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Cell type-specific roles of APOE4 in Alzheimer disease

Jessica Blumenfeld^{1,2,7}, Oscar Yip^{1,3,7}, Min Joo Kim^{1,3}, Yadong Huang^{1,2,3,4,5,6,†}

¹Gladstone Institute of Neurological Disease, Gladstone Institutes, San Francisco, CA, USA

²Neuroscience Graduate Program, University of California, San Francisco, CA, USA

³Biomedical Sciences Graduate Program, University of California, San Francisco, CA, USA

⁴Gladstone Center for Translational Advancement, Gladstone Institutes, San Francisco, CA, USA

⁵Department of Neurology, University of California, San Francisco, San Francisco, CA, USA

⁶Department of Pathology, University of California, San Francisco, San Francisco, CA, USA

⁷These authors contributed equally: Jessica Blumenfeld, Oscar Yip

Abstract

The *ε4* allele of the apolipoprotein E gene (*APOE*) is the strongest genetic risk factor for late-onset Alzheimer disease (AD). Within the CNS, APOE is produced by a variety of cell types under different conditions, posing a challenge for studying its roles in AD pathogenesis. However, through powerful advances in research tools and the use of novel cell culture and animal models, researchers have recently begun to study APOE4's roles in AD in a cell type-specific manner and at a deeper and more mechanistic level than ever before. In particular, cutting-edge omics studies have enabled APOE4 to be studied at the single-cell level and have allowed for the identification of critical APOE4 effects in AD-vulnerable cellular subtypes. Through these studies, it has become evident that APOE4 produced in various types of CNS cells — including astrocytes, neurons, microglia, oligodendrocytes and vascular cells — has diverse roles in AD pathogenesis. Here, we review these scientific advances and propose a novel cell type-specific APOE4 cascade model of AD. In this model, neuronal APOE4 emerges as a crucial pathological initiator and driver of AD pathogenesis, instigating glial responses and, ultimately, neurodegeneration. In addition, we provide perspectives on future directions for APOE4 research and related therapeutic developments in the context of AD.

Introduction

Alzheimer disease (AD) is a neurodegenerative disorder that is clinically characterized by a progressive decline in cognitive functions. Pathologically, AD is characterized by loss of

[†] yadong.huang@gladstone.ucsf.edu .
Author contributions

The authors contributed equally to the development of the general concept, structure, and graphics of the article. YH provided the original idea and draft of the cell type-specific APOE4 cascade model of AD, and all authors together modified and finalized the model.

Competing interests

YH is a co-founder and scientific advisory board member of GABAeron, Inc. Other authors declare no competing financial interests.

synapses and neurons, extracellular deposition of amyloid- β (A β) peptides in the form of amyloid plaques, neuritic dystrophy, intraneuronal accumulation of hyperphosphorylated tau (p-tau) in the form of neurofibrillary tangles (NFTs), vascular alterations and inflammatory responses mediated by microglia and astrocytes^{1,2}. AD starts with a long preclinical stage, lasting 20–30 years, during which amyloid plaques form without clinical symptoms^{3–5}. This is followed by a prodromal stage, usually lasting 3–5 years, characterized by mild cognitive impairment (MCI). Finally, some but not all cases progress to dementia^{3–5}. Worldwide, the total number of people with preclinical AD, prodromal AD and AD dementia is estimated to be approximately 416 million⁶ (Fig. 1a).

Despite over a century of AD research, we lack effective treatments or cures and those that have been proposed remain controversial. This is likely due to the complex etiology of AD pathogenesis, which involves interactions among diverse genetic, epigenetic and environmental factors. Causative genetic mutations have been identified for early-onset autosomal dominant AD (ADAD)^{7,8}; however, ADAD accounts for fewer than 1% of all AD cases. For late-onset AD, which accounts for the vast majority of AD cases, researchers so far have identified only genetic loci that can alter disease risk, the strongest of which is the apolipoprotein E (*APOE*) locus^{9,10}.

The human *APOE* gene is polymorphic, comprising three common alleles (*APOE* *e4*, *e3* and *e2*) that translate into three APOE isoforms: APOE4, APOE3 and APOE2^{1,11}. APOE's canonical function is as a transporter of lipids between cells and organs. As a result of amino acid differences at residue 112 or 158¹², the three APOE isoforms exhibit different binding properties with the APOE receptors through which they mediate this transport¹³ (Fig. 1b). Carriers of the *APOE* *e4* allele, the strongest genetic risk factor for late-onset AD^{10,14,15}, account for approximately 24% of the healthy population¹⁶ (Fig. 1b) but make up 55–75% of AD dementia cases (as well as approximately 57% of those with prodromal AD and approximately 55% of those with preclinical AD) (Fig 1a)^{14,17}.

Along with its unique structural and functional properties^{12,13,18}, the presence of APOE4 has been linked to more severe AD pathologies^{1,2,19–21}. Having a copy of the *APOE* *e4* allele increases the formation of amyloid plaques^{1,2,19,20} and NFTs in humans and animal models^{1,2,22–24}. APOE4 is also associated with increased gliosis and elevated levels of proinflammatory cytokines^{23–26}. This APOE4-enhanced gliosis is, in turn, associated with other pathological phenotypes such as lipid accumulation in glial cells^{27–29}, neurodegeneration^{23,24,28,30} and hippocampal atrophy^{23,24,28,30}. Moreover, humans with AD, especially those carrying the *APOE* *e4* allele, and AD mouse models carrying the same allele exhibit oligodendrocyte dysfunction and myelination defects^{24,31–33} as well as blood-brain barrier (BBB) impairments^{34–36}. Given this complicated picture, it is critical to dissect the detrimental effects of APOE4 at a cell type-specific level and to identify the underlying molecular mechanisms that drive AD pathogenesis in the most relevant cell populations. Although numerous studies and recent reviews have examined the pathobiological effects of APOE4 in general^{20,25,37,38}, a comprehensive review of APOE4's roles in AD pathogenesis at a cell type-specific level is lacking: this is therefore the focus of this Review.

Several cell types in the mammalian CNS can produce APOE, including astrocytes, neurons, microglia, oligodendrocytes and vascular cells, although the production levels vary by cell type and condition^{1,20,39} (Fig. 1c). Studies leveraging new research tools and experimental models have revealed that the effects of APOE4 on AD pathologies depend on its cellular sources and expression levels. Here, we review the roles of APOE4 derived from various CNS cell types, summarize the potential mechanisms underlying the cell type-specific effects of APOE4, present a novel cell type-specific APOE4 cascade model of AD, and provide perspectives on future APOE4 research and related therapeutic developments.

Astrocytic APOE4 in AD pathogenesis

Expression and regulation of astrocytic APOE

Astrocytes produce most of the APOE in the CNS^{39–41} (Fig. 2a), contributing approximately 75–80% of the total APOE in the mouse brain^{42–44} (Fig. 1c). Although the regulation of astrocytic APOE production remains to be fully elucidated, the transcription factor C/EBP β is known to bind the *APOE* promoter and regulate APOE expression in astrocytes⁴⁵. Mitochondrial function may also modulate APOE levels, as treatment of human astrocytes with an inhibitor of mitochondrial respiration increased APOE mRNA and protein levels⁴⁶. After production, approximately 60% of astrocytic APOE is secreted into the extracellular space^{47,48}. This process may be regulated by 25-hydroxycholesterol (25-HC), an oxysterol secreted by microglia, as treatment of primary mouse astrocytes expressing human *APOE* with 25-HC led to a 2-fold increase in secreted APOE⁴⁹. Since astrocytes are the major source of brain APOE, the roles that intracellular and extracellular astrocytic APOE may play in health and AD have been a major focus in APOE research (Fig. 2a–c).

Astrocytic APOE4 in inflammation, metabolism and BBB function

Under normal conditions, astrocytic APOE helps astrocytes maintain homeostatic lipid metabolism, controls synaptic pruning, regulates clearance of extracellular A β aggregates and cell debris, and helps to preserve BBB integrity^{50–58} (Fig. 2a). However, APOE can also promote astrocytic inflammatory responses in an isoform-dependent manner^{43,50,51,59} (Fig. 2b,c). In primary mouse astrocytes and astrocytes derived from human induced pluripotent stem cells (hiPSCs), expression of APOE4 increased the secretion of inflammatory cytokines, including interleukin-1 β (IL-1 β), IL-6, and inducible NO synthase (NOS2), as compared to expression of APOE3 or APOE2^{50,51,59}. In a tauopathy model in which mice express mutant human tau (PS19 mice), additional expression of APOE4 (PS19-APOE4 mice) enhanced the reactive astrogliosis elicited by tau pathology, while its selective removal from astrocytes induced a more physiological state⁴³, suggesting a role of astrocytic APOE4 in tauopathy-induced reactive astrogliosis.

APOE isoforms also differentially affect astrocytic lipid metabolism and accumulation (Fig. 2b,c). APOE4 expression increased cholesterol synthesis and accumulation in astrocytes of human *APOE4* knock-in (*APOE4*-KI) mice and astrocytes derived from APOE4 hiPSCs^{31,53–55,60}. Sterol regulatory element-binding protein 2, a key activator of cholesterol synthesis, was elevated in APOE4 hiPSC-derived astrocytes, suggesting a potential pathway through which astrocytic APOE4 may promote cholesterol production⁶⁰. Primary astrocytes

from *APOE4*-KI mice exhibited changes in lipid pathway proteins, including reduced levels of peroxisome proliferator-activated receptor- γ (PPAR γ , a transcription factor involved in maintaining lipid homeostasis) and altered levels of receptors and transporters involved in lipid uptake and efflux⁵⁵. Compared with *APOE3* hiPSC-derived astrocytes, *APOE4* hiPSC-derived astrocytes exhibited increased localization of cholesterol to lysosomes⁵¹. Furthermore, a recent preprint has reported that immortalized astrocytes from *APOE4*-KI mice developed larger lipid droplets and greatly impaired lipid turnover compared to their *APOE3*-KI counterparts⁶¹. In addition, selectively removing astrocytic *APOE* in mice markedly decreased the number of lipid-accumulating reactive astrocytes⁶². Beyond affecting lipid composition intracellularly, astrocytic *APOE4* also reduces the lipidation of secreted lipoprotein particles, compared to *APOE3*⁵⁶.

The differential effects of the *APOE* isoforms also extend to astrocytic glucose metabolism. Primary astrocytes from *APOE4*-KI mice demonstrated reduced mitochondrial membrane potential and respiration compared to those from *APOE3*-expressing controls⁶³. Similarly, immortalized astrocytes from *APOE*-KI mice, primary *APOE*-KI mouse astrocytes, and hiPSC-derived astrocytes displayed changes in glucose utilization, with *APOE4*-expressing astrocytes showing elevated levels of glycolytic activity relative to their *APOE3* counterparts^{64–66}. Interestingly, treatment with cholesterol-depleting agents restored mitochondrial respiration and activity in *APOE4* hiPSC-derived astrocytes, suggesting that *APOE4*-induced cholesterol accumulation may contribute to glucose metabolism shifts in astrocytes⁶⁶.

