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APOE and Alzheimer's Disease: Advances in Genetics, Pathophysiology, and Therapeutic Approaches.

Alberto Serrano-Pozo, MD PhD[1,2,3], **Sudeshna Das, PhD**[1,2,3], **Bradley T. Hyman, MD PhD**[1,2,3]

[1]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114 (USA)

[2]Massachusetts Alzheimer's Disease Research Center, Charlestown, MA 02129 (USA)

[3]Harvard Medical School, Boston, MA 02115 (USA)

SUMMARY

The *APOE* ε4 allele remains the strongest genetic risk factor for sporadic Alzheimer's disease and the *APOE* ε2 allele the strongest genetic protective factor after multiple large scale genome-wide association studies and genome-wide association meta-analyses. However, no therapies directed at APOE are currently available. Although initial studies causally linked APOE with amyloid-β peptide aggregation and clearance, over the past 5 years our understanding of APOE pathogenesis has expanded beyond amyloid-β peptide-centric mechanisms to tau neurofibrillary degeneration, microglia and astrocyte responses, and blood-brain barrier disruption. Because all these pathological processes can potentially contribute to cognitive impairment, it is important to use this body of knowledge to develop therapies directed at APOE. Several therapeutic approaches have been successful in mouse models expressing human *APOE* alleles, including increasing or reducing APOE levels, enhancing its lipidation, blocking the interactions between APOE and amyloid-β peptide, and genetically switching APOE4 to APOE3 or APOE2 isoforms, but translation to human clinical trials has proven challenging.

Keywords

Alzheimer's disease; apolipoprotein E; amyloid beta peptide; tau; microglia; astrocytes; blood-brain barrier; drug development

INTRODUCTION

Even after multiple large-scale genome-wide association studies (GWAS) and GWAS meta-analyses[1], the ε4 allele of the *APOE* gene (compared to the most common ε3 allele) continues to be the strongest genetic risk factor associated with sporadic Alzheimer's

Correspondence and reprints to: Bradley T. Hyman, MD PhD. 114 16th Street, Charlestown, MA 02129, Suite 2900. Tel: +1 617-726-2299. Fax: +1 617-726-1480, bhyman@mgh.harvard.edu.

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disease since its discovery in 1993. Moreover, the relatively rare *APOE* ε2 allele remains by far the strongest genetic protective factor against sporadic Alzheimer's disease (Panel 1), emphasising the importance of APOE's role in Alzheimer's disease pathogenesis. Because Alzheimer's disease is defined by the accumulation of two hallmark pathological protein aggregates: amyloid-β peptide (Aβ) plaques and neurofibrillary tangles containing hyperphosphorylated tau, one postulate is that APOE affects these lesions. Although solid evidence supports this view, emerging advances are changing our understanding of APOE involvement in Alzheimer's disease. First, new genetic modifiers and the *APOE* local ancestry (i.e., the population-specific genetic variation in the *APOE* region) have been associated with a differential *APOE* ε4-linked increased risk of Alzheimer's disease. Second, although *APOE* modification of Alzheimer's disease risk has been long attributed to its effects on Aβ, systematic neuropathological examination of large autopsy cohorts has suggested that the *APOE* genotype also correlates with the presence and severity of other proteinopathies, pointing to new causal links. Third, technological advances in the past decade —including mouse models genetically engineered to express human *APOE* alleles; virally-mediated gene transfer; proteomics and transcriptomics; patient-derived human-induced pluripotent stem cells; plasma, CSF, PET and MRI biomarkers— have implicated *APOE* in other aspects of Alzheimer's disease pathophysiology, such as tau-induced neurodegeneration, microglial and astrocyte reactions (including neuroinflammation), and blood-brain barrier disruption. Lastly, although no APOE-based therapy is yet available, several APOE-directed therapeutic approaches have been shown to be effective in mouse models and hold promise for translation to human clinical trials. In this Review, we discuss the advances made in genetics, pathophysiology, and therapeutic approaches related to APOE and Alzheimer's disease.

GENETIC DISCOVERIES RELATED TO *APOE*

Over the past 3 years, human genetic studies have suggested risk modifiers that mitigate or increase *APOE* ε4-associated Alzheimer's disease risk, and identified haplotypes with heterogeneous effects. Understanding the risk variation in *APOE* ε4 carriers has the potential to shed further light on APOE pathobiology and mechanisms of resilience and resistance to Alzheimer's disease, which could have therapeutic value.

APOE ε2 homozygosity

In an analysis[2] of a US cohort with approximately 5,000 neuropathologically confirmed Alzheimer's disease and control subjects, *APOE* ε2 homozygosity was associated with much lower odds of Alzheimer's disease than was *APOE* ε3 homozygosity (odds ratio [OR] 0.13 [95% CI 0.05–0.36]), and the *APOE* ε2/ε3 genotype (0.39 [0.30–0.50]). The contrast of *APOE* ε2 homozygosity versus *APOE* ε4 homozygosity was even greater (0.004 [0.001–0.014]), and *APOE* ε2 was also associated with milder Alzheimer's disease neuropathological changes (i.e., less widespread Aβ plaques and neurofibrillary tangles) in this autopsy cohort. However, these exceptionally low Alzheimer's disease ORs in *APOE* ε2 homozygotes were not found in the larger clinically defined but neuropathologically unconfirmed group (23,857 individuals; 10,430 with probable Alzheimer's disease and

13,426 cognitively unimpaired), suggesting a stronger protection against Alzheimer's disease neuropathology.

APOE Christchurch mutation

A single case report[3] described an approximately 70-year-old Colombian woman who, despite carrying a fully penetrant autosomal dominant E280A mutation in *PSEN1*, which is linked to familial Alzheimer's disease, and abundant fibrillary Aβ deposits in her PET scan, remained cognitively healthy well beyond her expected year of symptom onset (age 44 years). After whole exome sequencing, it was concluded that a rare homozygous *APOE* ε3 Christchurch (R136S) mutation conferred her resilience to Alzheimer's disease. Mechanistically, the APOE3 R136S mutation appears to inhibit Aβ oligomerization, disrupt APOE binding to low-density lipoprotein receptor, and interfere with APOE affinity for heparan sulfate proteoglycans, which are involved in toxic tau uptake by neurons, perhaps explaining the lower than average radioligand uptake observed in her tau PET scan[3].

Other genetic modifiers

A meta-analysis of 22 studies has revealed that *KLOTHO*-VS heterozygosity —a polymorphism previously associated with longevity— might attenuate the increased Alzheimer's disease risk associated with the *APOE* ε4 allele, because *APOE* ε4 carriers older than 60 years with *KLOTHO*-VS heterozygosity had a reduced Alzheimer's disease risk (OR 0.75 [95% CI 0.67–0.84]; $p=7.4\times10^{-7}$), reduced risk of conversion from mild cognitive impairment to dementia (hazard ratio [HR] 0.64 [95% CI 0.44–0.94]; $p=0.02$), higher CSF Aβ levels, and lower Aβ PET burden; the results were significant specifically in the group of individuals aged 60–80 years[4]. A whole genome sequencing on a mainland Chinese cohort identified nine potential causal variants in two genes located in the vicinity of the *APOE*, *PVRL2* and *APOC1*[5], which increased the risk of developing Alzheimer's disease independently of the *APOE* ε4 allele. The risk haplotypes associated with these variants correlated with some Alzheimer's disease endophenotypes such as worse cognition, more severe hippocampal atrophy, lower plasma Aβ levels, and higher brain *APOE* mRNA levels. Another analysis of whole genome sequencing data stratified by *APOE* genotype identified three genes significantly associated with Alzheimer's disease in *APOE* ε4 carriers only: *OR8G5* ($p=4.67\times10^{-7}$), *IGHV3–7* ($p=9.75\times10^{-16}$), and *SLC24A3* ($p=2.67\times10^{-12}$)[6]. Conversely, a systematic review investigating the genetic basis of resilience to Alzheimer's disease among *APOE* ε4 homozygotes revealed that *CASP7* (encoding caspase 7) rs10553596 and *SERPINA3* (encoding α1-antichymotrypsin) rs4934-A/A polymorphisms possibly reduce Alzheimer's disease risk[7].

