Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies

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Abstract

Polymorphism in the apolipoprotein E (*APOE*) gene is a major genetic risk determinant of late-onset Alzheimer disease (AD), with the *APOE*ε4* allele conferring an increased risk and the *APOE*ε2* allele conferring a decreased risk, relative to the common *APOE*ε3* allele. Strong evidence from clinical and basic research suggests that a major pathway by which APOE4 increases the risk of AD is by driving earlier and more abundant amyloid pathology in the brains of *APOE*ε4* carriers. The list of amyloid-β (Aβ)-dependent and Aβ-independent pathways that are known to be differentially modulated by APOE isoforms is increasing. For example, evidence is accumulating that APOE influences tau pathology, tau-mediated neurodegeneration, and microglial responses to AD-related pathologies. In addition, APOE4 is either pathogenic or shows reduced efficiency in multiple brain homeostatic pathways, including lipid transport, synaptic integrity and plasticity, glucose metabolism, and cerebrovascular function. Here, we review the recent progress in clinical and basic research into the role of APOE in AD pathogenesis. We also discuss how APOE can be targeted for AD therapy using a precision medicine approach.

Introduction

Polymorphism in the apolipoprotein E (*APOE*) gene is a major risk determinant of late-onset Alzheimer disease (AD), the symptoms of which develop after the age of 65 years (Box 1)[1]. AD is the leading cause of dementia in elderly individuals[2], and its pathological hallmarks include the deposition of extracellular amyloid-β (Aβ) aggregates as amyloid plaques and intracellular hyperphosphorylated tau aggregates as neurofibrillary tangles, along with neuronal loss and glial activation[3]. Given that individuals with late-onset AD account for more than 95% of the total AD population, efforts to elucidate the role of *APOE*, in particular its links to the pathological hallmarks of the disease, are relevant to the vast majority of patients with AD seeking new therapies.

Of the three major *APOE* allelic variants, *ε2, ε3* and *ε4*, *APOE*ε4* is associated with an increased risk[4,5] and *APOE*ε2* is associated with a decreased risk[6,7] of AD relative to the common *APOE*ε3* allele. Carrying one *APOE*ε4* allele increases the risk of late-onset AD 3–4-fold, and carrying two alleles increases the risk 9–15-fold[6,8,9]. Among individuals with AD, *APOE*ε4* is also associated with lower age of disease onset (Box 1)[4,10]. Mounting evidence suggests that the APOE4 protein isoform drives amyloid pathology and impairs multiple aspects of normal brain function, thereby increasing the risk of AD[11] (Table 1). Studies have also shed light on the associations between *APOE* genotype and tau-mediated neurodegeneration[12], the risk of dementia with Lewy bodies (DLB)[13–15], Parkinson disease dementia (PDD)[15–18] and the degree of TAR DNA-binding protein 43 (TDP43) pathology in the brains of individuals with AD[19–21]. In addition, advances in disease modelling and molecular profiling technology have provided insights into the role of APOE in AD pathogenesis at the molecular, cellular and organism level.

In light of these findings, in this Review we describe the emerging link between *APOE* genotype and pathogenic proteins found in the brains of individuals with AD. We summarize recent progress in understanding the mechanisms by which different APOE isoforms contribute to or counteract AD pathogenesis via Aβ-dependent and Aβ-independent pathways. Finally, we discuss opportunities and strategies for developing APOE-targeted AD therapies in the future.

Biology of APOE

Human APOE is a 34 kDa glycoprotein[22] that is composed of 299 amino acids after cleavage of the 18-amino-acid signal peptide. In the CNS, APOE is abundantly expressed in astrocytes, microglia, vascular mural cells and choroid plexus cells, and to a lesser extent in stressed neurons[23,24]. Once APOE has been secreted from the cells, the cell surface ATP-binding cassette transporters ABCA1[25] and ABCG1[26] transfer cholesterol and phospholipids to nascent APOE to form lipoprotein particles. The size and density of APOE lipoprotein particles in the cerebrospinal fluid (CSF) are similar to those of HDL[27]. APOE plays a critical role in redistributing cholesterol and other lipids to neurons through binding to cell-surface APOE receptors. The LDL receptor (LDLR) family members, including LDLR and LDLR-related protein 1 (LRP1), are major APOE receptors involved in APOE-mediated lipid metabolism. APOE has two main functional regions (FIG. 1): the receptor-binding region in the amino-terminal domain and the lipid-binding region in the carboxy-terminal domain[28,29].

Computer-assisted modelling has been performed to examine the conformational changes in APOE that potentially occur on lipid binding. Structural modelling of human APOE3 was accomplished using data on APOE from the Protein Data Bank[30–37]. The modelling technique has been well documented[38–44] and enables composite protein structures to be built from multiple structural templates. The resulting lipid-free and lipid-bound structures are shown in FIG. 1[45–48]. The structures show that the lipid-binding region (residues 244–272) of APOE interacts directly with the lipid particle. Lipid binding to APOE increases the accessibility of the receptor-binding region (residues 136–150), thereby enabling cellular lipid delivery. The structural changes to APOE that are predicted to take place on lipid

binding are consistent with those documented in the existing structural literature for this protein[30,31,34,35,37,49–54].

The APOE isoforms encoded by the three corresponding gene alleles differ from one another only at positions 112 and 158 (APOE2: Cys112, Cys158; APOE3: Cys112, Arg158; APOE4: Arg112, Arg158). However, these single amino acid polymorphisms substantially alter the structure and function of APOE, thereby modulating its binding properties with regard to both lipids and receptors. For example, the binding of APOE2 to LDLR is more than 50 times weaker than the binding of APOE3 or APOE4 to this receptor[55]. In the periphery, the relatively low affinity of APOE2 for LDLR impairs the clearance of triglyceride-rich lipoprotein remnant particles and, as a consequence, APOE2 contributes to the onset of type III hyperlipoproteinaemia[22,56]. By contrast, enhanced binding of APOE4 to VLDL particles, as compared with the other APOE isoforms[55], impairs lipolytic processing of VLDL in the periphery; thus, APOE4 is associated with pro-atherogenic changes in lipoprotein distribution[56].

In addition to their role in lipid homeostasis in the periphery, APOE isoforms differentially modulate multiple pathways in the brain, including lipid transport, synaptic integrity and plasticity, glucose metabolism and cerebrovascular function. However, the correlation between the structure of individual APOE isoforms and their modulation of these pathways in the brain is less clear than for their actions on peripheral lipid metabolism. Possible mechanisms by which structural differences between APOE isoforms could modulate these brain pathways include differences in protein conformation, post-translational modification, lipoprotein preference and binding affinity for receptors[29].

Studies have also shed light on the potential role of plasma APOE in brain homeostasis. Synaptic dysfunction in *Apoe*-knockout mice can be partially restored, and cognition improved, by genetic restoration of peripheral APOE[57]. These effects occur despite the presence of the blood–brain barrier (BBB), which blocks APOE influx from the periphery. The relative ratio of APOE4 to APOE3 in plasma positively correlates with the loss of regional grey matter volume and abnormal cerebral glucose metabolism in cognitively healthy *APOE***ε3/ε4* carriers[58], linking the isoform composition of plasma APOE to structural and metabolic changes of the brain. Therefore, plasma APOE is a potential determinant of brain structure and function.

In *APOE***ε3/ε*4 individuals, the APOE4:APOE3 ratio is >1 in the brain and CSF but <1 in the plasma[59,60], suggesting that the metabolic pathways of APOE isoforms differ between the CNS and plasma. Thus, further studies exploring the similarities and differences in APOE metabolism between the CNS and periphery, and the functional crosstalk between brain and plasma APOE, would aid a better understanding of the impact of APOE isoforms on brain physiology.

APOE4 and Aβ pathology

Although evidence from clinical and pathological studies shows a link between *APOE* genotype and multiple proteinopathies, including those involving tau, α-synuclein and

TDP43, the best-established association is between *APOE* genotype and Aβ. Pathological studies of post-mortem brain tissue from patients with AD have found that *APOE*ε4* exacerbates the intra-neuronal accumulation of Aβ[61], plaque deposition in the brain parenchyma[62–65], formation of neurotoxic Aβ oligomers[66] and the severity of cerebral amyloid angiopathy (CAA)[67,68]. By contrast, *APOE*ε2* is associated with reduced numbers of neuritic plaques[69] but somewhat increased risks of CAA and CAA-related intracranial haemorrhage[70–73].

Imaging and CSF biomarker studies have shown that *APOE*ε4* is consistently associated with greater Aβ deposition in the brains of cognitively healthy elderly individuals[74–78], individuals with mild cognitive impairment (MCI)[79] and individuals with AD[80]. A longitudinal study also showed that *APOE*ε4* carriers exhibit increased Aβ deposition in the cortex compared with *APOE*ε4* non-carriers[81]. Of note, *APOE*ε4* is associated with an increased rate of longitudinal Aβ accumulation among cognitively healthy individuals who are amyloid-negative, whereas no differences in rates of Aβ deposition are observed among amyloid-positive individuals with different *APOE* genotypes[82]. Assuming that longitudinal Aβ accumulation represents an early phase of amyloid deposition in amyloid-negative individuals and a later phase in amyloid-positive individuals, these results suggest that the effect of *APOE*ε4* on Aβ metabolism and aggregation is most pronounced during the initiation phase of Aβ deposition. By contrast, in cross-sectional studies, *APOE*ε2* was found to be associated with reduced brain Aβ deposition in cognitively healthy elderly people[77,83,84] and individuals with MCI[85], particularly those without an *APOE*ε4* allele. *APOE*ε2* is also protective against longitudinal Aβ accumulation, especially among individuals without amyloid deposition[82]. Together, multiple layers of evidence indicate that the *APOE* genotype, in particular the presence of *APOE*ε4*, modulates amyloid pathology in the brain.

