observations in human brain[46]. In mice expressing astrocyte specific APOE isoforms, no C-terminal APOE fragments were detected in brain tissue. The neuron specific APOE ε4 mice showed increased phosphorylated tau and intraneuronal tau inclusions when compared to APOE ε3 mice. These effects on tau where not observed when APOE expression was restricted to astrocytes[116]. C-terminal truncated APOE fragments (1−272) from AD brain samples induce NFTs in neuronal cultures. These C-terminal fragments are more likely to be generated from APOE ε4 isoforms than APOE ε3[116].

The effects of APOE isoforms on tau biology and the interaction between the two are apparent. A link between tau, Aβ, and neuroinflammation is evident and discussed below.

3.3. *Neuroinflammation*

APOE has a role in innate immunity. Glial cells, or microglia and astrocytes, are the main innate immunity components within the brain. Microglia respond to damage and pathogen associated molecular patterns in addition to other functions, such as synaptic pruning[117]. Microglial response can be attributed to repair or pro-inflammatory functions. Astrocytes are phagocytic like microglia, but also support neurons metabolically through interactions between neurons and vascular components[117]. Aberrant astrocyte and microglia phenotypes are observed in AD and are referred to disease associated phenotypes[117]. Astrocytes are the main APOE expressing cell type within the brain, however microglia also express APOE[117].

Microglia upregulate APOE expression in response to amyloid and tau pathologies. APOE ε4 likely increases the microglial proinflammatory phenotype in transgenic tau mice. APOE binds to triggering receptor on myeloid cells 2 (TREM2)[118−121]. The R47H TREM2 variant, although rare, is associated with increased AD risk[122−124]. TREM2 functions to downregulate the pro-inflammatory response through clearance of damaged cells or other debris (including Aβ) by promoting phagocytosis in microglia[125−127]. This function appears to be disrupted in AD[128]. The association of APOE with amyloid plaques is dependent on TREM2 and appears to originate from microglia[129].

In AD transgenic mice, data show a role for APOE in promoting gliosis. In transgenic mutant *APPV717F* mice administered chronic LPS APOE promoted gliosis and Aβ deposition[129]. Further *in vivo* studies using humanized *APOE* mice administered chronic LPS, showed that APOE ε4 increased pro-inflammatory cytokine expression when compared to APOE ε3[130]. APOE ε4 increases nitric oxide (NO) release by microglia in humanized *APOE* mice and in human microglia[131]. In mice, microglia expressing APOE ε4 have increased pro-inflammatory cytokine expression and NO production when compared to APOE ε3 expressing microglia[132]. These effects are gene dosage dependent and observed in peripheral macrophages of *APOE ε4* mice[132].

APOE is required for inflammatory responses against Aβ and tau. *APPswe/PSEN1ΔE9* and *APPswe/PSEN1*L166P* transgenic mice lacking APOE showed reduced Aβ pathology, altered Aβ plaque morphology, but increased size of Aβ plaques[133]. A reduction in microgliosis was also observed especially associated with Aβ plaques. Despite reduced microgliosis in *APOE* knockout mice, an increase in dystrophic neurites was observed surrounding Aβ plaques[133]. Tau pathology and inflammatory response are also modulated by APOE. In humanized *APOE ε4 P301S Tau* transgenic mice, depletion of microglia resulted in reduced tau pathology[134]. Increasing APOE expression in *P301S Tau* transgenic mice correlated with increased phosphorylated tau and insoluble tau[134]. Depletion of microglia in *APOE ε4* humanized *P301S Tau* transgenic mice decreased brain atrophy[134]. In a separate study using *P301S Tau* transgenic mice with either humanized *APOE* or *APOE* knockout, it was apparent that APOE modulated tau pathology[113]. In humanized *APOE ε4* mice on the *P301S Tau*, background higher levels of tau, increased brain atrophy, and neuroinflammation were observed when compared with APOE ε2 and APOE ε3 isoforms[113]. *APOE* knockout mice appeared protected from these phenotypes. Co-cultures of APOE ε4 expressing astrocytes with P301S Tau expressing neurons lead to increased pro-inflammatory cytokine expression when compared to other APOE isoforms[113]. These studies highlight the interactions between APOE, Aβ, tau, and neuroinflammatory pathologies.

