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# Apolipoprotein E and Alzheimer's Disease: Findings, Hypotheses, and Potential Mechanisms

Nicole Koutsodendris,[1,2,*] Maxine R. Nelson,[2,3,*] Antara Rao,[1,2] and Yadong Huang[1,2,3,4]

[1]Developmental and Stem Cell Biology Graduate Program, University of California, San Francisco, California 94131, USA; email: Nicole.koutsodendris@gladstone.ucsf.edu, Yadong.huang@gladstone.ucsf.edu

[2]Gladstone Institutes of Neurological Disease, San Francisco, California 94158, USA

[3]Biomedical Sciences Graduate Program, University of California, San Francisco, California 94143, USA

[4]Department of Neurology, University of California, San Francisco, California 94158, USA

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*These authors contributed equally to this article.



## Keywords

Alzheimer's disease, apoE, biomarker, Aβ, tau, neuroinflammation, neural network deficit

## Abstract

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder that involves dysregulation of many cellular and molecular processes. It is notoriously difficult to develop therapeutics for AD due to its complex nature. Nevertheless, recent advancements in imaging technology and the development of innovative experimental techniques have allowed researchers to perform in-depth analyses to uncover the pathogenic mechanisms of AD. An important consideration when studying late-onset AD is its major genetic risk factor, apolipoprotein E4 (apoE4). Although the exact mechanisms underlying apoE4 effects on AD initiation and progression are not fully understood, recent studies have revealed critical insights into the apoE4-induced deficits that occur in AD. In this review, we highlight notable studies that detail apoE4 effects on prominent AD pathologies, including amyloid-β, tau pathology, neuroinflammation, and neural network dysfunction. We also discuss evidence that defines the physiological functions of apoE and outlines how these functions are disrupted in apoE4-related AD.

## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by extensive brain atrophy and loss of cognitive functions. As a progressive disorder, AD is thought to begin decades before symptom onset (1). It is only after a considerable amount of damage occurs to vital biological components that clinicians are able to detect even the earliest symptoms of AD. Although researchers cannot conclusively pinpoint the initiating step that triggers a cascade of detrimental processes, there is a substantial body of scientific work that has identified key players in AD pathogenesis.

According to epidemiological and genome-wide association studies, apolipoprotein E4 (apoE4) is the single greatest genetic risk factor for late-onset AD (**Figure 1*a***) (2, 3). The human apolipoprotein E (*APOE*) gene has three alleles, ε2, ε3, and ε4, which encode three apoE protein isoforms, apoE2, apoE3, and apoE4 (4). In terms of AD risk, apoE3 is considered neutral, whereas apoE2 is protective and apoE4 is detrimental (5). The presence of the *APOE ε4* allele dose-dependently increases the risk of AD and lowers the age of onset (2, 5). The lifetime risk estimate of developing AD by the age of 85 is ∼10% in apoE3 homozygotes, ∼30% in apoE4 heterozygotes, and ∼65% in apoE4 homozygotes (6). ApoE4 homozygosity also decreases the mean age of onset for AD from 84 to 68 years old, emphasizing the importance of apoE4 in AD pathogenesis (2).

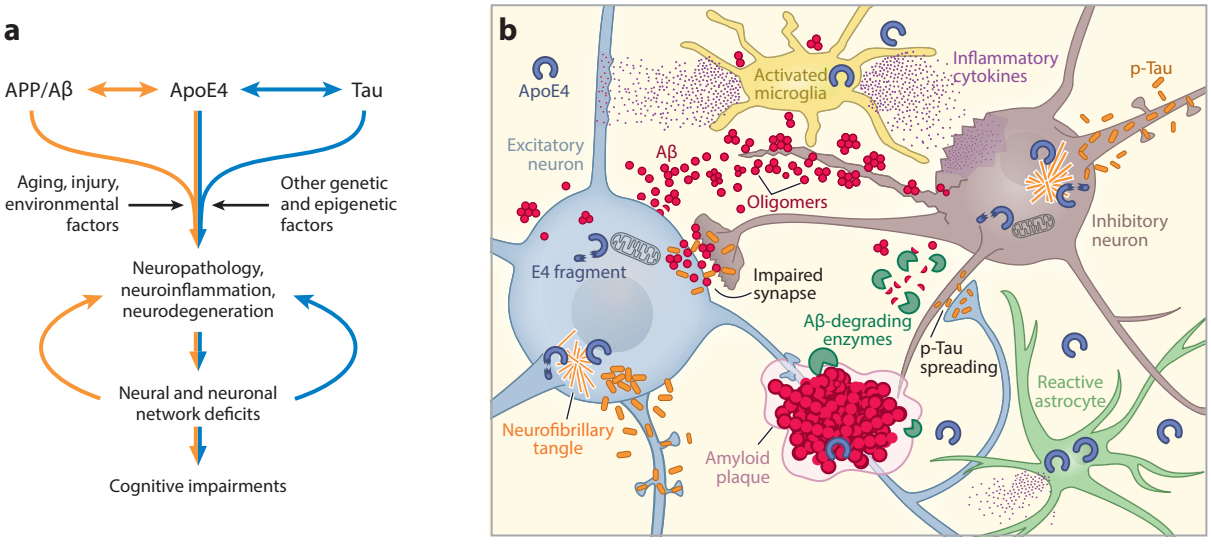



**Figure 1**

Multifactorial etiology and diverse pathologies of AD. (*a*) There are several key contributing factors that play major roles in AD pathogenesis, including apoE4, Aβ, and tau. In combination with other comorbidities, these three factors work in concert to contribute to a pathogenic cascade that disrupts a myriad of biological processes and results in neuropathology, neuroinflammation, and neurodegeneration. This leads to neural or neuronal network deficits and, ultimately, clinically observable cognitive decline. How exactly apoE4 conspires with Aβ and tau to elicit AD pathogenesis and clinical dementia remains to be determined. (*b*) The diverse AD pathologies include extraneuronal aggregation and accumulation of Aβ peptides and formation of amyloid plaques; intraneuronal tau hyperphosphorylation, aggregation, mislocalization, and spread to connected neurons and formation of neurofibrillary tangles; and responsive neuroinflammation mediated by astrocytes and microglia that release proinflammatory cytokines. ApoE4 worsens Aβ and tau pathologies and promotes the detrimental activation of glial cells. ApoE4 also exhibits gain-of-toxic-function effects when aberrantly cleaved into truncated fragments in neurons, causing mitochondrial dysfunction, tau pathology, and neuronal death. Figure adapted from Reference 7. Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; apoE4, apolipoprotein E4; APP, amyloid precursor protein; p-tau, phosphorylated tau.

A defining feature of AD is the presence of two distinct pathological hallmarks: extracellular amyloid-beta (Aβ) plaques composed of aggregated Aβ peptides and intraneuronal neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein (**Figure 1*b***). Since the discovery of these characteristic pathologies by Alois Alzheimer in 1906, Aβ plaques and NFTs have been at the center of AD diagnostics. Mounting evidence has shown that apoE4 significantly affects both of these pathologies, in addition to many other important cellular and molecular processes within the central nervous system (CNS) (7). This review gives prominence to some of the most noteworthy discoveries in apoE4-related AD research and calls attention to outstanding questions about the pathogenic mechanisms of AD.

## PATHOLOGICAL HALLMARKS, BIOMARKERS, AND GENETIC RISK FACTORS OF ALZHEIMER'S DISEASE

### Neuropathological Hallmarks and Biomarkers of Alzheimer's Disease

Traditionally, postmortem neuropathological examination is considered the gold standard for a diagnosis of AD. Recent advances in radiological imaging technology have provided important tools for diagnosing AD by allowing detection of protein lesions within living patients' brains. The development of selective amyloid radiotracers, such as $^{11}$C-Pittsburgh Compound B and $^{18}$F-florbetapir, allows detection of Aβ plaques in patients using positron emission topography (PET) imaging (8, 9). In AD patients, amyloid accumulation initially occurs diffusely in the neocortex and then spreads to the medial temporal lobe (10), which contains structures important for cognitive function such as the hippocampus and entorhinal cortex (11). Longitudinal studies illustrate that amyloid-PET scans are useful to detect early, preclinical AD prior to symptom onset (12). A high amyloid burden in cognitively normal individuals is associated with progression to symptomatic AD roughly 2.4 years later (13). These findings were further validated in a large cross-sectional study of asymptomatic older individuals; the study found that elevated amyloid levels are significantly associated with high risk factors for AD, such as age, the *APOE ε4* allele, and family history of dementia (14). This opens the possibility of a preventative clinical trial aimed at slowing cognitive decline during the preclinical stages of AD based on amyloid-PET scans of high-risk asymptomatic individuals. Still, there have been increasing concerns regarding the accuracy of using Aβ as a biomarker for AD, as ∼30% of cognitively normal elderly individuals have Aβ deposition in their brains (15).

Radiological imaging of tau has been made possible in recent years with the development of selective tau tracers, such as THK5117, $^{18}$F-flortaucipir, and $^{18}$F-AV-1451. Comparison of antemortem $^{18}$F-flortaucipir PET to postmortem neuropathology shows that this tau tracer reliably detects end-stage Braak VI tau pathology in advanced AD (16). PET scan studies using tau tracers have found that the pathological aggregation of tau is closely linked to cognitive decline and onset of neurodegeneration in AD patients (17, 18). Unlike the diffuse accumulation of amyloid throughout the neocortex, tau has been shown to spread throughout the brain in a stepwise fashion that tightly correlates with the patterns of neurodegeneration and the manifestations of clinical symptoms of AD (10, 18, 19). Tau accumulation in the anterior-temporal regions of the brain was found to be the most predictive measure of subsequent memory decline in individuals with a high Aβ burden, indicating that tau plays a primary role in memory decline in the early stages of AD (20). Interestingly, a longitudinal study found that the global intensity of the tau-PET signal predicted the degree of brain degeneration 15 months later and that the specific distribution of the tau-PET signal strongly indicates the topography of future atrophy at the individual patient level (21). Taken together, PET imaging studies on human patients using amyloid and tau

radiotracers have shown that amyloid and tau are useful biomarkers for AD diagnosis, although tau is emerging as a more precise biomarker than Aβ since it more accurately follows disease onset and progression. Notably, many patient data sets that are collected and utilized by researchers define AD status in part by these biomarker measurements; thus, the accuracy of these biomarker measurements is critically important for AD diagnostics and research.

Diagnostic testing of AD biomarkers has extended beyond radiological imaging to include cerebrospinal fluid (CSF) biomarkers. Proteolytic cleavage of amyloid precursor protein (APP) produces an assortment of Aβ peptides with varying significance to AD pathology. The accumulation of the Aβ 42 peptide ($A\beta_{42}$) is hypothesized to be the initial trigger of neurodegeneration and is used as a CSF biomarker to diagnose AD (22). Assessment of $A\beta_{42}$ in the CSF predicts future development of AD in patients with mild cognitive impairment (MCI) (23). Although the amount of $A\beta_{40}$ alone has no impact on the disease, the ratio of $A\beta_{42}/A\beta_{40}$ in the CSF is useful for discriminating AD (24, 25).

Measurement of tau in the CSF is also used as a diagnostic test for AD. As a phosphoprotein, tau protein has more than 80 potential phosphorylation sites (26). When tau has significantly more phosphorylation than physiological levels, it is categorized as hyperphosphorylated tau (26). Increased phosphorylation of tau at several AD-linked residue sites is used as a biomarker in diagnostic testing. These phosphorylation sites include threonine 181, 217, and 231 and serine 199, 231, 396, and 404 (27–33). In particular, phosphorylation of tau at threonine 181 is the gold standard for tau CSF biomarkers that are used to diagnose AD (30–32). However, a recent study using quantitative mass spectrometry demonstrated that phosphorylation at threonine 217 may be a more sensitive marker (33).

Beyond these two traditional AD hallmarks, assessment of brain activity using functional magnetic resonance imaging (fMRI) is proving to be valuable in AD diagnostics. This imaging technique takes advantage of the fascinating discovery that cerebral blood flow and neuronal activation are coupled, as blood flow to a particular brain region increases when it is in use (34). On the basis of recent fMRI studies, neural network connectivity appears to be disrupted in AD. There is an age-dependent reduction in connectivity between the entorhinal cortex and the hippocampus, and these functional alterations correlate with memory deficits (35). Furthermore, AD is associated with loss of global information integration, causing a decrease of functional long-distance links between frontal and caudal brain regions (36). There is also an intriguing relationship between functional connectivity, amyloid burden, and tau pathology. Early amyloid accumulation is associated with increased functional connectivity, whereas increasing tau pathology is correlated with a progressive decline in functional connectivity (37). Comparison of cognitively normal individuals to patients with MCI and AD shows that the early prodromal phase of AD initiates with increased activation of the medial temporal lobe and is followed by a subsequent decrease in activation as the disease progresses (38). These insightful studies illustrate the utility of neural network activity as a potential biomarker for AD. It is worth considering that combining PET imaging of AD pathologies with fMRI data could improve the accuracy of AD diagnostics, although further studies are needed to determine the validity of this hypothesis.