The effect of APOE4 in driving amyloid pathology is likely to outweigh the protective effect of APOE2 (FIG. 2, Table 2)[85], as *APOE*ε2/ε4* and *APOE*ε3/ε4* individuals have comparable probabilities of exhibiting amyloid-β (Aβ) deposition on an amyloid-PET scan. In addition, among cognitively healthy individuals and people with MCI, the prevalence of amyloid pathology is higher in those with *APOE*ε2/ε4* than in those with *APOE*ε3/ε3*. These findings indicate that APOE4 is a strong driver of Aβ deposition irrespective of the presence of APOE2 or APOE3. Importantly, the pattern of estimated probabilities of amyloid positivity associated with different *APOE* genotypes is similar to that of AD risk[6,8,9] (Table 2), reinforcing the idea that APOE4 increases the risk of AD largely by causing earlier and more abundant amyloid pathology in the brain. Likewise, the presence of *APOE*ε2* might reduce AD risk by decreasing amyloid pathology, especially in the absence of *APOE*ε4*. However, given that *APOE*ε2* is associated not only with intact cognition but also, paradoxically, with increased AD pathology[86] in the oldest old population, the protective effect of *APOE*ε2* might also be mediated by Aβ-independent mechanisms. Supporting this notion, *APOE*ε2* is associated with reduced cognitive decline during ageing even after adjustment for Aβ pathologies[87].

Aβ clearance.

As Aβ is continuously generated in the brain as a result of APP processing, efficient clearance is vital for preventing Aβ accumulation and subsequent aggregation[88]. The clearance of soluble Aβ from the brain interstitial fluid (ISF) occurs in an APOE isoform-dependent manner with APOE4 less efficient than APOE2 or APOE3[89]. In addition, deficiency of ABCA1, which lipidates APOE, decreases Aβ clearance in APOE4-targeted replacement (TR) mice, in which murine *Apoe* is replaced by a specific allele of human *APOE*, whereas no such effect is observed in APOE3-TR mice[90]. Thus, the isoform and lipidation status of APOE is likely to influence the overall efficiency of Aβ clearance in the brain. Major pathways by which Aβ is removed from the brain include clearance through the BBB, cellular uptake and subsequent degradation, enzymatic degradation, clearance via ISF bulk flow, and CSF absorption into the circulatory and lymphatic systems[88]. The effects of individual APOE isoforms on each of these processes (FIG. 3) could contribute in a synergistic manner to variations in Aβ metabolism rates, in particular under pathogenic conditions.

The removal of Aβ via transport through the BBB is mediated by APOE in an isoform-dependent manner. An in vivo study showed that APOE2–Aβ and APOE3–Aβ complexes were cleared by both VLDLR and LRP1 at the BBB, whereas APOE4 binding to Aβ switched the clearance pathway of Aβ at the BBB from LRP1 to the VLDL receptor (VLDLR)[91]. As VLDLR mediates the internalization of APOE–Aβ complex at a slower rate than LRP1, redirection of the clearance pathway for the APOE4–Aβ complex might contribute to the slower clearance of Aβ at the BBB, when compared with APOE2–Aβ and APOE3–Aβ complexes[91]. Consistent with the findings in vivo, a study utilizing a tissue engineering approach to generate a 3D model of human brain blood vessels showed that APOE4 was less efficient than APOE2 at promoting Aβ transport across vessel walls[92]. Furthermore, an in vitro study showed that clearance of Aβ aggregates by BBB-associated pericytes was impaired in the presence of astrocyte-derived APOE4 compared with APOE3[93]. The precise mechanisms by which APOE isoforms interact with Aβ receptors to mediate Aβ efflux from or influx into the brain require further investigation.

Cellular uptake and subsequent degradation of Aβ by glial cells represents a crucial Aβ clearance pathway in the brain. Human iPSC-derived astrocytes that are homozygous for *APOE*ε4* show impaired uptake of Aβ$_{1-42}$ in vitro compared with *APOE*ε3*-homozygous iPSCs[94]. A study using mouse brain slices indicated that astrocyte-mediated degradation of Aβ occurs through a mechanism that is dependent on both APOE and LRP1, suggesting that APOE is essential for receptor-mediated Aβ uptake by astrocytes[95]. However, another study showed that downregulation of LDLR reduced Aβ uptake, whereas upregulation of LDLR enhanced both the uptake and clearance of Aβ in astrocytes, independently of APOE[96]. A more recent study showed that the interaction between APOE and Aβ under physiological conditions is minimal, and that APOE might not be required for the cellular clearance of Aβ in astrocytes[97]. Instead, APOE competes with Aβ for the LRP1-dependent cellular uptake pathway, thereby blocking ~50% of the total Aβ cellular clearance by astrocytes *in vitro*[97]. Therefore, receptor-mediated internalization of Aβ in astrocytes might at least be partially

affected by the presence of different APOE isoforms and APOE receptors, with APOE4 impairing the uptake of Aβ by these cells.

Cellular uptake and degradation of Aβ by microglia is also differentially influenced by the various APOE isoforms. Degradation of Aβ peptides by neprilysin within microglia is markedly facilitated in the presence of APOE, with APOE4 being less efficient at promoting the degradation of soluble Aβ than APOE2 and APOE3[98]. In addition, APOE-mediated cholesterol efflux facilitates the delivery of Aβ to lysosomes and increases the efficiency of intracellular Aβ degradation in microglia[99]. The clearance of extracellular Aβ has been found to be impaired in human iPSC-derived microglia-like cells expressing *APOE*ε4* compared with those expressing *APOE*ε3*[94].

Studies have also investigated the effects of APOE isoforms on other clearance pathways, including perivascular drainage and proteolytic degradation of Aβ by Aβ-degrading proteases (Aβ-DPs). A wide array of Aβ-DPs, including neprilysin and insulin degrading enzyme (IDE), determine Aβ levels in the brain[100]. Expression of neprilysin in both brain parenchyma and vasculature and that of IDE in hippocampus are significantly lower in post-mortem brains from individuals with at least one copy of *APOE*ε4* than in those from individuals without *APOE*ε4*[101,102]. The perivascular drainage pathway has a major role in ISF bulk-flow clearance of Aβ[103]. In a study that examined this pathway, after intracerebral injections of $Aβ_{1-40}$, aggregation of $Aβ_{1-40}$ in the peri-arterial drainage pathway was observed in APOE4-TR mice but not in APOE3-TR mice[104]. Thus, the clearance of Aβ mediated by proteolytic degradation and perivascular drainage pathways seems to be impaired by the presence of APOE4.

The influence of APOE isoforms on the glymphatic clearance of Aβ[105] is less clear. In one study, APOE derived from the choroid plexus and CSF was shown to be delivered into the brain via the glymphatic fluid transporting system in an APOE isoform-dependent manner[106], although the relevance of these findings to disease needs to be further investigated.

Formation of Aβ fibrils.

Aβ aggregation occurs when Aβ is over-produced and/or inefficiently cleared. The formation of Aβ fibrils, a major component of amyloid plaques, follows three kinetic stages: a lag phase, a growth phase and a plateau phase[107]. Although the effects of APOE on the formation of Aβ fibrils are inconsistent in vitro[29,108], in vivo studies have shown a crucial role for APOE in the initial seeding stage of Aβ deposition. Through a novel approach involving conditional expression of different APOE isoforms in a mouse model of amyloid pathology, astrocytic overexpression of APOE4, but not APOE3, during the initial Aβ seeding stage was found to exacerbate amyloid pathology. However, expression of APOE4 or APOE3 during the period of rapid plaque growth had no effect on Aβ deposition[109]. In another study published in parallel, reduction of APOE expression by antisense oligonucleotide (ASO) treatment beginning at postnatal day 0 (Aβ seeding stage) led to a significant reduction in amyloid pathology in amyloid mouse models that were homozygous for *APOE*ε3* or *APOE*ε4*[110]. By contrast, ASO treatment beginning at the onset of amyloid deposition did not change the overall plaque load[110]. These *in vivo* results align

with the notion that APOE, and in particular APOE4, promotes the formation of Aβ fibrils by accelerating the initial seeding or nucleation of Aβ peptides (FIG. 3).

Aβ production.

Given that the generation of Aβ peptide from amyloid precursor protein (APP) represents a pivotal event in AD pathogenesis[111], researchers have sought to identify the role of APOE in this process. A study published in 2017 found that HEK293 cell-derived APOE induced *APP* transcription and Aβ production in human embryonic stem cell-derived and induced pluripotent stem cell (iPSC)-derived neurons. The effects were isoform-dependent, with APOE4 stimulating Aβ production more potently than other isoforms[112]. Aβ secretion is significantly higher in human iPSC-derived neurons carrying *APOE*ε4* than in those with *APOE*ε3*, probably as a result of increased APP transcription or processing[94,113]. However, transcriptomic analysis of cortical tissue from APOE-TR mice did not reveal the transcriptional regulation of *App* associated with APOE4[114]. Furthermore, in mouse models of amyloid pathology that expressed human APOE, no APOE isoform-dependent differences in the amount of APP fragment C99 or β-secretase activity were observed, suggesting that amyloidogenic processing of APP does not vary in the presence of different APOE isoforms *in vivo*[89]. Species-dependent (human versus mouse) differences in APOE isoform-regulated Aβ production[113] may have contributed to these conflicting results.

APOE in AD pathogenesis: beyond amyloid

APOE allele effects have also been implicated in proteinopathies characterized by tau, α-synuclein, and TDP43 pathology in the brains of individuals with AD. In addition, emerging evidence suggests that APOE can modulate tau-mediated neurodegeneration as well as microglial homeostasis, synaptic integrity, lipid transport, glucose metabolism and cerebrovascular function (FIG. 4). In this section, we discuss the disease relevance of these findings within the context of *APOE* genotype and its association with the risk of AD.

Tau, α-synuclein and TDP43 proteinopathies.