Influence of race in *APOE*-linked Alzheimer's disease risk

An interaction between race and the *APOE* genotype on Alzheimer's disease risk has long been known, with African American and Hispanic *APOE* ε4 carriers having lower risk than white *APOE* ε4 carriers, and Asian (i.e., Japanese) carriers having the highest ORs.[8–10] Studies have found that the local ancestry of *APOE* (i.e., the population-specific genetic variation within the *APOE* region), rather than global ancestry (i.e., the population-specific genetic variation in the entire genome) or environmental factors, explains these inter-racial differences in Alzheimer's disease risk. Specifically, an African local ancestry region

surrounding *APOE* underlies the smaller *APOE* ε4 allele effect on Alzheimer's disease risk observed in African American and Caribbean Hispanic (from Puerto Rico) populations[11,12]. Another study of 809 individuals identified a potentially protective African ancestral haplotype within *APOE* defined by the rs769449 SNP[13], but this was not been confirmed in a larger (7,997 individuals) study[14].

NEW PATHOLOGICAL CORRELATES OF *APOE* GENOTYPE

The classic post-mortem neuropathological correlates of the *APOE* genotype are a higher Aβ plaque burden and more severe cerebral amyloid angiopathy in *APOE* ε4 carriers, and a lower Aβ plaque burden in *APOE* ε2 carriers, relative to *APOE* ε3 homozygotes[15]. These differential effects of *APOE* alleles have been confirmed by Aβ PET imaging across preclinical and clinical stages of Alzheimer's disease (i.e., mild cognitive impairment and mild-to-moderate dementia)[16,17]. The *APOE* ε4 allele has also been associated with more severe tau pathology as defined by Braak neurofibrillary tangle stages[2,18], and the *APOE* ε2 allele with a lower Braak neurofibrillary tangle stages[15], independently of their effects on Aβ plaques. Cross-sectional data on tau PET imaging examining *APOE* effects on tau radioligand uptake after controlling for Aβ radioligand uptake are conflicting[19,20], but longitudinal combined tau and Aβ PET studies will elucidate this important question.

The *APOE* genotype can also impact the finding of comorbid brain pathologies at autopsy. On one hand, *APOE* ε4 partly drives (together with aging) the presence of Aβ plaques and neurofibrillary tangles in individuals with other primary neuropathological diagnoses such as amyotrophic lateral sclerosis, primary tauopathies, and Lewy body diseases[21]. On the other hand, in individuals with Alzheimer's disease the *APOE* ε4 allele appears to to correlate with the presence and severity of TDP-43 pathology[18,22], Lewy body diseases[23], and possibly cerebrovascular disease[24], independently of its effects on Aβ plaques and neurofibrillary tangles. Lastly, *APOE* could be a genetic risk factor for neurodegenerative diseases other than Alzheimer's disease. Indeed, *APOE* ε4 has been associated with Lewy body diseases, independently of the Aβ plaque and neurofibrillary tangle burdens[25,26] (but see also[27]). Of note, *APOE* ε4 has been associated with an earlier age of symptom onset in patients with *MAPT*-linked or autopsy-proven frontotemporal lobar degeneration-tau independently of its effects on Aβ plaque burden[28], and with more severe neurodegeneration at post-mortem examination in primary tauopathies[29]. Paradoxically, *APOE* ε2 might increase the risk of progressive supranuclear palsy[30], but results are conflicting[23]. The validation and expansion of PET imaging and CSF biomarkers for other neurodegenerative diseases will help confirm these correlations between *APOE* genotype and non-Alzheimer's pathologies.

APOE PATHOPHYSIOLOGICAL MECHANISMS

Although traditionally the *APOE* ε4 allele was represented as a trigger of Aβ accumulation at the top of the sporadic Alzheimer's disease amyloid cascade, numerous new data show that the *APOE* alleles have differential downstream effects in many other pathophysiological processes beyond Aβ metabolism (Figure).

Cellular sources of APOE in brains with and without Alzheimer's disease

Brain and peripheral pools of APOE are independent from each other because liver transplantation changes APOE isoforms towards the donor's in the recipient's blood but not CSF[31], and depleting APOE from hepatocytes —its main source cell type in the periphery— does not affect brain APOE (or Aβ) levels in mice[32]. Understanding the cell types expressing APOE in the brain is relevant because APOE is a secreted glycoprotein and could have autocrine effects on the secreting cell, but also paracrine effects on neighbouring cells. Although astrocytes are the main source of APOE in the normal brain, in the Alzheimer's disease brain reactive astrocytes around Aβ plaques were reported to be devoid of APOE, whereas Aβ plaque-associated microglia express high levels of APOE[33] (Figure). Single nuclei RNA-sequencing studies in human Alzheimer's disease and control brains have confirmed a down-regulation of *APOE* expression in reactive astrocytes[35,36] and an up-regulation in activated microglia[35–37]. Neuropathological studies also reported APOE staining in pyramidal neurons in neurodegenerating areas such as the hippocampus, but rare colocalisation between APOE and tangles, suggesting little direct interaction between APOE and tau[34]. The presence of APOE in pyramidal neurons suggested the internalization of APOE lipoparticles from the interstitial space through the APOE receptor LRP1 (Panel 1, Figure), which is highly expressed in neurons among other cell types. Expression of APOE has also been shown in vascular cells from the human brain, specifically pericytes[38].

Effects on Aβ

The disparate impact of APOE isoforms on Alzheimer's disease risk was attributed to a differential effect — deleterious for APOE4 and protective for APOE2, with respect to APOE3)— on both Aβ plaque burden and cerebral amyloid angiopathy severity[15]. These well-established autopsy neuropathological correlates of *APOE* alleles and the early observation that compact (dense-core, fibrillar, Thioflavin-S-positive), but not diffuse (amorphous, Thioflavin-S-negative), Aβ plaques contain APOE[39], supported the idea that APOE interacts with Aβ and promotes its aggregation and deposition in insoluble fibrillar deposits (Figure). Indeed, genetic deletion and haploinsufficiency of APOE reduces dense-core Aβ plaque burden in various mouse models of cerebral β-amyloidosis[40–42]. Of note, APOE deficiency inhibits diffuse Aβ deposits in some of these models[40] but increases them in others[42], further reinforcing the requirement of APOE for plaque compaction. When these Aβ-plaque depositing mice were crossed with *APOE* targeted replacement mice expressing human *APOE* alleles in place of the murine *Apoe* coding sequence (knock-in mice; Panel 1), *APOE*4 knock-in mice consistently exhibited higher Aβ plaque burden than did *APOE*3 knock-in mice, and these *APOE*3 knock-in mice had higher Aβ plaque burden than did *APOE*2 knock-in mice[43–45], thus recapitulating the allele-specific differences observed in human post-mortem autopsy and Aβ PET studies.