Neuroinflammation, including astrocytic and microglial gliosis, is another pathological feature of AD (**Figure 1*b***) and is emerging as a useful AD biomarker. Many studies have illustrated that neuroinflammation is an important contributing factor to AD development (39). Early evidence shows that activated microglia (40) and astrocytes (41) colocalize with senile plaques in AD patients and that the number of activated astrocytes in the parahippocampal cortex of AD patients correlates with the number of tangles and the stage of NFT formation (42). A PET study using a marker of activated microglia, [11C](R)PK11195, found a significant 20–35% increase in microglial activation in AD patients (43). Analysis of CSF from AD patients showed increased

levels of several inflammatory molecules (44). However, more studies are needed to fully characterize neuroinflammatory-related changes that occur in AD and to determine their usefulness as biomarkers for AD diagnosis and research.

## The Impact of ApoE4 on Alzheimer's Disease Biomarkers and Neurodegeneration

Longitudinal brain imaging studies have provided invaluable insight into the effects of apoE4 on neurodegeneration in human patients. Using structural MRI, apoE4 carriers demonstrate accelerated hippocampal volumetric loss in early life and accelerated cortical atrophy in midlife (45). Moreover, *APOE ε4* exerts a gene-dosage effect that correlates with the severity of hippocampal morphological deformation (46). ApoE4 also accelerates the rate of cognitive decline with aging, as it is associated with worsened memory (47). The impact of apoE4 on cognition and neurodegeneration has even been observed in healthy apoE4-carrier children by impairing their working memory and causing age-dependent thinning of the entorhinal cortex, which is the initial seeding site of tau pathology (48).

As radiological imaging of AD biomarkers is one of the most accurate methods for diagnosing AD, it is important to determine the effects of apoE4 on the accumulation of Aβ plaques and NFTs in human patients throughout various stages of the disease. Emerging patient data measuring apoE4-dependent amyloid and tau burdens offer real-time clarity of apoE4's effects on disease progression, as highlighted below.

Measuring Aβ plaques in vivo using PET imaging revealed that apoE4 is associated with an increased Aβ deposition rate (9) and a diffuse accumulation of Aβ pathology throughout the cortex of AD patients (45). A longitudinal study of cognitively normal individuals with a low Aβ plaque load found that apoE4 is a predictor of longitudinal Aβ accumulation (49). Interestingly, a recent study suggests that there is an interactive effect between apoE4 and Aβ to increase tau aggregation (50). However, amyloid-PET scans may not be reliable methods of AD diagnosis in older apoE4 patients, as the positive predictive value of amyloid-PET scans is highest in young apoE4 patients and is lower in older apoE4 patients (51). It has been proposed that knowledge of apoE status may be helpful when considering clinical amyloid assessment in older patients, as amyloid-PET scans were found to be less informative in apoE4 carriers than noncarriers. The prevalence of amyloid positivity is ∼90% in apoE4 carriers regardless of their age, whereas apoE4 noncarriers exhibit an age-dependent change in amyloid positivity (52).

On the basis of tau-PET measurements, apoE4 carriers present unique tau uptake patterns relative to apoE4 noncarriers. ApoE4 carriers have greater uptake of tau tracer $^{18}$F-AV-1451 in the temporal and parietal lobes, which are selectively vulnerable brain regions in late-onset AD (18). ApoE4 alters disease expression by promoting a more medial temporal lobe–predominant pattern of tau pathology in symptomatic AD patients (53). Intriguingly, apoE4 carriers also have a greater tau load in the entorhinal cortex than do apoE4 noncarriers (54); this finding is significant given that tau pathology in AD initiates in the entorhinal cortex (55). A recent study of two cross-sectional cohorts found that apoE4 is associated with an increased tau burden in the entorhinal cortex and hippocampus independently of Aβ, sex, clinical status, and age (56). These findings were corroborated in a longitudinal study that shows apoE4 enhances tau accumulation even after adjusting for the global cortical Aβ burden, indicating that the effect of apoE4 on tau is independent of Aβ (57). These important studies contribute to an evolving hypothesis in which the detrimental effects of apoE4 in AD go far beyond its impact on Aβ.

In addition to these typical AD biomarkers, apoE4 has been shown to cause network hyperactivity in AD patients. On the basis of fMRI data, cognitively normal apoE4 carriers exhibit

decreased task-induced deactivation of the default mode network (DMN) (58). This reveals that apoE4 carriers have hyperactivity in a network that is normally disengaged during task performance in healthy individuals. Reduced deactivation of the DMN during memory encoding correlates with worse task performance (59) and is observed in AD patients (60), suggesting that apoE4-induced deficits in DMN deactivation are linked to memory deficits observed in AD. Furthermore, healthy apoE4 carriers show dysfunctional hyperactivation in the CA1, CA3, and dentate regions of the hippocampus in the absence of structural deficits relative to apoE3 carriers, suggesting that network hyperactivity is an early manifestation of apoE4-related AD prior to neurodegeneration (61). These observed differences in brain activity between apoE4 carriers and noncarriers highlight the importance of understanding network dysfunctions that occur in AD.

Studies focusing on the association between apoE4 and neuroinflammatory biomarkers are just beginning to emerge. Within human AD patients, apoE4 is associated with a proinflammatory state. This was illustrated in a study involving intravenous lipopolysaccharide (LPS) injections to elicit inflammatory responses in healthy patients (62). ApoE4 carriers had higher plasma levels of proinflammatory markers tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) compared with apoE3 carriers, indicating that apoE4 promotes inflammation as compared with apoE3 (62). In addition, quantitative genotype-phenotype analysis of postmortem AD brains shows that apoE4 patients exhibit extensive microgliosis in the frontal and temporal cortices and astrogliosis in gray matter relative to apoE3 patients (63, 64). A study examining gliosis in human postmortem samples found that AD patients with apoE4 have increased levels of $CD68^{+}$, a marker of reactive microglia, relative to apoE3 and apoE2 (65). On the basis of these findings, apoE4 shows a clear effect on increasing neuroinflammation and gliosis in AD brains.

Radiological imaging of glucose metabolism is also used as a biomarker of AD, since measures of cerebral metabolic rate for glucose (CMRglc) show that its reduction occurs early in AD, correlates with disease progression, and predicts histopathological diagnosis of AD (66). Using $^{18}$F-fluorodeoxyglucose (FDG) as a radiotracer, apoE4 carriers exhibit regional glucose hypometabolism relative to apoE3 carriers (67). There also appears to be a link between hypometabolism and brain atrophy in apoE4 carriers, as they exhibit reduced CMRglc and reduced MRI gray matter volume (68). Interestingly, apoE4's effects on amyloid deposition and glucose metabolism appear to be reversed, as apoE4 is associated with more amyloid deposition in the frontal lobe and a more profound metabolic impairment in the posterior cortex (69). A longitudinal FDG PET study suggests a novel brain-region-specific glucose metabolism pattern associated with apoE4 in MCI patients, as apoE4 carriers demonstrate longitudinal decline in glucose uptake in eight forebrain and limbic brain regions relative to apoE3 carriers (70). Within cognitively normal apoE4 carriers, a reduction in posterior cingulate glucose metabolism precedes reduced hippocampal volume, suggesting that glucose metabolism is a valuable early biomarker of AD (71). These studies indicate that apoE4 has a clear impact on glucose metabolism in human patients and that detection of hypometabolism by FDG PET could potentially serve as a useful biomarker of cognitive decline in AD, especially in the context of apoE4.

To summarize, advancements in PET and MRI imaging techniques have revealed that apoE4 negatively impacts a plethora of biological processes associated with AD in human patients. Namely, apoE4 accelerates neurodegeneration and cognitive deficits, increases deposition of Aβ and accumulation of tau pathology, disrupts network activity within specific brain regions and functional connectivity between brain regions, amplifies gliosis and inflammation, and reduces CNS glucose metabolism. Now that these biomarkers of apoE4-related AD have been identified, the next steps are to determine which combinations of biomarker analyses will allow the most accurate diagnosis of AD from other dementia disorders and which tests will allow for the earliest detection of AD to maximize the efficacy of therapeutic interventions.

## APOE BIOLOGY AND PHYSIOLOGICAL FUNCTIONS

### ApoE Isoforms and Structural Differences

ApoE is a 34-kDa glycoprotein consisting of 299 amino acids and it has two structural domains: an N-terminal domain and a C-terminal domain (72–74). The three major isoforms of apoE are closely related in primary sequence and differ from one another by single amino acid substitutions (75). ApoE4 contains two arginines at positions 112 and 158, whereas apoE3 contains a cysteine at position 112 and apoE2 contains cysteines at positions 112 and 158 (76). Interestingly, this seemingly minuscule difference in primary sequence leads to a drastic change in the 3D structural conformation of apoE4 that distinguishes it from the other isoforms. Early studies using X-ray crystallography (77) and fluorescence resonance energy transfer (78) revealed that apoE4 has a unique domain interaction between its two structural domains, resulting in a more compact structure. The significant structural differences between apoE isoforms are thought to greatly impact their biological functions and make apoE4 much more susceptible to proteolytic cleavage, which is discussed further below. A nuclear magnetic resonance structure of apoE3 with multiple mutations at its C terminus has also been reported, although its physiological relevance is unclear (79).

### Physiological Functions of ApoE Within the Nervous System

Within the CNS, apoE is primarily responsible for the transport and metabolism of lipids and cholesterol (4). Under physiological conditions, apoE is mainly produced by astrocytes and is secreted into the extracellular matrix to bind lipids (80). Lipidated apoE then travels to neurons, binds to low-density lipoprotein receptors and other related receptors on the cell membrane, and enters the cell using receptor-mediated endocytosis (81). Transported lipids are released within neurons, and apoE either is exocytosed to undergo further lipidation (82) or undergoes degradation within lysosomes (83).

In addition to its role in lipid transport, apoE is also important for neuronal maintenance and repair. Following neuronal injury, astrocytic apoE production increases and lipidated apoE travels to regenerating nerve cells to facilitate the repair process (84, 85). Lipids released from apoE-containing lipoprotein particles are used to support synaptogenesis and aid axonal regeneration (86–88). The importance of apoE in synapse maintenance is evident from an in vivo study showing that apoE-knockout (apoE-KO) mice exhibit significant synaptic loss and dysfunction compared with apoE3 mice (89). In purified CNS neurons, the formation of mature synapses required apoE-containing lipoproteins, and insufficient availability of these lipoproteins limited synapse development (87). ApoE mRNA is also actively expressed in regenerating axons, and apoE-KO mice demonstrate attenuated axonal regeneration (90). Recent discoveries are further expanding our understanding of how apoE contributes to neuronal maintenance. Excitingly, apoE has been found to play a critical role in the development of adult newborn hippocampal neurons. It has been reported that adult mouse neural stem cells (NSCs) express *APOE* (91). ApoE-KO mice display impaired hippocampal neurogenesis, resulting in decreased numbers of NSCs and reduced numbers of mature granule cells in the dentate gyrus (92). This indicates that apoE is required for the maintenance of quiescent NSCs to prevent their depletion and is important for their maturation into granule cells. In addition, most newborn NSCs develop into astrocytes instead of neurons in apoE-KO mice relative to wild-type mice, suggesting that apoE is important for directing NSCs into a neuronal cell fate (91). Taken together, these studies suggest that apoE is a diverse protein that facilitates a wide variety of physiological functions, including lipid transport, synaptic homeostasis, neuronal repair, and NSC maintenance and differentiation. Understanding

the physiological roles of apoE within the CNS will help illuminate the loss- and gain-of-function effects that occur under pathological conditions in AD.

## ROLES OF APOE4 IN ALZHEIMER'S DISEASE PATHOGENESIS

Despite the high similarity in primary sequence between apoE isoforms, apoE4 is considered the detrimental allele because it drastically increases the risk of developing AD (4–7). Although the exact role of apoE4 in disease initiation and progression remains unclear, we highlight studies that reveal the cellular and molecular effects of apoE4 on AD pathogenesis.

### ApoE4 Effects on Neurodegeneration and Cognition In Vivo and In Vitro

The effects of apoE4 on neurodegeneration and cognition in human patients outlined above have also been replicated in human apoE knock-in (apoE-KI) mouse models and cell lines. Upon excitotoxic injury, apoE3-KI mice are protected against neurodegeneration, whereas apoE4-KI mice are not protected and exhibit cortical neuron loss, suggesting apoE4 promotes a loss-of-protective-function effect relative to apoE3 (93, 94). This loss-of-function effect is likely connected to the physiological role of apoE in neuronal maintenance and repair. For instance, apoE4 is associated with decreased lipidation capacity relative to apoE3, which may result in a reduced delivery of lipids to injured neurons to aid the repair process (95).

In addition, apoE4-KI mice display an age-dependent loss of hippocampal neurons and extensive learning and memory deficits relative to apoE3-KI mice, illustrating that apoE4 has detrimental effects on cognition and neuron survival (96, 97). Conditional deletion of apoE4 in neurons protects against neuronal loss and behavioral deficits (98). Within induced pluripotent stem cell (iPSC)-derived human neurons, apoE4 results in significant neuron degeneration and loss relative to apoE3 and apoE deficiency (99). ApoE4 has been shown to evoke apoptosis of neuronal cells in vitro in a time- and dose-dependent manner (100). Collectively, these studies indicate that apoE4 has gain-of-toxic-function effects on accelerating neurodegeneration and worsening cognitive functions.