Findings suggest that *APOE* alleles exert an effect on neurodegenerative diseases marked by the aggregation of tau, a microtubule-associated protein encoded by the *MAPT* gene[115]. Individuals with primary tauopathies, such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD)[115,116], exhibit tau but not amyloid pathology. AD, though characterized by extensive tau pathology, is considered a secondary tauopathy as the tau pathology is accompanied by earlier development of amyloid pathology. Of note, postmortem brains from individuals carrying two *APOE*ε4* alleles have more tau aggregates than those carrying either one or no *APOE*ε4* alleles when Aβ pathology is present[65], but no such association is observed in brains without Aβ[117,118]. Moreover, among patients with PSP, the presence of concomitant amyloid pathology is associated with an increased likelihood of carrying *APOE*ε4*[119]. Thus, *APOE*ε4* is likely to be a risk factor for amyloid pathology but not necessarily for tau burden in primary tauopathies[119,120]. Interestingly, although *APOE*ε2* is protective in the setting of AD, in the absence of amyloid pathology it has been implicated as a risk factor for tau-related neurodegeneration[121]. A study showed that *APOE*ε*2 was associated with increased risk

and greater severity of tau pathology in patients with PSP[122]. Taken together, these results indicate that the effects of *APOE* alleles on tauopathies are influenced by the presence of amyloid pathology.

The pathological aggregation of tau strongly correlates with patterns of neurodegeneration and clinical manifestations in AD[123–126]. Studies have shown that neuron-specific overexpression of APOE4 increases tau phosphorylation in mice[127]. Subsequently, Shi et al. crossed the Tau[P301S] transgenic mouse model, which overexpresses human tau containing the frontotemporal dementia-linked Pro301Ser mutation, with human APOE-TR mice. Tau[P301S]–APOE4-TR mice developed markedly more brain atrophy and neuroinflammation than Tau[P301S]–APOE2-TR and Tau[P301S]–APOE3-TR mice at 9 months of age[12]. By contrast, Zhao et al. introduced tau with another frontotemporal dementia-linked mutation, Pro301Leu, to APOE-TR mice via viral delivery, and found more severe tau pathology and behavioural deficits at 6 months of age in Tau[P301L]–APOE2-TR mice than in Tau[P301L]–APOE3-TR and Tau[P301L]–APOE4-TR mice[122]. These seemingly contradictory results are likely to be a result of differences in the experimental model systems that the two groups used. Furthermore, Shi et al. measured tau-mediated neurodegeneration, whereas Zhao et al. focused on tau pathology.

In addition to the findings from mouse models, human iPSC-derived *APOE*ε4*-expressing neurons have higher levels of tau phosphorylation than neurons expressing *APOE*ε3*[113]. Taken together, these results suggest that APOE influences tau pathology and tau-mediated neuronal toxicity in an isoform-dependent manner. Understanding the relevance of these findings to different neurodegenerative diseases with tauopathy as either a primary or concurrent pathology will require further studies using models that are more relevant to the specific disease conditions.

APOE*ε4 is also a genetic risk factor for DLB[13–15] and PDD[15–18]. These conditions are both classified as synucleinopathies, a spectrum of neurodegenerative disorders that also includes PD[128,129] and is characterized by the presence of hallmark accumulations of α-synuclein, termed Lewy bodies, within neuronal cell bodies[130,131]. Patients with DLB or PDD often have some degree of concomitant AD-type pathology[132,133], and 30–40% of individuals with AD also present with Lewy bodies[134]. Therefore, determining whether *APOE*ε4* contributes to α-synuclein pathology through an Aβ-dependent mechanism, similar to that proposed for tau pathology in the amyloid cascade hypothesis[135], or through an Aβ-independent mechanism[136] remains challenging. However, a study published in 2018 demonstrated that in individuals with Lewy body disease, *APOE*ε4* was associated with increased α-synuclein pathology irrespective of the degree of AD pathology[137], suggesting that *APOE*ε4* impacts the severity of α-synuclein pathology independently of tau and Aβ. Of note, the gene encoding LDLR-related protein 10 (LRP10), which is thought to play a role in the metabolism of APOE lipoproteins[138] and the trafficking of APP[139], is associated with the development of inherited forms of DLB and PD[140].

APOE*ε4 might also directly increase the risk of TDP43 pathology in the brains of people with AD. TDP43 proteinopathy is a core pathological hallmark of amyotrophic lateral sclerosis and of frontotemporal lobar degeneration with TDP43 pathology[141], and often

coexists with AD pathology. Of note, *APOE*ε4* is associated with the presence of comorbid TDP43 proteinopathy in the brains of individuals with AD[19], as well as the severity of TDP43 proteinopathy, even after adjusting for the presence of Aβ, tau and Lewy body pathologies[21]. These observations suggest that a direct association between *APOE*ε4* and TDP43 proteinopathy exists in AD. A study published in 2018 confirmed that *APOE*ε4* is associated with TDP43 pathology independently of Aβ in the brains of individuals with AD[20].

In summary, *APOE* allele-specific effects on tau and α-synuclein proteinopathies, and on TDP43 pathology in the brains of individuals with AD, have been identified; however, the relevance of these findings to AD risk and disease progression is currently unclear. Further studies addressing the associations between APOE isoforms and these pathogenic proteins, using a model that reflects disease-specific conditions, should increase our understanding of APOE pathobiology within the context of AD.

Neuroinflammation.

Evidence suggests that APOE has an important role in regulating the innate immune response to amyloid pathology and neurodegeneration. Microglia play a central role in the immune response in the brain, highlighted by the abundant reactive microgliosis surrounding Aβ plaques in post-mortem brain tissue from individuals with AD[142]. These disease-associated microglia (DAM)[143–145], also referred to as a microglial neurodegenerative (MGnD) phenotype[146], have a conserved transcriptional signature across mouse models of AD. *APOE* is among these DAM-associated genes, as *APOE* expression by microglia is upregulated in association with ageing and amyloid and tau pathology[14]. By contrast, APOE deficiency diminishes the DAM signature in AD mouse models[146,147], highlighting the crucial role of APOE in regulating DAM phenotypes.

The various APOE isoforms seem to modulate microglial functions differently in AD pathogenesis[148]. Human iPSC-derived microglia-like cells carrying *APOE*ε4* exhibit altered morphologies and reduced levels of Aβ phagocytosis when compared with those carrying *APOE*ε3*[94]. APOE4 also increases microglial reactivity against Aβ plaques in a mouse model of amyloid pathology[149]. Furthermore, APOE4 boosts microglial proinflammatory activation and neurodegeneration in a tau transgenic mouse model[12]. One suggestion is that the specific lipid composition of the APOE4 lipoprotein affects lipid raft structures on microglial cell membranes to induce a stronger DAM phenotype, which exacerbates Aβ or tau pathogenesis and neurodegeneration[150].

APOE is an endogenous ligand of triggering receptor expressed on myeloid cells 2 (TREM2)[151–153], a cell surface receptor expressed exclusively by microglia in the brain. A rare coding variant of *TREM2* is associated with increased risk of AD, with an effect size comparable to that of *APOE*ε4*[154,155]. Furthermore, TREM2 is likely to mediate the APOE-dependent molecular signatures in microglia[146,150,156]. As such, the APOE–TREM2 pathway might contribute to AD pathogenesis through the modulation of microglial homeostasis[157,158].

A study published in 2019 demonstrated that amyloid plaque seeding was increased in the absence of functional TREM2, both in animal models and in individuals with AD[159]. Lack of TREM2 was also associated with a reduction in plaque-associated APOE. Of note, levels of plaque-associated APOE were substantially reduced on microglial depletion in a mouse model of amyloid pathology, indicating that microglia represent a prominent source of amyloid plaque-associated APOE. Thus, microglial APOE seems to be induced in a TREM2-dependent manner, suggesting an important association of APOE with TREM2 in the context of AD pathogenesis.

Synaptic function.

Increasing evidence indicates that the presence of *APOE*ε4* is associated with increased synaptic degeneration[160] and synaptic accumulation of Aβ oligomers[66], along with reductions in dendritic density[161], plasticity[160] and numbers of glutamate receptors[162], in the brains of individuals with AD. In addition, the levels of the synaptic markers synaptophysin, syntaxin 1 and postsynaptic density protein 95 (PSD95) are reduced in the brains of *APOE*ε4* carriers, and levels of PSD95 are increased in the brains of *APOE*ε2* carriers[163]. Consistent with findings from post-mortem human brains, reduced expression of synaptic proteins[164,165], reduced dendritic spine density and length[166], and impaired synaptic transmission[167,168] have all been observed in APOE4-TR mice when compared with APOE3-TR mice. In addition, APOE4 reduces neuronal surface expression of the LDLR family member APOER2 (also known as LRP8), along with NMDA and AMPA receptors, by sequestering them in the intracellular endosomal compartments[169], leading to suppression of reelin-mediated synaptic transmission. Therefore, the isoform-dependent effect of APOE on the intracellular trafficking of lipoprotein and glutamate receptors represents a potential mechanism by which APOE4 can impair synaptic plasticity[170]. Furthermore, hippocampal neurogenesis and hilar GABAergic neurotransmission are impaired in mice carrying *APOE*ε4*[171–173].

Collectively, these results suggest that APOE4 is either less efficient at maintaining synaptic integrity and neurogenesis than other AOPE isoforms or has a toxic effect[113] on these spine-related and synapse-related pathways. Given that synaptic loss strongly correlates with cognitive decline in AD, the impact of APOE4 on synaptic function and neurogenesis might combine with other APOE4-mediated pathways and AD pathology to contribute to the earlier onset of symptoms.

Lipid transport.

Several lipid transport-related properties of APOE have been shown to be isoform-dependent. Although in vitro studies using cell systems with limited relevance to human brain physiology such as HEK293 cells and immortalized mouse astrocytes generated inconsistent results[174,175], a more recent study using iPSC-derived astrocytes showed that APOE4 is less lipidated than APOE3, which could potentially diminish the neurotrophic function of APOE4[176]. In addition, in the CSF, APOE particles are larger in *APOE*ε2/ε3* individuals and smaller in both *APOE*ε3/ε4* and *APOE*ε4/ε4* individuals than in *APOE*ε3/ε3* individuals[177]. Furthermore, cholesterol efflux capacity is impaired in the CSF of individuals who are homozygous for *APOE*ε4*[178]. The amount of poorly lipidated APOE

in the CSF is higher in *APOE*ε4* carriers than in *APOE*ε4* non-carriers[179]. Overall, the APOE isoform effects on the APOE–lipid interaction support a loss-of-function effect of *APOE*ε4*, with APOE4 being less efficient than APOE2 or APOE3 at transporting lipids to neurons.