Certain structural components of APOE are critical for neuroinflammatory modulation. APOE amino acids 1−185 have been shown to regulate levels of matrix metalloproteinase 9 (MMP9) and tissue inhibitor of metalloproteinase 1 (TIMP1)[135]. APOE modulates neuroinflammatory molecules by increasing IL1β and decreasing IL10 levels. Other truncated forms of APOE have been shown to be anti-inflammatory, such as a 133−149 APOE peptide[130]. Overall, more research is needed to understand the structure/function relationship between APOE and neuroinflammation.

The effects of APOE on MMP9 are important not only for neuroinflammation but also BBB integrity[136]. *APOE ε4* is associated with decreased BBB integrity[117]. The tight junctions responsible for maintaining BBB integrity are compromised with APOE ε4 expression[137]. Pericytes and MMP9 seem to mediate these effects[138]. A separate study using *APOE* humanized mice showed similar findings, that APOE ε4 activated a cyclophilin A/NF-κB/MMP9 pathway in pericytes[139]. In the context of other models, APOE has been shown to activate MMP9 and reduce BBB integrity, especially in the context of TBI[140]. APOE modulation of inflammatory pathways appears to also affect BBB integrity and therefore could modulate other AD risk factors like TBI.

3.4. Mitochondria and metabolism

Mitochondrial dysfunction and metabolism changes are observed within the brain and periphery in AD subjects. Brain glucose metabolism is decreased as observed through fluorodeoxyglucose positron emission tomography (FDG-PET)[71,141]. Systemic glucose metabolism is altered as insulin resistance and insulin levels are increased in AD[72,142]. An association of type two diabetes and AD risk is apparent[72,142]. Mitochondrial dysfunction is observed across *in vitro* models, *in vivo* models, and human AD subjects. Cytochrome oxidase (COX) or complex IV of the electron transport chain (ETC) has decreased maximum velocity (Vmax)[69,71,143−146]. Mitochondrial number and respiration are lower across models[71]. Mitophagy pathways are aberrant[71,147]. Overall, *APOE ε4* carriers appear to have worse metabolic and mitochondrial function when compared to non-carriers in both healthy and diseased states.

Individuals with *APOE ε4* alleles have reduced brain glucose metabolism and insulin signaling, independent of Aβ[148]. Mice with humanized *APOE ε4* show decreased brain glucose uptake, reduced cerebral blood volume, and impaired insulin signaling[149]. Humanized *APOE ε4* mice show changes in purine, glucose, and pentose-phosphate metabolism[149]. These *in vivo* effects are only observed with advanced age or introduction of a high fat diet. Separate studies show humanized *APOE ε4* mice have metabolic shifts towards lipid oxidation and thermogenesis. These mice also have increased insulin resistance and elevated insulin levels[150]. Overall, changes to brain metabolism appear consistent across human and animal studies.

The APOE receptor, LRP1, might play a critical role in brain glucose metabolism. Deletion of LRP1 in forebrain neurons of mice reduced brain insulin levels and glucose metabolism[151]. However, some studies suggest APOE itself plays a role in insulin signaling. APOE interacts with insulin receptors and influences their trafficking through endosomes. APOE ε4 impairs insulin receptor endosome trafficking and signaling *in vitro*[152]. The mechanism of altered brain glucose and insulin signaling with various APOE isoforms requires more study. The effects of APOE isoforms on brain metabolism are also observed within mitochondria as discussed below.

APOE fragments which originate from APOE ε4 specifically can directly affect mitochondrial function (Fig. 4). An APOE C-terminal fragment (1−272) localizes to mitochondria and induces mitochondrial dysfunction[153]. The 1−272 C-terminal fragment of APOE was shown to bind to components of complex III and complex IV (COX) of the ETC *in vitro* and reduce their activity[153]. Other studies have suggested that APOE ε4 increases the activity and association of mitochondria with the endoplasmic reticulum (ER), or mitochondrial associated endoplasmic reticulum membranes (MAM) *in vitro*[154]. Expression of a neuronal directed APOE C-terminal fragment 1−272 induced ER stress and the formation of MAMs *in vitro* and *in vivo*. The mechanism of this finding is supported through increased calcium loading of mitochondria through glucose regulated protein 75 (GRP75)[155]. APOE fragments derived from APOE ε4 specifically localize to mitochondria, alter mitochondrial function, and influence mitochondrial−ER interactions.