Astrocytic *APOE4* also affects many other cell types, especially under stressed and AD-like conditions (Fig. 2c). For instance, selectively removing *APOE4* from astrocytes prevented disease-associated gene signatures in neurons, oligodendrocytes and microglia in *PS19-APOE4* mice: fewer oligodendrocytes expressed AD-associated immune pathway genes and microglia showed less phagocytosis of synapses and disease-associated microglial (DAM) transcriptional signatures⁴³. These results suggest that astrocytic *APOE4* enhances glial reactivity⁴³. In addition, astrocytic *APOE4* seems to affect lipid metabolism in neurons. For example, primary neurons from wildtype (WT) mice showed elevated levels of cholesterol biosynthesis when treated with *APOE* derived from primary astrocytes of *APOE4*-KI mice, as compared to those treated with *APOE* from *APOE3*-KI mouse astrocytes⁶⁷. In a similar vein, in an organoid model containing hiPSC-derived neurons and astrocytes of various *APOE* genotype pairings, the presence of *APOE4*-producing human astrocytes elevated cholesterol levels and promoted lipid accumulation in human neurons⁶⁸. Additionally, astrocytic *APOE4* may also affect synaptic plasticity by impairing neuronal *APOE* receptor recycling via the endosomal pathway⁶⁹.

Astrocytic *APOE4* also influences BBB integrity and function (Fig. 2c). In an in vitro BBB model consisting of primary astrocytes derived from *APOE*-KI or *ApoE* knockout (*ApoE*-KO) mice together with WT primary brain endothelial cells and pericytes, astrocytic *APOE4* impaired endothelial cell tight junctions by reducing the levels of threonine-phosphorylated occludin⁵⁷. This effect was recapitulated in an in vivo model, in which selective removal of astrocytic *APOE4* prevented the impairments of the BBB, endothelial tight junctions and astrocytic end-foot coverage of blood vessels observed in *APOE4*-KI mice⁷⁰. In a mouse

ApoE-KO model, selective astrocytic expression of APOE4, but not APOE3, led to BBB leakiness⁵⁸. Taken together, these findings suggest that astrocytic APOE4 affects multiple CNS cell types that might contribute to AD pathogenesis.

Astrocytic APOE4 and hallmark AD pathologies

In mouse and cell culture models, astrocytic expression of APOE4 promotes amyloid plaque pathology and impairs astrocytic A β uptake, as compared to APOE3^{51,54,71–73} (Fig. 2c). Furthermore, selective removal of astrocytic APOE4 reduced plaque loads in a model of amyloidosis in which mice co-express APOE4, mutant amyloid precursor protein (APP) and mutant presenilin 1 (PSEN1, a protein involved in A β production) (APP/PS1–21 mice)⁷⁴. In another amyloidosis model (5xFAD mice) that co-expresses APOE4, selective ablation of astrocytic APOE4 reduced gliosis and BBB disruption and shifted A β deposition from the brain parenchyma to the vasculature⁴⁴.

The connection between astrocytic APOE4 and tau pathology is less straightforward. Selective expression of APOE4 in astrocytes on an *ApoE*-KO background caused little or no accumulation of endogenous mouse p-tau^{75,76}. However, selective removal of astrocytic APOE4 in PS19-APOE4 mice moderately lowered p-tau levels⁴³. Glypican-4 (GPC4), a protein that is secreted by astrocytes and has been shown to preferentially bind APOE4 (relative to APOE2)⁷⁷, may provide a link between astrocytic APOE4 and this effect. Notably, GPC4 treatment increased p-tau accumulation in primary neurons from PS19 mice⁷⁷. Similarly, inducing greater GPC4 expression in astrocytes of young PS19 mice increased hippocampal p-tau levels⁷⁷. Thus, the suggestion that astrocytic APOE4 might augment tau pathology warrants further studies.

APOE4 in disease-associated astrocytic subtypes

Single-nucleus RNA-sequencing (snRNA-seq) studies examining different types of cells in AD have identified a distinct group of disease-associated astrocytes (DAAs)^{78,79} (Fig. 2c). These DAAs generally have elevated APOE expression, suggesting an involvement of astrocytic APOE in their formation⁷⁸. DAAs also exhibit increased expression of the genes encoding cathepsin and serine protease inhibitor A3N, which are thought to be involved in AD pathogenesis^{80,81}, as well as genes involved in lipid and cholesterol pathways, the complement system, endocytosis and inflammatory responses⁷⁸. APOE4 hiPSC-derived astrocytes also showed upregulation of lipid metabolism-related genes and downregulation of lipid transport-related genes⁵⁴. Notably, selective ablation of neuronal APOE4 in PS19-APOE4 mice reduced the abundance of one DAA subtype, referred to as neuronal APOE4-promoted DAA (nE4-DAA), which showed increased expression of APOE and cathepsin genes²⁴. Intriguingly, the reduction of nE4-DAAs corresponded with ameliorated AD pathologies²⁴. Thus, the emergence of DAA subtypes may also be brought about indirectly by the effects of neuronal APOE4 expression (see below).

Taken together, these studies demonstrate that astrocytic APOE4 can enhance diverse aspects of AD pathogenesis directly or indirectly, through exacerbation of amyloid and tau pathology and the promotion of glial reactivity, adoption of a DAA phenotype,

proinflammatory cytokine production and release, altered metabolism and BBB disruption (Fig. 2b,c).

Neuronal APOE4 in AD pathogenesis

Expression and regulation of neuronal APOE

Under physiological conditions, mammalian neurons express low levels of APOE (Fig. 3a), contributing approximately 15–20% of total brain APOE in mice^{24,42} (Fig. 1c). However, they upregulate APOE expression in response to stress and aging^{39,82–89} (Fig. 3b). snRNA-seq studies of WT mice, human *APOE*-KI mice and human brains from young subjects or from older individuals with AD have shown that APOE is expressed in both inhibitory and excitatory neurons, albeit at lower levels than in astrocytes^{24,90}. Interestingly, unlike astrocytes, which secrete most of the APOE that they produce, neurons secrete only about 10% of the APOE that they generate⁴⁸, likely setting the stage for its intracellular accumulation.

The cellular and molecular mechanisms that control neuronal APOE expression are not fully understood. One study identified a neuron-specific splicing variant of *APOE* mRNA with an intron-3 retention (*APOE-I3*) that may help regulate neuronal APOE expression⁹¹ (Fig. 3a). The authors suggested that *APOE* transcripts are continuously produced in neurons under normal conditions, and the subsequently generated *APOE-I3* pre-mRNA is retained in the nucleus without being translated. However, in response to neuronal stress or injury, it is quickly processed into mature *APOE* mRNA for protein translation⁹¹ (Fig. 3b). Consistent with this model, neuronal APOE expression is increased in response to CNS injury, potentially enabling time-sensitive neuronal protection and repair^{92,93}. Indeed, neuronal APOE may be important for neurogenesis⁹⁴ and neuronal remodeling^{1,95} (Fig. 3a). RNA-seq analysis has shown that the abundance of *APOE-I3* transcripts in the brains of individuals with AD is related to the severity of tau and amyloid pathologies⁹⁶. Interestingly, the level of *APOE-I3* transcripts increased with the number of *APOE ε4* alleles that an individual possessed⁹⁶, possibly reflecting increasing levels of neuronal stress and injury. A recent study identified a stress-inducible transcriptional mechanism involving R-loops and RNA abasic sites that regulates an enhancer RNA that activates *APOE* transcription in non-neuronal cells⁹⁷. An interesting question is whether this enhancer RNA also regulates *APOE* expression in stressed neurons and, if so, whether it affects the production or splicing of *APOE-I3* pre-mRNA.

Like astrocytic APOE expression, neuronal APOE expression is also regulated by C/EBPβ^{45,98} (Fig. 3b). Notably, C/EBPβ promotes the expression of APOE4 more than APOE3^{45,98}. Interestingly, neuronal APOE has also been found to stimulate C/EBPβ activation, resulting in a feed-forward loop in which downstream AD pathology can be further exacerbated⁹⁸. In addition, astrocytes may secrete factor(s) that regulate neuronal APOE expression through an extracellular signal-regulated kinases (ERK)-dependent pathway⁴⁸, possibly by promoting the splicing of *APOE-I3* into mature *APOE* mRNA⁹¹. Clearly, further studies are needed to fully elucidate the mechanisms that regulate neuronal APOE expression.

Neuronal APOE4 causes cellular and network dysfunction

Neuronal APOE4 can have detrimental effects on synaptic and neuronal network functions (Fig. 3b,c) that occur early in life. Neuron-specific APOE4 expression in mice with a mouse *ApoE*-KO background resulted in synaptic loss as well as learning and memory deficits at 6–9 months of age^{99–101}. This APOE4-induced synaptic loss may be related to an increase in phosphorylation of vasodilator-stimulated phosphoprotein (VASP) S235 that disrupts its interactions with numerous actin cytoskeletal and microtubular proteins¹⁰². A recent preprint reported that ex vivo hippocampal neurons taken from 7–9-month-old *APOE4*-KI mice, compared to *APOE3*-KI controls, displayed hyperexcitability that was prevented by removal of neuronal, but not astrocytic, APOE4¹⁰³. This neuronal hyperexcitability persisted as the mice aged, probably due to an age- and APOE4-dependent loss of inhibitory tone^{103,104}. Recordings of local field potentials in 6–8-month-old *APOE4*-KI mice revealed reductions in hippocampal sharp wave ripple (SWR) activity and in the power of associated slow gamma oscillations, which are critical for memory consolidation^{105,106}. Strikingly, the presence of a SWR-associated slow gamma oscillation deficit at a younger age predicted the development of learning and memory impairments 10 months later in these mice^{105,106}. Moreover, selective removal of neuronal, but not astrocytic, APOE4 — especially from GABAergic interneurons — prevented these abnormalities^{42,103,105}, highlighting the pathogenic importance of neuronal APOE4. In fact, the extent of APOE4-induced GABAergic interneuron loss (especially somatostatin (SST)-expressing interneuron loss) correlated with the severity of learning and memory deficits in aged *APOE4*-KI mice^{42,107,108}. Interestingly, in *APOE*-KI mice, APOE expression in SST interneurons was ~40% higher than it was in dentate gyrus granule cells and CA1 neurons, suggesting that higher APOE expression (especially APOE4) renders SST interneurons selectively vulnerable to degeneration and loss in AD⁹⁰.

Although the mechanisms by which neuronal APOE4 impairs the functions and survival of neurons remain to be fully defined, several studies suggest that impairments of specific subcellular compartments may be involved. Neuronal APOE4 impairs the function and integrity of mitochondria^{95,109–111} (Fig. 3b,c), which could result in energy failure. Primary neurons from transgenic mice with neuron-specific expression of APOE4 had reduced levels of mitochondrial respiratory complexes, as compared to those expressing APOE3¹¹². Proteomic analysis of mouse neuroblastoma cell lines expressing human APOE4 versus APOE3 yielded compatible results¹¹³. Notably, no significant differences in the levels of neuronal mitochondrial complexes were detected in transgenic mice expressing APOE4 versus APOE3 specifically in astrocytes, making it unlikely that these changes result from the indirect effects of astrocytic APOE4¹¹². In a similar vein, neuronal (but not astrocytic) expression of APOE4 sensitized mice to excitotoxin-induced neurodegeneration¹¹⁴. In humans with early-stage AD¹¹⁵ and in cultured neuronal cells¹¹⁶, APOE4 is also associated with neuronal endo-lysosomal defects (Fig. 3c), which could contribute to its detrimental effect on receptor recycling and synaptic plasticity⁶⁹.