### ApoE4 Effects on Neuronal Structure and Function

While it is well documented that apoE4 leads to neuronal loss, recent investigations are beginning to uncover the detrimental effects of apoE4 on the structure and function of neurons. ApoE4 alters the architecture of neuronal cells by causing a reduction in dendritic arborization and length as well as a decrease in the density of dendritic spines relative to apoE3 (101, 102). In human AD patient iPSC-derived cerebral organoids, apoE4 exacerbates synapse loss by causing a decrease in levels of presynaptic synaptophysin and postsynaptic PSD95 relative to apoE3 (103). Transcriptional profiling of iPSC-derived neurons revealed that the top differentially expressed genes between apoE3 and apoE4 neurons were involved in synaptic function (104). This finding was corroborated in a study involving a novel in vivo chimeric disease model of AD, in which human iPSC-derived apoE4 excitatory neurons transplanted into apoE4-KI mice exhibit pronounced gene expression changes related to synaptic dysfunction (105). It is important to note that these gene expression changes were most prominent when apoE4 was produced both endogenously and exogenously, suggesting a unique role for each of these sources of apoE in synaptic dysfunction. Mechanistically, apoE4 is thought to alter synaptic function by interfering with Reelin signaling, which is a modulator of synaptic strength (106). Since dendrites and synapses are crucial for proper communication between neurons, it is plausible that the drastic effects of apoE4 on these structures lead to disrupted cellular signals and network dysfunction, which is discussed further below.

ApoE4 is also associated with functional deficits within neurons by causing dysregulation of the endosomal-lysosomal degradation pathway. This pathway is responsible for protein degradation, and its dysfunction underlies a variety of neurological disorders, including AD (107). It has been suggested that the endosomal-lysosomal pathway plays a critical role in AD pathophysiology, as impairment of this pathway leads to the accumulation of toxic proteins such as Aβ and NFTs (108). An interesting observation is that the acidic environment of endolysosomes causes apoE4 to unfold and form a molten globule that is prone to aggregation with other proteins (109). Most functional studies that assess the isoform-specific effects of apoE on endosomal trafficking involve Aβ clearance. ApoE4 neurons display reduced lysosomal trafficking and degradation of Aβ relative to apoE3 in vitro, as discussed further below (110, 111). RNA sequencing of apoE4-KI mouse brains demonstrates an enrichment of genes involved in endosomal-lysosomal processing relative to apoE3-KI (112). Further analysis shows an age-dependent increase in the number and size of early endosomes within neurons of apoE4-KI mice (112). This finding was validated in apoE4 human iPSC-derived neurons, which also display increased number and size of early endosomes compared with apoE3 neurons, suggesting a direct link between apoE4 and endosomal-lysosomal dysregulation in human cells (104). Considering the importance of the endosomal-lysosomal pathway in clearing toxic proteins and maintaining a healthy intracellular environment, the effects of apoE4 on this pathway should also be examined in the context of other CNS cell types and toxic proteins that are pertinent to AD.

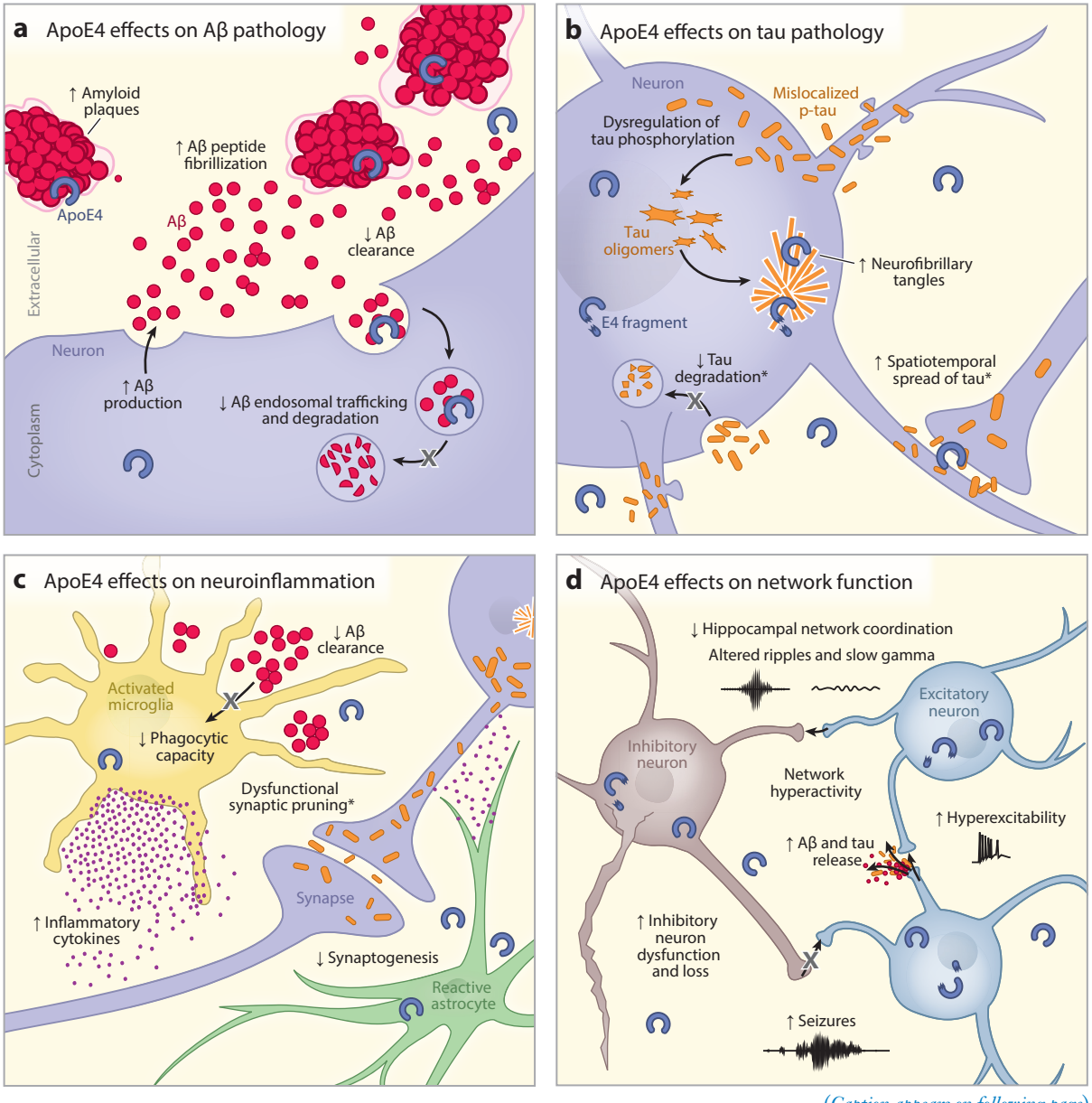
## ApoE4 Effects on Aβ Production, Deposition, and Clearance

Accumulation of Aβ is one of the earliest known pathological events that occurs in AD. As such, studies on Aβ have been the principal focus of AD research for the past few decades. Although recent failures of clinical trials targeting Aβ have raised concerns about the role of Aβ in disease progression, some studies suggest that it is still an important factor for disease initiation (113). Since the discovery of apoE4 as a major genetic risk factor for AD, a great effort has been made to understand how apoE4 affects Aβ aggregation and degradation. While the interaction between apoE4 and Aβ has already been extensively discussed elsewhere (7, 114, 115), we briefly highlight some of the most prominent findings (**Figure 2*a***).

It is widely accepted that apoE4 has a clear pathological impact on Aβ, as evidenced in the human PET and CSF studies mentioned above. In vivo studies using apoE/hAPP$_{FAD}$ mice show that apoE4 leads to a dramatic increase in the amount of fibrillar Aβ deposits and neuritic plaques relative to apoE3 (116). Human AD patients with apoE4 exhibit markedly elevated levels of oligomeric Aβ relative to apoE3, and apoE was found to colocalize with Aβ plaques at cortical synapses (117). Coimmunoprecipitation experiments illustrate a complex formation between apoE and Aβ in sodium dodecyl sulfate–stable synaptosomes, with an accumulation of synaptic Aβ occurring in apoE4 human AD samples (118). These studies provide evidence that apoE4 directly interacts with Aβ to promote its aberrant deposition in the CNS. Accordingly, apoE displays an isoform-dependent effect on Aβ accumulation in vivo, as hAPP$_{FAD}$ transgenic amyloid mice expressing two copies of the human *APOE ε4* gene had significantly higher levels of Aβ accumulation and plaque load than mice expressing one or two copies of the human *APOE ε3* gene (119). The dramatic effect of apoE4 on Aβ accumulation raises the question of whether decreasing apoE levels would attenuate Aβ-related pathology. Complete removal of apoE in apoE-KO/hAPP$_{FAD}$ mice results in a pronounced reduction in Aβ fibrillization and deposition (120). Furthermore, 12-month-old apoE4/hAPP$_{FAD}$ transgenic mice with one copy of the human *APOE* gene have significantly reduced Aβ levels compared with mice with two copies, suggesting a gene dose–dependent effect of apoE on Aβ accumulation (119). These findings were corroborated in a study using an apoE

haploinsufficiency mouse model, in which APPPS-21/apoE mice with decreased apoE levels exhibited significantly decreased amyloid plaque deposition (121). These studies indicate that decreasing apoE levels may be a valuable therapeutic strategy to decrease Aβ plaque levels in AD patients.

ApoE4 is also thought to play a role in the initiation of Aβ deposition during the early stages of AD. Analysis of postmortem human AD tissue revealed that apoE4 is involved in the deposition of newly formed plaques and that apoE4 carriers have higher deposition of apoE-Aβ complexes than




(*Caption appears on following page*)

**Figure 2** (*Figure appears on preceding page*)

Mechanisms of apoE4 effects on prominent AD pathologies. (*a*) ApoE4 has a profound effect on Aβ pathology by promoting the production and fibrillization of Aβ to form amyloid plaques. ApoE4 also reduces the clearance of Aβ by interfering with its cellular uptake and reducing its degradation via the endosomal-lysosomal degradation pathway. (*b*) Many features of tau pathology are evidently affected by apoE4. Although tau is typically localized to the axon, apoE4 causes it to be mislocalized to the soma and dendrites. ApoE4 promotes the aberrant hyperphosphorylation of tau by dysregulating tau kinases and phosphatases. Pathological tau is known to spread from diseased to healthy neurons, and it is hypothesized that apoE4 accelerates this spread. ApoE4 also enhances the aggregation of p-tau into insoluble neurofibrillary tangles. While it is unclear how apoE4 leads to the accumulation of aggregated tau, it is possible that apoE4 reduces the degradation of p-tau by disrupting the endosomal-lysosomal degradation pathway that is normally responsible for clearing tau. Asterisks indicate hypothesized mechanisms. (*c*) ApoE4 has a marked impact on neuroinflammation, causing activation of microglia and astrocytes that can release an array of proinflammatory cytokines. ApoE4 impairs microglial ability to clear Aβ plaques by reducing their phagocytic capacity. ApoE4 also disrupts the physiological functions of astrocytes to maintain synapses, resulting in decreased synaptogenesis and synaptic pruning. The asterisk indicates a hypothesized mechanism. (*d*) GABAergic interneurons are selectively vulnerable to apoE4 in AD. The dysfunction or loss of inhibitory neurons leads to excitatory neuron hyperexcitability and network hyperactivity that stimulate the release of pathological Aβ and tau from neurons. Neuronal network hyperactivity also leads to increased seizure activity. The dysfunction or loss of GABAergic interneurons also impairs hippocampal network coordination, resulting in reduced SWR events and SWR-associated slow gamma power, which are critical for memory consolidation. Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; apoE4, apolipoprotein E4; p-tau, phosphorylated tau; SWR, sharp-wave ripple.

apoE3 carriers (122). These findings were validated by an in vivo study that found that increased expression of apoE4 during the initial seeding stage of Aβ enhanced its deposition (123). It is possible that the ability of apoE4 to initiate Aβ deposition is due to its effects on Aβ aggregation. The Aβ aggregation process involves soluble Aβ peptides changing conformation to form insoluble fibrils (114). In vitro studies indicate that apoE acts as a pathological chaperone to promote the conformational shift of soluble Aβ into insoluble Aβ fibrils, with apoE4 proving more efficient at enhancing Aβ fibril formation than apoE3 (124).

A major Aβ clearance pathway involves the cellular uptake and degradation of Aβ via the endosomal-lysosomal degradation pathway. In vitro studies using mouse neuroblastoma Neuro-2a cells show that apoE plays a critical role in the neuronal uptake of Aβ and facilitates Aβ trafficking and degradation (111). Fascinatingly, apoE isoforms differentially affect $Aβ_{42}$ binding to the cell surface, with apoE4 exhibiting an impaired ability to facilitate the cellular uptake of Aβ relative to apoE3 (111). Also notable is the finding that apoE4 is less efficient at facilitating the endosomal trafficking and lysosomal degradation of Aβ than apoE3 (125). This reduced ability to clear Aβ leads to the accumulation of toxic Aβ species, likely causing cellular toxicity. ApoE4 also impairs Aβ clearance by glial cell types in the CNS, which is discussed further below.