Humanized *APOE* mice expressing *APOE ε4* have lower expression of sirtuin 3 (SIRT3) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α)[156]. These two proteins are critical for coordinating mitochondrial biogenesis and ultimately lower expression can result in decreased

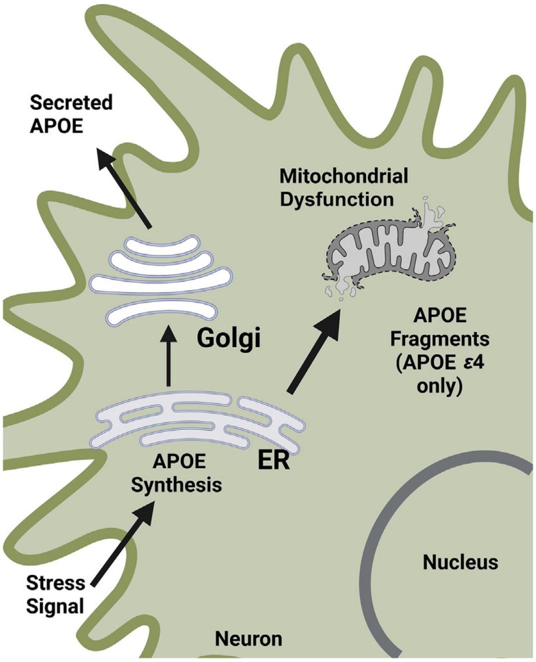

Figure 4 APOE effects on mitochondria in neurons. APOE synthesis is stimulated by stress signals in neurons. Synthesis occurs in the endoplasmic reticulum (ER) and if sorted through the Golgi APOE is secreted. Otherwise APOE is cleaved to generate toxic fragments which cause mitochondrial dysfunction. Fragmentation of APOE occurs only with APOE ε4 isoforms. Figure created with Biorender.com.

mitochondrial mass. *APOE ε4* mice also show reduced ATP production, altered NAD/NADH ratios, reduced synaptic markers, and lower cognition[156]. Overall, *APOE ε4* mice have altered proteomic profiles of mitochondrial proteins[157]. In astrocytes, *APOE ε4* increased mitochondrial fission, reduced autophagy and mitophagy, and reduced ATP levels[158]. Globally APOE affects brain metabolism, however some effects could be cell type specific.

In postmortem human brain, *APOE ε4* carriers showed changes to expression levels of proteins involved in mitochondrial structure, oxidative stress, and synaptic integrity[159]. The levels of these proteins were correlated with cognitive function within these subjects[159]. Postmortem brain samples from young *APOE ε4* carriers (prior to potential AD onset) had reduced COX activity with no changes to Aβ or tau pathology in the posterior cingulate cortex[160]. Further studies in young *APOE ε4* carrier postmortem brain tissue showed changes to metabolic pathway protein expression. Increased glucose and monocarboxylate transporters, hexokinase (glycolysis), ketone metabolism proteins (SCOT and AACS), and ETC enzymes from complex I, II, and IV were observed[161]. Overall, these data suggest a metabolic compensatory mechanism in *APOE ε4* carriers decades before AD onset.

Astrocytes play a crucial role in metabolic support in the brain, where they provide lactate, lipids, amino acids, and neurotransmitters to neurons. Disruption of the astrocyte/neuron energy coupling has been a proposed AD pathological mechanism[162]. Astrocytes expressing APOE ε4 when compared to ε2 and ε3 have impaired glucose uptake, with specific deficits in early glycolysis but increased lactate production[163]. APOE ε4 in astrocytes increased flux through the pentose phosphate pathway, gluconeogenesis, lipid, and nucleotide biosynthesis. Astrocytes with APOE ε4 likely increase carbon flux through the tricarboxylic acid (TCA) cycle leading to cataplerosis, as compared to increased oxidation and anapleurosis in ε2 and ε3 expressing astrocytes[163]. These findings suggest an overall energy deficit in astrocytes expressing APOE ε4 and are consistent with studies completed in peripheral cell types as discussed below.

APOE genotype effects blood-based measures of mitochondrial function and neuroinflammation[164]. *APOE ε4* carriers have decreased mitochondrial COX Vmax in platelets from blood compared to non-carriers[164,165]. These studies utilized only AD patients to control for potential confounding factors related to AD medications on mitochondrial function. Lymphocytes from AD *APOE ε4* carriers showed increased expression of inflammatory pathways and altered bioenergetic pathways suggesting an energy stress response[164]. Overall, APOE ε4 expression results in an energy deprivation/starvation state in numerous cell types.

