Review

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Role of Apolipoprotein E in Alzheimer's Disease Pathogenesis, Prognosis and Treatment

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Alzheimer's disease (AD) is an incurable and progressive neurodegenerative disease with increasing prevalence worldwide. Previous trials of anti-amyloid and anti-tau immunotherapy indicate that additional research needs to be conducted on other mechanisms to find curative or disease-modifying therapy. This review focuses on apolipoprotein E (ApoE), a critical protein in brain lipid metabolism that acts specifically in the clearance and transport of lipids and cholesterol. The ApoE4 allele confers substantial gene dose-dependent risk of developing AD and lowers the age of onset of AD, although the mechanisms of influence remain incompletely understood. The other isoforms bring different levels of AD risk. ApoE2 is protective while ApoE3 is the most common isoform and is considered neutral. An overview is presented of the latest information on the role of ApoE in AD pathogenesis with an emphasis on pathways that are involved in AD development and interactions with crucial processes in different cell types in the brain. Elucidating the key interactions of ApoE with multiple aspects of brain function can be useful for designing novel ApoE-targeted therapeutic approaches.

Keywords: Alzheimer's disease; neuroinflammation; apolipoprotein E; blood brain barrier; drug development; Alzheimer's disease prevention; lipids

Introduction

Alzheimer's disease (AD) is an incurable, progressive neurodegenerative disorder that manifests after an extended pre-symptomatic period as a deterioration in memory, thinking and language skills, and spatial reasoning [1]. As the disease advances, it interferes more and more with the ability to conduct the activities of daily living. As of 2020, dementia has been diagnosed in approximately 55 million individuals worldwide and AD is the most common form of dementia, accounting for about 60–80% of all dementia cases [2–4]. AD usually affects individuals older than age 65, and incidence increases with age [5]. Neurocognitive testing and exclusion of other disorders combined with newer biochemical markers of dementia measured in serum or cerebrospinal fluid (CSF) are improving the accuracy of diagnosis [6]. However, a definitive diagnosis can only be made post mortem by examination of brain tissue, where cortical atrophy and ventricle enlargement would be present in addition to sulcal widening [7,8]. The pathology of AD under the microscope is characterized by the presence of extracellular plaques formed by the accumulation of aggregated amyloid-β peptide and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein [9,10]. However, human trials of anti-amyloid and anti-tau immunotherapy indicate that these misfolded proteins are not the cause of AD and have spurred the investigation of other mechanisms in an effort to find curative or disease-modifying therapy [11–14]. Other than the new anti-amyloid drugs, the pharmacologic approach to AD is limited and consists of non-disease-modifying treatment with either acetylcholinesterase inhibitors, memantine or both combined [15].

Much attention is being given to apolipoprotein E (ApoE) because polymorphisms in the gene encoding this protein have been linked to AD for over 25 years, even though the mechanism underlying their contribution to the disease is unknown [16]. Homozygosity for the *ApoE4* gene has recently been confirmed as a distinct genetic type of AD [17]. This review provides the latest information on the relationship between inheritance of specific ApoE alleles and development of AD as a basis for uncovering new treatment approaches.

Table 1. ApoE properties, production and regulation in the central nervous system (CNS).

Property	Key features
Molecular weight	35 kDa
Residue count	299 amino acids
Structure	N-terminal domain (receptor binding), C-terminal domain (lipid binding)
Alleles	ApoE2 (protective against AD), ApoE3 (neutral), ApoE4 (greatest genetic risk factor for AD)
Amino acid sequence differences	ApoE2 – Cys112, Cys 158; ApoE3 – Cys112, Arg158; ApoE4 – Arg112, Arg158
Allele population frequency	ApoE2 – 6.4%; ApoE3 – 78.3%; ApoE4 – 14.5%
CNS sources of production (unstressed)	Astrocytes, microglia, vascular mural cells, choroid plexus
Added source of production (stress conditions)	Neurons
Blood brain barrier	Cannot cross, CNS ApoE separate from liver-synthesized ApoE
Receptors	LDL receptor-related protein 1 (LRP1), ApoE receptor 2, very low density lipoprotein receptor (VLDLr)

AD, Alzheimer's disease; ApoE, apolipoprotein E; LDL, low density lipoprotein.

ApoE Structure and Function

ApoE is a 35 kDa, 299-residue glycoprotein involved in cholesterol transport and fatty acid metabolism [18–20]. ApoE is encoded on chromosome 19 and the full-length protein is composed of an N-terminal domain and a C-terminal domain that are linked with a flexible hinge region. The N-terminal domain has the low density lipoprotein (LDL) receptor binding region while the C-terminal domain has the lipid binding region in order to perform the function of lipid transport [21].

ApoE is the most abundant lipoprotein in the central nervous system (CNS) where it is produced predominantly by astrocytes, microglia vascular mural cells, and choroid plexus, and, under excitotoxic stress conditions, by neurons as well [22–25]. Since it cannot cross the blood brain barrier (BBB), CNS-produced ApoE is separate from that synthesized in the liver and is regulated independently [26–28]. In mice, ApoE deficiency reduces brain cholesterol content, primarily by affecting astrocytes [29]. ApoE provides cholesterol to cells of the brain through its receptors, including LDL receptor-related protein 1 (LRP1), a critical transmembrane cell surface receptor that binds ApoE with high affinity [30]. LRP1 is expressed in neurons, glia, and vascular smooth muscle and is believed to act as a scavenger, endocytosing waste products in the brain [31,32] (Table 1).