The overall lipid-transporting capacity of APOE could also be affected by the amount of this molecule within the brain[180]. In mice, the quantity of APOE in CSF[181,182], ISF[183] and brain parenchyma[182] shows an isoform-dependent gradient, with APOE2 being the most abundant and APOE4 the least abundant of the three isoforms. By contrast, studies utilizing a mass spectrometry-based approach in young control individuals (aged 22–60 years, average 34.5 ± 10.3 years)[60], cognitively healthy people (aged 43–80 years, average 61 years) and patients with AD (aged 60–94 years, average 78 years)[59] found no isoform-dependent differences in APOE levels in the CSF. However, one of the studies found that in a cohort of Aβ-positive individuals composed of both cognitively healthy individuals and patients with MCI, APOE levels in the CSF were significantly lower in *APOE*ε4* carriers than in non-carriers[60]. Thus, the presence of Aβ in *APOE*ε4* carriers might further exacerbate APOE4-mediated loss of neuroprotective function by decreasing the amount of APOE4 available to transport lipids to neurons.

Glucose metabolism and insulin signalling.

Several studies have highlighted the need to consider the *APOE*ε4* genotype when assessing the role of glucose metabolism and insulin signalling in AD. Cerebral glucose hypometabolism is an early biomarker of AD and exists in pre-symptomatic individuals long before the clinical onset of AD[184–187]. Altered levels of insulin, insulin receptors and insulin signalling are observed in the brain of individuals with AD compared with cognitively healthy individuals[188–190], and epidemiological studies have confirmed that diabetes and midlife insulin resistance are risk factors for AD[191,192]. Interestingly, the *APOE*ε4* genotype, independently of Aβ, contributes to age-related reductions in cerebral glucose metabolism and insulin signalling[193–197]. In the brain areas frequently affected in AD, *APOE*ε4* carriers, both with and without dementia, have lower cerebral glucose metabolism than non-carriers[195,197–203]. Phase II clinical trials of an insulin nasal spray in individuals with AD have shown that the preventative effects of insulin on cognitive decline are modulated by *APOE* genotype status[204–206]. In the CSF, increased levels of insulin are associated with increased levels of total tau and phosphorylated tau among *APOE*ε4* non-carriers[207]. Fasting plasma insulin levels and free fatty acid levels are also increased in *APOE*ε4* non-carriers[208].

Consistent with the clinical findings, preclinical studies using APOE-TR mice have also revealed that APOE isoforms differentially influence brain glucose metabolism and insulin signalling. Compared with APOE3-TR mice, APOE4-TR mice display downregulation of brain peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ coactivator 1 (PGC1α) signalling, which is involved in the regulation of glucose uptake and metabolism[209]. Following treatment with a high-fat diet, APOE4-TR mice show more profound cognitive impairment, reduced cerebral blood volume, decreased glucose uptake, and impaired insulin signalling compared with APOE3-TR mice[194,210], providing additional

evidence that human APOE isoforms differentially modulate brain bioenergetic metabolism. Furthermore, APOE4-TR mice exhibit impaired brain insulin signalling and insulin resistance compared with APOE3-TR mice in an age-dependent manner[194]. Importantly, APOE4 interacts with insulin receptors and traps them in the endosomes, thus impairing insulin receptor trafficking and related signalling[194]. APOE receptors are also involved in brain glucose metabolism and insulin signalling. Conditional deletion of the gene encoding a major APOE receptor, LRP1, in mouse forebrain neurons leads to impaired brain insulin signalling and a reduced capacity to metabolize glucose[211].

Cerebrovascular integrity and function.

APOE*ε4 is a risk factor for vascular cognitive impairment[212,213], suggesting a possible link between this allele and neurovascular unit dysfunction. Imaging studies have shown that the presence of *APOE*ε4* is associated with increased severity of white matter hyperintensities independently of AD diagnosis[214,215]. In cognitively healthy individuals, *APOE*ε4* carriers show a higher CSF:plasma albumin quotient than *APOE*ε4* non-carriers[216], suggesting that BBB integrity is disrupted in the carriers. *APOE*ε4* carrier status is also associated with accelerated pericyte degeneration and loss of BBB integrity in AD, as observed in post-mortem brain tissue[217]. Thus, multiple layers of clinical evidence suggest a link between *APOE*ε4* and impaired cerebrovascular integrity and function, both in the presence and absence of AD pathology.

Consistent with these findings, reduced cerebral blood flow, vascular density and neurovascular coupling are observed in APOE4-TR mice compared with APOE2-TR and APOE3-TR mice[218–220]. Loss of BBB integrity has also been observed in APOE4-TR mice[218,219], although conflicting results have been obtained[221]. Another study showed that cerebral hypoperfusion, white matter damage and cognitive impairment induced by bilateral carotid artery stenosis are more severe in APOE4-TR mice than in APOE3-TR mice[220]. Overall, these APOE isoform effects on cerebrovascular integrity and function suggest that APOE4 is less efficient than APOE2 or APOE3 at maintaining cerebrovascular homeostasis. Of note, analysis using data sets from the Alzheimer's Disease Neuroimaging Initiative (ADNI)[222], and a CSF biomarker study[223] both indicated a role for vascular dysregulation in the early stages of the AD cascade. Thus, determining how the cerebrovascular effects of APOE4 drive AD pathogenesis could represent an important area for future studies.

The cerebrovascular contribution to cognitive decline and synaptic dysfunction probably combines with the accumulation of pathogenic proteins, such as Aβ[224,225], in both a synergistic and an additive manner. Therefore, the role of cerebrovascular effects of APOE4 in AD pathogenesis needs to be investigated in the context of both Aβ-dependent and Aβ-independent mechanisms. Peripheral APOE4 might modulate cerebrovascular function by exerting direct actions on the endothelial cells[226] that form the BBB, which exists as an interface between the CNS and the peripheral circulation. In addition, peripheral APOE4 could indirectly modulate the functions of brain endothelial cells and neurons through its effects on lipid metabolism, atherosclerosis and peripheral inflammation, perhaps acting synergistically with other vascular risk factors[227].

Perhaps surprisingly, *APOE*ε2* is associated with increased risk of CAA and CAA-related intracranial haemorrhage[70–73] and severity of cerebral small vessel disease[215,228] despite its protective effect against AD risk. However, the cerebrovascular effects of APOE2 remain relatively unexplored.

APOE-targeted therapies for AD

The current strategies for targeting of APOE to treat AD fall into three main categories: modulating APOE quantity and lipidation, targeting APOE structural properties and interactions with Aβ, and targeting APOE receptors.

Modulating APOE quantity and lipidation.

As discussed above, APOE has crucial roles in lipid transport and maintenance of synaptic homeostasis. Therefore, AD-related pathways might be modulated by increasing the quantity and/or degree of lipidation of APOE. In particular, administration of agonists of the liver X receptor (LXR) and the retinoid X receptor (RXR) has been shown to positively regulate transcription and secretion of APOE[229], reduce Aβ deposition[98,230–233] and restore cognitive function[98,231,233,234] in mouse models of amyloid pathology. Oral administration of the RXR agonist bexarotene reduced Aβ deposition and improved cognitive function in amyloid mouse models[235], including those that also expressed human *APOE*ε3* and *APOE*ε4*[236], and in APOE4-TR mice[237]. Bexarotene also seems to combat ageing-related loss of synaptic proteins in mice[238]. However, conflicting results with respect to the effects of bexarotene on amyloid pathology in mouse models have also been reported[236,239–241]. In addition, systemic adverse effects including liver toxicity are observed on bexarotene treatment in mice[238,242].

Bexarotene treatment does not reduce amyloid burden in patients with AD[243]. Oral administration of bexarotene to healthy individuals induced a 25% increase in CSF APOE levels but had no effect on CSF Aβ metabolism[244] and showed poor CNS penetration. The clinical utility of bexarotene as a drug for AD might also be hampered by systemic adverse effects, such as hypertriglyceridaemia[243], which are mediated by the permissive action of the RXR–LXR heterodimer on target genes, leading to activation of specific metabolic pathways in the liver[245]. Therefore, the potential clinical application of bexarotene has major limitations. From the perspective of gaining insight into APOE-targeted therapeutic strategies, the extent to which these RXR and LXR treatment-related phenotypes are mediated by an increase in APOE lipidation remains unclear. High-throughput screening for APOE agonists using human astrocytes may help to identify APOE-inducing compounds with pharmacological actions that do not depend on the nuclear receptor–APOE axis[246].

Studies utilizing virus-mediated gene transfer approaches have also provided insights into whether increasing the expression of APOE isoforms that are considered protective halts AD pathogenesis. Virus-mediated human *APOE* gene expression in mice has been shown to have APOE isoform-dependent effects on brain Aβ pathology[247–249] and APOE lipidation[250]. Adeno-associated virus (AAV)-mediated expression of *APOE*ε4* in a mouse model of amyloid pathology exacerbated synaptic loss and Aβ deposition, whereas expression of *APOE*ε2* in the same model caused a reduction in brain Aβ levels[249].

Consistent with this finding, AAV-mediated delivery of *APOE*ε2* reduced Aβ deposition in two different amyloid mouse models, crossed with *APOE*ε4* knock-in mice[248]. Furthermore, overexpression of *APOE*ε4* in APOE4-TR mice increased the levels of poorly lipidated APOE in the brain, whereas overexpression of *APOE*ε2* in these mice enhanced APOE lipidation[250]. Therefore, increasing the expression of *APOE*ε2*, but not *APOE*ε4*, might be beneficial with regard to increasing APOE lipidation and reducing Aβ pathology.

The therapeutic potential of decreasing the expression levels of specific APOE isoforms in order to reduce Aβ deposition has also been investigated in mouse models. Decreasing APOE expression by *APOE* haploinsufficiency in an amyloid mouse model carrying *APOE*ε3* or *APOE*ε4* led to a reduction in Aβ deposition, which was independent of APOE isoform[251,252]. Furthermore, a study published in 2017 showed that reduction of APOE expression by ASOs significantly decreased Aβ pathology in an amyloid mouse model that was homozygous for *APOE*ε3* or *APOE*ε4*[110]. Thus, decreasing the levels of APOE3 and APOE4, particularly during the initial Aβ seeding stage, may be beneficial.