Furthermore, apoE has been shown to influence the processing of APP and the production of Aβ. APP is typically cleaved by β and $\gamma$ secretases to generate Aβ peptides. ApoE4 increases Aβ production relative to apoE3 by stimulating APP recycling (126) and increasing the levels of β-site APP-cleavage enzyme 1 (BACE1) (127). The effect of apoE4 on Aβ production has also been confirmed in human cells, as iPSC-derived human neurons with apoE4 secreted twofold more $Aβ_{40}$ and $Aβ_{42}$ than apoE3 neurons (99). Studies of embryonic stem cell–derived human neurons illustrate that binding of apoE to its receptor initiates a signaling cascade that stimulates the AP-1 transcription factor and leads to enhanced APP transcription (128). Taken together, these studies provide compelling evidence that apoE4 is capable of stimulating Aβ production.

## ApoE4 Effects on Tau Phosphorylation, Aggregation, and Propagation

Over the past two decades, key discoveries have linked the combined toxic effects of apoE4 and tau in humans as well as in mouse and neuronal cell models (**Figure 2*b***). Encoded by the microtubule-associated protein tau (*MAPT*) gene, tau exists in the human brain as six main isoforms determined by alternate mRNA splicing (26, 129, 130). Tau isoforms are distinguished by the number of

N-terminal inserts and the presence of three or four repeats in the microtubule binding region, which is thought to contribute to protein aggregation (26, 129, 130). Tau acts in a myriad of cellular processes including neurite outgrowth, axonal stability, neuronal polarity, axonal transport, and synaptic integrity (26, 130). These are tied to its physiological functions of supporting the neuronal cytoskeleton by promoting microtubule assembly and stability (26, 129). The phosphorylation of tau regulates its affinity for binding microtubules, and aberrant tau phosphorylation strongly attenuates its binding abilities (26, 129). While physiological tau protein is soluble, hydrophilic, and natively unstructured, hyperphosphorylated tau forms classic, highly ordered, insoluble aggregates that make up NFTs (26, 129, 130). These aggregates are pathologically mislocalized to the soma and dendrites of neurons, whereas physiological tau is found primarily in axons (26, 129–131).

Early studies on apoE4 and tau revealed that expression of apoE4 in Neuro-2a cells induced intracellular NFT-like inclusions in vitro (132) and that neuronal expression of apoE4 in transgenic mice led to intraneuronal tau pathology (133). These tau inclusions were also found in Neuro-2a cells when C-terminal-truncated apoE, apoE4(Δ272–299), was either endogenously expressed or exogenously added (132). Transgenic mice expressing apoE4(Δ272–299) in neurons had higher phosphorylated tau (p-tau) in the hippocampus and cortex as well as impaired learning and memory at a young age (96, 134). Interestingly, removal of endogenous tau from apoE4(Δ272–299) mice rescued apoE4-driven learning and memory deficits and mitigated GABAergic interneuron loss (96). This finding demonstrates that tau is required for the detrimental phenotype of apoE4 and its fragments. It is worth noting that this phenotype resulted from apoE4 fragments that include the lipid binding region of the protein, since the shorter apoE4(Δ241–299) expression in mice did not cause neurodegeneration or intracellular tau inclusions (134). The lipid-binding region on the C terminus of apoE is necessary for neurotoxicity (135), although it remains unknown how this specifically causes tau-dependent neurodegeneration.

The effects of apoE isoforms on tau pathology have recently been studied in PS19 mice with endogenously expressed apoE2, apoE3, or apoE4 (136). PS19 is a widely studied tauopathy mouse model that expresses the aggregation-prone human tau-P301S mutant. At 3 months of age, PS19-E4 mice exhibited higher levels of p-tau and greater somatodendritic tau redistribution as compared with PS19-E3 and PS19-E2 mice. By 9 months of age, four distinct p-tau staining patterns emerged, and PS19-E4 mice displayed an increase in p-tau staining patterns that are associated with greater brain atrophy. Sequential biochemical extraction of tau from brain lysates revealed that PS19-E4 mice contain a greater amount of insoluble tau relative to PS19-E3 and PS19-E2 mice. When primary neurons expressing tau-P301S were treated with recombinant apoE isoforms, apoE4 caused the most damage, suggesting that exogenous apoE4 can lead to neurodegeneration in the context of mutant tau-P301S. Intriguingly, PS19-EKO mice, which lack apoE, were largely protected from the tau pathology and brain atrophy observed in PS19-E4 mice. Taken in conjunction with an early study showing that tau removal rescues apoE4-induced toxicity (96), these findings suggest that the pathological mechanisms underlying AD require both apoE and tau.

An important consideration is that PS19 mice express a tau-P301S mutant found in frontotemporal dementia but not in AD. While this tauopathy model reveals interesting biomolecular mechanisms underlying the interactive roles of apoE4 and tau in neurodegeneration, it may not precisely represent phenotypes that occur in AD patients with wild-type tau. This limitation highlights the value of studying human neurons derived from AD patients in vitro, which affords the opportunity to understand the interplay between tau and apoE4 in AD using a more human-relevant disease model.

Advancements in iPSC technology and gene-editing techniques have provided powerful tools for studying neurons from AD patients. Two recent studies showed that apoE4 specifically causes

increased tau phosphorylation in human neurons. A comparison of iPSC-derived neurons from apoE4-carrier AD patients with those from apoE3-carrier healthy donors revealed that apoE4 neurons have increased GABAergic neuron degeneration and higher levels of p-tau, independent of Aβ levels (99). While apoE-KO neurons phenotypically resemble apoE3 neurons, viral expression of apoE4 in apoE-KO neurons recapitulated the pathological phenotypes of apoE4 neurons, supporting a gain-of-toxic-function effect of apoE4 in human neurons. Converting apoE4 into apoE3 by gene editing rescued the detrimental phenotypes observed in apoE4 neurons. This finding was further validated when the deleterious effects of apoE4 were rescued following treatment with a small molecule structure corrector capable of converting the apoE4 structure to resemble apoE3. In another study, iPSCs derived from an apoE3-carrier healthy donor were converted to apoE4 using clustered regularly interspaced short palindromic repeats (CRISPR) technology and differentiated into cerebral organoids. ApoE4 organoids consisting of neurons and astrocytes aged to 6 months exhibited more than twice the levels of p-tau compared with apoE3 organoids (104). Similar to the previous study, CRISPR-based conversion of apoE4 to apoE3 ameliorated the tau pathology within these organoids. These important studies have advanced our understanding of how wild-type human tau and apoE4 together lead to neuronal toxicity in AD.

To further parse out how apoE and tau interact on a molecular and cellular level in disease, it is essential to understand the molecular pathogenesis of tau. Accumulation of pathological tau throughout the brain occurs in a consistent spatiotemporal pattern, as described above (18). After diseased neurons secrete pathological tau, the aggregated extracellular tau can enter interconnected healthy neurons and induce misfolding of intracellular tau (137), which can then recruit properly folded endogenous tau monomers and cause them to misfold and subsequently aggregate in a prion-like fashion (138, 139). As evidenced by the tau-PET studies mentioned above, apoE4 plays a major role in facilitating tau accumulation throughout the brain. Still, there is a considerable gap in knowledge surrounding the mechanistic role of apoE4 in tau misfolding and aggregation. Although apoE has been shown to bind to tau in NFTs, there is no apparent difference in NFT binding between apoE3 and apoE4 (140). A mechanism of interest that may link apoE4 to tau aggregation is its effect on the aberrant phosphorylation of tau. As a phosphoprotein, tau is typically phosphorylated and dephosphorylated by a variety of tau kinases and phosphatases. ApoE4 has been shown to increase p-tau via extracellular signal-regulated kinase 1/2 activation (141, 142). Additionally, apoE isoforms differentially regulate a key tau phosphatase, protein phosphatase 2A (143, 144). Overall, the mechanisms underlying the observed effects of apoE4 on tau misfolding and aggregation are relatively understudied, and uncovering how apoE4 facilitates tau aggregation may reveal viable therapeutic targets for AD.

To better understand the role of apoE4 in tau spread, it is important to study how apoE facilitates the neuronal uptake and internalization of tau. Roughly half of tau internalization by neurons occurs via binding to heparin sulfate proteoglycans (HSPGs) on the cell surface (145, 146). Importantly, HSPGs are known cell-surface receptors for apoE, but the impact of different apoE isoforms on tau uptake via HSPG receptors is understudied. Blockage of HSPGs using heparinase in a coculture of rat astrocytes and neurons reversed the increased tau accumulation caused by apoE4, suggesting that apoE4 regulates tau uptake within cells using HSPGs (147). A recent study found that another apoE cell-surface receptor, lipoprotein receptor-related protein 1 (LRP1), also regulates tau uptake. Exogenous apoE selectively inhibited tau uptake in neuroglioma cells, with apoE4 being the least effective isoform at inhibiting the direct interaction between tau and LRP1 and subsequent tau spreading (148). Follow-up studies on apoE4's ability to facilitate neuronal tau uptake via apoE receptors are needed to decipher how apoE4 promotes tau spreading and whether this process can be targeted therapeutically to prevent pathological tau propagation in disease.

A recent game-changing discovery of an AD-protective mutation in *APOE* has elucidated the link between apoE and tau pathogenesis. A woman carrying a causal AD mutation in presenilin 1 (*PSEN1*) developed MCI in her seventies, which is 30 years later than the expected age of onset (149). Despite having a high amyloid plaque burden, the patient exhibited limited tau spread and brain atrophy. Most intriguingly, whole-genome sequencing revealed homozygosity for a rare R136S mutation in the receptor binding region of *APOE ε3*, termed the Christchurch mutation (149). Further biochemical analysis of this novel apoE variant revealed a lower binding affinity for HSPG, while apoE4 had the highest binding affinity. Discovery of this protective mutation is monumental in dissecting how apoE and tau interactively contribute to AD, as it suggests a potential mechanism by which apoE may be involved in tau spreading—potentially through the interaction of apoE with HSPG and/or other cell-surface receptors critical for tau uptake.

A dominant theory in AD research has been that increased Aβ levels, Aβ aggregates, or amyloid plaques lead to tau pathologies and, subsequently, to AD-related cognitive decline. Clearly, the *APOE*-R136S-related findings in AD provide evidence that Aβ/amyloid accumulation alone is not sufficient to cause AD, at least in a *PSEN1*-mutation carrier with an exceedingly high Aβ plaque burden. Alternatively, Aβ might induce tau pathologies and cognitive decline only in the presence of normally functional apoE. Further understanding of this chain of causality will be vitally important for better understanding AD pathogenesis and improving drug development.

## ApoE4 Effects on Function and Activation of Neuroinflammatory Cells

While astrocytes are the main producers of apoE protein within the CNS, studies have also shown that homeostatic microglia can express apoE at very low levels (150). Since microglia and astrocytes are the most widely studied CNS immune cell types as they relate to apoE4 and AD, we focus this section of the review on understanding the impact of apoE4 on neuroinflammation in the context of these two types of glial cells (**Figure 2*c***).

Studies on mouse models of AD have been invaluable in expanding our understanding of apoE4's role in neuroinflammation (151, 152). Similar to findings in human patients, analysis of apoE-KI mice revealed that apoE3 acts as an anti-inflammatory agent while apoE4 acts as a proinflammatory agent (153). ApoE4 mice exhibit increased glial activation and cytokine release and greater synaptic protein loss after LPS injection (154). In mice that coexpress apoE and 5XFAD, apoE4 mice have greater microglial dystrophy and higher density of reactive cells surrounding cortical plaques compared with apoE3 mice, suggesting that apoE4 modulates immune cell reactivity to pathological protein aggregates (155).

Astrocytes and microglia are the primary cells responsible for the immune response in the CNS. Under homeostatic conditions, astrocytes support a wide range of neuronal functions, including synaptic pruning and guidance (156), synaptic plasticity (157), and neuronal signaling (158). Conversely, under conditions of stress or injury, astrocytes play an essential role in activating the CNS neuroinflammatory response. In response to CNS injury, astrocytes typically undergo reactive astrogliosis, a term used to describe the cells' shift into detrimental molecular, functional, and morphological phenotypes (159). Reactive astrocytes are able to produce cytokines, chemokines, and other soluble factors that impact both the innate and adaptive immune responses of the CNS.

Astrocytes are a highly phagocytic cell type and participate in homeostatic synapse pruning and turnover. It was recently discovered that apoE presents a novel allele-dependent role in controlling the phagocytic capacity of astrocytes, as apoE4 decreases the rate of astrocytic phagocytosis of synapses relative to apoE3 (160). In addition to synapse phagocytosis, astrocytes are also known to secrete neurotrophic factors to maintain synaptic integrity. A study involving a coculture of human iPSC-derived astrocytes and neurons found that apoE3 astrocytes promote homeostatic neuronal

synaptogenesis, whereas apoE4 astrocytes were ineffective in promoting neuronal synaptogenesis (161). These studies suggest that apoE4 disrupts the homeostatic functions of astrocytes to eliminate synapses and promote synaptogenesis and that astrocytic apoE4 may contribute to the previously described detrimental effects of apoE4 on synapse maintenance and repair. Astrocytes are also thought to play a prominent role in the degradation of proteins within the CNS. Investigation of autophagy within mouse-derived astrocytes showed that apoE4 astrocytes exhibit lower autophagic flux and are less effective at elimination of Aβ plaques than apoE3 astrocytes (162). This indicates that the accumulation of toxic proteins within apoE4 AD patients may be, at least partially, due to impaired astrocytic autophagic function caused by apoE4.