In-vitro and in-vivo studies have shown that, relative to APOE2 and APOE3, APOE4 promotes the seeding of Aβ peptide into Aβ oligomers, protofibrils, and fibrils[46–48], but also inhibits Aβ clearance from the brain prolonging its half-life in the interstitial fluid[45,48] and inhibiting its enzymatic degradation[49]. The intimate mechanism underlying these APOE isoform-driven differences in Aβ metabolism remains debated. On one hand, it has been proposed that APOE and Aβ direct interaction in the brain extracellular space might be

negligible in physiological conditions (i.e., Aβ monomers and lipidated APOE), but that both APOE and Aβ can compete for the same receptors, namely LRP1[50], which is involved in Aβ clearance by neurons[51], astrocytes[52], endothelial cells, vascular smooth muscle cells[53], and pericytes[54]. On the other hand, there is evidence supporting a direct interaction between APOE and oligomeric and fibrillar Aβ. First, APOE colocalises with synaptotoxic Aβ oligomers at the synapses in the vicinity of Aβ plaques and leads to synapse loss in an isoform-dependent manner (APOE4 more than APOE3)[55]. Second, in-vivo experiments in which *APOE* expression by astrocytes was conditionally deleted, or *APOE* expression globally silenced with antisense oligonucleotides at different stages of Aβ deposition, have shown that APOE influences Aβ plaque burden mainly during the seeding phase of Aβ aggregation, but has a lesser effect during the exponential growth phase (i.e., when fibrillar Aβ deposits are already formed)[48,56]. Third, a subtle difference in tertiary conformation across APOE isoforms (i.e., closer N-terminus and C-terminus in APOE4 versus APOE3 and more open in APOE2) could affect both the affinity of the Aβ and APOE interaction (higher for APOE4 *vs* APOE3 and APOE2) and the APOE propensity to enzymatic cleavage in its hinge region between the N-terminus and the C-terminus, rendering presumably toxic C-terminal fragments (also higher for APOE4 *vs* APOE3 and APOE2)[57–59].

Effects on tau

Unlike Aβ, there is little overlap between APOE-immunoreactive neurons and neurons that have neurofibrillary tangles[34]. No direct interaction between APOE (primarily secreted) and the microtubule-associated protein tau (primarily intraneuronal and axonal) has been shown in vivo[30]. However, studies in transgenic *Apoe* knockout or *APOE* knock-in mice overexpressing the P301S mutant of tau have shown that the human APOE isoforms do affect tau downstream pathology[29,60]. Specifically, APOE4 promotes tau-induced neurodegeneration and atrophy compared to APOE3, whereas APOE2 is protective with respect to these outcomes[29]. The mechanism underlying these effects is indirect, mediated by APOE effects on microglia, rather than a direct interaction between APOE and tau; transcriptome profiling and cytokine measures indicate that APOE4 microglia is primed towards a proinflammatory phenotype compared to APOE3, whereas APOE2 exhibits a more homeostatic phenotype[29,60]. Of note, LRP1 has been recently shown to be a receptor for tau uptake by neurons, and APOE affects the ability of tau to bind LRP1 (Figure), although in vitro all APOE isoforms reduced tau uptake to a similar extent[61]. Additionally, knocking down neuronal LRP1 reduced neuronal tau spreading in mice, but some astrocytes took up the human tau[61]. Whether different receptors have a role in tau uptake into different cell types remains unknown. Moreover, since LRP1 is a recycling receptor delivered into endosomal/lysosomal compartments, it remains unclear how tau escapes to the cytoplasm to interact with endogenous tau in neurons or to accumulate as glial fibrillary tangles in astrocytes, but it is plausible that APOE affects tau intracellular trafficking in an isoform-dependent manner[62].

Effects on glia

Astrocytes and microglia are known to react to plaques, neurofibrillary tangles, and neurodegeneration. Although quantitation of reactive (GFAP+) astrocytes and activated (IBA1+, CD68+) microglia per Aβ plaque in post-mortem sections of the temporal

neocortex has shown no difference between *APOE* ε4 carriers and non-carriers[63], transcriptomic studies have reported that *APOE* influences glia reactions. Microglia from *APOE*4 knock-in mice is primed towards a proinflammatory response compared with those from *APOE*3 knock-in mice[64] and *APOE* ε4 microglia derived from human-induced pluripotent stem cells exhibit a proinflammatory gene expression programme and impaired Aβ phagocytosis relative to *APOE* ε3 microglia[65]. These *APOE*-mediated differential effects on microglia phenotype appear to be at least partially mediated by the triggering receptor expressed on myeloid cells 2 (TREM2), which is another receptor for both Aβ and APOE expressed by microglia[66]. Loss of function mutations in *TREM2* (e.g., R47H, R62H) have been associated with a 2–3 times increased risk of developing Alzheimer's disease and with less compact Aβ plaques that have more neuritic dystrophies, less coverage by microglia, and less APOE content[67]. The Aβ plaque features of Alzheimer's disease mouse models deficient in TREM2 or APOE are phenocopies[42,67], suggesting that APOE and TREM2 are both involved in chemotaxis of microglia towards plaques and that plaque-associated microglia has a neuroprotective role minimising neuritic dystrophies. The transcriptomic changes associated with the conversion from homeostatic to Alzheimer's disease microglia require both APOE and TREM2 because genetic deletion of either in Alzheimer's disease transgenic mice precludes such transition[68,69], and *TREM2* loss of function mutations partially abrogate the microglia transcriptomic changes observed in the brains of patients with Alzheimer's disease[37]. Regarding APOE effects on astrocytes, *APOE* ε4 astrocytes derived from human-induced pluripotent stem cells exhibit impaired cholesterol metabolism and Aβ phagocytosis[65], reduced neurotrophic support[70], and impaired synaptic pruning[71], relative to *APOE* ε3 and *APOE* ε2 astrocytes.

Effects on blood brain barrier

Another area of growing interest is the effects of *APOE* ε4 allele on the blood-brain barrier. Traditionally, *APOE* ε4 had been associated with a more severe cerebral amyloid angiopathy[15] (Figure), resulting in a higher risk of lobar intracerebral haemorrhage, but also focal subarachnoid haemorrhage and cortical superficial siderosis, cortical microinfarcts, and white matter ischemic changes. *APOE* ε4 effects on the blood-brain barrier were also shown in the first randomised clinical trials with anti-Aβ monoclonal antibodies, which reported a higher incidence of MRI findings (brain oedema, microbleeds, and cortical superficial siderosis) in the treatment versus placebo groups —collectively termed amyloid-related imaging abnormalities. Only occasionally symptomatic (e.g., headaches, confusion, and seizures), these amyloid-related imaging abnormalities are indicative of an increased blood-brain barrier permeability presumably caused by the antibody-mediated Aβ efflux from the brain parenchyma into the bloodstream[72]. Because amyloid-related imaging abnormalities are twice as probable in *APOE* ε4 carriers, their occurrence has been attributed to a more severe pre-existing cerebral amyloid angiopathy in *APOE* ε4 carriers *vs* *APOE* ε3 homozygotes[72]. Supporting this interpretation, immunotherapy with an anti-Aβ monoclonal antibody is associated with higher numbers of cerebral microbleeds in *APPswePSEN1dE9* × *APOE*4 knock-in mice versus *APOE*3 knock-in and *APOE*2 knock-in mice[73]. A post-mortem quantitative neuropathological study on individuals who participated in a phase 2 anti-Aβ active immunotherapy trial (NCT00021723) also showed that Aβ plaque clearance is associated with a redistribution of Aβ and APOE from plaques to