Given that APOE4 is associated with reduced levels of lipid in the CNS[179,253], which could contribute to the APOE4-mediated loss of neuroprotective function, the question of whether an increase in APOE lipidation can reduce Aβ pathology has been investigated. Deletion of the gene encoding ABCA1, which lipidates APOE in the CNS, resulted in a decrease in APOE lipidation and an increase in Aβ deposition in amyloid mouse models[254,255]. Conversely, overexpression of ABCA1 in an amyloid mouse model decreased Aβ deposition — an effect that was probably mediated by an increase in APOE lipidation[256]. In addition, an *Abca1* hemizygous knockout exacerbated memory deficits and Aβ deposition in an amyloid mouse model carrying human *APOE*ε4*, but not in mice carrying *APOE*ε3*[90]. The exacerbation of Aβ pathology in the mice carrying *APOE*ε4* could have been attributable to an increase in the amount of poorly lipidated APOE4 in the absence of one allele of *Abca1*. Whether, and to what extent, increasing APOE4 lipidation is beneficial in the presence of Aβ pathology remains to be fully determined.

APOE-mediated plaque formation might also be targeted via APOE immunotherapy. Treatment with the anti-mouse APOE antibody HJ6.3 induced a significant reduction in Aβ deposition in a mouse model of amyloid pathology that expressed murine APOE[257,258]. In another study, Aβ deposition was reduced in an *APOE*ε4*-positive amyloid mouse model following treatment with an anti-human APOE antibody, HAE-4, which preferentially binds the non-lipidated, aggregated form of APOE[259]. By binding to aggregated APOE in Aβ plaques, HAE-4 is likely to induce activation of plaque phagocytosis by microglia, thereby reducing Aβ deposition. Importantly, peripheral administration of HJ6.3 or HAE-4 does not change the total amount of APOE in plasma and brain parenchyma[257–259]. Thus, the targeting of APOE that is specifically associated with amyloid plaques is an attractive approach that would have minimal impact on the physiological function of APOE.

Treatment with peptides that mimic the structural and biological properties of native APOE reduces Aβ deposition[260,261], tau hyperphosphorylation[262] and glial activation[260–262] in mouse models of amyloid pathology. However, the effects of these peptides on Aβ

deposition and other AD-related pathologies in the context of human APOE isoforms have not been fully determined.

Targeting APOE structural properties and Aβ interaction.

The pathological conformation of APOE4 is proposed to result from an interaction between its amino-terminal and carboxy-terminal domains[263,264,265], and the use of small molecules to block this interaction has been explored in vitro[264,266,267]. The APOE4 structure corrector PH002, at a final concentration of 100 nM in the culture medium, was shown to decrease APOE4 fragmentation and reduce the effects of APOE4 on Aβ production, tau phosphorylation and GABAergic neuron degeneration in human iPSC-derived neurons[113]. Targeting the abnormal biophysical properties of APOE4 represents a potential therapeutic approach; however, the efficacy of this approach in vivo, in the context of human APOE isoforms and amyloid pathology, remains to be investigated.

Conversion of the APOE4 amino acid sequence into that of APOE3 or APOE2 seems to be a more straightforward approach to modulate the pathobiology of APOE4. Conversion of *APOE*ε4* to *APOE*ε3* by gene editing considerably alters cellular phenotypes[94,113]. Reductions in APOE fragmentation, Aβ production, tau phosphorylation and GABAergic neuron degeneration are observed in iPSC-derived neurons when *APOE*ε4* is converted to *APOE*ε3*, suggesting that the detrimental effect of APOE4 could be abolished by gene editing[113]. Similarly, converting *APOE*ε4* to *APOE*ε3* attenuates several AD-related phenotypes in glial cells and organoids: this intervention enhances the ability of glial cells to endocytose extracellular Aβ and significantly reduces the amount of Aβ deposition in organoids after 6 months of culture[94]. Despite these promising in vitro findings, the in vivo feasibility and clinical translatability of this therapeutic concept remain to be determined.

Inhibition of the APOE–Aβ interaction by the synthetic peptide Aβ12–28P, which is homologous to the APOE binding site on the full-length Aβ molecule, reduced Aβ deposition[268–270] and insoluble tau accumulation[270] in AD mouse models, and intra-neuronal Aβ accumulation[271] in primary hippocampal neurons. In addition, treatment with Aβ12–28P decreased brain Aβ accumulation, co-deposition of APOE within Aβ plaques and neuritic degeneration in amyloid mouse models with APOE2-TR and APOE4-TR backgrounds[272]. Therefore, blocking APOE-mediated facilitation of Aβ assembly and/or deposition with a synthetic peptide seems to be beneficial in reducing Aβ pathology, irrespective of APOE isoforms.

Targeting APOE receptors.

Given that clearance of Aβ in the brain is partially mediated by APOE receptors, including LDLR and LRP1[28,29,273], increasing the expression of these receptors is a potential therapeutic strategy to reduce Aβ pathology. In mouse models of amyloid pathology, *Ldlr* deficiency is associated with increased Aβ deposition[274,275], whereas overexpression of *Ldlr* leads to enhanced Aβ clearance and decreased Aβ deposition[276]. Conditional knockout of *Lrp1* in neurons[277], astrocytes[278] and vascular smooth muscle cells[279] led to increased Aβ deposition in amyloid mouse models. Furthermore, treatment of animal models with compounds[280] that have been clinically explored for AD therapy, including fluvastatin[281],

decreases Aβ deposition and/or enhances Aβ clearance, probably by increasing *Lrp1* expression. Whether these APOE receptor-related effects are mediated by APOE remains to be elucidated.

Challenges and considerations.

As with any attempts to target proteins that have essential physiological functions, a major challenge for APOE-targeted therapy is to minimize potential adverse effects on APOE-dependent brain homeostasis and systemic physiology. One individual with an ablative homozygous *APOE* frameshift mutation did not exhibit substantial neurocognitive deficits at the age of 40 years[282]. However, the impact of APOE deficiency on brain physiology in the context of ageing and AD development has not been documented. Thus, it will be crucial to monitor the effects of modulating APOE levels not only on AD pathogenesis, but also on brain physiology at different stages of AD development. The potential impact of APOE-targeted therapy on peripheral lipid metabolism and related physiology should also be considered. For example, modulating the amount of APOE or the expression of APOE receptors in the periphery could increase the risk of hyperlipidaemia, atherosclerosis and cardiovascular events, owing to impaired lipoprotein metabolism. Although increasing APOE2 levels in the brain might be beneficial, long-term expression of APOE2 could increase the risk of CAA, CAA-related intracerebral haemorrhage[70–73] and perhaps primary tauopathy[122].

These adverse effects could be managed by optimizing the treatment strategy for each *APOE* genotype, taking into consideration the treatment duration, the therapeutic window, and the specific biochemical properties and in vivo distribution of the pathogenic forms of APOE. Furthermore, the identification of APOE downstream effectors[219] that modulate AD pathogenesis could provide therapeutic options with a limited impact on APOE-related physiology. Finally, the development and integration of novel technologies, such as targeted in vivo gene editing and more efficient drug delivery methods, could accelerate the clinical translation of APOE-targeted therapeutic concepts that have been uncovered in basic studies.

Conclusions

Strong evidence suggests that human APOE isoforms modulate AD pathogenesis primarily through their differential effects on Aβ clearance and aggregation. However, APOE isoforms also differentially affect multiple pathways that are not necessarily dependent on Aβ, with APOE4 exhibiting either a gain of toxic function or a loss of physiological function. Associations between APOE isoforms and tau-mediated neurodegeneration, and the risk of multiple neurodegenerative proteinopathies found in the brains of individuals with AD, are also recognized. Furthermore, a growing body of evidence links APOE with TREM2, highlighting the crucial role of APOE in the innate immune response in the brain.

To elucidate the precise pathological mechanism by which the *APOE* polymorphism determines the risk of AD (Table 1), several important yet underappreciated aspects of APOE pathobiology need to be determined. These aspects include the structure–function relationships for the different APOE isoforms, the impact of peripheral APOE on brain neurobiology and pathobiology, Aβ-dependent and independent protective pathways

activated by APOE2, similarities and differences in the role of APOE in late-onset and early-onset AD (Box 2), sex–*APOE* genotype interactions (Box 3), and the role of APOE in immune responses and cerebrovascular pathways. As APOE is expressed by multiple cell types in the brain and periphery, the specific contributions of cell-autonomous versus cell-non-autonomous effects of APOE at different stages of AD development will also need to be investigated, perhaps using conditional mouse models and human cerebral organoids[283], combined with human studies. Finally, a precision medicine approach based on *APOE* genotype status could facilitate the development of different AD treatment strategies (Box 4, FIG. 5). Establishment of APOE-based therapeutics is a considerable challenge; however, the targeting of APOE and its pathogenic interconnections offers great promise for the prevention and/or treatment of AD.

Acknowledgements

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Key points

- The apolipoprotein E (*APOE*) gene has three major allelic variants, *ε2, ε3* and *ε4*; *APOE*ε4* is associated with an increased risk and lower age of onset of Alzheimer disease (AD), whereas *APOE*ε2* seems to confer protection against AD.

- Increasing evidence suggests that the effect of *APOE*ε4* on AD risk is exerted through inhibition of amyloid-β (Aβ) clearance and promotion of Aβ aggregation.

- APOE influences tau pathology and tau-mediated neurodegeneration in an isoform-dependent manner, although the relevance of this observation to AD pathogenesis requires further investigation.

- APOE4 also contributes to AD pathogenesis by impairing microglial responsiveness, lipid transport, synaptic integrity and plasticity, glucose metabolism, and cerebrovascular integrity and function; some of these effects are independent of Aβ-related pathways.

- Current research into APOE-targeted AD therapeutic strategies aims to modulate APOE quantity and lipidation, APOE structural properties, APOE–Aβ interaction, and APOE receptor expression.