Lipids serve as essential components for energy storage and transportation. They facilitate the efficient mobilization and utilization of energy, ensuring the proper functioning of various physiological processes. Additionally, lipids contribute significantly to the structural integrity and functionality of cell membranes, which are fundamental for cellular communication and regulation [33]. Disruptions in lipid metabolism can lead to brain injury due to the accumulation of lipids in peripheral tissues like the brain and blood vessels [34]. ApoE is a critical protein in CNS lipid metabolism, specifically in the clearance and transport of lipids and cholesterol via interactions with microglial cells [35,36]. The different ApoE isoforms vary in efficiency, with ApoE4 being the least effective with the lowest capacity for lipid uptake [37]. Clearance of lipids in the brain is reduced with ApoE4, impacting neuronal protection and repair [38]. In mouse studies, ApoE4 expression causes brain region-specific lipid changes such that the entorhinal cortex, an AD-vulnerable area, exhibits pronounced lipid alterations [39]. Microglia and astrocytes harboring ApoE4 accumulate lipid droplets and triglycerides and conditioned media from the lipid droplet-containing ApoE4 microglia is neurotoxic, promoting tau phosphorylation [40–42].

ApoE interacts with many other proteins in the CNS in order to tightly regulate cholesterol content and distribution. Adenosine triphosphate (ATP) binding cassette transporter A1 (ABCA1), expressed in all cell types in the brain, transfers cholesterol to ApoE-containing lipoprotein particles similar to high-density lipoproteins (HDL) [43,44]. Astrocytes are the primary synthesizers of brain cholesterol and ApoE-containing particles within astrocytes are loaded with cholesterol and then conveyed to neurons where they are internalized via neuronal lipoprotein receptors, allowing for proper brain cholesterol allocation [45,46]. Neurons use cholesterol to maintain normal physiology, membrane integrity and synaptic processes [47]. In *ApoE* knockout mice, hippocampal ABCA1 is reduced [45]. When comparing ABCA1 expression in a murine astrocyte cell line harboring either human ApoE3 or ApoE4, the ApoE4 astrocytes have less ABCA1, which may account, to some extent for poorer lipidation in the presence of ApoE4 [48]. When lipidation is poor, ApoE is more likely to aggregate or to degrade into toxic fragments [49,50]. ABCA1 is also important not only in lipid transport, but also in protecting against neuroinflammation and fostering synaptogenesis [51–53].

ApoE Alleles and Alzheimer's Risk

The three distinct alleles for ApoE are ApoE2, ApoE3, and ApoE4 and they have frequencies in the population of

6.4%, 78.3% and 14.5% respectively, with variations based on ethnic origin that have been shown to have different effects on the risk of developing AD [54]. Depending on which combination of the alleles one inherits, they are considered low, moderate, or high risk. ApoE2 is shown to have the most protective effects against AD, as it has poor receptor binding properties in the N-terminal region [55–57]. ApoE3 is the normal allele and is present in approximately 78.3% of the population and hasn't been shown to increase or decrease the risk of the condition. ApoE4 has been found to increase the amounts of both amyloid plaques and hyperphosphorylated tau proteins, and therefore decreases the age of late-onset Alzheimer's disease [56,58]. The ApoE4 allele is associated with a faster rate of hippocampal atrophy and a reduced grey matter volume in the brain, particularly in the hippocampal region [59–62].

The Effects of the ApoE4 Isoform on Different Brain Cell Types

ApoE influences a variety of processes related to the development of AD, including cholesterol transport, glucose metabolism, amyloid-β accumulation, and tauopathy. ApoE interacts with these processes in an isoform-specific manner, with ApoE4 exhibiting particular neurotoxicity [63,64].

In addition to ApoE isoform-related differences in function and interaction with lipids, some of the ApoE4 toxicity may be due to the way in which ApoE4 itself is subject to proteolytic cleavage and fragmentation [56]. ApoE4 generates fragments that can be neurotoxic while ApoE3 has been found to generate more of a specific 25 kDa neuroprotective fragment [65–67].

The following subsections are devoted to the key ways in which ApoE4 affects molecular pathways involved in AD pathogenesis.

Neurons

ApoE4 in neurons contributes to the upregulation of cholesterol precursor levels and an increased intracellular cholesterol concentration [68]. These elevated levels of cholesterol are a result of the decreased affinity of the ApoE4 protein for cholesterol relative to the higher affinities of the ApoE3 and ApoE2 isoforms. Thus, ApoE4 has a reduced lipidation ability with inefficient transport leading to inadequate redistribution of lipids in the brain [69,70]. A disruption of cholesterol homeostasis by ApoE4 presents itself in the form of an accumulation of lipid droplets in neurons, which often store esterified cholesterol, despite lipid droplets being uncommonly found in neurons [71,72]. Abnormal accumulation of lipid droplets has been detected in conditions of hypoxia, inflammation or oxidative stress [73].

High concentrations of intracellular cholesterol have neurodegenerative effects characteristic of AD. In particular, elevated cholesterol can contribute to increased cholesterol oxidation producing high concentrations of oxysterols in the brain as a mechanism of preventing hypercholesterolemia [74]. Heightened oxysterol concentrations can result in an oxysterol-induced astrocytic reactivity, which is linked to the release of pro-inflammatory cytokines by astrocytes, synaptotoxic effects, and an increase in cleaved or activated caspase-3 protein, a zymogen associated with apoptosis, in neurons [75,76]. This suggests that induced neuroinflammation, synaptotoxicity, and cell death are neurodegenerative consequences of the reduced cholesterol transport capability of the ApoE4 isoform in AD.