3.5. *Synaptic function and axonal repair*

APOE may play a role in axonal repair through the redistribution of lipids to Schwann cells for remyelination[1,166–170]. APOE ε4 may impair neurite outgrowth. APOE ε4 destabilizes microtubules and increases NFT formation through tau hyperphosphorylation[1,108,171,172]. *In vitro* studies show that APOE ε4 alters cytoskeleton structure and impairs neurite outgrowth when compared to APOE ε3[173–175]. While more recent studies in new models are lacking, nonetheless these findings are of interest to the AD field.

Further studies have focused on the mechanism of APOE effects on memory and neurite outgrowth. Humanized *APOE* mice expressing APOE ε2, ε3, or ε4 were crossed with humanized *LDLR* or *LDLR* knockout mice. *LDLR* knockout mice and humanized *LDLR/APOE ε4* mice showed spatial memory deficits[176]. LRP appears to mediate the effects of APOE on neurite outgrowth. APOE ε3 associated β-VLDL and HDL particles stimulate neurite outgrowth more efficiently than those containing APOE ε4 *in vitro*[177,178]. APOE containing lipid particles act as a ligand for LRP *in vitro*[177,178]. Overall, APOE appears to affect neurite outgrowth *in vitro* and *in vivo* through an LRP mediated mechanism.

The effects of APOE on synaptic function and memory are also attributed to effects on neurotransmitter receptor expression and recycling. APOE ε4 reduces neurotransmitter receptor expression, including *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. The mechanism of this is likely through effects on receptor trafficking resulting in impaired synaptic function[179]. NMDA receptor function is also affected by its phosphorylation through Reelin. APOE ε4 reduces the expression of a receptor for both APOE and Reelin (apolipoprotein E receptor 2 or ApoER2). The reduced expression of ApoER2 and the reduced cell trafficking of NMDA/AMPA receptors leads to reduction of long-term potentiation (LTP) and reduced synaptic function[179]. In humanized *APOE* mice, APOE ε4 appeared to increase LTP in the hippocampus through NMDA receptors[180]. While acute exposure of hippocampal slices to APOE ε4 reduced LTP through NMDA receptors[180]. A separate study using hippocampal slices from humanized *APOE* mice showed reduced LTP in APOE ε4 expressing slices[181]. Overall, the *in vivo* effects are not consistent but APOE appears to influence synaptic function through NMDA receptors.

3.6. *Sex specific effects of APOE in AD*

The prevalence of LOAD is higher in females even when adjusted for age and lifespan sex differences[182,183]. Females with MCI and LOAD have higher rates of disease progression and higher burden of neuropathology (such as Aβ and tau)[184,185]. Female heterozygote *APOE ε4* carriers show similar AD risk to male homozygous *APOE ε4* carriers[186,187]. Menopause or loss of estrogen was thought to play a role in the increased risk of LOAD and *APOE ε4* associated LOAD in females[187].

APOE ε4 alleles have been shown to influence the onset of menopause[188,189]. *APOE ε4* increases the perimenopause period which leads to reduced glucose metabolism[187]. *APOE ε4* also reduces the protective effects of estrogen and can worsen the effects of the loss of sex hormones during menopause[187]. Some studies suggest that the energy shift towards ketosis during menopause leads to reductions in white matter and loss of myelination in *APOE ε4* carriers[190–192]. These studies have been replicated in ovariectomy transgenic AD mouse models[193,194].

Both APOE and estrogen are important for cholesterol metabolism. While APOE is discussed above, estrogen regulates cholesterol synthesis and transport, through LRP1, LDLR, and hydroxymethylglutaryl-CoA reductase (HMG-CR)[195,196]. APOE also appears to control hormone levels during menopause including estradiol. Other studies show that estradiol mediates APOE expression and neuroprotective effects[187]. *In vitro* and *in vivo* studies show that estrogen can increase APOE expression[197,198].