Box 1:

APOE polymorphism as a risk determinant for AD

Numerous susceptibility genes (or loci) and coding variants associated with the risk of developing Alzheimer disease (AD) have been identified[284,285]. However, owing to its large effect size and high prevalence, the apolipoprotein E (*APOE*) polymorphism is considered to be the strongest genetic risk determinant for late-onset AD (Table 1). According to a meta-analysis, which included African American, white, Hispanic and Japanese individuals, the odds ratio (OR) for AD development in individuals with one allele of APOE2 is 0.621 (95% CI 0.456–0.85) compared with individuals homozygous for *APOE*ε3. By contrast, the OR for AD development in individuals carrying one *APOE*ε4 allele is 3.68 (95% CI 3.30–4.11) compared with individuals homozygous for *APOE*ε3[286]. Importantly, *APOE*ε4 both increases the risk of AD[6,8,9] and lowers the age of disease onset[4,10] in an allele number-dependent manner. A study that included >17,000 white individuals showed that the ORs for AD development were 2.64 and 3.63, respectively, in individuals with the *APOE*ε2/ε4 and *APOE*ε3/ε4 genotypes, in relation to those with the *APOE*ε3/ε3 genotype[9]. The OR increased dramatically to 14.49 for individuals with the *APOE*ε4/ε4 genotype (Table 2)[9]. In addition, compared with *APOE*ε4 non-carriers, carrying one *APOE*ε4 allele brings the onset of AD forward by 2–5 years[4,10], and carrying two *APOE*ε4 alleles brings onset forward by 5–10 years. The average allele frequency of *APOE*ε4 in cognitively healthy individuals across African American, white, Hispanic and Japanese populations is 9–20%[286]; however, it is dramatically increased to ~40% among patients with AD (Table 1)[6,10], further highlighting the strong association of *APOE*ε4 with the risk of AD. A study published in 2018 showed that the prevalence of *APOE*ε4 was 66% in individuals with biomarker-confirmed AD-type dementia[287].

Ethnicity might influence the magnitude of the *APOE*ε4-associated risk of developing AD. For example, when compared with white individuals[6], the association of *APOE*ε4 with AD risk is weaker among African American and Hispanic individuals, but is stronger in Japanese individuals. Understanding the mechanism by which traits such as ethnicity and sex (Box 3) influence the association of *APOE*ε4 with AD risk might lead to the identification of modifiable factors that promote cognitive resilience in certain *APOE*ε4 carriers.

Box 2:

APOE polymorphism and early-onset AD

The apolipoprotein E ε4 allele (*APOE*ε4*) is associated with an increased risk of early-onset Alzheimer disease (EOAD)[9,288], the symptoms of which develop at ≤65 years of age. The presence of two *APOE*ε4* alleles is sufficient to increase the risk of EOAD regardless of family history of dementia[289]. By contrast, carrying one allele of *APOE*ε4* increases the risk of EOAD only in individuals with a positive family history of the disease[289]. The allele frequency of *APOE*ε4* is higher in individuals with EOAD than in healthy controls[290,291], further supporting an association of *APOE*ε4* with the risk of EOAD. Furthermore, *APOE*ε4* decreases the age of onset of EOAD in families carrying amyloid precursor protein (*APP*)[292], presenilin 1 (*PSEN1*)[293] or *PSEN2*[294] mutations, whereas *APOE*ε2* delays age of onset in those carrying *PSEN1* mutations[295]. Overall, the impact of *APOE* polymorphism on the risk and age of onset of EOAD seems to be similar to that of *APOE* polymorphism on late-onset AD, at least with respect to the directionality of effect. However, the available evidence is insufficient to draw conclusions about the relative dose effects of *APOE*ε4* and *APOE*ε2* alleles in individuals with EOAD who have different genetic backgrounds. Of note, in individuals with autosomal dominant AD, family history and mutation type explain substantial portions of the observed variance in the age of symptom onset[296], suggesting that the impact of *APOE* polymorphism on age of onset differs among subtypes of individuals with EOAD.

Box 3:

Sex-specific association of *APOE*ε4* with AD

The apolipoprotein E ε4 allele (*APOE*ε4*) seems to interact with sex to modify the risk of Alzheimer disease (AD). Women carrying one or two copies of *APOE*ε4* have a greater risk of developing AD than men with the same *APOE* genotype[6]. In addition, cognitively healthy women carrying *APOE*ε4* are more likely to progress to mild cognitive impairment and Alzheimer disease (AD) than are men carrying *APOE*ε4*[297]. One study showed that among *APOE*ε4* carriers aged 65–75 years, women had a greater risk of AD than did men[8]. A post hoc analysis of imaging data sets from cognitively healthy individuals who carried *APOE*ε4* and were positive for amyloid-β (Aβ) deposition on an amyloid-PET scan suggested that age-related cognitive decline was faster in the women than in the men[298]. A stronger association between *APOE*ε4* and elevated cerebrospinal fluid tau levels[297,299,300] was observed in women than in men, particularly among Aβ-positive individuals[299,300]. Thus, the increased risk of AD in women carrying *APOE*ε4* might be attributed to increased susceptibility to Aβ pathology, leading to accelerated neuronal damage. By contrast, no sex difference was observed in the association of *APOE*ε4* with regional tau deposition in clinically healthy individuals[301]. Comprehensive studies investigating sex differences in the association of *APOE*ε4* with AD-related biomarkers at different stages of AD development[302] would help us to better understand the mechanisms by which *APOE*ε4* confers an increased risk of AD in women.

Box 4:

APOE genotype as a guide for precision medicine

The aim of precision medicine is to tailor therapeutic interventions to an individual's predisposition to disease, disease progression and response to therapy. A strategy in which medical interventions for Alzheimer disease (AD) are guided by apolipoprotein E (*APOE*) polymorphisms, within the framework of precision medicine, might hold promise. In cognitively healthy individuals, the presence of *APOE*ε4* correlates with earlier and greater memory decline[303–305] and progression to mild cognitive impairment or AD[306]. On the other hand, advanced education, active leisure activities and good vascular health are all likely to reduce the risk of *APOE*ε4*-mediated cognitive decline[307]. A sedentary lifestyle is associated with higher amyloid-β (Aβ) deposition in cognitively healthy individuals carrying *APOE*ε4*[308]. Thus, at least in the cognitively healthy population, *APOE* genotyping, when performed as part of the polygenic risk score analysis[309,310] and/or combined with pathological biomarker status, might be helpful in assessing the potential risk of AD and age-related cognitive decline. The early detection of latent pathological processes is an integral part of precision medicine[311] as it provides greater opportunities for effective intervention, including lifestyle changes. If the initiation of Aβ pathology is detected, different types of interventions could be considered depending on the individual's *APOE* genotype (FIG. 5).

In several clinical studies, *APOE*ε4* carriers and non-carriers responded differently to treatment for AD. In phase III trials of bapineuzumab, a humanized anti-Aβ monoclonal antibody, in individuals with mild-to-moderate AD, *APOE*ε4* status was associated with differences in key biomarker outcomes including Aβ metabolism and tau pathology[312]. In *APOE*ε4* carriers Aβ deposition detected by PET imaging remained unchanged in individuals treated with bapineuzumab (0.5 mg kg^{-1}), whereas individuals receiving placebo showed increases in Aβ deposition over the course of 71 weeks. By contrast, in individuals not carrying *APOE*ε4*, no increase in Aβ deposition was observed in the placebo groups, and there was no difference between the placebo group and individuals receiving 0.5 mg/kg or 1.0 mg/kg bapinezumab. In addition, *APOE* genotype seems to influence the outcomes of treatment with intranasal insulin[204–206,313,314] and cholinesterase inhibitors[315] in individuals with AD. For example, *APOE*ε4* non-carriers, but not *APOE*ε4* carriers, show memory facilitation after intravenous insulin administration[313]. Acute administration of short-acting intranasal insulin improves verbal memory in *APOE*ε4* non-carriers but not in *APOE*ε4* carriers[314]. Interestingly, long-acting intranasal insulin treatment in patients with AD leads to memory improvement in *APOE*ε4* carriers but worsening in *APOE*ε4* non-carriers[206]. Further understanding the relationship between *APOE* genotype and treatment response will improve delivery of individualized AD treatment. As such, the *APOE* genotype could serve as a guide for precision medicine. Given its strong AD risk-determining effect, however, there are ethical considerations pertaining to human genomic-based medicine that need to be taken into consideration when testing the *APOE* genotype.
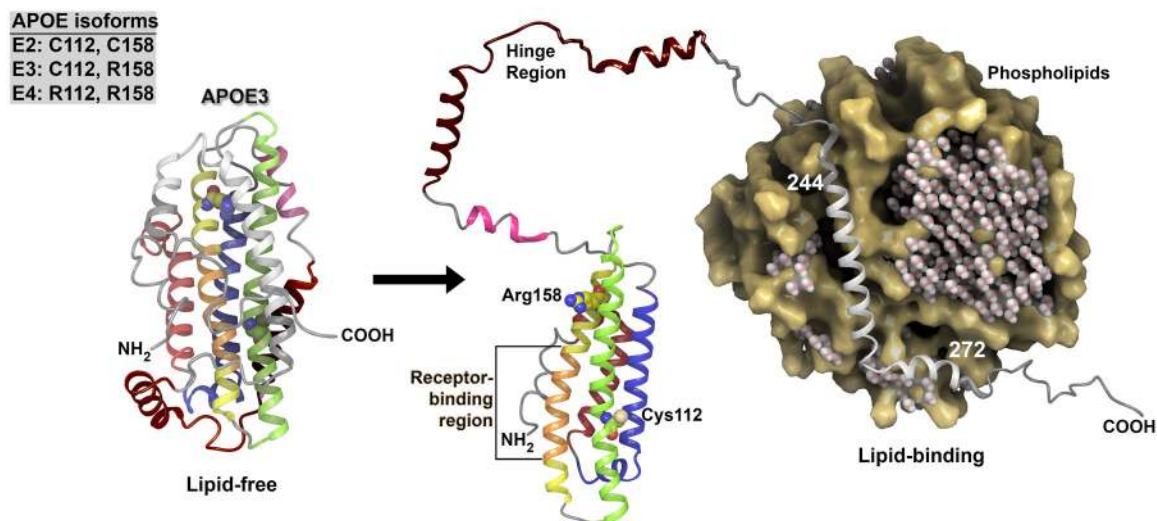
Figure 1: Structure of lipid-free and lipid-bound APOE3.