vessels, and more severe cerebral amyloid-angiopathy-related vasculopathic changes[74]. Pericytes, which are another cellular source of APOE, are gaining attention for their implication in cerebral amyloid angiopathy pathogenesis (Figure). Pericyte loss resulted in increased cerebral amyloid angiopathy and Aβ plaques in a mouse model of Aβ deposition[75]. Pericytes take up Aβ via LRP1 in an APOE isoform-dependent manner, with APOE4 interfering with this uptake compared to APOE3[54]. Human *APOE* ε4 pericytes express higher levels of APOE mRNA and protein than *APOE* ε3 pericytes, resulting in increased Aβ vascular accumulation[38]. Pericytes exposed to Aβ oligomers constrict capillaries via endothelin-1 receptor ET_A activation, leading to reduced blood flow[76].

APOE4 can also increase blood-brain barrier permeability with respect to APOE3 in an Aβ-independent manner, as shown in *APOE*4 versus *APOE*3 knock-in mice[77] and confirmed with dynamic contrast-enhanced MRI in the medial temporal lobe of cognitively healthy (clinical dementia rating score 0) and mildly impaired (clinical dementia rating score 0.5) *APOE* ε4 carriers versus *APOE* ε3 homozygotes[24]. The underlying proposed mechanisms include the activation of cyclophilin A, resulting in increased levels of MMP9 and pericyte injury[78], and disruption of the capillary basement membrane (i.e., collagen IV)[77].

APOE-BASED THERAPEUTIC OPPORTUNITIES

Experimental in-vivo studies in Alzheimer's disease mouse models that have a human *APOE* knock-in background have suggested promising approaches to ameliorate phenotypes related to Alzheimer's disease (Table 1). However, there are only a few APOE-directed clinical trials completed or underway (Table 2), highlighting a lag in therapeutic translation for this target.

Increasing APOE levels and its lipidation

Because brain APOE4 is less lipidated and stable than APOE3 and APOE2[57,79], increasing brain APOE levels and lipidation has been proposed as a therapeutic approach. Genetic deletion of ABCA1 results in poor APOE lipidation and increased Aβ plaque burden[80], whereas ABCA1 overexpression reduces Aβ deposition[81]. ABCA1 and ABCG1 expression is induced by the stimulation of the retinoid X receptor. Bexarotene is a US Food and Drug Administration approved retinoid X receptor agonist for use in cutaneous T-cell lymphoma and was reported to cause a rapid reduction of Aβ plaque burden and restoration of cognitive functioning in Alzheimer's disease mouse models by inducing *ABCA1* and *ABCG1* expression, enhancing APOE lipidation, and increasing APOE levels (Table 1)[82]. This result was, at least partly, replicated by some investigators but not others, and led to examine bexarotene for Alzheimer's disease in human clinical trials. A phase 1b proof-of-mechanism trial in young (21–49 years) volunteers revealed poor penetration of bexarotene in the CNS according to its plasma versus CSF levels. Bexarotene was able to increase CSF APOE levels by 25% although it had no effects on CSF Aβ levels as measured by stable isotope labelling kinetics (Table 2)[83]. In a proof of concept, double-blind, placebo-controlled clinical trial in 20 patients with moderate Alzheimer's disease (Mini Mental State Examination range was 10–20; Table 2), bexarotene 150 mg twice daily for 4 weeks was

associated with a significant reduction in Aβ PET burden and a proportional increase in serum $A\beta_{42}$ levels but, contrary to the prediction, only in *APOE* ε4 non-carriers[84].

A non-toxic small peptide derived from the C-terminus of APOE, CS-6253, has been shown to increase ABCA1 levels and, subsequently, APOE lipidation without changing brain APOE levels, in *APOE*4 but not *APOE*3 knock-in mice. These effects correlated with a reduction of hippocampal Aβ and phosphorylated tau and improved learning and memory in *APOE*4 knock-in mice[85].

Probucol, the now abandoned non-statin lipid-lowering drug, has been shown to counteract hippocampal synaptic loss and cognitive impairment in Aβ-injected wild-type mice[86], increase APOE and LRP1 levels in the hippocampus of aged rats[87] and, while results from a phase 1/2 clinical trial (NCT02707458) are awaited, might also increase CSF APOE levels in humans (Table 2)[88].

Blocking APOE and Aβ interaction

Another therapeutic strategy is to interfere with the APOE and Aβ interaction, because this is thought to stabilise toxic oligomeric and fibrillar Aβ species existing within and around Aβ plaques[46,55,47,48]. This strategy has been achieved in Alzheimer's disease mouse models with both monoclonal anti-APOE antibodies and small molecules that act as Aβ mimetics.

Chronic intraperitoneal administration of an anti-APOE monoclonal antibody (HJ6.3) to *APPswePSEN1dE9* mice led to a statistically significant reduction of insoluble Aβ levels and Aβ plaque burden, and APOE levels in the brain, which correlated with improved learning and memory and higher cortical network connectivity in the resting state. While the Aβ plaque reduction was larger when administered before plaque deposition, in older mice with substantial plaque deposition this antibody appears to prevent the formation of new plaques and clear the smallest previously existing plaques by binding APOE within them. Of note, systemic treatment with this anti-APOE antibody increased plasma Aβ levels, but did not have systemic (i.e., unchanged plasma cholesterol and APOE levels) or local (i.e., cerebral amyloid angiopathy did not worsen) adverse side effects[89,90]. Another anti-APOE monoclonal antibody specific for non-lipidated APOE (HAE-4) reduced the plaque burden in *APPPS1–21* × *APOE*4 knock-in mice through microglia-mediated clearance, without affecting the levels of plasma APOE, which is mostly lipidated[91]. Therefore, anti-APOE immunotherapy has promise for testing in future trials.

Aβ12–28P, a small peptide corresponding to the APOE-binding motif within Aβ except for a Val18Prol substitution, reduced soluble and insoluble Aβ levels and Aβ plaque burden in *APPswePSEN1dE9* × *APOE*3 knock-in and *APPswePSEN1dE9* × *APOE*4 knock-in mice and improved memory deficits in *APPswePSEN1dE9* × *APOE*4 knock-in mice. Moreover, Aβ12–28P reduced soluble and insoluble APOE levels and the deposition of APOE into Aβ plaques. Of note, this improvement was not due to an active immunisation effect, because these mice did not generate antibodies against this Aβ fragment[92]. Aβ12–28P efficacy remains to be tested in clinical trials.