Oligodendrocytes

Oligodendrocytes are central in the process of myelination of axons in the CNS. Cholesterol is needed in large quantities as a component of lipid-rich myelin membranes and is either made by oligodendrocytes or transferred to them from nearby cells [77,78]. Blanchard *et al*. [79] performed a series of studies in mice to complement analysis of post mortem human brain tissue to examine the role of ApoE4 in myelination and found that the presence of ApoE4 heightened expression of cholesterol biosynthesis genes, while compromising the cholesterol trafficking and clearance genes that promote transport of cholesterol out of the cells. This combination leads to intracellular cholesterol accumulation and interference with utilization of cholesterol to produce myelin. A decrease in myelin-associated gene expression was found to accompany ApoE4 genotype in human oligodendrocytes. In their transgenic mouse model, ApoE4 decreased myelination while pharmacologic manipulation to increase cholesterol transport improved myelination and cognition. In addition, in cultured oligodendroglia homozygous for the ApoE4 allele compared to cultured oligodendroglia homozygous for the ApoE3 allele, they detected an increase in lysophosphatidylcholine and lysophosphatidic acid, which has been linked to brain inflammation and demyelination [79,80]. Poor myelination and myelin breakdown are known pathological features of AD [81,82].

Astrocytes

Astrocytes provide metabolic support to neurons by performing glycolysis and thereby utilizing about 80% of the glucose available to them to generate lactate and pyruvate. When astrocytes release lactate, neurons can derive energy via lactate shuttle, and in addition to acting as a vehicle to provide energy, lactate is also neuroprotective [83]. Astrocytes not only synthesize apolipoproteins for cholesterol transport, they also accept neurotoxic fatty acids produced in excess by overactive neurons and ApoE serves as the carrier for transfer of these fatty acids [84]. ApoE is produced in neurons when there is stress or injury [85]. In a series of cell culture experiments using embryonic hippocampal neurons and astrocytes from humanized ApoE3

or ApoE4 mice, Qi *et al*. [86] showed that ApoE4 neurons are less able to form lipid droplets and are unable to efficiently move fatty acids into neuronal lipid droplets, leading to decreased transfer of fatty acids to astrocytes and lipotoxic effects on neurons. Adding to the burden, ApoE4 astrocytes are limited in their fatty acid oxidation capabilities, preventing them from degrading fatty acids and making them less able to act as a support for neurons. Overall, the disruption of coupling in lipid metabolism between neurons and astrocytes by the presence of ApoE4 leads to impaired neuronal bioenergetics. Patil and Kuehn [87] used cultured human ApoE4-expressing astrocytes to examine the relationship between glucose metabolism and lipidation of ApoE4 and found that higher glucose concentrations resulted in increased ApoE4 lipidation and increased lactate production. Co-treatment with a glycolytic inhibitor significantly inhibited ApoE lipidation. Their study suggests that ApoE4 may cause poor lipidation due to glucose hypometabolism.

Microglia

Microglia, considered the immune cells of the CNS, also participate in immune surveillance, synaptic pruning and phagocytosis of pathogens and debris [88,89]. Microglia play a crucial role in lipid transport and consumption. ApoE4 microglia have a reduced capacity for lipid uptake, increasing the amount of extracellular lipids, which deleteriously affects neuronal activity [90]. Increased intracellular lipids in ApoE4 microglia with impairment of fatty acid oxidation leads to microglial dysfunction, interferes with their ability to support neurons and fosters a proinflammatory state [91–93]. ApoE4 microglia are flexible in their energy source and can switch from oxidative phosphorylation to glycolysis in the presence of inflammatory stimuli, affecting the rate of fatty acid catabolism and further enhancing inflammation [90,94]. ApoE4 microglia exhibit a higher propensity toward glycolysis [95].

Haney *et al*. [41] reported that one mechanism through which ApoE genotype affects microglial lipids is through the enzyme acyl-CoA synthetase long-chain family member 1 (ACSL1), which is important in lipid droplet biogenesis. Using human induced pluripotent stem cell-derived microglia, they found that lipid droplet accumulation is greater in cells homozygous for ApoE4 compared to cells homozygous for ApoE3 and this difference is magnified in the presence of fibrillar amyloid-β. The pattern was the same for ACSL1 expression and thus, increased lipid droplets correlated with upregulation of ACSL1. Further, the increase in lipid droplets in the microglia was abrogated by an ACSL1 inhibitor. ACSL1 is important for lipid droplet accumulation in microglia and is influenced by ApoE genotype. Since ACSL1 is not upregulated in neurons, the authors postulate that lipid accumulation in AD neurons originate from ApoE4 microglia.

Fig. 1 provides an overview of the multitude of ways in which ApoE4 influences processes that contribute to AD pathogenesis in cell types found in the brain.

The Pathways Affected by the ApoE4 Isoform

Amyloid-β Peptide

An accumulation of amyloid plaque generates neurotoxic effects linked to cognitive impairment in AD [96]. ApoE is involved in the regulation and clearance of amyloid-β in the brain and the interaction is complex and the focus of intense scrutiny. In a molecular modeling study, Lewkowicz *et al*. [97] examined ApoE binding to fibrillar amyloid-β and found that binding occurs at exposed hydrophobic surfaces of the amyloid and changes fibril morphology with either anti- or pro-amyloid effects depending on a multitude of factors. The ApoE4 isoform has a particularly high affinity for amyloid-β oligomers [98]. ApoE4 plays a disruptive role by increasing the amyloid-β half-life, stabilizing oligomers, promoting aggregation and preventing amyloid-β clearance over time [99–101]. In a murine model in which human ApoE3 or ApoE4 is inducibly expressed, early astrocyte expression of ApoE4 was most effective at promoting amyloid-β brain deposition [102]. Further, brain half-life of amyloid-β in the hippocampus was longer in the ApoE4 mice. Additionally, ApoE4 has been found to stimulate amyloid precursor protein (APP) production as demonstrated by increased *APP* gene transcription in human neurons with ApoE4 [35,103].