Neuronal APOE4 and hallmark AD pathologies

Neuronal APOE4 also affects major AD pathologies, including amyloid and tau pathologies, gliosis, neurodegeneration and myelin deficits (Fig. 3c). APOE4 hiPSC-derived neurons

produced more A β , had higher levels of APOE fragments and p-tau and more degeneration of GABAergic neurons than APOE3 controls⁸⁵. These pathologies were rescued by gene-editing of APOE4 to APOE3⁸⁵. In another study, APOE4 hiPSC-derived neurons were more susceptible to cytotoxicity than APOE3 controls and had higher p-tau levels¹¹⁷. Although the mechanisms that link neuronal APOE4 and p-tau remain to be fully defined, several interesting leads have emerged. For example, heparan sulfate proteoglycans (HSPGs) are cell-surface receptors that interact more strongly with APOE4 than APOE3^{13,118} and are involved in the inter-neuronal spread of tau^{119–121}. Treatment of APOE4 neurons with an HSPG inhibitor reduced their p-tau levels¹¹⁷. Moreover, APOE4 hiPSC-derived neurons carrying the APOE-R136S mutation, which strongly protects against ADAD^{122,123}, had lower p-tau levels and reduced tau uptake via HSPG than isogenic APOE4 neurons without this mutation²⁸. Taken together, these results identify HSPGs as a critical mediator of copathogenic interactions between APOE4 and tau.

The effects of neuronal APOE4 on AD pathologies are also evident in vivo. In transgenic mice, expressing APOE4 in neurons increased neuronal p-tau levels, whereas expressing APOE4 in astrocytes did not^{75,76}, indicating that neuronal APOE4 is more directly linked than astrocytic APOE4 to neuronal p-tau accumulation. In PS19-APOE4 mice, selective removal of APOE4 from neurons markedly reduced tau pathology²⁴, whereas selective removal of astrocytic APOE4 in a similar model lowered p-tau levels only moderately⁴³ (an effect that might have resulted secondarily from a reduction in astrocytosis and microgliosis¹²⁴). Moreover, neuronal, but not astrocytic APOE4 promoted excitotoxic neuronal death¹¹⁴. A recent study further suggests that neuronal APOE4 activates C/EBP β , which regulates the expression of *APP*, *MAPT* (the gene encoding tau) and beta-secretase 1 (*BACE1*, encoding an enzyme involved in APP processing), promoting A β deposition, tau aggregation, and neurodegeneration⁹⁸. A recent preprint has reported that, in experiments in which APOE4 hiPSC-derived neurons were transplanted into the hippocampi of *APOE4*-KI mice in conjunction with microglial depletion, both human neuronal APOE4 and microglia were important for the formation of dense-core A β plaques and tau aggregates¹²⁵.

Although the mechanisms linking neuronal APOE4 to the pathological hallmarks of AD are likely multifarious, diverse lines of evidence suggest several mechanisms that have a prominent role and may make interesting targets for therapeutic interventions. One of these mechanisms involves the intraneuronal proteolytic cleavage of APOE, to which APOE4 is much more susceptible than APOE3, and which results in the generation of C-terminally truncated, neurotoxic APOE fragments²². Such APOE4 fragments can elicit the formation of intraneuronal p-tau accumulation and neurodegeneration in neuronal cultures and transgenic mice^{22,126} and reduce A β clearance in mutant APP transgenic mice¹²⁷. Transgenic mice expressing full-length APOE4 in neurons showed an age-dependent accumulation of APOE4 fragments in the hippocampus, whereas transgenic mice expressing APOE4 in astrocytes did not⁷⁵. Furthermore, APOE4 hiPSC-derived neurons⁸⁵ and AD brains from APOE4 carriers^{126,128} had higher levels of APOE fragments than those from non-carriers. Taken together, these findings indicate that APOE4 proteolysis in neurons promotes the intraneuronal accumulation of p-tau as well as other AD-related neuronal deficits (Fig. 3b,c).

Another mechanism linking APOE4 to such deficits is the interaction between neuronal APOE and major histocompatibility complex class 1 (MHC1) molecules (Fig. 3b,c). Neuronal expression of APOE and MHC1 molecules have been shown to be tightly and positively correlated, and reducing neuronal MHC1 molecule levels resulted in reduced intraneuronal accumulation and mislocalization of p-tau in mouse primary neurons overexpressing APOE4⁹⁰. It was proposed that the APOE4-induced increase in neuronal MHC1 molecule expression serves as an ‘eat me’ signal to recruit microglia (and potentially T cells) to engage with the ‘tagged’ neurons, causing synaptic and neuronal loss⁹⁰. In line with this model, selective removal of APOE4 from neurons in PS19-APOE4 mice markedly reduced not only tau pathology, but also gliosis, neurodegeneration and myelin deficits in the hippocampus²⁴. More specifically, PS19-APOE4 mice exhibited significantly higher levels of pathological tau accumulation, astrogliosis, microgliosis, hippocampal neuronal loss and myelin deficits compared to PS19-APOE4 mice in which neuronal APOE4 was selectively removed, suggesting that neuronal APOE4 is an upstream initiator and potent driver of these AD-related pathologies and that its removal is sufficient to attenuate these phenotypes²⁴.

In addition to MHC1 molecules, another proinflammatory molecule that may promote pathogenic neuronal–glial interactions is high mobility group box 1 (HMGB1). Compared to APOE3, neuronal expression of APOE4, together with tau pathology, increased the nucleo-cytoplasmic translocation and cellular release of HMGB1. This triggered gliosis, likely by binding to toll-like receptors (TLRs) on glial cells¹²⁹ (Fig. 3c). Clinical studies of individuals with MCI have revealed an increase in HMGB1 levels in their cerebrospinal fluid (CSF)¹³⁰ and an interactive association of APOE4 and plasma HMGB1 levels with widespread cortical thinning¹³¹. Notably, pharmacological inhibition of HMGB1 release from neurons ameliorated neuroinflammation, neurodegeneration and myelin deficits in PS19-APOE4 mice¹²⁹. Interestingly, HMGB1 can upregulate MHC1 molecule expression in response to cellular stress^{132,133}. Thus, in response to neuronal APOE4 expression, HMGB1 and MHC1 molecules may act in concert to trigger glia-mediated synaptic and neuronal degeneration (Fig. 3c).

APOE4 in Disease-associated neuronal subtypes

snRNA-seq studies have identified neuronal states that are enriched in disease, known as disease-associated neuron (DAN) phenotypes. A comparative snRNA-seq analysis identified two excitatory neuronal clusters with abnormally high expression of genes encoding major heat shock proteins, calmodulin, and ubiquitin B in the hippocampus of PS19-APOE4 mice that were not seen in PS19-APOE4 mice with neuronal APOE4 removal (these were referred to as neuronal APOE4-promoted DAN or nE4-DAN)²⁴. nE4-DAN had high levels of APOE4 expression, as compared to other excitatory neuron subpopulations, and were associated with high levels of tau pathology and hippocampal atrophy²⁴ (Fig. 3c).

Relative levels of APOE expression may also provide insight into the vulnerability of different subtypes of neurons in AD. In *APOE4*-KI mice, snRNA-seq revealed higher levels of APOE4 expression in inhibitory than excitatory neurons⁹⁰, paralleling the earlier onset of the detrimental effects of APOE4 on inhibitory neurons^{42,107,108}. In a similar vein, snRNA-seq analyses of human cortices from individuals with AD revealed that

disease progression was related to the proportion of neuronal cells, especially inhibitory interneurons, expressing high levels of APOE^{90,134}. Specifically, the proportion of APOE-expression-high neurons, especially inhibitory neurons, was relatively low in individuals with no cognitive impairment, was highest in individuals with MCI, and was relatively low in individuals with AD. This pattern suggests that neuronal (especially inhibitory neuronal) APOE expression is upregulated early in the clinical progression of AD and declines thereafter as neurodegeneration advances⁹⁰, which is in line with the observation that vulnerable inhibitory neuron subtypes were selectively depleted in the brains of individuals with AD¹³⁵.

In summary, in response to stress, aging or disease, some (but not all) neurons increase their APOE expression (Fig. 3b). In APOE4 carriers (to a greater extent than in APOE3 carriers), this process results in the intraneuronal generation of neurotoxic APOE fragments, neuronal dysfunction, the emergence of nE4-DAN and the overproduction or increased release of proinflammatory molecules, triggering glial responses that further promote synaptic and neuronal degeneration (Fig. 3b,c). Specific subpopulations of inhibitory neurons produce more APOE and may also be more vulnerable to detrimental APOE4 effects than other types of neurons. Their dysfunction and loss lead to excitation-inhibition imbalance and network hyperexcitability, which is associated with faster cognitive decline in AD^{136,137}. Thus, neuronal APOE4 is likely to be a critical upstream pathogenic initiator and driver that directly or indirectly affects many AD-related cell types, genes and pathways (Fig. 3c).

Microglial APOE4 in AD pathogenesis

Expression and regulation of microglial APOE

Like neurons, microglia can express APOE in a stress and injury-dependent manner^{39,47,138–140} (Fig. 4a,b). While APOE expression is low in microglia under normal conditions (Fig. 4a), microglia in both aging and diseased brains have been shown to greatly upregulate their APOE expression^{39,138–142} (Fig. 4b). For instance, microglial APOE expression was increased in aged human *APOE*-KI mice¹⁴¹, PS19 mice³³, and 5xFAD mice¹³⁹. Notably, microglia surrounding A β plaques displayed the greatest increase in APOE expression^{124,140,143}.

The low-density lipoprotein receptor (LDLR) pathway may directly or indirectly contribute to the regulation of APOE expression in microglia, as LDLR overexpression in microglia in mice suppressed microglial activation and expression of APOE³³. APOE produced by other CNS cells may also affect microglia, as selective removal of neuronal APOE4 in PS19-APOE4 mice decreased the sizes of populations of microglia exhibiting high levels of APOE expression²⁴. This suggests that neuronal APOE4, or the pathological alterations that it causes, augment microglial APOE expression. Since microglia have been implicated in a variety of APOE4-related AD pathologies^{23,144}, it is crucial to understand the effects of microglia-derived APOE4.

Microglial APOE4 in metabolism and lipid accumulation

APOE genotype influences microglial metabolism and lipid accumulation (Fig. 4b). Homeostatic microglia have been shown to rely on oxidative phosphorylation for energy production, while activated microglia with high APOE expression shift toward the glycolytic pathway and increase their expression of hypoxia inducible factor 1-alpha (HIF1 α), a gene highly upregulated during glycolysis^{141,145}. These findings are in line with observations indicating that aged *APOE4*-KI mice had increased aerobic glycolysis and higher HIF1 α expression¹⁴¹.