APOE mimetics

Another approach is to use APOE N-terminal fragments including its receptor-binding motif, so called APOE mimetics. Chronic subcutaneous administration of CN-105, a pentapeptide corresponding to the receptor binding face of APOE, reduced both soluble Aβ and Aβ plaque burden and improved cognition in *APP1–21 × APOE*4 knock-in mice before plaque deposition, but not after this[93]. CN-105 is being tested to prevent delirium after major surgery in a phase 2 clinical trial (NCT03802396; Table 2). APOE mimetics spanning the APOE receptor binding motif such as COG1410 (12 amino acids) and COG112 (34 amino acids) have been shown to ameliorate Aβ levels and Aβ plaque burden, tau hyperphosphorylation, and neuroinflammation in various Alzheimer's disease mouse models[94,95], but have not been tested in human clinical trials.

Lowering APOE levels

Lowering brain APOE levels has also been proposed as a therapy because *Apoe* genetic deletion or haploinsufficiency reduces Aβ deposition in mouse models of cerebral β-amyloidosis[40–42] and rescues neurodegeneration induced by tau in tauopathy mouse models[29]. Additionally, null mutations in the *APOE* gene do not seem to have adverse effects on cognition in humans, although they are associated with familial dyslipoproteinemia (also known as type III hyperlipoproteinemia)[96]. One way of reducing brain APOE levels is increasing the expression of its receptors. Over-expression of LDLR reduced Aβ plaque deposition[97] due to increased efflux of Aβ from the brain through the blood-brain barrier[98]. A more specific approach is to silence *APOE* expression with specific antisense oligonucleotides. In *APPPS1–21 × APOE*3 knock-in mice and *APPPS1–21 × APOE*4 knock-in mice, a reduction in soluble APOE levels by half with anti-*APOE* antisense oligonucleotides resulted in lower soluble and insoluble Aβ levels and lower total and dense-core plaque burden when administered intracerebroventricularly at birth, but did not change much these Aβ measures when applied at the onset of Aβ plaque deposition (i.e., 6 weeks in this mouse model)[56]. However, both treatments resulted in fewer plaque-associated dystrophic neurites, suggesting less neuronal toxicity of existing plaques and some beneficial effect of APOE reduction on microglia and astrocyte responses to plaques, and lending support for testing in patients with Alzheimer's disease in clinical trials.

Genetic switch of APOE isoforms

Gene therapy has become a reality in several diseases, including neurodegenerative diseases such as spinal muscular atrophy. The application of CRISPR-Cas9 editing technology to switch *APOE* alleles has been successful in a dish with neurons and glial cells derived from human-induced pluripotent stem cells[65], but remains to be shown in *APOE* knock-in mice. However, the application of gene therapy to express *APOE* ε2 and increase APOE2 levels in *APOE* ε4 carriers (or even *APOE* ε3 homozygotes) has become feasible and the first phase 1 clinical trial with this approach has been initiated (NCT03634007; Table 2). In mice, intraventricular transfer of human *APOE* alleles with an adeno-associated virus type-4 leads to sustained expression of human APOE in the choroid plexus and ependymal cell lining, that diffuses to the brain parenchyma reaching a concentration of 10% of mouse endogenous APOE[99]. Adeno-associated virus type-4-mediated delivery and expression of *APOE* ε2 in 7-

month-old *APPswePSEN1dE9* mice (i.e., after Aβ plaque deposition) resulted in reduced soluble and insoluble Aβ levels and enhanced plaque clearance, whereas delivery of *APOE* ε4 had the opposite effects. Plasma $A\beta_{40}$ concentrations were decreased in the *APOE* ε3 and *APOE* ε4 treated mice versus *APOE* ε2 treated mice, suggesting a reduced efflux from brain to plasma through the blood-brain barrier, relative to *APOE* ε2 treated mice. Monitoring of Aβ plaque growth by in-vivo multiphoton microscopy in living mice showed a significant effect on plaque growth rate, slower in *APOE* ε2 treated mice and faster in *APOE* ε4 treated mice. Moreover, *APOE* ε2 ameliorated plaque-associated dystrophic neurites and synapse loss, which were more severe in *APOE* ε4 treated mice. Similarly, intracerebroventricular AAV8-mediated astrocyte-specific expression of human *APOE* ε2 in *APOE*4 knock-in mice from birth increased APOE lipidation and decreased endogenous murine Aβ, whereas *APOE* ε4 delivery had opposite deleterious effects[79].

CONCLUSIONS AND FUTURE DIRECTIONS

New insights in genetic modifiers, neuropathological and gene expression correlates, and pathophysiological mechanisms in different brain cell types are broadening our understanding of the implications of APOE in Alzheimer's disease and offering previously unforeseeable opportunities for therapeutic and preventative interventions. Because of this mounting evidence, strategies to lower APOE4 levels and to increase APOE2 levels in the brain hold the greatest promise (Table 2). Against this remarkable momentum, there remains a paucity in translation of APOE-based therapies to human clinical trials, especially when compared with the expedited cases of anti-Aβ and anti-tau immunotherapies. What are the hurdles slowing APOE-based drug development programmes down? First, further development of small molecules that reliably change APOE4 conformation to APOE3 or APOE2 has proven to be difficult because the variable degree of lipidation of APOE might influence its tertiary conformation. Second, the new data implicating a variety of non-Aβ and non-tau targets in APOE pathophysiology raises new questions, such as determining what the best downstream therapeutic target should be and monitoring the consequences of target engagement. Third, there are some unique problems related to APOE separate peripheral (liver generated) and CNS pools and its inability to cross the blood-brain barrier. This means that affecting CNS APOE (levels, isoforms, or interactions) will require drugs with adequate blood-brain barrier penetration. Moreover, potential systemic off-target adverse effects of some of these approaches should be carefully considered: rare *APOE* ε2 homozygotes and Christchurch mutant carriers, and even more rare individuals with homozygous *APOE* null mutations, suffer from type III hyperlipoproteinemia[3,96,100], resulting in accelerated atherosclerosis; in fact, *APOE*2 knock-in and *Apoe* knockout mice are widely used to model atherosclerosis. Therefore, gene therapies to lower APOE levels or switch APOE4 to APOE2 should probably be targeted specifically to the CNS (i.e., via direct injection or with viral capsids that penetrate the blood-brain barrier and appropriate promoters), which poses its own challenges.

Notwithstanding all these barriers, the risk and protective profiles of *APOE* genotype in human populations across the globe reinforce the robustness of the effects of subtle variations in this gene, and encourage the field to redouble its efforts at further

understanding the pathophysiology of APOE effects in Alzheimer's disease (Panel 2), and attempts at translating that knowledge into therapeutics.

Panel 1: Apolipoprotein E basic facts.