Amyloid-β clearance is specifically mediated by ApoE binding and transport into the systemic circulation and induction of its enzymatic degradation [104,105]. The ApoE4 isoform has a lower affinity for amyloid-β and is less efficient at promoting its clearance and proteolytic degradation than other isoforms, enabling deposition of amyloid plaque [104,106,107]. Amyloid-β phagocytosis by microglia is reduced in an ApoE4 isoform-specific manner [108,109].

Chernyaeva *et al*. [110] found that when amyloid-β42 forms complexes with ApoE, complement regulator factor H (FH) does not bind as well to ApoE4/amyloid-β42 complexes as to ApoE2 or ApoE3/amyloid-β42 complexes. Since the FH binding is protective against amyloid-β oligomerization and neuroinflammation, reduced binding in an isoform-specific manner to ApoE4 may contribute to AD pathology [11].

Since APP, ApoE and amyloid-β are all ligands for LRP1, the role of ApoE isoform in transport via LRP1 is considered relevant to AD pathogenesis [111]. LRP1 is involved in clearing amyloid-β from neurons and astrocytes [112]. In mouse models, LRP1 knockout in brain endothelial cells leads to reduced egress of amyloid-β from the brain across the BBB [113]. ApoE4 may compete with amyloid-β for occupation of LRP1 in glial cells [114,115].

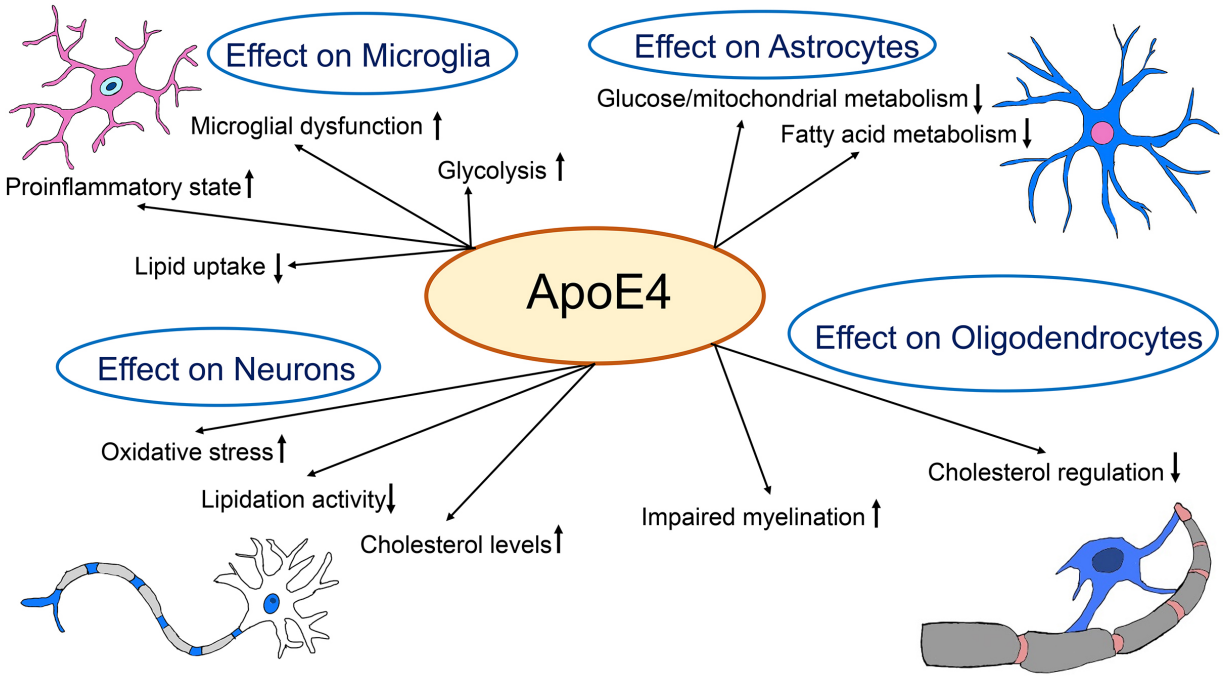

Fig. 1. Schematic representation of the key effects of ApoE4 on neurons and glial supporting cells in the brain. ApoE4 increases microglial dysfunction due to impairment of fatty acid oxidation which interferes with their ability to support neurons and fosters a proinflammatory state. ApoE4 impacts astrocytes by impairing fatty acid metabolism and inducing poor lipidation due to glucose and mitochondrial hypometabolism. In oligodendrocytes, ApoE4 disrupts cholesterol regulation and impairs myelination. Overall, the effect on neurons of the ApoE4 polymorphism is increased oxidative stress, reduced lipidation activity, and elevated levels of cholesterol. ↑ = increases, ↓ = decreases. Drawn by PowerPoint version 2021 (Microsoft Corporation, Redmond, WA, USA) .

In transgenic mice that form plaques of amyloid-β and have been engineered to exhibit neuron-specific *LRP1* knockout, Tachibana *et al.* [116] compared the effects of an ApoE4 versus an ApoE3 phenotype and found that increased brain amyloid deposition with ApoE4 was abolished with knockout of *LRP1*. This indicates that detrimental effects of ApoE4 on amyloid brain pathology are dependent on neuronal LRP1.