The structure of apolipoprotein E3 (APOE3) in the lipid-free state is shown on the left. APOE consists of multiple helices: a 4-helix bundle that consists of helix 2 (red), helix 3 (blue), helix 4 (green) and helix 5 (yellow and orange), hinge helices (pink and brown), and carboxyl-terminal domains that include lipid-binding residues and helices (translucent grey). The receptor-binding region (orange) is located on helix 5. The APOE isoforms differ at two residues only, and the residue composition for each isoform is given in the inset box. Isoform-specific residues (Arg158 and Cys112) are indicated on the structure of APOE3, with labels and carbons, shown as a van der Waals representation and coloured to match the helix with the residue. The lipid-bound structure of APOE3 is shown on the right. In its lipid-bound state, APOE3 demonstrates release of both the hinge region and the lipid-binding region, which causes the receptor binding region to be exposed. The crucial lipid-binding region includes residues 244–272. The lipid particle is shown in cross-section to allow better visualization of the APOE binding region. Water molecules depicted within the lipid particle illustrate the aqueous conditions and demonstrate the exclusion of water on binding of APOE3. The positively charged residues within the receptor-binding region in helix 5 (residues 136–150) interact with the negatively charged residues in the ligand-binding domains of LDL receptor family members; however, the precise binding structure of APOE with its receptors remains to be determined.
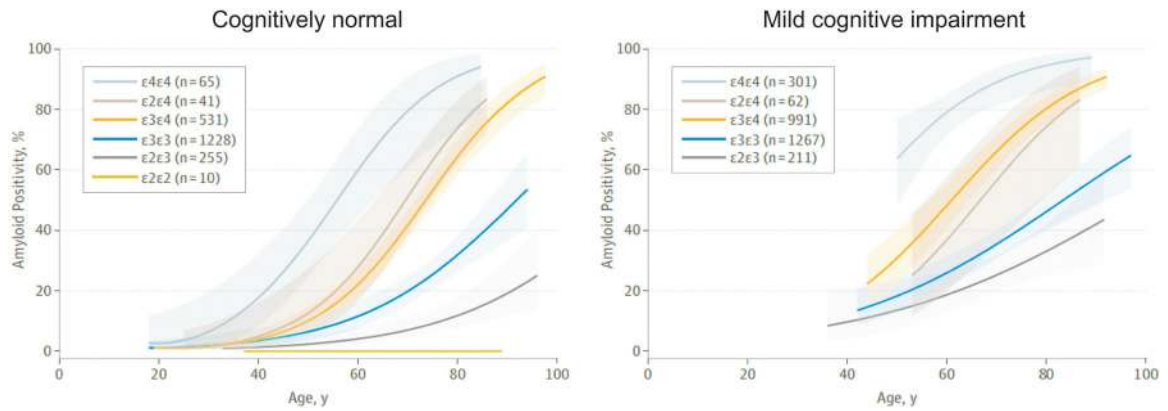
Figure 2: *APOE* **genotype and amyloid positivity.**
Estimated probabilities of amyloid positivity according to apolipoprotein E (*APOE*)
genotype, plotted against age in cognitively healthy individuals and individuals with mild
cognitive impairment (MCI). Shaded areas represent 95% CIs. In both groups, *APOE***ε4/ε4*
individuals are more likely to be positive for amyloid pathology than individuals with any
other genotype, whereas *APOE***ε2/ε2* individuals have the lowest probability of amyloid
positivity. Also note that *APOE**ε4 is a strong driver of Aβ positivity irrespective of the
presence of *APOE**ε2 or *APOE**ε3. Data for *APOE***ε2/ε2* individuals are not shown in the
right panel owing to a small sample size. Adapted with permission from REF.[85], American
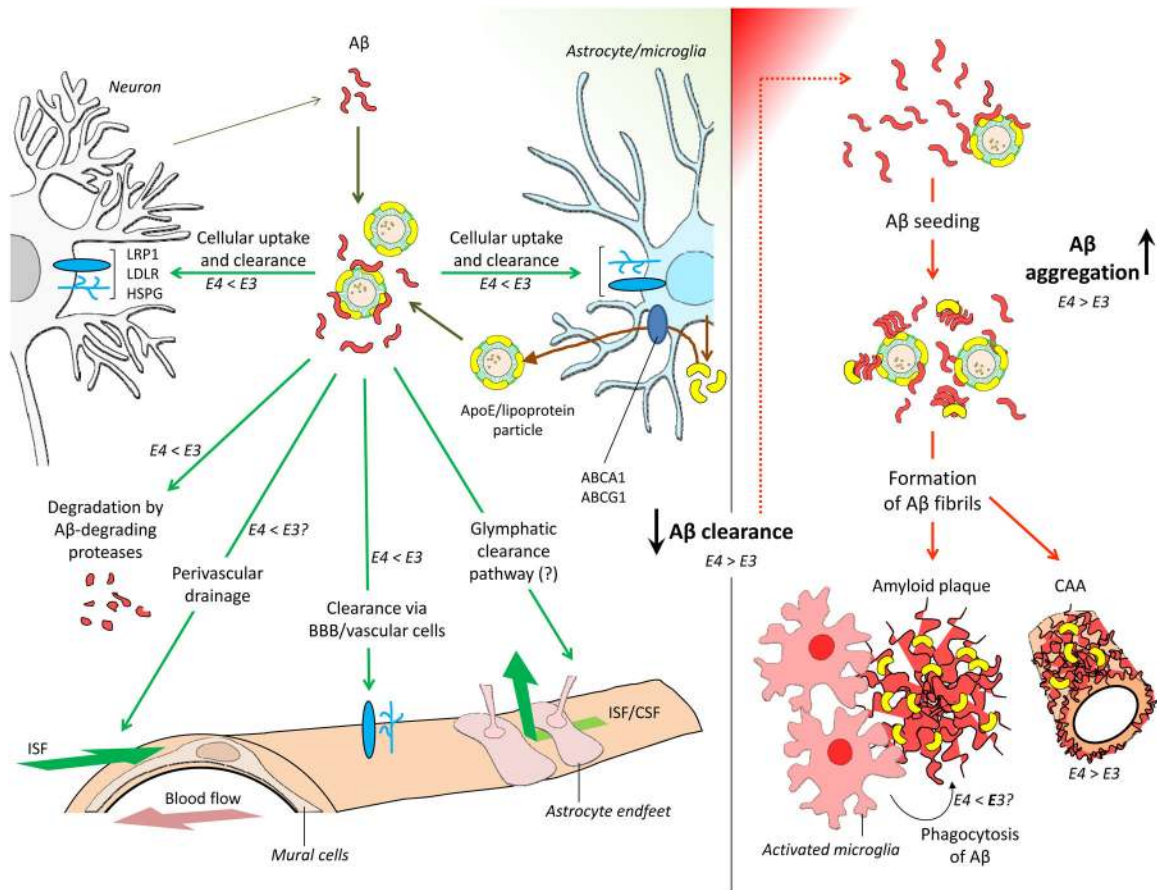Medical Association.

Figure 3: APOE isoforms and Aβ aggregation and clearance.