Emerging evidence highlights the role of APOE in microglial lipid metabolism and accumulation (Fig. 4b). APOE is a major lipid transporter between neurons and glial cells^{11,19}. APOE-containing lipid particles produced by *APOE4*-KI mouse microglia were smaller than those produced by astrocytes, implying cellular source-dependent differences in the lipidation state of APOE¹⁴⁶. Furthermore, lipid droplet accumulation was a major phenotype observed in aged and diseased microglia^{27,29,147,148} (Fig. 4b,c). Similarly, *APOE4* hiPSC-derived microglia (hiMGL) accumulated more lipid droplets than *APOE3* microglia^{27,149}. Additionally, when human APOE was selectively expressed in microglia in an amyloidosis model in which mice expressing APP harboring the Swedish FAD mutation are crossed with mice in which PSEN1 exon 9 is deleted (*APP^{swe}/PS1^{E9}* mice) on a mouse *ApoE*-KO background, *APOE4* microglia showed increased lipid accumulation compared to *APOE3* microglia¹⁴⁹. *APOE4* microglia also demonstrated excessive buildup of lipid droplets in *APOE*-KI mice fed with cuprizone, which induces demyelination, compared to *APOE3* and *APOE2* microglia, indicating an impairment in lipid metabolism in *APOE4* microglia in response to demyelination¹⁵⁰.

Notably, in hiMGL, human post mortem brains, and *APOE*-KI mouse brains, *APOE4* increased de novo cholesterol synthesis in microglia and impaired cholesterol trafficking, possibly through lysosomal sequestration⁶⁰. Several other studies also showed that microglial *APOE4* expression caused lysosomal dysfunction through aberrant lysosomal trafficking¹⁵¹ and impaired endosomal-lysosomal activities^{86,151,152}. Additionally, during de novo fat synthesis, *APOE4* hiMGL exhibited lipogenesis that promoted proinflammatory signals and reduced the uptake of extracellular fatty acids and lipoproteins, thereby disrupting neuronal network activity¹⁴⁷.

Microglial APOE4, functional deficits and AD pathologies

Microglial APOE appears to be a key regulator of microglial function and reactivity, which consequently affect global brain health and disease progression (Fig. 4b,c). Ingenuity pathway analysis (IPA), a pathway analysis application used for high throughput omics data analysis, on isolated microglia from *APP*-*PS1* mice revealed that APOE affected the relative proportions of homeostatic microglia versus microglia associated with neurodegeneration¹⁴³. In *APOE*-KI mice, *APOE4* microglia displayed increased activation and exacerbated pro-inflammatory responses^{86,152} and, in postmortem human brains, *APOE4* carriers had increased numbers of microglia compared to non-carriers^{153,154}. In hiMGL, *APOE4* promoted a more reactive morphology and enhanced inflammatory gene signatures compared to *APOE3*⁵⁴. Lastly, when stimulated with lipopolysaccharide

(LPS), microglia from *APOE4*-KI mice exhibited higher levels of proinflammatory signals compared to microglia from *APOE3*-KI mice¹⁵⁵.

APOE genotype also influences vital microglial functions such as phagocytosis and chemotaxis^{53,156} (Fig. 4a,b). For instance, in murine N9 microglia expressing human *APOE* isoforms, those expressing *APOE4* showed impaired phagocytosis of A β ₄₂ (an aggregation prone form of A β peptides) and dysfunctional motility¹⁵⁶. Moreover, in APP^{swe}/PS1 E9 mice that received bone marrow transplants from human *APOE4*-KI mice (*APOE4*-KI-BMT), microglia exhibited reduced association with A β deposits and dystrophic changes and amyloid plaques were larger and more abundant than in APP^{swe}/PS1 E9 mice that received *APOE3*-KI-BMT¹⁵⁷. Meanwhile, selectively removing microglial *APOE4* in APP/PS1 mice led to significant reduction in plaque loads compared to *APOE4*-APP/PS1 mice¹⁵⁸, suggesting a 'gain-of-toxic' effect of *APOE4* on microglia-mediated A β clearance. Similarly, when *APOE* was selectively expressed in microglia in APP^{swe}/PS1 E9 mice on a mouse *ApoE*-KO background, the presence of *APOE3* microglia sufficiently protected against the development of amyloid pathology, whereas *APOE4* microglia did not¹⁴⁹. This defective response of *APOE4* microglia to A β pathology seemed to be mediated by the *APOE4*-ITGB8-TGF β pathway, which acted as a negative regulator¹⁵⁸. A similar defective response of *APOE4* microglia to A β was also observed in a chimeric model in which hiPSC-derived neurons were transplanted into *APOE*-KI mouse hippocampus: here, *APOE4* microglia tended to colocalize less with A β deposits than *APOE3* microglia¹⁵⁹. Mechanistically, IL-33 has been shown to mediate microglial migration towards *APOE*-associated A β plaques by inducing microglial VCAM1, and when this VCAM1-*APOE* interaction was disrupted, A β clearance was reduced in APP/PS1 mice¹⁶⁰.

In contrast to these effects, *APOE4*-expressing microglia actually exhibited increased phagocytosis of apoptotic cells in culture (N9 murine microglia overexpressing *APOE4*)¹⁵⁶ and increased synaptic engulfment in PS19-*APOE4* mice¹⁶¹ (Fig. 4c). Some studies even suggest that microglia may actively participate in tau spreading and seeding¹⁶²⁻¹⁶⁴. According to these studies, reactive microglia may phagocytose tau or tau-containing synapses and/or neurons. Due to *APOE4*-dependent lysosomal defects and resulting inefficiencies in processing the internalized tau^{86,151,152}, pathological tau aggregates then begin to accumulate within the microglia and are released via exosome transfer to spread and seed tau pathology¹⁶²⁻¹⁶⁴ (Fig. 4c). Consistent with this model, a human brain imaging study found greater levels of microglial activation and tau accumulation in medial temporal structures of *APOE4* carriers than in non-carriers, possibly contributing to their increased neurodegeneration and clinical impairment¹⁶⁵. Likewise, a recent preprint has reported that, in a chimeric AD model in which *APOE4* hiPSC-derived neurons were transplanted into *APOE4*-KI mouse hippocampus, microglial depletion reduced human neuronal *APOE4*-driven tau pathology¹²⁵. Interestingly, selectively removing microglial *APOE4* in PS19-*APOE4* mice restored microglia to a neuroprotective phenotype¹⁵⁸ and microglial depletion in these mice reduced tau pathology and neurodegeneration, possibly by reducing cerebral infiltration by T cells responding to MHC2 molecule-expressing microglia¹⁶⁶ (Fig. 4c).

APOE4 in disease-associated microglial subtypes

Microglia are an extremely heterogeneous cell type whose roles and functions in the CNS confer unique spatial-temporal gene signatures. The transcriptional profile of microglia is markedly altered in AD and in the presence of APOE4, with an increased proportion of microglia showing disease-associated rather than homeostatic profiles^{140,152,167}.

Disease-associated subtypes or states of microglia include disease-associated microglia (DAM)^{140,152}, lipid-droplet-accumulating microglia (LDAM)²⁹, interferon responsive microglia (IRM)¹⁶⁸, and antigen-presenting microglia (MHC2-M)^{125,168}. Among these, DAM and LDAM have been studied most extensively in the context of APOE4 (Fig. 4c).

DAM are characterized by increased expression of genes associated with proinflammatory immune responses, phagocytosis and lipid metabolism, and decreased expression of genes associated with surveillant microglia^{140,152}. Interestingly, even in the absence of overt AD pathology, the combination of APOE4 and aging was sufficient to elicit changes in microglia of *APOE4*-KI mice that strongly resembled the DAM phenotype¹⁴¹. In AD-related mouse models with amyloid or tau pathology, DAM localized around A β plaques and NFT-bearing neurons¹⁶⁹. Remarkably, selectively ablating endogenous microglial APOE using *Cx3cr1-Cre:ApoE* mice prevented the emergence of microglia with a neurodegenerative phenotype (MGnD, another type of DAM), compared to WT mice¹⁴³. The same study showed that global *ApoE*-KO conferred no additional protection, suggesting that microglial APOE is particularly crucial in initiating the appearance of MGnD¹⁴³. However, selectively removing microglial APOE4 in APP/PS1/APOE4 or PS19-APOE4 mice increased MGnD¹⁵⁸ and, conversely, selectively expressing APOE4 in microglia in APP^{swe}/PS1 E9 mice on a mouse *ApoE*-KO background decreased activated response microglia (ARM, another type of DAM), which highly expressed MHC genes¹⁴⁹. Together, these findings suggest that mouse APOE and human APOE4 might affect MGnD and ARM differently, which warrants further in-depth study. It should be noted that the proportion of DAM was not reduced in PS19-APOE4 versus PS19-APOE3 mice in other studies^{24,28,43,129}.

As may be expected, DAM (or MGnD) have increased APOE expression, which precedes the decrease in homeostatic markers (such as P2Y purinoceptor 12 (P2RY12) and C-X-C motif chemokine ligand 3 (CXCL3)) and increase in disease-associated signatures (including lipoprotein lipase (LPL), cystatin-F (CST7), and secreted phosphoprotein-1 (SPP1))^{140,143}, suggesting that APOE influences microglial gene expression. Human *APOE4* carriers and human *APOE*-KI mice crossed with APP/PS1 mice or stimulated with LPS demonstrate that APOE4 primes microglia for conversion into the phagocytic and proinflammatory state of DAM or promotes the proliferation of such cells¹⁶⁷. In PS19-APOE4 mice with or without neuronal APOE4 removal, neuronal APOE4-promoted DAM (nE4-DAM) subtypes were identified and found to positively associate with tau pathology and hippocampal degeneration²⁴. Furthermore, introduction of the APOE-R136S mutation, which protects against ADAD^{122,123}, markedly reduced the number of DAM as well as tau pathology and neurodegeneration in PS19-APOE4 mice²⁸. Interestingly, removing APOE4 selectively from astrocytes⁴³ or neurons²⁴ in PS19-APOE4 mice also decreased DAM and nE4-DAM, respectively, suggesting that both astrocytic and neuronal APOE4 promote the emergence of DAM subtypes.

LDAM are another emerging subtype of diseased microglia, defined by an increased expression of genes, such as *ACSL1*, that are involved in pathways related to lipid handling, transport and metabolism²⁹. LDAM also exhibit an accumulation of lipid droplets in the cell soma, which may indicate increased endogenous lipid production, excessive uptake of extracellular lipids from degenerating neurons, or an inability to degrade lipids²⁹. Notably, APOE4 hiMGL show more lipid droplet accumulation than APOE3 microglia^{27,147,149}. Recent research has raised the possibility that dysfunctional lipid metabolism and lipid droplet formation in APOE4 microglia impair microglial surveillance of neuronal network activity¹⁴⁷. Furthermore, a recent preprint has reported that this can promote neuronal p-tau accumulation and neurotoxicity²⁷. Interestingly, introduction of the protective APOE-R136S mutation markedly reduced lipid droplet accumulation in microglia of PS19-APOE4 mice²⁸.