Female rodents do not undergo menopause but do have reduced levels of estrogen during aging[187,199]. As such AD associated changes are more pronounced in female AD transgenic mice than male mice[187]. Humanized *APOE ε4* female mice show worse cognition than males[200]. A recent study compared female humanized *APOE ε4* mice with samples from human plasma[201]. It was found that in mice *APOE ε4* is associated with reduced oxidative phosphorylation and increased glycolysis in astrocytes suggesting a shift towards aerobic glycolysis[201]. Human plasma samples and indirect calorimetry measures supported the data found in mice[201]. Overall, more research needs to focus on sex differences in AD, especially with regards to *APOE ε4*.

4. APOE specific therapeutic development in AD

Therapeutic approaches targeting APOE directly focus on genome editing, antisense oligonucleotides, modulators of APOE structure and interactions, antibodies against APOE, and efforts to alter the lipidation levels of APOE[202,203]. Other therapeutic efforts are indirect and focus on modulating lipid levels and energy metabolism through changes to life-style factors. The method of therapeutic targeting for APOE in AD is dependent on the mechanism of how APOE modulates risk. Some therapeutic strategies assume *APOE ε4* results in a gain of toxic function and others assume a loss of function consequence. These therapeutic approaches are reviewed below.

Several therapeutic approaches aim to change the expression levels and direct receptor interactions of APOE using distinct mechanisms. One approach is to treat with antisense oligonucleotides to disrupt APOE or receptor protein synthesis and expression levels. This therapeutic approach has only been examined in animal models to date. However, in AD transgenic mice antisense oligonucleotides targeting *Apoer2* improved synaptic function and cognition[204]. Antisense oligonucleotides against *APOE* reduce Aβ burden in AD transgenic mice[103]. Small molecules which modulate APOE and receptor expression have been tested in animal models and clinical trials. GW3965 increases APOE and ABCA1 expression levels and shows cognitive benefit with reduced Aβ in AD transgenic mice[205]. Bexarotene is an RXR agonist that modulates APOE expression and has showed varying results in AD mouse models[202,206]. In one study, Bexarotene reduced Aβ pathology in AD transgenic mice but several other studies failed to replicate these findings. In one clinical trial (BEAT-AD), Bexarotene failed to show any cognitive benefit but did reduce Aβ levels in AD subjects[207]. In healthy subjects, Bexarotene increased APOE levels but did not affect Aβ within the central nervous system (CNS)[208]. Furthermore, side effects of Bexarotene include increased risk of stroke and cardiovascular disease due to altered lipid homeostasis and further studies suggest that the drug has low CNS penetrance[202]. Overall, antisense oligonucleotides may prove to have reduced side effects and better CNS penetrance when compared to small molecule modulators.

APOE ε4 is hypo-lipidated due to its domain−domain interactions and salt bridge between the C- and N-terminus. This structural feature reduces APOE ε4 lipid interactions. One therapeutic approach is to increase the lipidation of APOE using the small molecule CS-6253[209]. This small peptide molecule activates ABCA1 and increases APOE lipidation in AD mouse models while also reducing Aβ/tau pathologies and increasing cognitive function[209]. Antisense oligonucleotides targeting microRNAs (miRNA) which reduce ABCA1 expression have been tested *in vivo* and *in vitro* where they reduce Aβ[210,211]. Other peptide mimetics have been shown to alter the lipidation of APOE while also increasing its secretion. 4F binds to LDL and HDL and is an 18 amino acid peptide. *In vitro* 4F increases APOE lipidation and secretion but no other studies have examined its affects in AD models[212]. Other mimetics, COG112 and COG113, showed beneficial effects in both *Drosophila* and mouse models of AD[132,213,214]. CN-105 is an APOE mimetic that has been tested in a clinical trial for intracerebral hemorrhage (ICH) and showed beneficial effects on neuroinflammation in mouse models[215]. In the phase I trial, CN-105 showed safety, tolerability, and reduced disability in a small cohort of patients with ICH[216]. Overall, clinical trials are lacking in AD for targeting APOE lipidation as a therapeutic target.

Targeting the domain−domain interaction of APOE ε4 has been tested in AD animal models. This approach uses small molecules identified in high-throughput screening approaches and was shown to stabilize APOE ε4. Two small molecules (CB9032258 and PH-002) restore COX levels within the mitochondria and mitochondrial function while inhibiting the APOE ε4 domain interaction *in vitro*[217]. One additional *in vitro* study showed PH-002 reduced APOE fragmentation and reduced Aβ and tau pathologies[104]. However, no *in vivo* studies have followed up on these findings to date.