Tau Protein

ApoE4 contributes to the development of tauopathy, thereby facilitating AD progression [117]. Tauopathy is a pathological condition characterized by the aggregation of hyperphosphorylated tau proteins in the cytoplasm of neurons, leading to neurodegeneration [118,119]. ApoE4 demonstrates a low affinity for the microtubule-binding regions of tau proteins, increasing the availability of these regions and susceptibility of tau proteins to phosphorylation by glycogen synthase kinase 3 (GSK3), a serine/threonine kinase highly expressed in the CNS and most concentrated in axons [120,121]. While the ApoE3 isoform is superior at blocking tau phosphorylation by GSK3, ApoE4 leaves tau vulnerable to phosphorylation and disassembly [122–124]. Furthermore, GSK3 will display an increase in activity through the phosphorylation of the majority of tau protein sites [125]. This hyperphosphorylation of tau proteins drives aggregation to form cytotoxic neurofibrillary tangles (NFTs) [126]. Hyperphosphorylation and aggregation of tau proteins prevent normal functioning of the individual proteins in binding microtubules and promote cytoskeleton stability through their microtubule-binding domains [127].

Kang *et al.* [128] showed another mechanism through which ApoE4 promotes tau toxicity. It is known that tau proteolytic cleavage at N368 by asparagine endopeptidase leads to tau aggregation [129]. Kang *et al.* [128] found that this endopeptidase is more highly activated in ApoE4-expressing neurons compared to ApoE3-expressing neurons. Blocking or reducing asparagine endopeptidase attenuated tau cleavage at N368 and reduced hyperphosphorylation and neuronal death.

Nelson *et al.* [130] examined the effects of a rare variant of ApoE called ApoE3-Christchurch on ApoE4-mediated AD risk. ApoE3-Christchurch contains the R136 mutation confers changes in the receptor-binding region of ApoE that, although leading to high amyloid-β accumulation, lowers tau burden and provides resistance to development of AD [131]. Nelson and colleagues [130] generated mice harboring ApoE4 containing the R136 mutation

in heterozygous and homozygous dosage and found that mice carrying 2 copies of ApoE4 with the R136 mutation, but not mice carrying only a single copy, were rescued from ApoE4-induced tau pathology and neurodegeneration. This work provides direction for future studies aimed at reducing negative effects of tau in persons with AD.

In summary, tau aggregates are involved in synapse loss and dysfunction, microtubule destabilization and disassembly, neurodegeneration, and ultimately neuronal apoptosis, which can be linked to AD progression [132].

Insulin Signaling and Glucose Metabolism

Insulin and glucose metabolism are central to brain function and neurons have particularly high energy demands [133,134]. Cerebral glucose hypometabolism is a known feature of AD and disruption of glucose metabolism in AD occurs in an ApoE isoform-specific manner [135, 136]. In mice with targeted replacement of mouse *ApoE* with the human *ApoE3* or *ApoE4* gene, ApoE4 protein fostered endosomal retention of the insulin receptor, interfering with its trafficking and leading to impaired insulin signaling in neurons [137]. Even in young mice, there are differences in insulin signaling between *ApoE3*-expressing and *ApoE4*-expressing mice [138]. Hees *et al*. [139] found that abnormal insulin signaling promoted by ApoE4 can contribute to mitochondrial dysfunction in AD by interfering with mRNA localization and activation of the mitophagy regulator phosphatase and tension homologous gene-induced putative kinase 1 (PINK1) [139,140]. Onos *et al*. [141] looked at cerebral metabolism in mice with targeted replacement of mouse ApoE with human ApoE alleles over a timespan between ages 8 and 12 months and found that female mice homozygous for ApoE4 showed the most severe decrease in glucose metabolism in specific brain regions including the cingulate cortex, corpus callosum, and fornix.

Inflammatory Pathways

Neuroinflammation plays an important role in AD and research suggests that inflammation can act in opposing ways with either exacerbating or ameliorating effects on disease pathology [142,143]. Neuroinflammation is characterized by activation of microglia and astrocytes, increased levels of chemokines and cytokines, production of reactive oxygen species (ROS) and infiltration of peripheral leucocytes across the BBB into the CNS [144,145]. ApoE regulates neuroinflammation in an isoform specific manner with ApoE4 bringing greater inflammatory activity in some studies and less in others relative to ApoE3 [146–148]. ApoE4 has been shown to increase inflammatory cytokine production in mouse models with knock in of the human ApoE4 allele [149–151].

Vitek *et al*. [146] demonstrated that microglia derived from homozygous ApoE4 targeted replacement mice show a pro-inflammatory phenotype, including higher pro-inflammatory cytokine production, altered cell morphology, and increased NO production compared to microglia derived from ApoE3 targeted replacement mice. Targeted replacement mice expressing human ApoE2, ApoE3 or ApoE4 isoforms given intraventricular lipopolysaccharide to induce inflammation were compared and the ApoE4 mice show increased cytokine levels and enhanced induction of a pro-inflammatory state in microglial cells [152,153]. Guo *et al*. [154] examined the effect of exogenous ApoE3 and ApoE4 in rat glial cell cultures including astrocytes and microglia. The study showed higher levels of IL-1β production in rat glial cells when exposed to exogenous ApoE4 as compared to ApoE3 [154]. Fernandez *et al*. [22] established that the presence of recombinant or endogenous ApoE4 increases pro-inflammatory cytokine production in blood, brain and microglia across rodent and human species. Tao *et al*. [155] performed a population based study and demonstrated that chronic low grade inflammation is associated with increased risk of AD pathology in human ApoE4 carriers. On the other hand, exposure to ApoE2 via a gene therapy approach ameliorates neurodegenerative and neuroinflammatory phenotypes and reduces microglial activation in the APP/presenilin 1 mouse model of AD despite continued expression of human ApoE4 [156]. In aggregate, these findings indicate that ApoE plays an important role in glial cell activation and neuroinflammation, which further has a regulatory role in AD pathogenesis. Since the effect of neuroinflammation and microglial activation can differ based on the stage of disease and other factors that are not entirely known, the ultimate impact of ApoE phenotype remains to be determined [157].