Beyond DAM and LDAM, other disease-associated microglial subtypes continue to be identified. For example, in a chimeric AD model in which APOE4 hiPSC-derived neurons were transplanted into *APOE4*-KI mouse hippocampus, there was an enrichment in microglial subpopulations highly expressing MHC2 genes (MHC2-M) that contributed to neuronal APOE4-promoted A β and tau pathologies¹²⁵. Comparing to the data from APP^{swE}/PS1 E9 mice selectively expressing APOE4 in microglia on a mouse *ApoE*-KO background¹⁴⁹, the data from the chimeric AD model suggest that APOE4 microglia might respond differently to human neuron-produced versus mouse neuron-produced AD pathologies, which warrants further in-depth study. MHC2-M have been found to be enriched in human AD patient brains, close to amyloid plaques and tau tangles^{170,171}. MHC2-M were also enriched in the hippocampus of PS19-APOE4 mice, which was important for recruiting infiltrating T cells¹⁶⁶.

Altogether, these findings have begun to highlight the heterogeneity of microglia and their differential contributions to AD pathogenesis, especially in the context of APOE4 (Fig. 4c). Clearly, microglial APOE4 promotes the emergence of DAM subtypes. Notably, APOE4 in DAM and LDAM may increase the phagocytosis of neuronal synapses containing pathological tau, leading to increased synaptic loss and neurodegeneration. APOE4-associated endo-lysosomal defects in microglia may impede the degradation of pathological tau and promote the accumulation of tau in microglia, a process that may contribute to tau spreading and seeding (Fig. 4c). APOE4 also seems to enhance MHC2-M-dependent T-cell infiltration, which may further exacerbate tau pathology and neurodegeneration.

Oligodendrocytic APOE4 in AD pathogenesis

Expression and regulation of oligodendrocytic APOE

Like the other glial cells of the CNS, oligodendrocytes can express APOE^{24,31,90} (Fig. 5a). In PS19-APOE4 mice, a subgroup of oligodendrocytes exhibited increased expression of APOE compared to PS19-APOE3 mice²⁴. This suggests that increased APOE expression is part of their injury response, and that this is augmented in PS19-APOE4 mice because they have more tauopathy and neurodegeneration than PS19-APOE3 mice (Fig. 5b). Consistent with this interpretation, rat primary oligodendrocytes increased their expression and secretion of APOE when they were treated with conditioned medium from mechanically injured neurons¹⁷². Notably, oligodendrocytic APOE expression is also affected by neuronal

APOE, as selective ablation of APOE4 from neurons reduced oligodendrocytic APOE expression in PS19-APOE4 mice²⁴.

Oligodendrocytic APOE4 in lipid metabolism and myelination

APOE isoforms differentially affect oligodendrocytic lipid metabolism and myelination (Fig. 5a,b). Notably, oligodendrocytes of the prefrontal cortex of human APOE4 carriers exhibited elevation of cholesterol biosynthesis-associated gene expression compared to oligodendrocytes of non-carriers³¹. Interestingly, this APOE4 effect was gene dose-dependent³¹. Similarly, APOE4 hiPSC-derived oligodendrocytes displayed increased cholesterol synthesis and intracellular cholesterol storage compared to APOE3 oligodendrocytes³¹. The APOE4 oligodendrocytes also had reduced cholesterol localization in the plasma membrane, increased cholesteryl esters and lipid droplets, and elevated endoplasmic reticulum (ER) stress³¹. When co-cultured with hiPSC-derived neurons, APOE4 oligodendrocytes displayed less myelin basic protein (MBP) and axonal colocalization than their APOE3 counterparts³¹ (Fig. 5b). Furthermore, aged *APOE4-KI* mice showed fewer cells in the oligodendrocyte lineage and an abnormal accumulation of oligodendrocyte precursors, suggesting that APOE4 disrupts oligodendrocyte differentiation and maturation¹⁷³. Indeed, neural stem/progenitor cell differentiation into oligodendrocytes is affected by interactions between APOE and LDLR-related protein-1¹⁷⁴. Together, these findings raise the possibility that alterations in the differentiation and cholesterol metabolism of oligodendrocytes may causally contribute to the link between APOE4 and AD (Fig. 5b), although this hypothesis requires further testing.

APOE4 in disease-associated oligodendrocyte subtypes

snRNA-seq analyses have identified subpopulations of oligodendrocytes that are enriched in brains of patients with neurodegenerative diseases and related mouse models, termed disease-associated oligodendrocytes (DAO)¹⁷⁵ (Fig. 5b). In a variety of pathological conditions, including animal models of multiple sclerosis, amyloidosis, tauopathy and aging brains, DAO show increased expression of immune-related genes¹⁷⁵, suggesting neuroinflammation as a common driver of DAO formation¹⁷⁵.

Selective neuronal ablation of APOE in PS19-APOE4 mice identified a specific subtype of DAO (neuronal APOE4-promoted DAO or nE4-DAO)²⁴. nE4-DAO had increased expression of genes encoding APOE, heat shock protein, calmodulin, or ubiquitin B, and reduced expression of genes encoding MBP or myelin-associated oligodendrocyte basic protein, as compared to other oligodendrocyte subpopulations²⁴. The number of nE4-DAO was negatively related to hippocampal volume and MBP coverage area and positively related to the extent of tau pathology²⁴. Removal of neuronal APOE4 diminished the number of nE4-DAO, demonstrating their dependence on neuronal APOE4 effects. Interestingly, the protective APOE-R136S mutation markedly reduced the number of DAO in PS19-APOE4 mice²⁸.

Taken together, these findings suggest that oligodendrocytes respond to APOE4 effects from other types of brain cells, especially neurons (Fig. 3c), but are also directly impacted by

the APOE isoforms that they express (Fig. 5b). The detrimental effects of APOE4, either directly or indirectly, on oligodendrocytes can lead to myelin impairments in AD.

Vascular cell APOE4 in AD pathogenesis

Expression and regulation of APOE in vascular cells

Multiple vascular cell types, including endothelial cells, pericytes, and smooth muscle cells, express APOE^{39,176–182} (Fig. 5c). Several transcription factors, including C/EBP β and NFAT, may regulate APOE expression in these cells¹⁷⁸. Pericytes seem to secrete large amounts of APOE that they produce¹⁸¹ and are located in a position in which they can affect the neurovascular junction. Interestingly, both cultured APOE4 hiPSC-derived pericytes and pericytes in the hippocampus of human APOE4 carriers expressed higher levels of APOE than APOE3 pericytes¹⁷⁸. Though APOE from vascular cells has been studied much less than APOE produced by other CNS cell types, it is possible that it may also affect brain health and disease in an isoform-dependent manner (Fig. 5c,d).

Vascular cell APOE4 in stress and AD

APOE4 produced by vascular cells has detrimental effects on endothelial cell function, pericyte mobility, A β -driven cytotoxicity and BBB integrity^{176–178,183} (Fig. 5d). Human endothelial cells co-cultured with pericytes from *APOE4*-KI mice exhibited reduced extracellular matrix protein production, impaired formation of normal tube-like structures and decreased barrier formation compared to those cultured with APOE3 pericytes¹⁸³. In cultures of primary human brain vascular pericytes in which endogenous APOE3 was 90% knocked down via small interfering RNA treatment, addition of exogenous APOE3 led to typical levels of pericyte migration, whereas cells treated with APOE4 had aberrantly high pericyte mobility¹⁷⁶. Conceivably, this effect could contribute to BBB dysfunction in AD, as the BBB becomes increasingly vulnerable when pericytes migrate away towards sites of pathology¹⁷⁶. By increasing pericyte mobility, APOE4 may promote leakiness of this important barrier. In addition, APOE4 increases and activates the BBB-degrading pro-inflammatory cyclophilin A (CYPA)–matrix metalloproteinase 9 (MMP9) pathway in pericytes, leading to breakdown of the BBB via degradation of BBB tight junction proteins^{36,184}. Increased activity of this pathway in the CSF of human APOE4 carriers was associated with high baseline levels of soluble platelet-derived growth factor receptor beta (PDGFRB), a marker of pericyte injury linked to BBB breakdown, and this was predictive of future cognitive decline³⁵.

APOE isoforms may also differentially affect the susceptibility of pericytes to A β (Fig. 5d). When treated with exogenous A β , primary human APOE4-expressing brain pericytes showed higher levels of cell death than their APOE3 counterparts¹⁷⁷. Similarly, in a 3-dimensional in vitro BBB model that included hiPSC-derived pericyte-like cells, amyloid deposition was highly elevated only in cultures containing APOE4 pericytes¹⁷⁸. Inhibition of NFAT–calcineurin signaling in pericytes decreased their APOE4 expression and reduced amyloid accumulation in this model¹⁷⁸. These findings suggest a role for pericyte-derived APOE4 in cerebral amyloid angiopathy (CAA) and pinpoint calcineurin–NFAT signaling as a potential therapeutic target. APOE4 hiPSC-derived endothelial cells exhibited increased

binding of platelets and higher secretion of cytokines and A β compared to endothelial cells expressing APOE3¹⁸⁰, indicating that APOE4 may exert detrimental effects also on this cell type (Fig. 5d).

Beyond these in vitro models, human APOE4 and APOE3 have been expressed in *ApoE*-KO mice under the control of the smooth muscle cell-specific promoter SM22alpha in vivo¹⁸⁵. In this model, smooth muscle cell-produced APOE4 reduced cerebral blood flow, decreased synaptic plasticity, and increased astrocyte reactivity¹⁸⁵. These mice also exhibited increased anxiety-like behavior and impaired spatial learning¹⁸⁵.

APOE4 in disease-associated vascular cell subtypes

In a transcriptomic study, pericytes demonstrated a number of differentially expressed genes in APOE4 versus APOE3 mice at young and old ages³⁴. These included genes critical to cell adhesion, BBB function, inflammatory response, DNA damage and apoptosis³⁴. Transcriptomic and protein analyses of hiPSC-derived pericyte-like cells revealed that, relative to APOE3, APOE4 was associated with increased expression of genes related to protein targeting to the membrane and endoplasmic reticulum and decreased expression of genes related to mitosis and cell cycle progression¹⁷⁸. In post-mortem brain tissues from individuals with AD, pericytes showed altered expression of genes related to blood flow and vasoconstriction¹⁸², while pericytes from APOE4 carriers also had downregulation of genes involved in cell migration regulation, transport across the BBB, and cell junction maintenance¹⁸⁶. In contrast to non-carriers, endothelial cells of APOE4 carriers had increased expression of interferon genes¹⁸², and decreased expression of genes related to transport across the BBB and cell junction organization¹⁸⁶. In conjunction with the findings from cell culture and animal models, these single-cell data suggest that vascular cell APOE4 could contribute to AD pathogenesis through multiple mechanisms (Fig. 5d).

Cell type-specific APOE4 cascade model of AD

Based on the literature reviewed above, it is evident that, in AD pathogenesis, the APOE4 produced in each of several CNS cell types (astrocytes, neurons, microglia, oligodendrocytes and vascular cells) exerts distinct roles, probably in a spatiotemporally specific and interactive manner.