As another prominent immune cell type of the CNS, microglia are responsible for eliminating microbes, dead cells, redundant synapses, protein aggregates, and other potentially harmful particles (163). During homeostasis, microglia remain relatively quiescent and are responsible for monitoring the brain microenvironment and engaging in cross-cell signaling with neurons and astrocytes (164, 165). In response to injury, microglia become activated, prompting cell proliferation and secretion of proinflammatory molecules such as cytokines and chemokines (e.g., IL-1β, IL-6, IL-12, TNFα, and CCL2) (163). In addition to secreting soluble factors, activated microglia show a harmful tendency to form scar tissue (166), which encompasses the region undergoing inflammatory response and can prevent local axonal regrowth. Recent studies indicate that in the context of AD, microglia increase proliferation and inflammatory molecule secretion while reducing their clearance-related phagocytic activity (167, 168). One possible explanation is that microglia cells could influence AD depending on the duration of their signaling—microglia that are acutely activated might reduce Aβ accumulation through increased phagocytosis, whereas chronic activation of the same microglia could induce proinflammatory cascades that in turn cause neurotoxicity and synapse loss (167).

An increasing amount of evidence shows that apoE4 has a profound effect on microglia function and reactivity. Microglia derived from apoE4-KI mice present a reactive cell morphology and produce more proinflammatory cytokines than those derived from apoE3-KI mice (169). ApoE4-expressing microglia also display increased phagocytosis of apoptotic neurons, suggesting that apoE4 alters the phagocytic behavior of microglia (170). Transcriptomic analysis of human iPSC-derived microglia-like cells revealed that apoE4 microglia have 329 upregulated immune response genes compared with apoE3, likely rendering apoE4 microglia to promote inflammation (104). Strikingly, when human iPSC-derived neurons were transplanted into the brains of apoE-KI mice and produced Aβ aggregates, microglia in apoE4-KI mice exhibited impaired phagocytosis of Aβ aggregates relative to those in apoE3-KI mice, leading to an accumulation of Aβ within apoE4-KI mouse brains (105). This finding was corroborated by an in vitro study of iPSC-derived microglia-like cells that monitored the uptake of fluorescently tagged $A\beta_{42}$ in real time. Microglia harboring apoE4 had impaired phagocytosis of $A\beta_{42}$, with a much slower rate of uptake than that of apoE3 microglia (104). Mechanistically, APOE signaling within microglia is activated by TREM2, and this TREM2-APOE pathway was identified as a major regulator of microglial functional phenotypes in AD (171). These findings suggest that apoE4 negatively impacts microglia function by causing increased activation and interfering with their ability to phagocytose toxic CNS protein aggregates.

Interest has grown in understanding how apoE4-induced microglial dysfunction affects tau, another important pathological hallmark of AD. A recent study using mice that coexpress human tau-P301S and apoE isoforms (PS19-E) revealed that PS19-E4 mice have significantly higher p-tau, greater neurodegeneration, and increased neuroinflammation compared with PS19-E3 mice (136). Following LPS treatment, apoE4-expressing cultured microglia displayed higher innate immune reactivity than apoE3 microglia. Coculturing tau-P301S-expressing neurons with

apoE3- or apoE4-expressing mixed glia revealed significantly higher levels of proinflammatory marker TNFα in the apoE4 glial condition. This indicates that apoE4 and tau work in concert to activate microglia. In a follow-up study, when microglia were depleted by treating the PS19-E4 mice with a CSF-1R inhibitor (PLX3397), PS19-E4 mice displayed brain volumes equal to PS19-EKO mice, suggesting that removal of microglia impedes neurodegeneration and that apoE, especially apoE4, regulates neurodegeneration by modulating microglial activation, at least in the context of a mutant tau. Intriguingly, tau pathology progression was halted to some extent in PS19-E4 mice upon microglial depletion. This indicates that microglial-mediated damage is a driving force of neurodegeneration in a PS19-E mouse model expressing mutant tau and suggests that apoE exerts its effect on neurodegeneration, at least partially, by regulating microglial functions. Still, it would be interesting to see if this apoE- and microglia-dependent effect on tau pathology holds true in wild-type human tau models, since studies on human neurons in vitro show that apoE4 can directly affect tau pathology even in the absence of microglia (99).

Interestingly, a recent study found an association between apoE4, tau, and microglia when performing single-cell RNA sequencing on human tissue (172). Examination of the dorsolateral frontal cortex from deceased patients revealed that the cellular density of $Iba1^+$ microglia was positively associated with tau pathology only in apoE4 carriers. In addition, the cytokines IL-10, IL-13, IL-4, and IL-1α were negatively associated with tau pathology only in apoE4 noncarriers, suggesting a protective effect in these patients. These results indicate that apoE4 mediates an altered inflammatory response and increases tau pathology and provide evidence that apoE exerts a modulatory role in neuroinflammation and glial cell function.

Taken together, these important studies illustrate that apoE4 has a significant effect on neuroinflammation by incapacitating the function of critical immune cells within the brain (**Figure 2*c***). Further studies need to be performed to understand the exact mechanism by which apoE4 causes dysfunction in these cells. Revealing these mechanisms could provide novel therapeutic targets to reduce neuroinflammation in AD.

## Cellular Source–Dependent and Gain-of-Toxic-Function Effects of ApoE4

Besides the observed effects of apoE4 on prominent AD pathologies, studies have also shown that apoE4 exhibits some interesting disease-associated properties depending on the cellular origin of its production and its posttranslational processing.

Under physiological conditions, apoE is mainly produced by astrocytes within the brain (80, 173). However, conditions of stress or injury can induce apoE production within neurons (174, 175). Using enhanced green fluorescent protein (GFP) as a real-time location marker of apoE expression in vivo, it was observed that kainic acid treatment induces intense apoE-GFP expression in injured neurons, demonstrating that neurons upregulate apoE expression in response to excitotoxic injury (174). This begs the question of whether the cellular source of apoE determines its pathogenicity. To determine if the cellular sources of apoE differentially cause detrimental effects, an in vivo study analyzed mice expressing apoE3 or apoE4 under a neuronal (NSE) or astrocytic (GFAP) promoter. This work demonstrated that mice expressing apoE4 solely in neurons exhibited an age-dependent increase in tau hyperphosphorylation (133). The effect of apoE on p-tau levels was absent when apoE was expressed in astrocytes, regardless of isoform. Under excitotoxic injury, astrocyte-derived apoE4 behaves like apoE3 and protects against excitotoxicity, whereas neuron-derived apoE4 is not protective and results in neuronal loss (94). Similarly, human apoE-KI mice with deletion of astrocytic apoE4 still had neuronal loss and cognitive deficits typical of apoE4-KI mice, whereas deletion of apoE4 in neurons protected the mice from both deficits (98). This evidence suggests that the cellular source of apoE does determine its pathological activities.

Many studies have focused on the loss-of-function effects of apoE4 relative to apoE3; however, apoE4 also exhibits gain-of-toxic-function effects when aberrantly cleaved into fragments in neurons (**Figures 1*b*** and **2*b***). In response to stress or injury, neurons induce apoE expression to aid membrane repair and remodeling (176). When produced in neurons, apoE4 undergoes proteolytic cleavage to produce truncated fragments (132). Analysis of mice expressing apoE4 under NSE or GFAP promoters found that apoE4 fragments are produced only when apoE4 is expressed in neurons, not when it is produced within astrocytes (133). Compared with apoE3, apoE4 is much more susceptible to proteolysis due to its unique 3D structure containing a domain interaction (74, 133). ApoE4 fragments are neurotoxic and cause detrimental intracellular effects. While the exact amino acid sequences of these apoE4 fragments are unknown, it is hypothesized that a C-terminal-truncated (Δ272–299) fragment is among the most toxic fragments (132). Endogenously expressed and exogenously added apoE4(Δ272–299) resulted in intracellular NFT-like inclusions containing p-tau and high-molecular-weight phosphorylated neurofilaments in Neuro-2a cells (132). ApoE4(Δ272–299) was also shown to interact with mitochondria and cause mitochondrial dysfunction (135). Mechanistically, these apoE4 fragments have a high binding affinity for components of mitochondrial respiratory complex III and IV and were shown to impair the activity of these complexes (177). Transgenic mice expressing apoE4(Δ272–299) displayed neurodegeneration, increased levels of p-tau, and significant behavioral deficits (134). These studies indicate that apoE4 fragments have potent neurotoxic effects and may play a pivotal role in apoE4-related AD pathogenesis. Given the gain-of-toxic-function effects that occur when apoE4 is cleaved into fragments, identification of the protease responsible for apoE4 truncation would provide immense therapeutic value as a drug target.

## ApoE4 Effects on GABAergic Interneurons and Network Dysfunction

The findings in the previously described studies make it probable that AD in apoE4 carriers is phenotypically distinct, at least to some extent, from AD in apoE4 noncarriers. This is made even more evident by the fact that apoE4 has a selective toxic effect on GABAergic interneurons, leading to neuronal network dysfunction (**Figure 2*d***). Studies in apoE4-KI mice show that apoE4 results in an age- and tau-dependent decrease in hilar GABAergic somatostatin-positive interneurons in the hippocampus (96) and that the degree of interneuron loss correlates with enhanced learning and memory deficits (96, 178). Interestingly, studies show that this apoE4-related loss of interneurons can be rescued either by deletion of apoE4 specifically in GABAergic interneurons or by removal of tau (96, 98). Additionally, the systemic addition of a GABA agonist or hippocampal transplantation of mouse-derived inhibitory interneuron progenitors can also rescue learning and memory deficits in aged apoE4-KI mice with or without mutant $hAPP_{FAD}$ expression (96, 179). A recent study using human iPSC-derived neurons demonstrated that apoE4 GABAergic interneurons have increased phosphorylated tau levels and degeneration relative to apoE3 GABAergic interneurons (99). These interneuron pathologies were rescued either by genetically editing *APOE4* to *APOE3* or by treating apoE4 neurons with a small molecule structure corrector that alters apoE4's protein conformation to more closely resemble apoE3. Furthermore, transplantation of human iPSC-derived neurons into apoE-KI mice showed that inhibitory neurons are more susceptible to apoE4-mediated gene expression changes than excitatory neurons (105). There also appeared to be a neuronal-subtype-specific response to apoE4 toxicity, as inhibitory neurons were uniquely prone to dysregulation of unfolded protein response, oxidative stress, and RNA degradation. These findings support the conclusion that GABAergic inhibitory neurons are particularly vulnerable to apoE4-induced toxicity.

The effects of apoE4 on GABAergic interneurons have further ramifications for overall network dysfunction. It is clear that apoE4 has a profound effect on network hyperactivity and higher-level coordination. In humans, even healthy young and middle-aged apoE4 carriers show increased brain activity during rest and increased hippocampal activity during memory encoding (180, 181). Aged apoE4-KI mice demonstrate increased field potential synchrony and pyramidal cell firing in the entorhinal cortex, both of which are signs of increased network excitability (182). Aged apoE4-KI mice also show impaired hippocampal network coordination in the form of a reduced abundance of sharp-wave ripples (SWRs) and reduced levels of SWR-associated slow gamma power, processes that are critical for spatial memory consolidation (183). These phenotypes can accurately predict future spatial memory deficits, in some cases months beforehand (184). GABAergic interneurons play a key role in apoE4-induced network dysfunction. Loss of slow gamma power does not manifest in young apoE4-KI mice before the onset of significant interneuron loss, and deleting apoE4 from inhibitory interneurons alone rescues slow gamma power and learning and memory deficits in aged apoE4-KI mice (183). In light of these studies, a recent review suggests a model whereby the toxic effects of apoE4, through a tau-dependent mechanism, cause GABAergic interneuron-specific dysfunction and death. The resulting interneuron loss in the hippocampus then leads to network dysfunction and hyperexcitability, which give rise to the learning and memory deficits seen in AD (185).

## CONCLUSION AND PERSPECTIVE

As evidenced by the outlined studies, apoE4 plays a major role in the pathogenesis of AD. The complexity of AD is made apparent by the sheer number of cellular and molecular processes that are disrupted in the presence of apoE4, yet it is possible that we are just beginning to scratch the surface. Distinguishing the pathogenic factors that are critical for disease initiation and progression from those that are innocuous by-products of the disease is vital for efforts to develop therapies to combat AD.