ApoE and the Blood Brain Barrier

The BBB is one of the most important physiological systems we have to protect the brain from pathogens and toxic substances in the peripheral circulation [158]. It also regulates the transport of essential nutrients and waste products. Microvascular endothelial cells with proteins forming tight junctions between them are the main components responsible for the integrity of the BBB [159].

Individuals with the ApoE4 allele have increased BBB permeability, which can be due to the degradation of tight junction proteins and the destruction of pericytes, a cell type that is crucial for BBB maintenance because of their supportive role in regulating blood flow and response-to-injury [160,161].

The activation of the cyclophilin A (CypA)–metalloproteinase-9 (MMP-9) pathway by nuclear factor-κB (NF-κB), has been shown to cause BBB breakdown in association with ApoE [162–164]. This pro-inflammatory pathway uses the CypA as the main activator to trigger the release of toxins from the blood vessels, which causes the tight junction proteins zonula occludens-1, occludin and claudin-5 to be broken down, leading to the eventual destruction of the basement membrane and the neurodegen-

erative changes of AD [165]. In mouse studies, the CypA pathway also activates MMP-9 in pericytes, fostering BBB breakdown [166]. CSF levels of CypA and MMP-9 can be considered biomarkers indicating breakdown of the BBB and studies have shown that in those harboring an ApoE4 allele, these levels are greater compared to ApoE3 homozygotes or ApoE3/ApoE2 carriers, even when all groups are cognitively unimpaired [167]. Further, microscopic examination of post mortem brain tissue from persons with AD carrying ApoE3 alleles compared to persons with AD heterozygous or homozygous for the ApoE4 allele showed greater pericyte coverage of capillary walls in the AD ApoE3 specimens compared to the AD ApoE4 specimens with greatest coverage in the non-AD control specimens. The level of CypA in pericytes was high in AD ApoE4 carriers, lower in AD ApoE3 carriers and lowest in non-AD controls. The level of CypA in endothelial cells followed the same pattern as in pericytes [163].

ApoE phenotype influences BBB integrity and ApoE4 allows for leakage and breach of neurovascular integrity [168,169]. This impairment exacerbates neuroinflammatory and neurodegenerative processes and may be a therapeutic target.

Interactions with ApoE Phenotype of Cholinesterase Inhibitors and Memantine

Cholinesterase inhibitors and memantine are the currently approved drugs for the management of the cognitive, behavioral, and functional symptoms of AD [170]. The available cholinesterase inhibitors are donepezil, galantamine and rivastigmine [171]. Memantine is a partial antagonist of N-methyl-D-aspartate receptors (NMDAR), which are overactivated by high synaptic concentrations of glutamate [172]. Under normal conditions, NMDAR channels are blocked by magnesium ions settled within each channel, and only activated briefly [173]. The strict regulation of these channels by magnesium ions is disrupted under pathological conditions such as elevated synaptic glutamate levels, leading to prolonged activation and opening of these ion channels [174]. NMDAR overactivation contributes to an abnormal influx of calcium ions into neurons and subsequent excitotoxicity [175]. This occurs through the activation of caspases and prevention of ATP synthesis by mitochondria that both result in the neurodegeneration observed in AD [176]. Memantine acts to non-competitively block NMDAR channels and influx of calcium ions by replicating the physiological function of magnesium ions, improving brain glutamate homeostasis and reducing synaptic background noise so that true signals can predominate [177].

de Oliveira *et al*. [178] assessed the effects of psychotropic drugs on cognitive functional changes in AD based on ApoE4 carrier status. Patients were followed over 1 year and cognitive tests administered included the Mini-Mental State Examination (MMSE) and the Severe Mini-Mental State Examination (SMMSE) with concomitant assessment of caregiver burden and activities of daily living. The results showed that patients given the combination of memantine and a cholinesterase inhibitor had a slower worsening of SMMSE scores than patients who took neither. They found that cholinesterase inhibitors benefited basic functionality and caregiver burden only for ApoE4 non-carriers, while memantine was harmful with regard to SMMSE score changes [178]. On the other hand, ApoE4 carriers did not experience any of the effects of isolated psychotropic drugs on their clinical status while for non-carriers of ApoE4, second-generation antipsychotics were associated with poorer SMMSE scores. This data signifies the importance of stratifying patients according to ApoE4 carrier status in clinical trials since cognitive and functional changes brought about by psychotropic drugs differed based on ApoE4 carrier status.

A study by Belitskaya-Lévy *et al*. [179] showed the importance of ApoE genotypes in the design of future trials. They conducted a randomized controlled trial of vitamin E and memantine in 613 veterans with mild-to-moderate AD and determined the effect of their ApoE genotype on the efficacy of vitamin E and memantine treatments in slowing functional decline. The study found that when a combination of vitamin E and memantine was given, ApoE4 carriers had significantly slower functional decline from baseline than noncarriers [179]. The mechanism through which ApoE genotype influenced response to vitamin E was unclear and future work should expand on the continued role of ApoE in AD.