Thus, we propose a cell type-specific APOE4 cascade model (Fig. 6) to help explain how the different cellular sources of APOE4 may act in a stepwise and interactive manner to drive AD pathogenesis. During the first stage (neuronal initiation), stress and aging trigger increased APOE4 expression in neurons, leading to neuronal dysfunction and the appearance of nE4-DAN. The dysfunctional neurons may start to overproduce and release proinflammatory molecules, such as MHC1 molecules and HMGB1. Simultaneously, dysfunctional neurons may also increase their A β production and secretion and accumulate pathological p-tau intracellularly. During the second stage (glial response), astrocytes and microglia become activated in response to proinflammatory molecules released by dysfunctional neurons and to the elevated levels of pathological A β and p-tau assemblies. As this stage evolves, disease-associated glia (such as DAA, DAM, LDAM, IRM and MHC2-M) in which APOE expression and secretion are increased start to appear, disrupting

the BBB and further promoting the formation and spread of A β and tau pathologies. The combination of these processes, in concert with elevated APOE4 expression in neurons, oligodendrocytes and vascular cells, ultimately triggers the third stage (degeneration), which is characterized by the loss of synapses, neurons, and myelin. These cellular impairments and degeneration further augment the expression of APOE4 in neurons and other cell types, promoting vicious cycles and ultimately leading to the fourth stage (AD dementia).

Additional studies are needed to further test this model and to address the following questions: How is APOE4 expression regulated in neurons, astrocytes, microglia, oligodendrocytes and vascular cells? What protease is responsible for neuron-specific APOE4 cleavage? How does APOE4 in dysfunctional neurons promote the neuronal production and release of proinflammatory molecules? How exactly do the distinct cellular sources of APOE4 affect amyloid and tau pathologies? What are the mechanisms underlying the formation of disease-associated glial subtypes? To what extent is the emergence of these glial subtypes pathogenic or adaptive? Do they cause neurodegeneration, BBB impairments, and oligodendrocyte and/or myelin deficits? Answering these questions will help further elucidate the cell type-specific roles of APOE4 in AD pathogenesis and foster the development of novel therapeutics for this challenging condition.

Potential therapeutics targeting APOE4

Although there has been progress on AD therapeutic development with the recent US Food and Drug Administration (FDA) approval of Lecanemab and Aducanumab (anti-A β monoclonal antibodies), APOE4 carriers have unfortunately been found to be at high risk for developing serious side effects, such as amyloid-related imaging abnormalities (ARIA), to these therapies^{187,188}. In addition, *APOE* genotype seems to also differentially modulate the amyloid lowering efficacy and microglial response to anti-A β immunotherapy in an AD mouse model¹⁸⁹. Given the high proportion of APOE4 carriers in the AD population, there is an urgent need to devise therapeutic strategies that take into account the APOE4 carrier status, such as therapies directly targeting the detrimental effects of APOE4 expression.

A key question in developing APOE4-targeting therapies has been and continues to be whether APOE4 differs from the other APOE isoforms primarily through loss-of-function mechanisms or through gain-of-function mechanisms in AD-relevant contexts. In hiPSC-derived neuronal cultures, APOE-deficient neurons resembled those expressing APOE3, whereas APOE4-expressing neurons showed a range of AD-relevant pathological phenotypes, suggesting an adverse gain-of-function at least for APOE4 produced by neurons⁸⁵. In vivo studies also support a gain-of-function mechanism for APOE4 produced by neurons or other cell types. Genetically reducing APOE4 by 50% markedly lowered amyloid pathology in mutant human APP transgenic mice with hemizygous APOE4 expression^{190,191}. Furthermore, ablation of APOE4 specifically in neurons or astrocytes protected against tau pathology, gliosis, and degeneration in PS19-APOE4 mice^{24,43}. Human case studies also support the conclusion of predominant gain-of-function effects of APOE4. For example, a recent preprint has reported that an APOE3/4 heterozygote with the *APOE4* allele-specific loss-of-function mutation did not develop AD at old age¹⁹² and an older study reported a similar effect in one person with complete APOE deficiency¹⁹³. Likewise,

genetic studies revealed that APOE4 carriers with European ancestry have higher APOE4 expression levels in various types of CNS cells than those with African ancestry and, interestingly, the former have a relatively higher AD risk than the latter^{194,195}. Together, these findings highlight the potential therapeutic benefit of reducing APOE4 levels. Indeed, anti-APOE immunotherapies and antisense oligonucleotides (ASO) targeting APOE are in development. In amyloidosis mouse models, such therapies efficiently inhibited the formation of amyloid plaques when initiated before the onset of pathology^{196,197}. Treatment with an APOE-ASO also reduced tau pathology, neuroinflammation, and neurodegeneration in a tauopathy mouse model¹⁹⁸.

Based on the cascade model we proposed above (Fig. 6), one could also envision directing therapeutic interventions at APOE4 in specific cell types, which may yield more precise outcomes and help avoid unexpected side effects from broader APOE reductions (Box 1). Potential AD-relevant targets include neuronal APOE4 and its proteolytic cleavage as well as the APOE4-dependent neuronal production and release of proinflammatory molecules, astrocytic and microglial APOE4, and oligodendrocytic or vascular cell APOE4. The rapidly evolving development of viral vectors and gene editing [G] approaches^{199,200} may also enable the isoform-specific deletion or conversion of APOE4 in neurons or other CNS cell types. To monitor the effects of APOE4-targeting therapies and for the stratification of AD patient populations in general, it will also be important to develop better APOE-related biomarkers. Potential examples include the detection and quantification of neuron-derived APOE4 fragments and of APOE4-induced molecules released from specific CNS cell types, such as MHC1 molecules and HMGB1, in the CSF and plasma.

Summary

Recent scientific advances have revealed the importance of cell type-specific roles of APOE4 in AD pathogenesis. APOE4 produced in various CNS cell types, such as neurons, astrocytes, microglia, oligodendrocytes, and vascular cells, exert differential effects on amyloid and tau pathology, altered lipid metabolism, glial reactivity, neurodegeneration, myelin deficits, and blood-brain barrier dysfunction, likely in a spatiotemporal and interactive manner. The cell type-specific APOE4 cascade model that we outline above poses neuronal APOE4 as a critical upstream initiator and driver of AD pathogenesis, instigating glial response and subsequent degeneration. This model also indicates that targeting specific cellular sources of APOE4 may provide new therapeutic strategies for the prevention or treatment of AD.

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Glossary:

APOE fragments

Neurotoxic fragments of the APOE protein that are generated as a result of APOE expression in neurons, with more fragments being generated from APOE4, due to its unique conformation, than from APOE3 via neuron-specific proteolysis

Cytokines

Classes of small secreted proteins that serve to signal and activate immune functions, including triggering of glial responses in the CNS

Dementia

a general term for loss of memory, language, problem-solving and other thinking abilities that are severe enough to interfere with daily life. Diseases grouped under the general term dementia are caused by abnormal brain changes, with Alzheimer disease being the most common cause

Disease-associated gene signatures

refers to specific patterns or sets of genes that are consistently found to be altered or expressed differently in a disease context compared to physiological contexts. These gene signatures are often identified through various transcriptomic analyses, such as microarray or RNA sequencing experiments

Exosome

small, membrane-bound vesicles that are released by cells into the extracellular environment. These vesicles play a crucial role in intercellular communication by facilitating the transfer of various molecules between cells

Gene editing

a type of genetic engineering by which specific changes can be made to DNA, including inserting, deleting, or altering DNA sequences

Gliosis

the proliferation or hypertrophy of microglia and/or astrocytes in response to stressors, insults, injury, or diseases

Human induced pluripotent stem cells (hiPSCs)

a type of pluripotent stem cell derived from adult somatic cells that have been genetically reprogrammed to an embryonic stem (ES) cell-like state through the forced expression of genes and factors important for maintaining the defining properties of ES cells

Lysosomes

Lysosomes are membrane-bound organelles that are part of the endo-lysosomal system within cells and play a crucial role in cellular digestion and waste removal. Lysosomes contain various enzymes that are capable of breaking down different types of macromolecules, including proteins, lipids, nucleic acids, and carbohydrates

Mild cognitive impairment (MCI)

an early stage of memory loss or other cognitive ability loss (such as language or visual/spatial perception) in individuals who maintain the ability to independently perform most

activities of daily living. For neurodegenerative diseases, such as Alzheimer disease, MCI can be an early stage of the disease continuum

Pericytes

contractile cells located at intervals along the walls of capillaries, playing a role in blood vessel formation, maintenance of the blood–brain barrier, regulation of immune cell entry to the central nervous system and control of brain blood flow

Single-nucleus RNA-sequencing (snRNA-seq)

a molecular biology technique used to analyze the gene expression profiles of individual cell nuclei within a complex tissue or organ. Traditional RNA sequencing methods usually require intact cells, but snRNA-seq allows researchers to study gene expression in individual nuclei, providing insights into cellular diversity and heterogeneity within tissues

Tauopathy

refers to a group of clinically, biochemically, and morphologically heterogeneous neurodegenerative diseases, including Alzheimer disease, characterized by the abnormal hyperphosphorylation of microtubule-associated protein, tau, that leads to the formation of neurofibrillary tangles in the brain

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Box 1:**Cell type-specific therapeutic APOE4 targeting: strategies, advantages and challenges.**

Targeting the E4 isoform of apolipoprotein E (APOE4) produced by neurons may be an effective way to reduce Alzheimer disease (AD) risk (Fig. 6b) because neuronal APOE4 promotes all major pathological hallmarks of AD^{24,75,76,85,98,125} and may also drive APOE4 expression in other cell types²⁴. Reducing neuronal APOE4 through targeted gene editing, neuron-specific CRISPR-interference (CRISPRi) delivery or by blocking neuronal APOE4 proteolysis could limit the initiation of the pathological cascade shown in Fig. 6. To have the greatest therapeutic impact, however, suppression of neuronal APOE4 would probably have to begin in early stages of disease development. Highlighting the potential of targeting neuronal APOE4, treatment of APOE4-expressing human induced pluripotent stem cell (hiPSC)-derived neurons with an APOE4 structure corrector^{201,202} decreased neurotoxic APOE4 fragments, p-tau levels, and amyloid β ($A\beta$) production and secretion and reduced the loss of GABAergic neurons in a dose-dependent manner⁸⁵. In an in vivo study, selective removal of neuronal APOE4 protected against tau pathology, gliosis, neuronal dysfunction, neurodegeneration and oligodendrocyte and/or myelin deficits in PS19-APOE4 mice²⁴. Additionally, targeting neuronal APOE4-mediated inhibitory neuron loss, especially somatostatin (SST)-expressing interneuron loss, by stem cell-based replacement therapy is another avenue to pursue²⁰³.

Targeting APOE4-induced neuronal production or release of proinflammatory molecules, such as high mobility group box 1 (HMGB1) and major histocompatibility complex class 1 (MHC1) molecules, should also be considered, as this strategy could block downstream glial responses to APOE4-dependent neuronal dysfunctions and pathologies and prevent the escalation of pathogenic neuroinflammatory processes. Relevant therapeutics could be monoclonal antibodies or small molecule inhibitors.

Targeting astrocytic APOE4 could also prove to be valuable, as astrocytic APOE4 correlates with gliosis, $A\beta$ accumulation, blood-brain barrier (BBB) vulnerability, and neurodegeneration^{43,51,54,70–74}. However, astrocytic APOE4 does not seem to exhibit a correlation to tau pathology or neuronal dysfunction that is as strong as that of neuronal APOE4 and does not seem to contribute to neural network hyperexcitability or myelin deficits. There is also evidence that reducing astrocytic APOE4 might increase cerebral amyloid angiopathy (CAA)⁴⁴. Additionally, since astrocytes normally produce and secrete high levels of APOE that serves a host of homeostatic functions, it is conceivable that targeting astrocytic APOE4 could result in some side effects, which warrants further studies.