- Two single nucleotide polymorphisms (SNPs) —rs429358 and rs7412— define the three alleles of *APOE*, located in chromosome 19q13.2: ε2, ε3, and ε4. Relative to the most common *APOE* ε3/ε3 genotype (reference group), possessing one *APOE* ε4 allele increases the risk of developing Alzheimer's disease by approximately 3.7 times and being homozygous for the *APOE* ε4 allele increases the risk up to 12 times, whereas carrying a single *APOE* ε2 allele reduces the risk by approximately 40%, and being homozygous for *APOE* ε2 reduces the risk even further[2,39].
- Besides Alzheimer's disease risk, the *APOE* genotype mainly affects the age of onset of cognitive impairment, with *APOE* ε4 carriers having an earlier age of onset and *APOE* ε2 carriers a later age of onset than *APOE* ε3 homozygotes. By contrast, the effect of *APOE* genotype on the rate of cognitive decline after symptom onset remains controversial, with allele differences typically considered not clinically relevant[15].
- APOE is a 299-amino acid (MW 34 kDa) secreted glycoprotein that binds cholesterol and phospholipids through the C-terminus domain and to its receptors through the N-terminus domain[57].
- The three APOE isoforms differ in two amino acid residues at positions 112 (Cys in APOE2 and APOE3, and Arg in APOE4) and 158 (Cys in APOE2, and Arg in APOE3 and APOE4), and these polymorphisms cause significant differences across APOE isoforms in both lipid binding properties (i.e., APOE4 is hypolipidated compared to APOE3 and APOE2[57,79]) and receptor affinities.
- APOE transports lipids packed into HDL-like particles in the brain, or LDL particles in the peripheral blood. APOE main receptors in the brain are the LRP1, the LDL receptor, the very LDL receptor, and the apolipoprotein E receptor 2, all of which are also Aβ receptors[39,51–53,98].
- Lipidation of brain APOE is mediated by ATP-binding cassette transporters A1 and G1[80,81,85].
- APOE directly interacts with amyloid-β peptide[46,47,50,57,59], but there is no solid in-vivo evidence of a direct interaction between APOE and tau[30].
- Mouse models to study the effects of APOE isoforms on amyloid-β peptide and tau include mice deficient in APOE (*Apoe* knockout) and mice genetically engineered to replace the mouse *Apoe* with each of the human *APOE* alleles (*APOE*-targeted replacement or knock-in), crossed with either mice overexpressing one or more familial Alzheimer's disease-linked *APP* mutations —with or without one or *PSEN1* mutations (e.g., *APP*^{V717F} [43,45], *APPswePSEN1dE9*[42,99], *5xFAD*[44], *APPPS1–21*[42,56])— or mice overexpressing

frontotemporal lobar degeneration-tau-linked *MAPT* mutations (e.g., *MAPT*^{P301S})[29,60].

Panel 2: Areas of uncertainty and research priorities.

- Possible differential independent effects of *APOE* alleles on Alzheimer's disease progression through preclinical and clinical stages with longitudinal multimodal imaging, CSF, and plasma or serum biomarkers.
- Influence of genetic modifiers of *APOE*-linked Alzheimer's disease risk, including the intimate mechanisms of local ancestry, interaction with longevity genetic polymorphisms such as *KLOTHO*-VS heterozygosity, and *APOE* mutations such as R136S (Christchurch), as plausible substrates of resistance or resilience to Alzheimer's disease.
- Possible Aβ-independent mechanisms of APOE on tau seeding and propagation through neuronal circuits.
- Influence of *APOE* genotype on other neurodegenerative proteinopathies, such as primary tauopathies (e.g., Pick's disease, progressive supranuclear palsy, corticobasal degeneration), TDP-43 proteinopathies (amyotrophic lateral sclerosis, frontotemporal lobar degeneration-TDP-43), and α-synucleinopathies (Parkinson's disease, dementia with Lewy bodies, multiple system atrophy), as well as on other neurological disorders in which the blood-brain barrier or the immune system play a substantial role.
- Autocrine versus paracrine effects of APOE on each brain cell type (astrocytes, microglia, neurons, oligodendrocytes, vascular smooth muscle cells, endothelial cells, and pericytes).
- In-vivo applications of gene therapy, including genetic editing of *APOE* alleles with CRISPR-Cas9 technology and strategies for viral vector delivery to specific brain cell types.

Search strategy and selection criteria

We searched PubMed articles in English published between Jan 1, 1993 and May 15, 2020 using the search terms "APOE AND Alzheimer's disease", "APOE AND blood-brain barrier", "APOE AND Lewy body disease", "APOE AND alpha-synuclein", "APOE AND TAR DNA-binding protein 43", "APOE AND tau", "APOE AND microglia", "APOE AND astrocytes", "APOE AND TREM2", "APOE AND immunotherapy", and "APOE AND gene therapy". Only human, mouse model, and human-induced pluripotent stem cell studies were reviewed. In-vitro studies using recombinant APOE and synthetic or recombinant Aβ or tau species and in-cellulo studies using cultured cell lines or primary neuron, astrocyte, or microglial cultures were excluded. The final reference list was generated on the basis of relevance and originality with regards to the topics covered in this Review.

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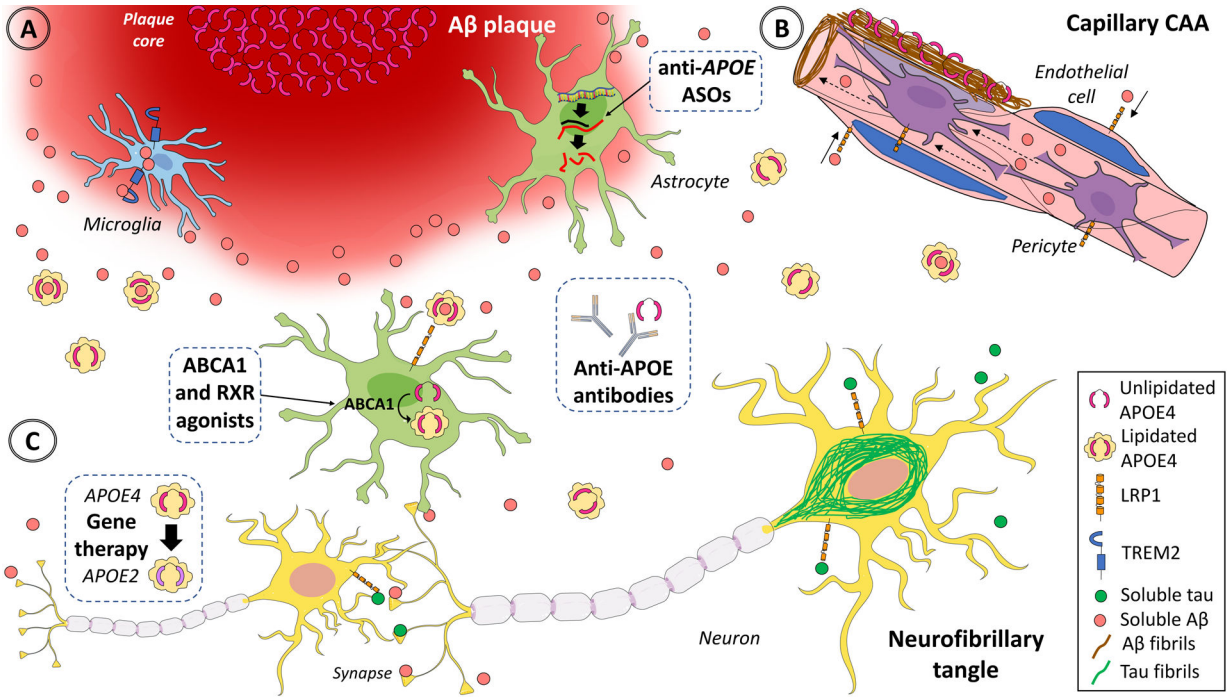

Figure. Multifaceted effects of APOE in the brain and potential strategies to decrease APOE4 and increase APOE2 levels.