Unlike these previously cited studies, Waring *et al*. [180] show that ApoE genotype had no statistically significant effect on cognitive response to donepezil. The analysis included three similarly designed 12-week clinical studies that enrolled patients with mild-to-moderate AD and examined correlations between ApoE4 carrier status and Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) scores. The results showed no appreciable interaction between donepezil response and ApoE4 carrier status [180]. Both groups experienced significant improvements from baseline in ADAS-cog scores versus the placebo group. This inconsistency in the association between presence or absence of the *ApoE4* genotype and its impact on cholinesterase inhibitor treatment outcomes is analyzed in a systemic review and meta-analysis by Cheng *et al*. [181]. This meta-analysis screened 30 studies and included continuous data for assessing the association between ApoE4 and cognitive outcomes of cholinesterase inhibitor treatment from 18 of these studies. This meta-analysis concluded that the association between ApoE4 carrier status and cholinesterase inhibitor treatment in patients with AD was insignificant and the inhibitors had a positive therapeutic effect compared with placebo regardless of the carrier status [181].

Overall, the significance of the association between ApoE4 and cognitive outcomes of cholinesterase inhibitor treatment is inconclusive and further research should be done to clarify possible interaction of ApoE4 status with specific AD treatment impact.

Cutting Edge Therapies

Anti-ApoE4 Immunotherapy

ApoE binding to amyloid-β plays a role in amyloid-β deposition and clearance. Since the ApoE4 isoform stabilizes amyloid-β oligomers and slows clearance, one potential therapy is to reduce levels of ApoE4, using anti-ApoE4 immunotherapies that can cross the BBB [115,116]. Passive immunotherapy with antibodies against ApoE4 could, in theory, neutralize the target ApoE4 and its toxic effect on amyloid-β. Several murine studies have demonstrated the utility of this therapy. Xiong *et al*. [182] generated 5XFAD mice expressing human ApoE4 with a phenotype of prominent cerebral amyloid angiopathy (CAA) and parenchymal amyloid-β pathology. Treatment of these 5XFAD mice expressing human ApoE4 with the anti-human ApoE antibody HAE-4 led to reduced amyloid-β deposition compared to mice given control IgG [182,183]. Despite early and acute upregulation of some inflammatory markers of astrocyte, microglial and vascular origin, chronic HAE-4 administration over 2 months dampened reactive microglial, astrocytic, and proinflammatory-associated genes in the cortex.

In another study in 5XFAD mice expressing human ApoE4, the mice were given either HAE-4 or control antibody intraperitoneally for 13 weeks starting at 5 months of age and then one week after treatment initiation, they were injected in the dentate gyrus and overlying cortex with tau aggregates extracted from human AD brain tissue to bring about tau seeding [184]. The HAE-4 antibody significantly decreased amyloid-β plaques in the hippocampus and cortex and reduced amyloid-β-mediated tau seeding and spreading compared to control untreated mice. However, while HAE-4 treatment lowered amyloid-β-mediated tau seeding and spreading to the side contralateral from the injection site in the male mice, there was no significant contralateral change in the female mice. This suggests that HAE-4 can protect against amyloid-β-driven tau spreading from one side of the brain to the other only in male mice. Further, when neuritic plaque tau was quantified on a per-plaque basis with and without HAE-4 treatment, there was a reduction in males, but not females, suggesting that HAE-4 affects tau pathology through a mechanism independent from the amyloid-β plaque reduction. These preclinical results support the therapeutic potential of reducing ApoE4, but do not address the need for ApoE by the brain which might be fulfilled with the introduction of a better-functioning isoform.

Antisense Oligonucleotides

Antisense oligonucleotides (ASOs) can be designed to diminish ApoE4 levels [185]. Litvinchuk *et al*. [186] found that in a transgenic mouse overexpressing both a mutated version of human tau and human ApoE4, intracerebroventricular injection of an ApoE ASO reduced ApoE4 levels by around 50%, and this protected against tau pathology and associated neurodegeneration.

Vandermeulen *et al*. [187] performed bilateral intracerebroventricular injection of an ASO to attenuate expression of ApoE in human microglia xenografted into the brain of a mouse model with amyloid pathology, but the ASO did not cause any change in microglial accumulation around amyloid-β plaques nor did it affect amyloid burden. The ASO caused knockdown of *ApoE* by about 40% after 1 week and 35% after 4 weeks and yielded some transcriptional changes such as lowering the expression of human leukocyte antigen genes, but did not alter phenotype.

ASOs have also been designed to correct deregulation in the splicing of the ApoE receptor ApoER2 so that exon 19 of this receptor is included, which leads to improved memory and synaptic function in a mouse model [188].

Altering ApoE Phenotype

Rosenberg and colleagues [189] engineered a vector carrying the full-length human ApoE2 cDNA and administered it into the CNS of African Green monkeys either surgically directly into the hippocampal parenchyma, intraventricularly to the frontal horn of the third ventricle via burr hole, intracisternally to the cisterna magna non-surgically or in a combination of intracisternal plus intraventricular routes. All of these methods were successful in producing ApoE2 protein, but intracisternal delivery was found to be the best and safest choice because of its less invasive nature.

Perhaps the most direct manipulation of ApoE attempted in humans is an ongoing small trial in which an adeno-associated viral vector carrying the ApoE2 allele is being given by intrathecal injection to ApoE4 homozygous persons with mild cognitive impairment or mild-to-moderate AD (https://clinicaltrials.gov/; NCT03634007) [190]. CSF ApoE2 protein levels rose in the initial part of the study and tau levels decreased compared to baseline.

Another way to affect ApoE expression is through gene editing. The clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR-associated protein 9 (CRISPR-Cas9) system is a gene editing technology that consists of three main components: the endonuclease Cas9, CRISPR RNA, and trans-activating crRNA. Cas9 cleaves the target DNA, and crRNA contains a 20 nucleotide guide sequence that directs Cas9 to a 20 base pair DNA target [191,192]. This system can be used for targeting genes pertaining to AD such as ApoE4. Viral vectors are a classical approach and are efficient, while non-viral vectors are known to be less immunogenic than viral vectors, but can be challenging to deliver to the brain because they do not

Table 2. Therapeutic approaches targeting ApoE in Alzheimer's disease animal models and humans.