Targeting microglial APOE4 may counteract the progression of tau and amyloid pathologies as well as inflammation-related neurodegenerative processes. Like neurons, microglia increase their APOE4 production in response to stress and disease^{39,47,138–140}. Although microgliosis appears to happen downstream of neuronal APOE4 effects, targeting microglial APOE4 and inflammation could synergize with strategies targeting

neuronal APOE4. Targeting oligodendrocytic APOE4 to protect against oligodendrocyte impairment and myelin loss, or targeting vascular cell APOE4 to alleviate pericyte and vascular dysfunction and BBB disruption, could also be beneficial.

With recent advances in gene editing technologies, gene therapy should also be considered to target APOE4, including correction of APOE4 to APOE3, APOE2, APOE-R136S, or APOE-KO in a cell type-specific manner. Since APOE2 markedly reduces AD risk compared to both APOE4 and APOE3^{204–206}, adeno-associated viruses have been used to deliver this isoform into the brains of APP/PS1/APOE4 mice, which reduced amyloid plaque burdens²⁰⁷. Further studies are warranted to investigate how increasing APOE2 expression in specific CNS cell types affects other key AD pathologies, including tau pathology and neurodegeneration. In a similar vein, understanding the mechanisms through which rare protective variants of APOE, such as APOE-R136S, confer protection against AD pathology could also lead to the development of novel therapeutic approaches²⁰⁸.

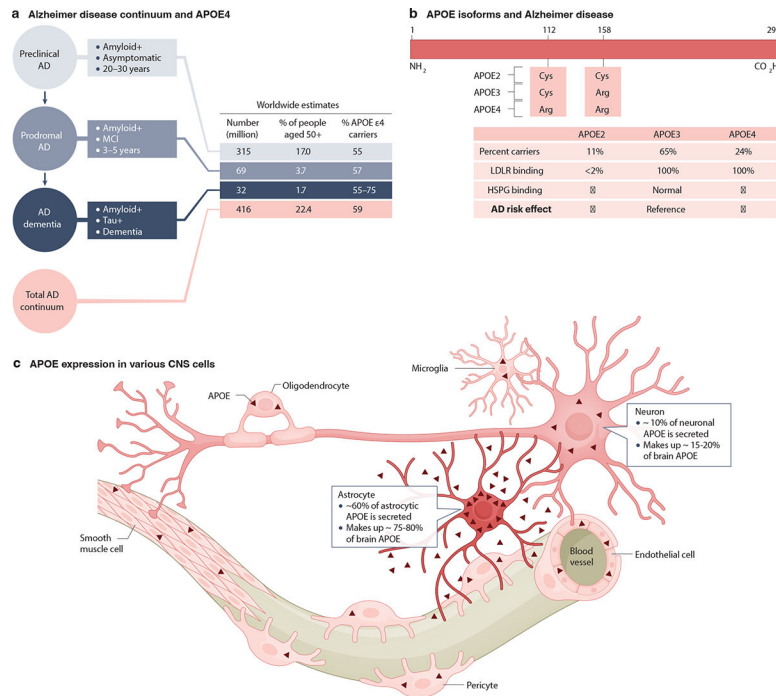


Fig. 1: CNS APOE expression and links to Alzheimer disease.

a, An overview of Alzheimer disease (AD) continuum³⁻⁵, worldwide estimates of numbers of individuals with preclinical AD, prodromal AD and AD dementia⁶, and their relationship with the E4 isoform of apolipoprotein E (APOE4)⁶. **b**, There are three common APOE isoforms: APOE2, APOE3, and APOE4^{1,11}. They exhibit sequence differences at amino acid residues 112 and 158¹², differential receptor binding properties¹³ and varying impacts on AD risk^{6,10,14-17}. Downward-pointing arrows indicate reduced heparan sulfate proteoglycan (HSPG) binding or AD risk; upward-pointing arrows indicate increased HSPG binding or AD risk. **c**, Multiple cell types in the CNS can produce APOE at different levels (indicated by the depth of red shading) under normal conditions^{1,20,39}. These include astrocytes, neurons, microglia, oligodendrocytes and vascular cells (endothelial cells, pericytes and smooth muscle cells). In studies in which astrocytic APOE was selectively removed in mice, there was a loss of 75-80% of brain APOE levels⁴²⁻⁴⁴, while removing neuronal APOE led to a 15-20% reduction of brain APOE levels^{24,42}. Thus, astrocytic and neuronal APOE seem to account for approximately 75-80%⁴²⁻⁴⁴ and 15-20%^{24,42} of total APOE in the brain, respectively. After production, approximately 60% of astrocytic APOE is secreted into the extracellular space^{47,48}, whereas most APOE produced in neurons remains intracellular⁴⁸. The proportions of total and secreted APOE in the brain that are contributed by microglia, oligodendrocytes and vascular cells have yet to be determined. LDLR, low-density lipoprotein receptor; MCI, mild cognitive impairment.

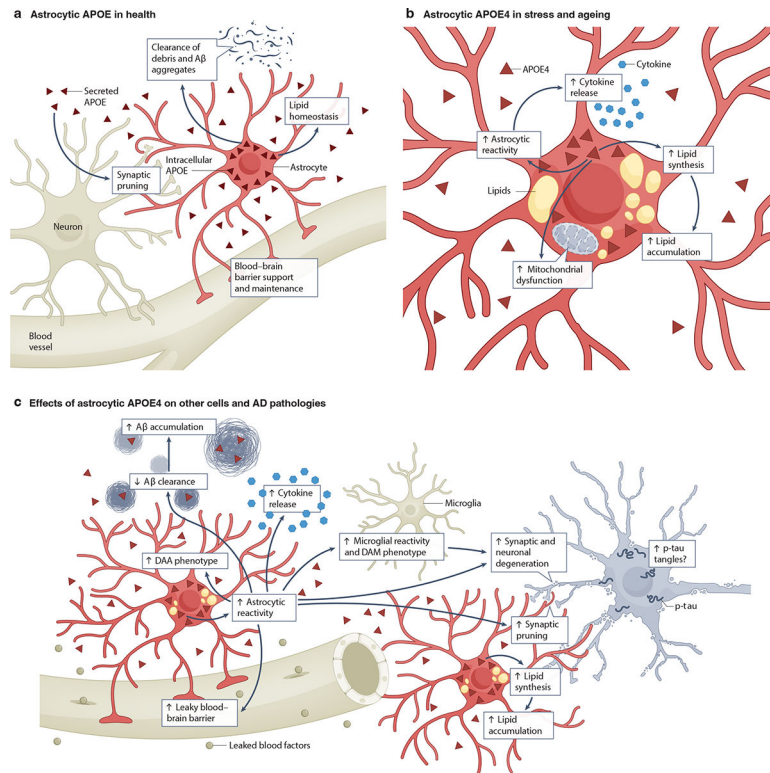


Fig 2: Expression of APOE4 in astrocytes and its roles in AD pathogenesis.

a. Healthy astrocytes produce and secrete most of the apolipoprotein E (APOE) found in the brain^{39–41}. This APOE participates in many astrocyte functions, including synaptic pruning, lipid homeostasis, clearance of amyloid β ($A\beta$) aggregates and cell debris and blood-brain barrier (BBB) support and maintenance^{50–58}. **b.** During stress and aging, astrocytic APOE4 contributes to astrocytic reactivity^{43,50,51,59}, elevated proinflammatory cytokine release^{50,51,59}, augmented mitochondrial dysfunction^{63–66}, and increased lipid synthesis^{31,53–55,60} and intracellular lipid accumulation^{31,51,53–55,60}. **c.** Astrocytic APOE4 also affects other CNS cells and can promote AD pathologies. For example, it drives an increase in microglial reactivity and adoption of a disease-associated microglia (DAM) phenotype⁴³. In addition, it contributes to AD pathogenesis by increasing astrocytic reactivity^{43,50,51,59}, the transition to a disease-associated astrocyte (DAA) phenotype⁷⁸, cytokine release^{50,51,59}, synaptic pruning^{43,52} and lipid accumulation^{31,51,53–55,60}. In turn, these changes may lead to BBB leakiness^{57,58,70}, $A\beta$ accumulation^{51,54,71,74}, tau accumulation^{43,77} and (in conjunction with neuronal APOE4) synaptic and neuronal degeneration⁴³. The potential relationships among the effects of astrocytic APOE4 on other cells and AD pathologies are depicted using arrows. Note that non-astrocytic sources of APOE4 have been omitted from this figure for clarity. p-tau, hyperphosphorylated tau.

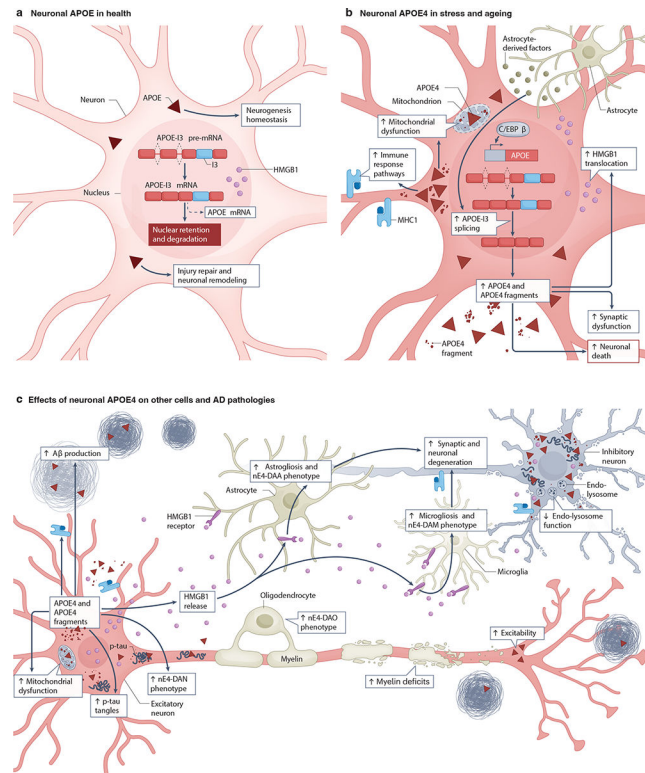


Fig. 3: Expression of APOE4 in neurons and its roles in AD pathogenesis.

a. Healthy neurons express low levels of apolipoprotein E (APOE)^{39,82–89}; the *APOE* mRNA they produce has an intron 3 retention (*APOE-I3* mRNA), which ensures that it is largely retained in the nucleus, where it is degraded, rather than being processed into mature *APOE* mRNA⁹¹. Neurons also retain high mobility group box 1 (HMGB1), a proinflammatory molecule, within the nucleus¹²⁹. Neuronal APOE is involved in the maintenance of neurogenesis⁹⁴, injury repair^{92,93} and neuronal remodeling^{1,95}. **b.** Under conditions of stress and aging, neurons increase APOE expression by active splicing of *APOE-I3* into mature *APOE* mRNA⁹¹. The transcription factor C/EBP β ^{45,98} and astrocyte-derived factor(s)⁴⁸ also upregulate APOE expression in neurons. Increased neuronal APOE4 production leads to increased APOE4 fragmentation^{22,75,85,126,128}, expression of immune response pathway genes (such as major histocompatibility complex class 1 (MHC1) genes)⁹⁰, nucleo-cytoplasmic translocation of HMGB1¹²⁹, mitochondrial dysfunction^{95,109–113}, synaptic dysfunction^{99,101}, and neuronal death^{24,85,90,98,114}. **c.** Neuronal APOE4 affects other CNS cells. For example, it increases astrogliosis and the adoption of a neuronal APOE4-promoted disease-associated astrocyte (nE4-DAA) phenotype²⁴, microgliosis and the adoption of a neuronal APOE4-promoted disease-associated microglial (nE4-DAM) phenotype²⁴, the adoption of neuronal APOE4-promoted disease-associated oligodendrocyte (nE4-DAO) phenotype²⁴ and myelin deficits²⁴. Neuronal APOE4 also increases intraneuronal tau tangles^{24,75,76,85,98}, endo-lysosomal dysfunction^{115,116}, neuronal hyperexcitability^{103–106}, amyloid β (A β) production and accumulation^{85,98}, the adoption of a neuronal APOE4-promoted disease-associated neuronal (nE4-DAN) phenotype²⁴, HMGB1 release (resulting in binding with its receptors on glial cells and consequently inducing gliosis)¹²⁹, neuronal MHC1-mediated microglia engagement⁹⁰ and synaptic

and neuronal degeneration^{24,85,90,98,114}. The potential relationship among these neuronal APOE4 effects on other cells and AD pathologies is depicted using arrows. Note that non-neuronal sources of APOE4 have been omitted for clarity.