In the healthy brain, APOE is expressed and secreted predominantly by astrocytes, and to a lesser extent by microglia. Most brain APOE is lipidated by the ATP-binding cassettes A1 (ABCA1) and G1 (ABCG1) and lipidated APOE is internalized via APOE receptors such low-density lipoprotein receptor-related protein 1 (LRP1), which is expressed in astrocytes, neurons, vascular smooth muscle cells, endothelial cells, and pericytes. In the Alzheimer's disease brain, astrocytes and microglia react to (A) dense-core Aβ plaques[63], (B) cerebral amyloid angiopathy-laden arteries and capillaries, and (C) neurofibrillary tangles, activating transcriptional programmes that include *APOE* mRNA up-regulation in microglia[35,37] and down-regulation in astrocytes[35,36] and lead to altered lipid metabolism (not shown). APOE directly interacts with both soluble and fibrillar Aβ. Relative to APOE3 and APOE2, APOE4 promotes Aβ seeding and aggregation in oligomers and fibrils[46–48] and reduces its clearance from the interstitial fluid[45], potentially leading to Aβ deposition as dense-core (Thioflavin-S positive) amyloid plaques and cerebral amyloid angiopathy together with APOE[39]. This evidence suggests that decreasing APOE (especially APOE4) expression or blocking the effects of APOE4 or enhancing the effects APOE2 would be beneficial (dashed boxes). Experimental approaches to achieving these outcomes include lowering APOE4 levels with isoform-specific antisense oligonucleotides[56] or antibodies[89–91], which could also target lipid-poor APOE associated with plaques[91]. Alternatively, APOE4 could be switched to APOE3 or APOE2[65], or APOE2 could be added[79,99], with gene therapy. Last, APOE lipidation could be enhanced with RXR[82–84] and ABCA1 or ABCG1[85] agonists to improve APOE4 receptor-mediated internalization and lower Aβ in the interstitial fluid. Dashed boxes illustrate the most promising therapeutic approaches. ASO=antisense oligonucleotides. Aβ=amyloid-β peptide. CAA=cerebral amyloid angiopathy. TREM2=triggering receptor expressed in myeloid cells 2. RXR=retinoid X receptor.

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Table 1.

APOE-directed therapeutic approaches tested in Alzheimer's disease mouse models.

Rationale	Treatment	Route	Mouse model	Age	Duration	Results	Comments / Limitations	Ref.
Increasing APOE levels	Bexarotene	p.o.	*APPswe/ PSEN1dE9*	6 & 11 mo	3, 7, 14 & 90 d	Increased APOE, ABCA1, ABCG1, and HDL; reduced s/i-Aβ and Aβ plaques; improved memory	Subsequent mouse studies by other groups yielded mixed results on the efficacy of bexarotene on Aβ phenotypes	Cramer et al. 2012[82]
			APPPS1–21	7–8 mo	20 d	Increased APOE, ABCA1, ABCG1, and HDL; reduced s/i-Aβ and Aβ plaques; improved memory		
			Tg2576	12–14 mo	3 & 9 d	Improved social and olfaction/ circuit connectivity		
	CS-6253	i.p.	*APOE*3 knock-in & *APOE*4-knock-in	2.5 mo	6 wks	*APOE*4 knock-in only: reduced Aβ and p-tau; increased ABCA1, APOE lipidation, APOER2, VGLUT1, and memory	ABCA1 agonist derived from APOE C-terminus, target engagement shown by CS-6253+ astrocytes in IHC	Boehm-Cagan et al. 2014[85]
	Probucol	p.o.	Wt male rats	26 mo	30 d	Increased hipp APOE, *Apoe* mRNA, HMGCoAR, LRP, and SNAP25; = chol, LDLR, and SYP; reduced GFAP	Aged Wt rats used as a model of normal cognitive aging	Champagne et al. 2003[87]
		i.p.	Wt mice, i.c.v. aggregated $A\beta_{1-40}$	90 d	2 wks	Reduced plasma chol; improved memory and synapses	Acute i.c.v. injection of Aβ in Wt mice does not model well the AD scenario of chronic Aβ deposition	Santos et al. 2012[86]
APOE mimetics	COG1410	s.c.	*SwDI-APP/ NOS2*$^{-/-}$	9 mo	3 mo	Reduced Aβ plaques, p-tau, and *Il6* mRNA; improved memory	*SwDI-APP* mice lacking NOS2 exhibit endogenous tau pathology and neuron loss besides Aβ plaque	Vitek et al. 2012[94]
	COG112	i.p.	AICD Tg (FeCγ25 line)	1 mo	3 mo	Reduced p-tau and CD45+/ IBA1+ mg; improved neurogenesis	APP intracellular domain (AICD)-overexpressing mice exhibit microglial activation but no Aβ plaques	Ghosal et al. 2013[95]
	CN-105	s.c.	*APPPS1–21* × *APOE*4 knock-in	14–18 wks	40 d	Reduced s-Aβ and Aβ plaques; improved memory	Greater benefits in young mice (e.g. improved fear conditioning but not spatial memory in older mice)	Krishnamurthy et al. 2020[93]
				25–28 wks	40 d	= s-Aβ; slightly improved memory		
Blocking APOE-Aβ interaction	HJ6.3 anti-APOE Ab	i.p.	*APPswe/ PSEN1dE9*	4 mo	14 wks	Reduced s/i-Aβ, Aβ plaques, IFN-γ, and IL-1α; increased CD45+ mg	Preventative use prior to Aβ plaques very effective, microglial activity modulation suggested	Kim et al. 2012[89]
				7 mo	21 wks	Reduced s/i-Aβ, Aβ plaques, brain APOE, CD45+ mg; increased plasma Aβ; improved memory and connectivity; =	Therapeutic use after Aβ plaque deposition also effective due to inhibition of plaque formation and growth plus removal of existing plaques	Liao et al. 2014[90]