Considering the substantial body of research supporting the interactive roles of apoE4 with Aβ, tau, and the immune system in AD pathogenesis, it is possible to hypothesize a new multiroute pathogenic cascade for AD. (*a*) ApoE4 promotes the production and fibrillization of Aβ and impairs pathways involved in its degradation/clearance, causing an accumulation of toxic Aβ species and the formation of amyloid plaques. (*b*) ApoE4 affects tau by increasing its phosphorylation, accelerating its spread to other neurons, and enhancing its fibrillization to form NFTs. Tau also affects apoE4 since its removal prevents apoE4-driven deficits, although the mechanism behind this is unknown. (*c*) ApoE4 exacerbates the neuroinflammatory response, impairs the ability of astrocytes to maintain synapses, and causes microglia to increase phagocytosis of neurons and decrease removal of toxic proteins, including Aβ and tau aggregates. (*d*) The toxicity of apoE4 is dependent on the cellular origin of its production, with neuronal apoE4 causing more neuronal and cognitive deficits than astrocytic apoE4. (*e*) ApoE4 exhibits gain-of-toxic-function effects when aberrantly cleaved into truncated fragments within neurons, leading to an accumulation of p-tau and mitochondrial dysfunction. (*f*) ApoE4 has a selectively toxic effect on GABAergic interneurons, whose death then leads to network hyperexcitability, impairment of hippocampal network coordination, and ultimately memory and learning deficits. When considering their contributions to AD pathogenesis, it is possible that these apoE4-induced detrimental effects could work independently or in concert with one another.

On the basis of these demonstrated detrimental effects of apoE4, different disease-modifying therapeutic approaches may be developed. (*a*) To reverse the specific toxicity of apoE4, using gene therapy to convert *APOE ε4* into the AD-protective *APOE ε2* or *APOE-R136S* allele may

prevent the development of AD pathologies. (*b*) Due to the unique apoE4-domain interaction, using a small molecule structure corrector to disrupt the domain interaction would cause apoE4 to resemble apoE3, both structurally and functionally. (*c*) One potential approach based on the gain-of-toxic-function effects of apoE4 is the reduction of apoE4, in all CNS cells or in specific types of cells, either with antisense oligonucleotides targeting *APOE* expression or with a monoclonal antibody treatment. (*d*) If the protease responsible for cleaving neuronal apoE4 into fragments is identified, another potential approach is to develop apoE4-cleaving protease inhibitors to block the production of neurotoxic apoE4 fragments. (*e*) To address the neurodegeneration and subsequent neural network dysfunction in apoE4-AD patients, a viable option may be cell-replacement therapy using a patient's stem cell–derived neurons to convert *APOE4* to *APOE3* using gene editing, once these technologies advance. What is still debatable is whether removal of microglia or apoE4 is a more viable therapeutic solution to prevent neurodegeneration. Both strategies appear to be effective, but further studies are needed to assess which target would prevent neuronal loss and cognitive deficits long term. It is possible that a combination of two or more approaches is more effective than any single approach. Of course, an important missing piece in all therapeutic development is pinpointing apoE4's exact role in the progression of pathobiology. Thus, it is essential to conduct early screening and diagnosis of apoE4 carriers to allow for therapeutic intervention before irreversible damage occurs.

## DISCLOSURE STATEMENT

Y.H. is a cofounder and scientific advisory board member of Escape Bio, Inc., GABAeron, Inc., and Mederon Bio, LLC. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## AUTHOR CONTRIBUTIONS

N.K., M.R.N., A.R., and Y.H. developed the concept and overall structure of the review. N.K., M.R.N., and A.R. drafted the manuscript, and Y.H. provided critical input on the contents. M.R.N. designed the figures with input from N.K., A.R., and Y.H. All authors reviewed, edited, and approved the final manuscript.

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## LITERATURE CITED

1. Beason-Held LL, Goh JO, An Y, Kraut MA, O'Brien RJ, et al. 2013. Changes in brain function occur years before the onset of cognitive impairment. *J. Neurosci.* 33:18008–14
2. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, et al. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921–23
3. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, et al. 2009. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat. Genet.* 41:1088–93
4. Huang Y, Mahley RW. 2014. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. *Neurobiol. Dis.* 72(Part A):3–12

5. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, et al. 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. *JAMA* 278:1349–56
6. Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, et al. 2011. *APOE* and Alzheimer disease: a major gene with semi-dominant inheritance. *Mol. Psychiatry* 16:903–7
7. Huang Y, Mucke L. 2012. Alzheimer mechanisms and therapeutic strategies. *Cell* 148:1204–22
8. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, et al. 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann. Neurol.* 55:306–19
9. Toledo JB, Habes M, Sotiras A, Bjerke M, Fan Y, et al. 2019. APOE effect on amyloid-β PET spatial distribution, deposition date, and cut-points. *J. Alzheimers Dis.* 69:783–93
10. Cho H, Choi JY, Hwang MS, Kim YJ, Lee HM, et al. 2016. In vivo cortical spreading pattern of tau and amyloid in the Alzheimer disease spectrum. *Ann. Neurol.* 80:247–58
11. Raslau FD, Mark IT, Klein AP, Ulmer JL, Mathews V, Mark LP. 2015. Memory part 2: the role of the medial temporal lobe. *Am. J. Neuroradiol.* 36:846–49
12. Hanseeuw BJ, Betensky RA, Jacobs HIL, Schultz AP, Sepulcre J, et al. 2019. Association of amyloid and tau with cognition in preclinical Alzheimer disease: a longitudinal study. *JAMA Neurol.* 76:915–24
13. Morris JC, Roe CM, Grant EA, Head D, Storandt M, et al. 2009. Pittsburgh Compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Arch. Neurol.* 66:1469–75
14. Sperling RA, Donohue MC, Raman R, Sun C-K, Yaari R, et al. 2020. Association of factors with elevated amyloid burden in clinically normal older individuals. *JAMA Neurol.* 77:735–45
15. Rowe CC, Ellis KA, Rimajova M, Bourgeat P, Pike KE, et al. 2010. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol. Aging* 31:1275–83
16. Soleimani-Meigooni DN, Iaccarino L, La Joie R, Baker S, Bourakova V, et al. 2020. $^{18}$F-flortaucipir PET to autopsy comparisons in Alzheimer's disease and other neurodegenerative diseases. *Brain* 143:3477–94
17. Schwarz AJ, Yu P, Miller BB, Shcherbinin S, Dickson J, et al. 2016. Regional profiles of the candidate tau PET ligand $^{18}$F-AV-1451 recapitulate key features of Braak histopathological stages. *Brain* 139:1539–50
18. Ossenkoppele R, Schonhaut DR, Schöll M, Lockhart SN, Ayakta N, et al. 2016. Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer's disease. *Brain* 139:1551–67
19. Schöll M, Lockhart SN, Schonhaut DR, O'Neil JP, Janabi M, et al. 2016. PET imaging of tau deposition in the aging human brain. *Neuron* 89:971–82
20. Chen X, Cassady KE, Adams JN, Harrison TM, Baker SL, Jagust WJ. 2021. Regional tau effects on prospective cognitive change in cognitively normal older adults. *J. Neurosci.* 41:366–75
21. La Joie R, Visani AV, Baker SL, Brown JA, Bourakova V, et al. 2020. Prospective longitudinal atrophy in Alzheimer's disease correlates with the intensity and topography of baseline tau-PET. *Sci. Transl. Med.* 12:eeau5732
22. Hardy JA, Higgins GA. 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256:184–85
23. Buchhave P, Minthon L, Zetterberg H, Wallin ÅK, Blennow K, Hansson O. 2012. Cerebrospinal fluid levels of β-amyloid 1–42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch. Gen. Psychiatry* 69:98–106
24. Shoji M, Matsubara E, Kanai M, Watanabe M, Nakamura T, et al. 1998. Combination assay of CSF tau, Aβ1–40 and Aβ1–42(43) as a biochemical marker of Alzheimer's disease. *J. Neurol. Sci.* 158:134–40
25. Lewczuk P, Esselmann H, Otto M, Maler JM, Henkel AW, et al. 2004. Neurochemical diagnosis of Alzheimer's dementia by CSF Aβ42, Aβ42/Aβ40 ratio and total tau. *Neurobiol. Aging* 25:273–81
26. Morris M, Maeda S, Vossel K, Mucke L. 2011. The many faces of tau. *Neuron* 70:410–26
27. Hu YY, He SS, Wang X, Duan QH, Grundke-Iqbal I, et al. 2002. Levels of nonphosphorylated and phosphorylated tau in cerebrospinal fluid of Alzheimer's disease patients: an ultrasensitive bienzyme-substrate-recycle enzyme-linked immunosorbent assay. *Am. J. Pathol.* 160:1269–78
28. Ishiguro K, Ohno H, Arai H, Yamaguchi H, Urakami K, et al. 1999. Phosphorylated tau in human cerebrospinal fluid is a diagnostic marker for Alzheimer's disease. *Neurosci. Lett.* 270:91–94
29. Kohnken R, Buerger K, Zinkowski R, Miller C, Kerkman D, et al. 2000. Detection of tau phosphorylated at threonine 231 in cerebrospinal fluid of Alzheimer's disease patients. *Neurosci. Lett.* 287:187–90

30. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, et al. 2020. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat. Med.* 26:379–86
31. Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, et al. 2018. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement*. 14:989–97
32. Thijssen EH, La Joie R, Wolf A, Strom A, Wang P, et al. 2020. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat. Med.* 26:387–97
33. Barthélemy NR, Bateman RJ, Hirtz C, Marin P, Becher F, et al. 2020. Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. *Alzheimers Res. Ther.* 12:26
34. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. 2001. Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412:150–57
35. Yassa MA, Mattfeld AT, Stark SM, Stark CEL. 2011. Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. *PNAS* 108:8873–78
36. Sanz-Arigita EJ, Schoonheim MM, Damoiseaux JS, Rombouts SARB, Maris E, et al. 2010. Loss of "small-world" networks in Alzheimer's disease: graph analysis of fMRI resting-state functional connectivity. *PLOS ONE* 5:e13788
37. Schultz AP, Chhatwal JP, Hedden T, Mormino EC, Hanseeuw BJ, et al. 2017. Phases of hyperconnectivity and hypoconnectivity in the default mode and salience networks track with amyloid and tau in clinically normal individuals. *J. Neurosci.* 37:4323–31
38. Dickerson BC, Salat DH, Greve DN, Chua EF, Rand-Giovannetti E, et al. 2005. Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. *Neurology* 65:404–11
39. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, et al. 2015. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 14:388–405
40. Uchihara T, Akiyama H, Kondo H, Ikeda K. 1997. Activated microglial cells are colocalized with perivascular deposits of amyloid-β protein in Alzheimer's disease brain. *Stroke* 28:1948–50
41. Beach TG, McGeer EG. 1988. Lamina-specific arrangement of astrocytic gliosis and senile plaques in Alzheimer's disease visual cortex. *Brain Res.* 463:357–61
42. Sheng JG, Mrak RE, T. Griffin WS. 1997. Glial-neuronal interactions in Alzheimer disease: progressive association of IL-1$\alpha^{+}$ microglia and S100$\beta^{+}$ astrocytes with neurofibrillary tangle stages. *J. Neuropathol. Exp. Neurol.* 56:285–90
43. Edison P, Archer HA, Gerhard A, Hinz R, Pavese N, et al. 2008. Microglia, amyloid, and cognition in Alzheimer's disease: an [11C](R)PK11195-PET and [11C]PIB-PET study. *Neurobiol. Dis.* 32:412–19
44. Sun Y-X, Minthon L, Wallmark A, Warkentin S, Blennow K, Janciauskiene S. 2003. Inflammatory markers in matched plasma and cerebrospinal fluid from patients with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 16:136–44
45. Mishra S, Blazey TM, Holtzman DM, Cruchaga C, Su Y, et al. 2018. Longitudinal brain imaging in preclinical Alzheimer disease: impact of *APOE* ε4 genotype. *Brain* 141:1828–39
46. Li B, Shi J, Gutman BA, Baxter LC, Thompson PM, et al. 2016. Influence of APOE genotype on hippocampal atrophy over time – an N=1925 surface-based ADNI study. *PLOS ONE* 11:e0152901
47. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, et al. 2009. Longitudinal modeling of age-related memory decline and the *APOE* ε4 effect. *N. Engl. J. Med.* 361:255–63
48. Chang L, Douet V, Bloss C, Lee K, Pritchett A, et al. 2016. Gray matter maturation and cognition in children with different *APOE* ε genotypes. *Neurology* 87:585–94
49. Lim YY, Mormino EC. 2017. *APOE* genotype and early β-amyloid accumulation in older adults without dementia. *Neurology* 89:1028–34
50. Therriault J, Benedet AL, Pascoal TA, Mathotaarachchi S, Savard M, et al. 2020. APOEε4 potentiates the relationship between amyloid-β and tau pathologies. *Mol. Psychiatry*. **https://doi.org/10.1038/s41380-020-0688-6**
51. Bergeron D, Ossenkoppele R, Laforce R. 2018. Evidence-based interpretation of amyloid-β PET results: a clinician's tool. *Alzheimer Dis. Assoc. Disord.* 32:28–34