a | Amyloid-β (Aβ) production and clearance pathways. Aβ is produced primarily in neurons through proteolytic cleavage of amyloid precursor protein (grey arrow). Aβ is then removed from the brain by multiple Aβ clearance pathways (green boxes), including cellular uptake and subsequent degradation, enzymatic degradation, clearance through the blood–brain barrier (BBB), and clearance via interstitial fluid (ISF) bulk flow and, potentially, the glymphatic pathway. LDL receptor-related protein 1 (LRP1), LDL receptor (LDLR) and heparan sulfate proteoglycan (HSPG) are major APOE receptors that mediate cellular uptake of Aβ. Apolipoprotein E (APOE) is produced and lipidated primarily by astrocytes (brown arrow). A sub-pool of APOE lipoprotein particles interacts with soluble Aβ released into the brain interstitial fluid by neurons. **b |** Insufficient Aβ clearance from the brain leads to Aβ accumulation. This accumulation initiates Aβ oligomerization and accelerates subsequent aggregation and fibrillogenesis, leading to deposition of insoluble Aβ in the brain parenchyma (amyloid plaques) and in the vascular wall (cerebral amyloid angiopathy). APOE promotes the formation of Aβ fibrils by accelerating the initial seeding or nucleation of Aβ peptides. APOE can influence Aβ clearance and aggregation, either directly or indirectly, in an isoform-dependent manner. The relative abilities of APOE3 and APOE4 to promote each pathway are indicated alongside the arrows. ABCA1, ATP-binding cassette sub-family A member 1; ABCG1, ATP-binding cassette sub-family G member 1; CSF, cerebrospinal fluid.
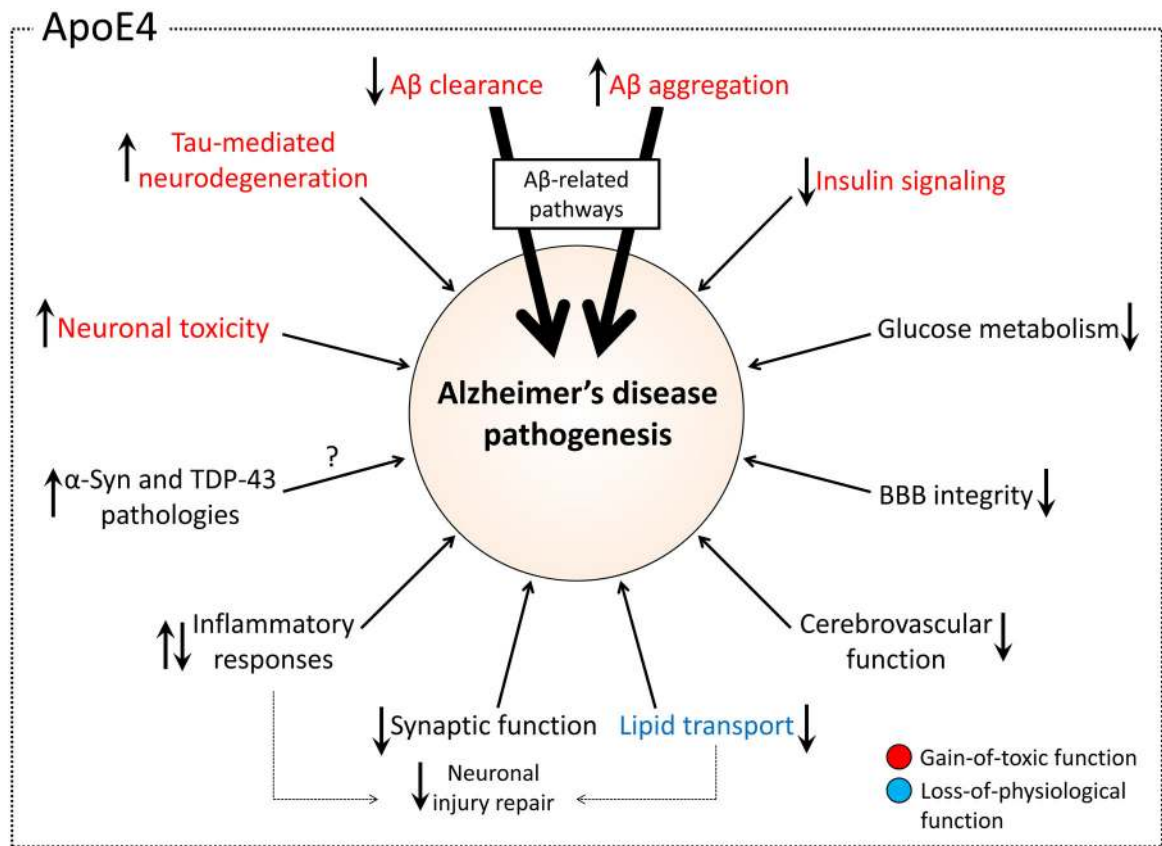
Figure 4: Effects of APOE4 on AD pathogenesis pathways.
Apolipoprotein E4 (APOE4) affects multiple different pathways in Alzheimer disease (AD) pathogenesis. Key functional pathways are shown, and the arrows within the boxes depict the effects of APOE4 compared with APOE3. Pathways are shown in red boxes if evidence suggests that APOE4 increases risk of AD via a gain of toxic function. The effects on the lipid transport pathway, shown in blue, represent a potential loss of physiological function of APOE4 relative to APOE3. Pathways shown in grey are unclassified either owing to insufficient evidence or the potential for both gain of toxic and loss of physiological function. The two thicker arrows indicate the importance of the Aβ-related pathway as the key mechanism by which APOE influences AD. Aβ, amyloid-β; α-syn, α-synuclein; BBB, blood–brain barrier; TDP43, TAR DNA-binding protein 43.
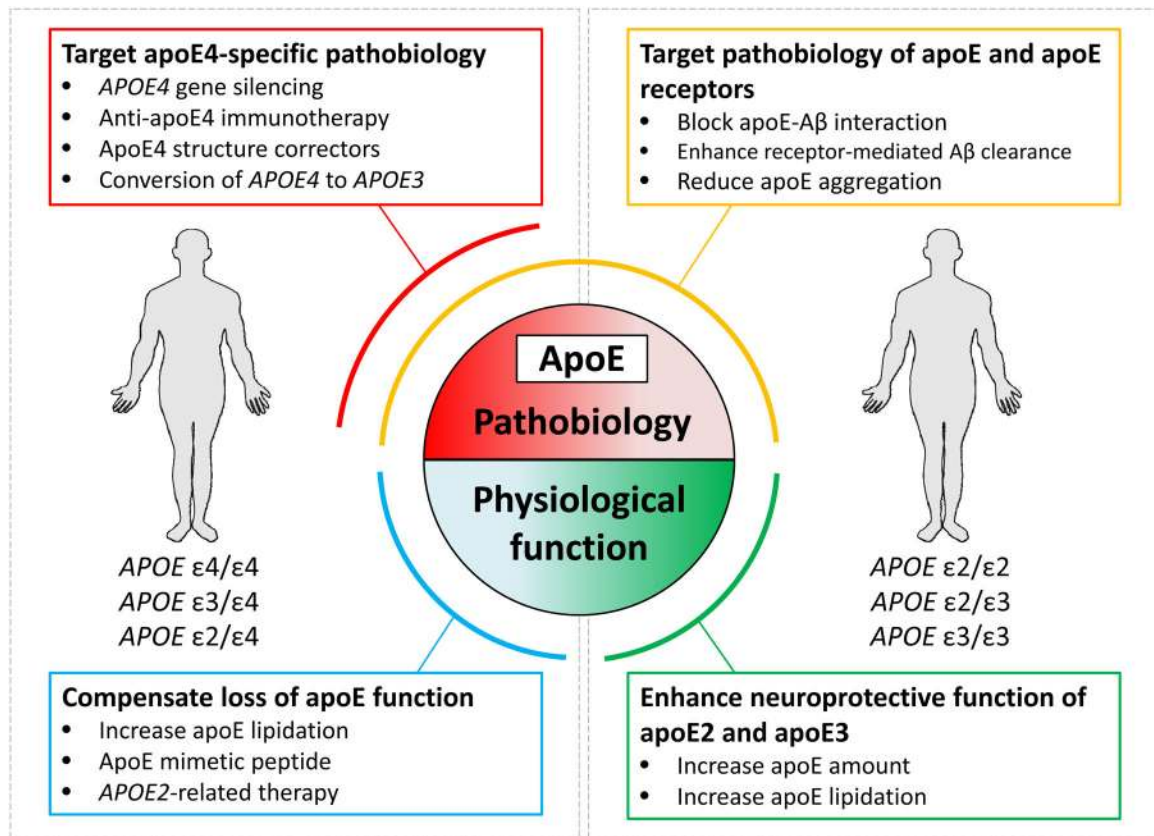
Target apoE4-specific pathobiology
- *APOE4* gene silencing
- Anti-apoE4 immunotherapy
- ApoE4 structure correctors
- Conversion of *APOE4* to *APOE3*

Target pathobiology of apoE and apoE receptors
- Block apoE-Aβ interaction
- Enhance receptor-mediated Aβ clearance
- Reduce apoE aggregation

ApoE
Pathobiology
Physiological function

APOE ε4/ε4
APOE ε3/ε4
APOE ε2/ε4

APOE ε2/ε2
APOE ε2/ε3
APOE ε3/ε3

Compensate loss of apoE function
- Increase apoE lipidation
- ApoE mimetic peptide
- *APOE2*-related therapy

Enhance neuroprotective function of apoE2 and apoE3
- Increase apoE amount
- Increase apoE lipidation

Figure 5: Model of precision medicine based on *APOE* genotype.
Although all apolipoprotein E (APOE) isoforms seem to promote amyloid-β (Aβ) deposition in the brain, the disease-driving effect is more pronounced with the presence of *APOE*ε4* than of *APOE*ε2* or *APOE*ε3*. Therefore, a precision medicine approach in which different treatment strategies are developed for individuals with different genotypes might be beneficial. The figure lists the potential therapeutic options according to the individuals who are most likely to benefit and whether the strategy attempts to correct APOE pathobiology or restore APOE physiological function. Modulation of Aβ pathology in individuals with different *APOE* genotypes is likely to require a combination of strategies.

Table 1.

APOE variants and key AD-related clinical features

APOE allelic variant	Allele frequency in cognitively healthy individuals (%)[286]	Allele frequency in patients with AD (%)[286]	Odds ratio for AD development[286]	Key clinical features
*APOE*ε2	7	4	0.621	• Associated with a reduced risk of AD[6–9] • Associated with slower cognitive decline during ageing, even after adjustment for amyloid-β pathologies[87] • Associated with an increased risk of cerebral amyloid angiopathy (CAA) and CAA-related intracerebral haemorrhage[70–73] • Contributes to the onset of type III hyperlipoproteinaemia[22,56] • Associated with increased tau pathology in progressive supranuclear palsy[122]
*APOE*ε3	79	58	1.000	• The most common *APOE* allele • *APOE*ε3/ε3 is considered to be a reference genotype in most studies
*APOE*ε4	14	38	3.680	• Associated with increased risk of both early-onset[9] (Box 2) and late-onset AD[4–6,8,9] • Shifts the onset of AD to 2–5 or 5–10 years earlier depending on *APOE*ε4 allele number[4,10] • Seems to interact with sex to modify AD risk (Box 3)[6,8,297] • Associated with pro-atherogenic changes in lipoprotein distribution[56] • Associated with an increased risk of CAA[67,68] • Associated with increased tau pathology in AD[65] • Associated with increased risk of dementia with Lewy bodies[13–15], Parkinson disease dementia[15–18] and TAR DNA-binding protein 43 (TDP43) pathology in AD[19–21] • Associated with a reduction in cerebral glucose metabolism[193,195–203] • Associated with greater synaptic pathology in the brains of patients with AD[66,160–163] • Associated with increased risk of vascular cognitive impairment[212,213] and pathologies that lead to neurovascular unit dysfunction[214–217]

AD, Alzheimer disease; *APOE*, apolipoprotein E.

Table 2.

APOE genotype, AD risk and amyloid-β deposition

APOE genotype	Frequency in cognitively healthy individuals (%)[286]	Frequency in patients with AD (%)[286]	Odds ratio for AD development[9]	Odds ratio for amyloid positivity at 70 years of age[85]	
				Cognitively healthy	Mild cognitive impairment
ε2/ε2	0.7	0.3	0.56	NA	NA
ε2/ε3	11.0	4.6	0.56	0.34	0.59
ε3/ε3	62.3	34.3	1.00	1.00	1.00
ε2/ε4	1.9	2.6	2.64	4.29	2.38
ε3/ε4	22.2	43.4	3.63	2.94	3.52
ε4/ε4	1.9	14.8	14.49	18.76	14.50

Carrying one or two apolipoprotein E ε4 (*APOE*ε4*) alleles increases the odds ratio for Alzheimer disease (AD) development 3–4-fold or 9–15-fold, respectively, regardless of the presence of *APOE*ε2* or *APOE*ε3*[6,8,9]. The pattern of estimated probabilities of amyloid positivity for the different *APOE* genotypes[85] is similar to that of the risk of AD. NA, not available.