Rationale	Treatment	Route	Mouse model	Age	Duration	Results	Comments / Limitations	Ref.
						CAA, plasma APOE, and plasma chol		
	HAE-4 anti-APOE Ab	i.c.v.	*APPPS1–21* × *APOE*4 knock-in	2 mo	6 wks	i.c.v.: reduced Aβ plaques; = i-Aβ i.p.: reduced i-Aβ and Aβ plaques; = brain s/i-APOE, plasma APOE, and plasma Aβ	Additionally, AAV2/8-mediated expression of HAE-4 and HAE-1 anti-APOE Abs via i.c.v. injection at birth reduced Aβ plaque and i-Aβ at age 3.5-mo in an Fc-dependent manner, implicating FcγR1 in microglia	Liao et al. 2018[91]
	Aβ12–28P	i.p.	*APPswe/PSEN1dE9* × *APOE*2 knock-in *APPswe/PSEN1dE9* × *APOE*4 knock-in	6 mo	4 mo	Reduced s/i-Aβ, s/i-APOE, Aβ plaques, DNs, and serum chol in both mice; improved memory (only E4 mice); = serum APOE	Greater cognitive benefit in E4 mice because only vehicle-treated *APPswe/PSEN1dE9* × *APOE*4 knock-in mice (but not *APPswe/PSEN1dE9* × *APOE*2 knock-in mice) exhibited impaired cognition	Pankiewicz et al. 2014[92]
Silencing *APOE*	Anti-APOE ASOs	i.p.	*APPPS1–21* × *APOE*3 knock-in *APPPS1–21* × *APOE*4 knock-in	Birth (P0)	16 wks	Reduced s/i-Aβ, Aβ plaques, DNs, and s-APOE; = i-APOE	Reduced Aβ plaque burden when treated at birth but no change or increased when treated at 6 wks suggests preventative rather than therapeutic use	Hyunh et al. 2017[56]
		i.c.v.		6 wks	10 wks	Reduced s-APOE and DNs; = s/i-Aβ, i-APOE, and CD45+ mg; = or increased Aβ plaques		
Switching *APOE*4 to *APOE*2	AAV4-*APOE*2/3/4	i.vent.	*APPswe/PSEN1dE9*	7 mo	2 mo & 5 mo	AAV4-*APOE*2 vs E3: reduced s/i Aβ, Aβ plaques, and DNs; = plasma Aβ; increased synapses AAV4-*APOE*4 vs E3: increased s/i Aβ, Aβ plaques, DNs, and plasma Aβ; reduced synapses	AAV4-mediated expression of APOE2 mainly in choroid plexus and ependymal cells via i.vent. injection can improve Aβ measures in aged mice after Aβ plaque deposition	Hudry et al. 2013[99]
		i.vent.	Tg2576	16–18 mo	3 mo	AAV4-*APOE*2 vs E3: reduced ISF Aβ and o-Aβ; = i-Aβ AAV4-*APOE*4 vs E3: increased ISF Aβ, o- Aβ and i-Aβ		
	AAV8-GFAP-*APOE*2/3/4	i.c.v.	*APOE*3 knock-in *APOE*4 knock-in	Birth (P2)	3 mo	AAV8-GFAP-*APOE*2 vs E3: increased APOE and APOE lipidation; reduced ms $A\beta_{40}$ (trend in *APOE*4 knock-in only); = APP-FL, APP-CTFs, ABCA1, ABCG1, *Apoe* mRNA AAV8-GFAP-*APOE*4 vs E3: increased ms $A\beta_{40}$ (*APOE*4 knock-in only); reduced APOE lipidation; = APP-FL, APP-CTFs, ABCA1, ABCG1, APOE and *Apoe* mRNA	Results suggest that APOE4 is less lipidated and stable than APOE2, and that exogenous expression of APOE4 in APOE4 carriers could be deleterious by increasing endogenous Aβ, whereas expression of APOE2 in APOE4 carriers would have opposing beneficial effects	Hu et al. 2015[79]

Abbreviations (excepting those shown in the main text): Ab = antibody; AICD = amyloid-β precursor protein (APP) intracellular domain; APP = amyloid-β precursor protein; APP-CTFs = APP C-terminal fragments; APP-FL = APP full length; *APPPS1–21* = Swedish *APP* mutation (K670N/M671L) and presenilin-1 L166P mutation; *APPswe* = Swedish *APP* mutation (K670N/M671L); *APPswePSEN1dE9* = Swedish *APP* mutation (K670N/M671L) and presenilin-1 exon 9 deletion; chol = cholesterol; d = days; DNs = dystrophic neurites; Fc = constant fraction of immunoglobulin/antibody; FcγR1 = Fc gamma receptor-1; GFAP = glial fibrillary acidic protein; HMGCoAR = 3-hydroxy-3-methylglutaryl coenzyme A reductase; HDL = high density lipoprotein; i = insoluble; i.c.v. = intracerebroventricular; IBA1 = ionized calcium binding adapter molecule-1; IFN-γ = interferon-γ; IL-1α = interleukin-1α; *Il6* = interleukin-6 mRNA; i.p. = intraperitoneal; i.vent. = intraventricular; LRP1 = low density lipoprotein related protein-1; mg = microglia; mo = months; mRNA= messenger RNA; ms = mouse; NOS2 = nitric oxide synthetase 2; o = oligomeric; p.o. = per os (orally); p-tau = phospho-tau; s = soluble; s.c. = subcutaneous; s/i = soluble and insoluble; SNAP25 = synaptosome associated protein-25; *SwDI-APP* = Swedish (K670N/M671L), Dutch (E693Q) and Iowa (D694N) *APP* mutation; SYP = synaptophysin; Tg2576 = Transgenic Swedish (K670N/M671L) *APP* mutation overexpressing mice; VGLUT1 = vesicular glutamate transporter-1; wks = weeks; Wt = wild-type.

Table 2.

Early development of *APOE*-directed therapeutic approaches in human clinical trials.

Drug	Rationale	ID	Status	Design	Phase	Subjects	Dose/route/ duration	Primary outcome	Secondary outcomes	Results	Ref.
Bexarotene	Increase APOE levels	NCT02061878	Completed	Randomised, double-blind, placebo-controlled	1b	Healthy young (aged 21–49) *APOE* ε3/ε3 volunteers	225 mg BID PO × 5 days	Newly generated Aβ in CSF (SILK)	Fractional clearance rate of Aβ from CNS (SILK)	Poor bexarotene brain penetration (not detectable in >95% CSF samples), 25% increase in CSF APOE levels, marginally significant increase in newly synthesized APOE, no change in Aβ synthesis/ clearance or levels	Ghosal et al. 2016[83]
Bexarotene	Increase APOE levels	NCT01782742	Completed	Randomized double blind placebo-controlled	2	Moderate AD (MMSE 10–20) with positive baseline amyloid PET scan	150 mg BID PO × 4 weeks	Aβ burden in amyloid PET imaging	Cognition (MMSE, ADAS-Cog, CDR), behavior (NPI), ADL, serum Aβ40/42	Significant reduction in Aβ burden only in *APOE* ε4 non-carriers which correlated with increased serum Aβ42 levels, increased triglyceride plasma level in bexarotene group, no efficacy in clinical outcomes	Cummings et al. 2016[84]
Probucol	Increase APOE levels	NCT02707458	Completed	Open label, dose finding	1 & 2	Cognitively intact at risk of AD by family history	Initial 600 mg QD PO, then individualized, 1 year of follow-up	Plasma probucol and CSF and plasma APOE levels	N.A.	N.A.	n.a.
CN-105	APOE mimetic	NCT03802396	Recruiting	Randomized double blind placebo-controlled	2	≥60 year-old undergoing major surgery	0.1 mg/kg vs 0.5 mg/kg vs 1 mg/kg IV Q6H × 4 days and 6 weeks of follow-up	Safety	CSF cytokine levels, change in cognition, post-	N.A.	n.a.

Drug	Rationale	ID	Status	Design	Phase	Subjects	Dose/route/ duration	Primary outcome	Secondary outcomes	Results	Ref.
									operative delirium		
Gene therapy (AAVrh.10 hPOE2 vector)	Switch APOE4 to APOE2	NCT03634007	Recruiting	Open label, dose ranging	1	Symptomatic (any stage), *APOE* ε4/ε4, positive CSF biomarkers or amyloid PET scan	8×10^{10} GC/kg vs 2.5×10^{11} GC/kg vs 8×10^{11} GC/kg, single intracisternal, injection and 2 years of follow-up	Safety	Maximum dose tolerated	N.A.	n.a.

AAV = adeno-associated virus; AD = Alzheimer's disease; ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive subscale; ADL = Activities of Daily Living; BID = *bis in die* (twice daily); CSF = cerebrospinal fluid; GC = genome copies; IV = intravenously; MMSE = Mini Mental State Examination; N.A. = not available. NPI = neuropsychiatric inventory; PET = positron emission tomography; PO = *per os* (orally); Q6H = *quaque sexta hora* (every 6 hours); QD = *quaque die* (once daily); SILK = stable isotope labelling kinetics.