Therapeutic approach	Results	Animal model	References
Immunothereapy	HAE-4 antibody decreased amyloid-β plaques in the hippocampus and cortex. Reduced amyloid-β-mediated tau seeding and spreading	5XFAD mice expressing human ApoE4	[182–184]
Antisense oligonucleotides	Intracerebroventricular injection reduced ApoE4 levels, protected against tau pathology and associated neurodegeneration	Transgenic mouse with amyloid-β pathology xenografted with human microglia	[187]
Antisense oligonucleotides	Correcting deregulated splicing of ApoE receptor (ApoER)2 leads to improved memory and synaptic function	TgCRND8 mice harboring a double mutant human APP695 transgene	[188]
Altering ApoE phenotype	Vector carrying ApoE2 produced protective ApoE2 protein in the CNS	African green monkeys	[189]
Altering ApoE phenotype	Intrathecal injection of LX1001, a virus equipped with the gene for the protective ApoE2 allele increases ApoE2 protein levels in CSF and decreased tau levels	Humans	[190]
Altering ApoE phenotype	Decrease of ApoE4 protein levels using CRISPR-Cas9 system	ApoE3/4 mice models	[194]
ApoE receptor-related approaches	CS-6253 promotes cholesterol efflux and ApoE lipidation by stabilizing ABCA1	ApoE-targeted-replacement mice	[201]
ApoE receptor-related approaches	Intraperitoneal injection of CS-6253 reduced amyloid-β pathology and memory loss in young male E3 mice	E3FAD and E4FAD overexpressung mutant amyloid-β and APP	[43]

CSF, cerebrospinal fluid; CRISPR-Cas9, clustered regularly interspaced palindromic repeats-associated protein 9; APP, amyloid precursor protein.

cross the BBB as efficiently [193]. Offen *et al.* [194] used mouse astrocytic cells expressing the human *ApoE3* or *ApoE4* gene and found that use of the CRISPR-Cas9 system with specific targeting of the ApoE4 led to a 56% decrease in ApoE4 protein levels, but no significant change in ApoE3 levels. Next steps include injecting lentiviruses containing CRISPR system into brains of ApoE3/4 mouse models [194].

Using the CRISPR system with their own unique changes to optimize the vector, Kantor *et al.* [195] showed that they could reduce expression of endogenous mouse ApoE by stereotaxically injecting vector with repressor into the dorsal hippocampus of C57BL/6 mice. Further preclinical investigation is planned.

ApoE Receptor-Related Approaches

LRP1, LDL receptor, and heparan sulfate proteoglycan (HSPG) are cell surface receptors for both ApoE and amyloid-β in the brain. They are important for regulating lipid metabolism and amyloid-β clearance [196,197]. One detrimental effect of ApoE4 is to lower the expression of ApoER2, the LDL receptor and LRP1 [48,198]. This leads to a potential therapeutic approach in which an appropriate vector is used to increase expression of one or more of these receptors [199,200]. Another approach could be to use ApoE mimetic peptides that correspond to a receptor binding domain and protect against amyloid-β and tau-driven pathology in various animal studies. One mimetic, CS-6253, is a novel alpha-helical peptide that was developed to bind and stabilize ABCA1 and induce its activity. This promotes cholesterol efflux, and ApoE lipidation [201]. Valencia-Olvera *et al.* [43] isolated and cultured glia from mice with amyloid pathology carrying either the human ApoE3 or ApoE4 alleles and treated these glia with CS-6253. The study found that CS-6253 treatment increased ApoE levels and cholesterol efflux. They then administered CS-6253 via intraperitoneal injection to these transgenic mice at both young and older ages, and measured amyloid-β, ApoE, ABCA1, and ApoE lipidation levels. They found that the peptide treatment reduced amyloid-β pathology and memory loss only in young male mice carrying ApoE3, but not in young mice carrying ApoE4 or in any of the older mice [43]. The authors interpreted the data as an indication that the degree of progression of amyloid-β pathology at the time of therapeutic intervention influences the effectiveness of this ApoE mimetic.

A summary of the approaches to AD treatment via ApoE manipulation that are being tested in preclinical and human studies are synopsized in Table 2 (Ref. [43,182–184,187–190,194,201]).

Conclusion

This review provides information about the relationship between inheritance of specific ApoE alleles and development of AD. The ApoE4 allele is the strongest AD genetic risk factor and is associated with earlier AD onset while the ApoE2 allele is considered protective. ApoE polymorphisms play an important role in determining cellular bioenergetics, glial cell activation and neuroinflammation, all of which contribute to AD pathogenesis. The pathologic consequences set in motion by ApoE4 are many and complex and likely related to the lipidation and conformational state of the ApoE molecule. As our knowledge continues to evolve, new therapeutic candidates that manipulate ApoE4 expression and activity in brain cells can be developed, tested preclinically and ultimately become the subject of future clinical trials.

Availability of Data and Materials

Not applicable.

Author Contributions

ABR, TH, AS, AP and JDL made substantial contributions to conception and design; ABR, MH, SG and BL performed the literature research; AP, AS, IHG and TW made substantial contributions to acquiring the data; IHG, TW and AS made substantial contributions to analysis and interpretation of data; ABR, AS, TH and TW were involved in drafting the manuscript. AP, IHG and JDL revised the manuscript and added critically important intellectual content. All authors contributed significantly to editorial changes of important content. All authors have given final approval for the version to be published. All authors have participated sufficiently in the work to take public responsibility for its content and agree to be accountable for all aspects of this work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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