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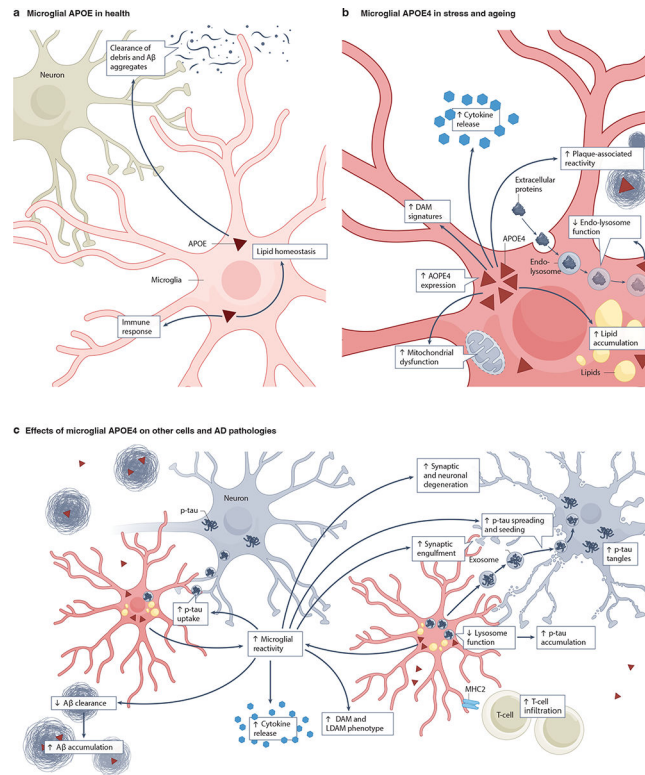


Fig. 4: Expression of APOE4 in microglia and its roles in AD pathogenesis.

a. Healthy microglia express low levels of apolipoprotein E (APOE)³⁹. This APOE is involved in immune responses, lipid homeostasis and in clearing amyloid β ($A\beta$) aggregates and cell debris^{23,53,143}. **b.** During stress and aging, microglia increase their APOE expression^{138–141}. Microglial APOE4 leads to an increase in amyloid plaque-associated reactivity⁵⁴, lipid accumulation^{27,148}, lysosomal defects^{151,152}, mitochondrial dysfunction¹⁴¹, disease-associated microglial (DAM) signatures^{140,167} and proinflammatory cytokine release¹⁶⁷. **c.** Microglial APOE4 affects other cells, including neurons and T cells¹⁶⁶, and contributes to AD pathogenesis by reducing $A\beta$ clearance¹⁵⁷ and lysosome function¹⁵¹ and by increasing synaptic engulfment¹⁶¹, p-tau uptake and accumulation^{86,151,152}, DAM signatures^{140,152}, lipid-droplet-accumulating microglia LDAM^{29,147}, and cytokine release¹⁶⁷. Together, these APOE4-induced microglial dysfunctions facilitate tau spreading and seeding^{162–164} and, ultimately, synaptic and neuronal degeneration (in conjunction with neuronal APOE4)^{140,152}. The potential relationships among these microglial APOE4 effects on other cells and AD pathologies is depicted using arrows. Note that non-microglial sources of APOE have been omitted for clarity.

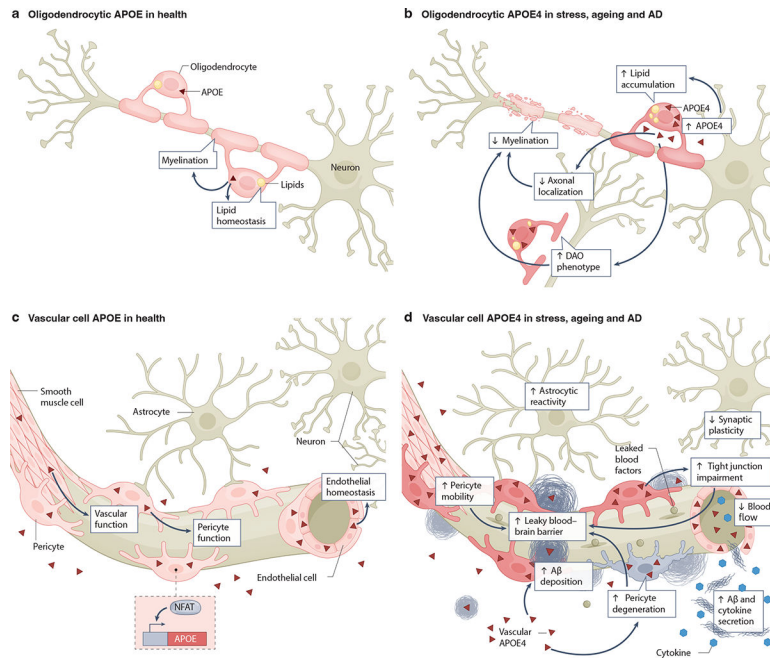


Fig. 5: Expression of APOE4 in oligodendrocytes and vascular cells and its roles in AD pathogenesis.

a, Healthy oligodendrocytes express low levels of apolipoprotein E (APOE)^{24,31,90}. Oligodendrocytic APOE is involved in lipid homeostasis and myelination of neurons³¹. **b**, Under conditions of stress, aging, and AD, oligodendrocytes increase their APOE4 expression^{24,172}. Oligodendrocytic APOE4 increases intracellular lipid accumulation and disease-associated oligodendrocyte (DAO) phenotypes^{24,31} and decreases oligodendrocytic axonal localization and myelination of neuronal axons³¹. The potential relationship among oligodendrocytic APOE4 effects on AD pathologies is depicted using arrows. **c**, Healthy cells of the brain vasculature, including smooth muscle cells, pericytes and endothelial cells, produce and secrete APOE^{39,176–182}. The transcription factor NFAT regulates APOE expression in pericytes¹⁷⁸. Vascular cell-produced APOE is involved in maintaining vascular function, pericyte function and endothelial homeostasis^{176–178,183}. **d**, Under conditions of stress, aging, and AD, vascular cell APOE4 leads to tight junction impairments^{36,183}, astrocyte reactivity¹⁸⁵, increased pericyte migration and degeneration^{176,177}, decreased synaptic plasticity¹⁸⁵, reduced blood flow¹⁸⁵, elevated secretion of amyloid β ($A\beta$) and cytokines¹⁸⁰, increased $A\beta$ deposition in the form of cerebral amyloid angiopathy (CAA)¹⁷⁸, and blood-brain barrier (BBB) leakage^{35,36,176}. The potential relationship among vascular APOE4 effects on other cells and AD pathologies is depicted using arrows. Note that non-oligodendrocytic and non-vascular sources of APOE4 have been omitted for clarity.

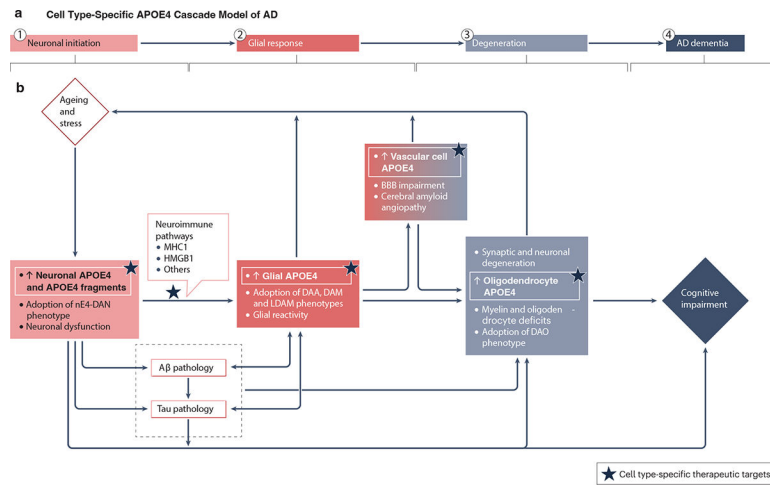


Fig. 6: Cell type-specific APOE4 cascade model of AD and related therapeutic strategies.

a. Overview of the four stages of the cascade model. **b.** During the first stage (neuronal initiation), stress and aging trigger increased neuronal expression of the E4 isoform of apolipoprotein E (APOE4), leading to neuronal dysfunction and the appearance of neuronal APOE4-promoted disease-associated neurons (nE4-DAN). The dysfunctional neurons may start to overproduce and release proinflammatory molecules (such as major histocompatibility complex class 1 (MHC1) molecules and high mobility group box 1 (HMGB1)) and increase their amyloid β ($A\beta$) production and secretion and intracellular accumulation of pathological p-tau. During the second stage (glial response), astrocytes and microglia become activated in response to proinflammatory molecules released by dysfunctional neurons and to the elevated levels of pathological $A\beta$ and p-tau assemblies. As this stage evolves, disease-associated glia (such as disease-associated astrocytes (DAA), disease-associated microglia (DAM), lipid-droplet-accumulating microglia (LDAM), interferon responsive microglia (IRM), and antigen-presenting microglia (MHC2-M)) with increased APOE expression and secretion start to appear, disrupting the BBB and further promoting the formation and spread of $A\beta$ and tau pathologies. The combination of these processes, in concert with elevated APOE4 expression in neurons, oligodendrocytes, and vascular cells, ultimately triggers the third stage (degeneration), which is characterized by the loss of synapses, neurons, and myelin. These cellular impairments and degeneration further augment the expression of APOE4 in neurons and other cell types, promoting vicious cycles and, ultimately, leading to the fourth stage (AD dementia). Based on the proposed cascade model, cell type-specific APOE4-guided AD therapeutic targets are highlighted, as indicated by stars.