Antibodies targeting APOE have shown positive effects in AD animal models but have not been tested in the clinic. In AD transgenic mice, APOE targeted antibodies reduced Aβ plaques, improved cognition, and did not affect plasma cholesterol levels[218,219]. In one study, administration of APOE antibodies after Aβ plaque formation showed beneficial effects in a transgenic AD mouse model[219]. Other antibody therapies (HAE-4) target non-lipidated forms of APOE regardless of isoform. HAE-4 showed beneficial effects in a transgenic mouse model harboring both mutant *APP* and *APOE ε4*. HAE-4 reduced Aβ pathology and was able to do so efficiently when administered systemically[220]. Thus far, no clinical trials examining the effects of APOE targeted antibodies are ongoing.

Genetic engineering approaches to modulate APOE expression and function include viral mediated gene transfer and CRISPR/Cas9 gene editing. The overall goal with this strategy is to modify the *APOE ε4* alleles to *APOE ε3* or *ε2*. CRISPR genome editing has been completed *in vitro* but not using *in vivo* models[92,221]. Adenoviral mediated delivery (AAV) of *APOE ε2* is currently enrolling an open label phase I clinical trial with expected completion in 2023. The goal of the study is to test the safety and toxicity of intracisternal administration of an AAV gene transfer vector expressing human *APOE ε2*. Intracisternal injection allows direct delivery of the AAV vector into the cerebrospinal fluid (CSF) and the intended population for the phase I trial are homozygous *APOE ε4* carriers with AD (ClinicalTrials.gov; Identifier: NCT03634007). Injection of AAV-*APOE ε2* into AD transgenic mouse models did reduce Aβ burden and is the basis for the phase I clinical trial[222].

Changes to lifestyle factors can indirectly target the effects of *APOE ε4* on AD risk. These lifestyle changes include diet and exercise. The beneficial effects of exercise on the brain and in AD models have been established and reviewed elsewhere[72,223−229]. Human clinical trial results are mixed but overall, the amount and type of exercise that is beneficial in AD needs to be established. Numerous clinical trials are currently enrolling with the goal to examine the type and regimen of exercise most beneficial in AD. A search of ClinicalTrials.gov yields 65 currently enrolling studies focused on exercise in AD. Diet modifications which are targeted for AD and *APOE ε4* include the ketogenic and Mediterranean diets, both of which show beneficial effects in AD mouse models. Four trials are currently enrolling for the ketogenic diet and three for the Mediterranan diet. The overall goal with diet and exercise modifications are to alter energy homeostasis by providing alternative fuel sources for the brain with added benefit of modulating lipid profiles and insulin resistance.

5. Concluding remarks

While APOE was originally identified as a lipid binding protein, its affects are pleiotropic. Polymorphisms in *APOE* modulate risk for vascular disease and AD. There are likely other unidentified associations of *APOE* isoforms with diseases across lifespan. It's also important to note that some *APOE* isoforms confer advantages early in life but are a disadvantage in aging. The role of *APOE* in the brain has largely focused on the effects of *APOE ε4*. While APOE is mostly expressed in glial cells (astrocytes and microglia), its effects are observed on other cell types, including neurons.

APOE ε4 influences pathologies observed in AD. APOE ε4 is associated with increased Aβ burden likely through reducing its clearance and degradation. Tau hyperphosphorylation and NFTs are increased in the presence of APOE ε4. Not surprisingly, APOE ε4 modulates neuroinflammation and this role directly impacts its effects on Aβ and tau pathologies. Mitochondrial function and metabolism are altered by the expression of APOE ε4, and these effects are observed in the periphery as well as the brain. Overall, the effects of APOE ε4 on AD associated pathologies are clear.

Therapeutic development targeting APOE in AD has focused thus far on mouse and cell models of disease. A critical issue is a lack of understanding of the disease modifying factor which leads to increased risk of AD with *APOE ε4*. Without this basic understanding, therapeutic strategies are difficult and rely on assumptions of gain or loss of function approaches.

An important question for the AD field remains, are the effects of APOE ε4 on AD pathologies a cause or effect? Of particular interest is the need to understand the role of metabolic deficits caused by APOE ε4 expression in driving AD pathology. Addressing this mechanistic question can drive therapeutic development.

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Conflicts of interest

The authors declare that they have no competing interests or conflicts.

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