52. Ossenkoppele R, Jansen WJ, Rabinovici GD, Knol DL, van der Flier WM, et al. 2015. Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis. *JAMA* 313:1939–49
53. La Joie R, Visani AV, Lesman-Segev OH, Baker SL, Edwards L, et al. 2020. Association of *APOE4* and clinical variability in Alzheimer disease with the pattern of tau- and amyloid-PET. *Neurology* 96:e650–61
54. Mattsson N, Ossenkoppele R, Smith R, Strandberg O, Ohlsson T, et al. 2018. Greater tau load and reduced cortical thickness in *APOE* ε4-negative Alzheimer's disease: a cohort study. *Alzheimers Res. Ther.* 10:77
55. Kaufman SK, Del Tredici K, Thomas TL, Braak H, Diamond MI. 2018. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol.* 136:57–67
56. Therriault J, Benedet AL, Pascoal TA, Mathotaarachchi S, Chamoun M, et al. 2020. Association of apolipoprotein E ε4 with medial temporal tau independent of amyloid-β. *JAMA Neurol.* 77:470–79
57. Baek MS, Cho H, Lee HS, Lee JH, Ryu YH, Lyoo CH. 2020. Effect of APOE ε4 genotype on amyloid-β and tau accumulation in Alzheimer's disease. *Alzheimers Res. Ther.* 12:140
58. Pihlajamäki M, Sperling RA. 2009. Functional MRI assessment of task-induced deactivation of the default mode network in Alzheimer's disease and at-risk older individuals. *Behav. Neurol.* 21:77–91
59. Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJS. 2009. Default-mode brain dysfunction in mental disorders: a systematic review. *Neurosci. Biobehav. Rev.* 33:279–96
60. Mevel K, Chételat G, Eustache F, Desgranges B. 2011. The default mode network in healthy aging and Alzheimer's disease. *Int. J. Alzheimers Dis.* 2011:535816
61. Sinha N, Berg CN, Tustison NJ, Shaw A, Hill D, et al. 2018. APOE ε4 status in healthy older African Americans is associated with deficits in pattern separation and hippocampal hyperactivation. *Neurobiol. Aging* 69:221–29
62. Gale SC, Gao L, Mikacenic C, Coyle SM, Rafaels N, et al. 2014. *APOε4* is associated with enhanced in vivo innate immune responses in human subjects. *J. Allergy Clin. Immunol.* 134:127–34
63. Egensperger R, Kösel S, Von Eitzen U, Graeber MB. 1998. Microglial activation in Alzheimer disease: association with APOE genotype. *Brain Pathol.* 8:439–47
64. Overmyer M, Helisalmi S, Soininen H, Laakso M, Riekkinen P, Alafuzoff I. 1999. Astrogliosis and the ApoE genotype: an immunohistochemical study of postmortem human brain tissue. *Dement. Geriatr. Cogn. Disord.* 10:252–57
65. Minett T, Classey J, Matthews FE, Fahrenhold M, Taga M, et al. 2016. Microglial immunophenotype in dementia with Alzheimer's pathology. *J. Neuroinflamm.* 13:135
66. Mosconi L, Berti V, Glodzik L, Pupi A, De Santi S, De Leon MJ. 2010. Pre-clinical detection of Alzheimer's disease using FDG-PET, with or without amyloid imaging. *J. Alzheimers Dis.* 20:843–54
67. Carbonell F, Charil A, Zijdenbos AP, Evans AC, Bedell BJ. 2014. β-Amyloid is associated with aberrant metabolic connectivity in subjects with mild cognitive impairment. *J. Cereb. Blood Flow Metab.* 34:1169–79
68. Chen K, Ayutyanont N, Langbaum JBS, Fleisher AS, Reschke C, et al. 2012. Correlations between FDG PET glucose uptake-MRI gray matter volume scores and apolipoprotein E ε4 gene dose in cognitively normal adults: a cross-validation study using voxel-based multi-modal partial least squares. *Neuroimage* 60:2316–22
69. Ossenkoppele R, van der Flier WM, Zwan MD, Adriaanse SF, Boellaard R, et al. 2013. Differential effect of *APOE* genotype on amyloid load and glucose metabolism in AD dementia. *Neurology* 80:359–65
70. Paranjpe MD, Chen X, Liu M, Paranjpe I, Leal JP, et al. 2019. The effect of ApoE ε4 on longitudinal brain region-specific glucose metabolism in patients with mild cognitive impairment: a FDG-PET study. *Neuroimage Clin.* 22:101795
71. Protas HD, Chen K, Langbaum JBS, Fleisher AS, Alexander GE, et al. 2013. Posterior cingulate glucose metabolism, hippocampal glucose metabolism, and hippocampal volume in cognitively normal, late-middle-aged persons at 3 levels of genetic risk for Alzheimer disease. *JAMA Neurol.* 70:320–25
72. Wetterau JR, Aggerbeck LP, Rall SC, Weisgraber KH. 1988. Human apolipoprotein E3 in aqueous solution. I. Evidence for two structural domains. *J. Biol. Chem.* 263:6240–48
73. Weisgraber KH. 1994. Apolipoprotein E: structure-function relationships. *Adv. Protein Chem.* 45:249–302

74. Mahley RW, Weisgraber KH, Huang Y. 2006. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *PNAS* 103:5644–51
75. Rall SC, Weisgraber KH, Mahley RW. 1982. Human apolipoprotein E. The complete amino acid sequence. *J. Biol. Chem.* 257:4171–78
76. Weisgraber KH, Rall SC, Mahley RW. 1981. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J. Biol. Chem.* 256:9077–83
77. Dong LM, Wilson C, Wardell MR, Simmons T, Mahley RW, et al. 1994. Human apolipoprotein E. Role of arginine 61 in mediating the lipoprotein preferences of the E3 and E4 isoforms. *J. Biol. Chem.* 269:22358–65
78. Hatters DM, Budamagunta MS, Voss JC, Weisgraber KH. 2005. Modulation of apolipoprotein E structure by domain interaction: differences in lipid-bound and lipid-free forms. *J. Biol. Chem.* 280:34288–95
79. Chen J, Li Q, Wang J. 2011. Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. *PNAS* 108:14813–18
80. Pitas RE, Boyles JK, Lee SH, Foss D, Mahley RW. 1987. Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochim. Biophys. Acta Lipids Lipid Metab.* 917:148–61
81. Ignatius MJ, Shooter EM, Pitas RE, Mahley RW. 1987. Lipoprotein uptake by neuronal growth cones in vitro. *Science* 236:959–62
82. Minagawa H, Gong JS, Jung CG, Watanabe A, Lund-Katz S, et al. 2009. Mechanism underlying apolipoprotein E (ApoE) isoform-dependent lipid efflux from neural cells in culture. *J. Neurosci. Res.* 87:2498–508
83. Deng J, Rudick V, Dory L. 1995. Lysosomal degradation and sorting of apolipoprotein E in macrophages. *J. Lipid Res.* 36:2129–40
84. Boyles JK, Zoellner CD, Anderson LJ, Kosik LM, Pitas RE, et al. 1989. A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J. Clin. Investig.* 83:1015–31
85. Ignatius MJ, Gebicke-Harter PJ, Skene JHP, Schilling JW, Weisgraber KH, et al. 1986. Expression of apolipoprotein E during nerve degeneration and regeneration. *PNAS* 83:1125–29
86. Pfrieger FW. 2003. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell. Mol. Life Sci.* 60:1158–71
87. Mauch DH, Nägler K, Schumacher S, Göritz C, Müller EC, et al. 2001. CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294:1354–57
88. Mahley RW. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240:622–30
89. Lane-Donovan C, Wong WM, Durakoglugil MS, Wasser CR, Jiang S, et al. 2016. Genetic restoration of plasma ApoE improves cognition and partially restores synaptic defects in ApoE-deficient mice. *J. Neurosci.* 36:10141–50
90. Yin C, Guo ZD, He ZZ, Wang ZY, Sun XC. 2019. Apolipoprotein E affects in vitro axonal growth and regeneration via the MAPK signaling pathway. *Cell Transplant.* 28:691–703
91. Li G, Bien-Ly N, Andrews-Zwilling Y, Xu Q, Bernardo A, et al. 2009. GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. *Cell Stem Cell* 5:634–45
92. Tensaouti Y, Stephanz EP, Yu TS, Kernie SG. 2018. ApoE regulates the development of adult newborn hippocampal neurons. *eNeuro* 5:ENEURO.0155-18.2018
93. Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, et al. 1999. Expression of human apolipoprotein E3 or E4 in the brains of Apoe$^{-/-}$ mice: isoform-specific effects on neurodegeneration. *J. Neurosci.* 19:4867–80
94. Buttini M, Masliah E, Yu G-Q, Palop JJ, Chang S, et al. 2010. Cellular source of apolipoprotein E4 determines neuronal susceptibility to excitotoxic injury in transgenic mice. *Am. J. Pathol.* 177:563–69
95. Boehm-Cagan A, Michaelson DM. 2014. Reversal of apoE4-driven brain pathology and behavioral deficits by bexarotene. *J. Neurosci.* 34:7293–301
96. Andrews-Zwilling Y, Bien-Ly N, Xu Q, Li G, Bernardo A, et al. 2010. Apolipoprotein E4 causes age- and tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. *J. Neurosci.* 30:13707–17

97. Salomon-Zimri S, Boehm-Cagan A, Liraz O, Michaelson DM. 2014. Hippocampus-related cognitive impairments in young apoE4 targeted replacement mice. *Neurodegener. Dis.* 13:86–92
98. Knoferle J, Yoon SY, Walker D, Leung L, Gillespie AK, et al. 2014. Apolipoprotein E4 produced in GABAergic interneurons causes learning and memory deficits in mice. *J. Neurosci.* 34:14069–78
99. Wang C, Najm R, Xu Q, Jeong DE, Walker D, et al. 2018. Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. *Nat. Med.* 24:647–57
100. Hashimoto Y, Jiang H, Niikura T, Ito Y, Hagiwara A, et al. 2000. Neuronal apoptosis by apolipoprotein E4 through low-density lipoprotein receptor-related protein and heterotrimeric GTPases. *J. Neurosci.* 20:8401–9
101. Rodriguez GA, Burns MP, Weeber EJ, Rebeck GW. 2013. Young APOE4 targeted replacement mice exhibit poor spatial learning and memory, with reduced dendritic spine density in the medial entorhinal cortex. *Learn. Mem.* 20:256–66
102. Jain S, Yoon SY, Leung L, Knoferle J, Huang Y. 2013. Cellular source-specific effects of apolipoprotein (Apo) E4 on dendrite arborization and dendritic spine development. *PLOS ONE* 8:e59478
103. Zhao J, Fu Y, Yamazaki Y, Ren Y, Davis MD, et al. 2020. *APOE4* exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids. *Nat. Commun.* 11:5540
104. Lin Y-T, Seo J, Gao F, Feldman HM, Wen H-L, et al. 2018. *APOE4* causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron* 98:1141–54.e7
105. Najm R, Zalocusky KA, Zilberter M, Yoon SY, Hao Y, et al. 2020. In vivo chimeric Alzheimer's disease modeling of apolipoprotein E4 toxicity in human neurons. *Cell Rep.* 32:107962
106. Chen Y, Durakoglugil MS, Xian X, Herz J. 2010. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *PNAS* 107:12011–16
107. Hu YB, Dammer EB, Ren RJ, Wang G. 2015. The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. *Transl. Neurodegener.* 4:18
108. Van Acker ZP, Bretou M, Annaert W. 2019. Endo-lysosomal dysregulations and late-onset Alzheimer's disease: impact of genetic risk factors. *Mol. Neurodegener.* 14:20
109. Morrow JA, Hatters DM, Lu B, Höchtl P, Oberg KA, et al. 2002. Apolipoprotein E4 forms a molten globule: a potential basis for its association with disease. *J. Biol. Chem.* 277:50380–85
110. Yamauchi K, Tozuka M, Hidaka H, Nakabayashi T, Sugano M, Katsuyama T. 2002. Isoform-specific effect of apolipoprotein E on endocytosis of β-amyloid in cultures of neuroblastoma cells. *Ann. Clin. Lab. Sci.* 32:65–74
111. Li J, Kanekiyo T, Shinohara M, Zhang Y, LaDu MJ, et al. 2012. Differential regulation of amyloid-β endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms. *J. Biol. Chem.* 287(53):44593–601
112. Nuriel T, Peng KY, Ashok A, Dillman AA, Figueroa HY, et al. 2017. The endosomal-lysosomal pathway is dysregulated by *APOE4* expression in vivo. *Front. Neurosci.* 11:702
113. Selkoe DJ, Hardy J. 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* 8:595–608
114. Kanekiyo T, Xu H, Bu G. 2014. ApoE and Aβ in Alzheimer's disease: accidental encounters or partners? *Neuron* 81:740–54
115. Huynh TPV, Davis AA, Ulrich JD, Holtzman DM. 2017. Apolipoprotein E and Alzheimer's disease: the influence of apolipoprotein E on amyloid-β and other amyloidogenic proteins. *J. Lipid Res.* 58:824–36
116. Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, et al. 2000. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *PNAS* 97:2892–97
117. Koffie RM, Hashimoto T, Tai H-C, Kay KR, Serrano-Pozo A, et al. 2012. Apolipoprotein E4 effects in Alzheimer's disease are mediated by synaptotoxic oligomeric amyloid-β. *Brain* 135:2155–68
118. Bilousova T, Melnik M, Miyoshi E, Gonzalez BL, Poon WW, et al. 2019. Apolipoprotein E/amyloid-β complex accumulates in Alzheimer disease cortical synapses via apolipoprotein E receptors and is enhanced by *APOE4*. *Am. J. Pathol.* 189:1621–36

119. Bien-Ly N, Gillespie AK, Walker D, Yoon SY, Huang Y. 2012. Reducing human apolipoprotein E levels attenuates age-dependent Aβ accumulation in mutant human amyloid precursor protein transgenic mice. *J. Neurosci.* 32:4803–11
120. Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, et al. 1997. Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. *Natl. Genet.* 17:263–64
121. Kim J, Jiang H, Park S, Eltorai AEM, Stewart FR, et al. 2011. Haploinsufficiency of human *APOE* reduces amyloid deposition in a mouse model of amyloid-β amyloidosis. *J. Neurosci.* 31:18007–12
122. Thal DR, Capetillo-Zarate E, Schultz C, Rüb U, Saido TC, et al. 2005. Apolipoprotein E co-localizes with newly formed amyloid β-protein (Aβ) deposits lacking immunoreactivity against N-terminal epitopes of Aβ in a genotype-dependent manner. *Acta Neuropathol.* 110:459–71
123. Liu CC, Zhao N, Fu Y, Wang N, Linares C, et al. 2017. ApoE4 accelerates early seeding of amyloid pathology. *Neuron* 96:1024–32.e3
124. Castano EM, Prelli F, Wisniewski T, Golabek A, Kumar RA, et al. 1995. Fibrillogenesis in Alzheimer's disease of amyloid β peptides and apolipoprotein E. *Biochem. J.* 306:599–604
125. Li J, Kanekiyo T, Shinohara M, Zhang Y, LaDu MJ, et al. 2012. Differential regulation of amyloid-β endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms. *J. Biol. Chem.* 287:44593–601
126. Ye S, Huang Y, Müllendorff K, Dong L, Giedt G, et al. 2005. Apolipoprotein (apo) E4 enhances amyloid peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. *PNAS* 102:18700–5
127. Dafnis I, Raftopoulou C, Mountaki C, Megalou E, Zannis VI, Chroni A. 2018. ApoE isoforms and carboxyl-terminal-truncated apoE4 forms affect neuronal BACE1 levels and Aβ production independently of their cholesterol efflux capacity. *Biochem. J.* 475:1839–59
128. Huang Y-WA, Zhou B, Wernig M, Südhof TC. 2017. ApoE2, apoE3, and apoE4 differentially stimulate APP transcription and Aβ secretion. *Cell* 168:427–41.e21
129. Lee VMY, Goedert M, Trojanowski JQ. 2001. Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* 24:1121–59
130. Chang C-W, Shao E, Mucke L. 2021. Tau: enabler of diverse brain disorders and target of rapidly evolving therapeutic strategies. *Science* 371:eabb8255
131. Spillantini MG, Goedert M. 2013. Tau pathology and neurodegeneration. *Lancet Neurol.* 12:609–22
132. Huang Y, Liu XQ, Wyss-Coray T, Brecht WJ, Sanan DA, Mahley RW. 2001. Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *PNAS* 98:8838–43
133. Brecht WJ, Harris FM, Chang S, Tesseur I, Yu G-Q, et al. 2004. Neuron-specific apolipoprotein E4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. *J. Neurosci.* 24:2527–34
134. Harris FM, Brecht WJ, Xu Q, Tesseur I, Kekonius L, et al. 2003. Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *PNAS* 100:10966–71
135. Chang S, Ma TR, Miranda RD, Balestra ME, Mahley RW, Huang Y. 2005. Lipid- and receptor-binding regions of apolipoprotein E4 fragments act in concert to cause mitochondrial dysfunction and neurotoxicity. *PNAS* 102:18694–99
136. Shi Y, Yamada K, Antony Liddelow S, Smith ST, Zhao L, et al. 2017. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* 549:523–27
137. Frost B, Jacks RL, Diamond MI. 2009. Propagation of tau misfolding from the outside to the inside of a cell. *J. Biol. Chem.* 284:12845–52
138. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, et al. 2009. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat. Cell Biol.* 11:909–13
139. Goedert M, Eisenberg DS, Crowther RA. 2017. Propagation of tau aggregates and neurodegeneration. *Annu. Rev. Neurosci.* 40:189–210
140. Richey PL, Siedlak SL, Smith MA, Perry G. 1995. Apolipoprotein E interaction with the neurofibrillary tangles and senile plaques in Alzheimer disease: implications for disease pathogenesis. *Biochem. Biophys. Res. Commun.* 208:657–63

141. Harris FM, Brecht WJ, Xu Q, Mahley RW, Huang Y. 2004. Increased tau phosphorylation in apolipoprotein E4 transgenic mice is associated with activation of extracellular signal-regulated kinase: modulation by zinc. *J. Biol. Chem.* 279:44795–801
142. Hoe H-S, Harris DC, Rebeck GW. 2005. Multiple pathways of apolipoprotein E signaling in primary neurons. *J. Neurochem.* 93:145–55
143. Braithwaite SP, Stock JB, Lombroso PJ, Nairn AC. 2012. Protein phosphatases and Alzheimer's disease. *Prog. Mol. Biol. Transl. Sci.* 106:343–79
144. Theendakara V, Bredesen DE, Rao RV. 2017. Downregulation of protein phosphatase 2A by apolipoprotein E: implications for Alzheimer's disease. *Mol. Cell. Neurosci.* 83:83–91
145. Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, et al. 2013. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *PNAS* 110:E3138–47
146. Rauch JN, Chen JJ, Sorum AW, Miller GM, Sharf T, et al. 2018. Tau internalization is regulated by 6-O sulfation on heparan sulfate proteoglycans (HSPGs). *Sci. Rep.* 8:6382
147. Jablonski AM, Warren L, Usenovic M, Zhou H, Sugam J, et al. 2021. Astrocytic expression of the Alzheimer's disease risk allele, *ApoE ε4*, potentiates neuronal tau pathology in multiple preclinical models. *Sci. Rep.* 11:3438
148. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, et al. 2020. LRP1 is a master regulator of tau uptake and spread. *Nature* 580:381–85
149. Arboleda-Velasquez JF, Lopera F, O'Hare M, Delgado-Tirado S, Marino C, et al. 2019. Resistance to autosomal dominant Alzheimer's disease in an *APOE3* Christchurch homozygote: a case report. *Nat. Med.* 25:1680–83
150. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, et al. 2014. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat. Neurosci.* 17:131–43
151. Kloske CM, Wilcock DM. 2020. The important interface between apolipoprotein E and neuroinflammation in Alzheimer's disease. *Front. Immunol.* 11:754
152. Shi Y, Holtzman DM. 2018. Interplay between innate immunity and Alzheimer disease: APOE and TREM2 in the spotlight. *Nat. Rev. Immunol.* 18:759–72
153. LaDu MJ, Shah JA, Reardon CA, Getz GS, Bu G, et al. 2001. Apolipoprotein E and apolipoprotein E receptors modulate Aβ-induced glial neuroinflammatory responses. *Neurochem. Int.* 39:427–34
154. Zhu Y, Nwabuisi-Heath E, Dumanis SB, Tai LM, Yu C, et al. 2012. *APOE* genotype alters glial activation and loss of synaptic markers in mice. *Glia* 60:559–69
155. Rodriguez GA, Tai LM, LaDu MJ, Rebeck GW. 2014. Human APOE4 increases microglia reactivity at Aβ plaques in a mouse model of Aβ deposition. *J. Neuroinflamm.* 11:111
156. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, et al. 2007. The classical complement cascade mediates CNS synapse elimination. *Cell* 131:1164–78
157. Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, et al. 2007. Glutamate exocytosis from astrocytes controls synaptic strength. *Nat. Neurosci.* 10:331–39
158. Bak LK, Schousboe A, Sonnewald U, Waagepetersen HS. 2006. Glucose is necessary to maintain neurotransmitter homeostasis during synaptic activity in cultured glutamatergic neurons. *J. Cereb. Blood Flow Metab.* 26:1285–97
159. Sofroniew MV, Vinters HV. 2010. Astrocytes: biology and pathology. *Acta Neuropathol.* 119:7–35
160. Chung WS, Verghese PB, Chakraborty C, Joung J, Hyman BT, et al. 2016. Novel allele-dependent role for APOE in controlling the rate of synapse pruning by astrocytes. *PNAS* 113:10186–91
161. Zhao J, Davis MD, Martens YA, Shinohara M, Graff-Radford NR, et al. 2017. *APOE* ε4/ε4 diminishes neurotrophic function of human iPSC-derived astrocytes. *Hum. Mol. Genet.* 26:2690–700
162. Simonovitch S, Schmukler E, Bespalko A, Iram T, Frenkel D, et al. 2016. Impaired autophagy in APOE4 astrocytes. *J. Alzheimers Dis.* 51:915–27
163. Colonna M, Butovsky O. 2017. Microglia function in the central nervous system during health and neurodegeneration. *Annu. Rev. Immunol.* 35:441–68
164. Junker A, Krumbholz M, Eisele S, Mohan H, Augstein F, et al. 2009. MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. *Brain* 132:3342–52
165. Ransohoff RM, Cardona AE. 2010. The myeloid cells of the central nervous system parenchyma. *Nature* 468:253–62

166. Reier PJ, Houle JD. 1988. The glial scar: its bearing on axonal elongation and transplantation approaches to CNS repair. *Adv. Neurol.* 47:87–138
167. Sarlus H, Heneka MT. 2017. Microglia in Alzheimer's disease. *J. Clin. Investig.* 127:3240–49
168. Kamphuis W, Orre M, Kooijman L, Dahmen M, Hol EM. 2012. Differential cell proliferation in the cortex of the APPswePS1dE9 Alzheimer's disease mouse model. *Glia* 60:615–29
169. Vitek MP, Brown CM, Colton CA. 2009. APOE genotype-specific differences in the innate immune response. *Neurobiol. Aging* 30:1350–60
170. Muth C, Hartmann A, Sepulveda-Falla D, Glatzel M, Krasemann S. 2019. Phagocytosis of apoptotic cells is specifically upregulated in ApoE4 expressing microglia in vitro. *Front. Cell. Neurosci.* 13:181
171. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, et al. 2017. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* 47:566–81.e9
172. Friedberg JS, Aytan N, Cherry JD, Xia W, Standring OJ, et al. 2020. Associations between brain inflammatory profiles and human neuropathology are altered based on apolipoprotein E ε4 genotype. *Sci. Rep.* 10:2924
173. Boyles JK, Pitas RE, Wilson E, Mahley RW, Taylor JM. 1985. Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Investig.* 76:1501–13
174. Xu Q, Bernardo A, Walker D, Kanegawa T, Mahley RW, Huang Y. 2006. Profile and regulation of apolipoprotein E (apoE) expression in the CNS in mice with targeting of green fluorescent protein gene to the apoE locus. *J. Neurosci.* 26:4985–94
175. Mahley RW, Huang Y. 2012. Apolipoprotein E sets the stage: response to injury triggers neuropathology. *Neuron* 76:871–85
176. Mahley RW, Rall SC. 2000. Apolipoprotein E: far more than a lipid transport protein. *Annu. Rev. Genom. Hum. Genet.* 1:507–37
177. Nakamura T, Watanabe A, Fujino T, Hosono T, Michikawa M. 2009. Apolipoprotein E4 (1–272) fragment is associated with mitochondrial proteins and affects mitochondrial function in neuronal cells. *Mol. Neurodegener.* 4:35
178. Leung L, Andrews-Zwilling Y, Yoon SY, Jain S, Ring K, et al. 2012. Apolipoprotein E4 causes age- and sex-dependent impairments of hilar GABAergic interneurons and learning and memory deficits in mice. *PLOS ONE* 7:e53569
179. Tong LM, Djukic B, Arnold C, Gillespie AK, Yoon SY, et al. 2014. Inhibitory interneuron progenitor transplantation restores normal learning and memory in apoE4 knock-in mice without or with Aβ accumulation. *J. Neurosci.* 34:9506–15
180. Leal SL, Landau SM, Bell RK, Jagust WJ. 2017. Hippocampal activation is associated with longitudinal amyloid accumulation and cognitive decline. *eLife* 6:e22978
181. Dennis NA, Browndyke JN, Stokes J, Need A, Burke JR, et al. 2010. Temporal lobe functional activity and connectivity in young adult *APOE* ε4 carriers. *Alzheimers Dement.* 6:303–11
182. Nuriel T, Angulo SL, Khan U, Ashok A, Chen Q, et al. 2017. Neuronal hyperactivity due to loss of inhibitory tone in *APOE4* mice lacking Alzheimer's disease-like pathology. *Nat. Commun.* 8:1464
183. Gillespie AK, Jones EA, Lin YH, Karlsson MP, Kay K, et al. 2016. Apolipoprotein E4 causes age-dependent disruption of slow gamma oscillations during hippocampal sharp-wave ripples. *Neuron* 90:740–51
184. Jones EA, Gillespie AK, SY Yoon, Frank LM, Huang Y. 2019. Early hippocampal sharp-wave ripple deficits predict later learning and memory impairments in an Alzheimer's disease mouse model. *Cell Rep.* 29:2123–33.e4
185. Najm R, Jones EA, Huang Y. 2019. Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease. *Mol. Neurodegener.* 14:24