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Apolipoprotein E, Receptors and Modulation of Alzheimer's Disease

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Abstract

Apolipoprotein E (apoE) is a lipid carrier in both periphery and the central nervous system (CNS). Lipid-loaded apoE lipoprotein particles bind to several cell surface receptors to support membrane homeostasis and injury repair in the brain. Considering prevalence and relative risk magnitude, the ε4 allele of the *APOE* gene is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). ApoE4 contributes to AD pathogenesis by modulating multiple pathways including but not limited to the metabolism, aggregation, and toxicity of amyloid-β (Aβ) peptide, tauopathy, synaptic plasticity, lipid transport, glucose metabolism, mitochondrial function, vascular integrity, and neuroinflammation. Emerging knowledge on apoE-related pathways in the pathophysiology of AD presents new opportunities for AD therapy. In this Review, we describe the biochemical and biological features of apoE and apoE receptors in the CNS. We also discuss the evidence and mechanisms addressing differential effects of apoE isoforms and the role of apoE receptors in AD pathogenesis, with a particular emphasis on the clinical and preclinical studies related to Aβ pathology. Finally, we summarize the current strategies of AD therapy targeting apoE, and postulate that effective strategies require an apoE isoform-specific approach.

Keywords

Apolipoprotein E; low-density lipoprotein receptor family; Alzheimer's disease; amyloid-β; tauopathy; synaptic plasticity

Alzheimer's disease (AD), the most common form of dementia, is a neurodegenerative disorder characterized by the appearance of extracellular senile plaques and intracellular neurofibrillary tangles, accompanying a progressive loss of memory, thinking and language skills, as well as behavioral changes (1). Mounting genetic and biochemical evidence

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support that the accumulation and aggregation in the brain of amyloid-β (Aβ), either 40 or 42 amino acids in length derived from proteolytic processing of amyloid precursor protein (APP), is a central and defining event in the pathogenesis of AD (2). The toxic soluble Aβ oligomers, intraneuronal Aβ, and extracellular amyloid plaques disrupt normal synaptic functions and trigger downstream toxic pathways which ultimately lead to neurodegeneration (1). The toxicity of Aβ-induced neuronal dysfunction likely depends on the presence of microtubule-associated protein tau, which aggregates and deposits in AD brains as neurofibrillary tangles (3).

Only a small percentage (<5%) of AD cases develop symptoms before the age of 65 commonly referred to as early-onset AD (EOAD). A portion of EOAD known as familiar AD (FAD) is caused by genetic mutations in *APP*, *presenilin 1 (PSEN1)* or *PSEN2* gene leading to Aβ overproduction (2). The vast majority of AD cases occur late in life (age 65 and older) known as late-onset AD (LOAD) (2). The impairment of Aβ clearance appears to be the leading cause of Aβ accumulation in LOAD (4). Considering prevalence and relative risk magnitude, the strongest genetic risk factor for LOAD is apolipoprotein E (*APOE*) gene allele, with the ε4 allele increasing AD risk and the ε2 allele being protective compared with the common ε3 allele (5–7). Although studies indicate that apoE4 enhances AD risk likely by both gain of toxic function and loss of neuroprotective function through several distinct pathways when compared to apoE2 and apoE3, the exact mechanisms by which apoE isoforms differentially contribute to AD pathogenesis are still not completely understood (6, 8). In this review, we will discuss the current status of clinical and basic evidences on how apoE and apoE receptors modulate AD pathogenic pathways with a particular focus on Aβ-related pathways, and update the recent advances in targeting apoE and apoE receptors for AD therapy.

Biochemical features of apoE

ApoE is a glycoprotein of 299 amino acids with a molecular mass of ~34 kDa. It transports and delivers cholesterol and other lipids in the plasma and the central nervous system (CNS) via binding to cell surface apoE receptors (Figure 1) (9, 10). The receptor-binding domain of apoE is in the N-terminal domain (residues 136–150), whereas the major lipid-binding region (residues 244–272) resides in the C-terminal domain (6, 8). The three isoforms, apoE2, apoE3 and apoE4, differ at position 112 and 158. ApoE2 has Cys residues at both positions 112 and 158; apoE3 has a Cys residue at 112 and an Arg residue at 158, and apoE4 has Arg residues at both positions (11).

ApoE is mainly produced by hepatocytes, macrophages and adipocytes in the peripheral tissues (12). In the CNS, apoE is highly expressed in astrocytes, as well as in microglia, vascular mural cells, and choroid plexus, and in neurons only under stress or injury conditions (12–14). The secreted apoE is lipidated by ATP-binding cassette transporter A1 (ABCA1) and ABCG1, which transport the cellular cholesterol and phospholipids to nascent apoE forming lipoprotein particles (15). ApoE in the plasma is preferentially associated with very low-density lipoprotein (VLDL) particles, whereas it is found in HDL-like particles in the CNS (16, 17). Brain apoE is primarily derived from *de novo* synthesis because the blood-brain barrier (BBB) limits the transport of apoE into and out of brain (18). Consistent

with this notion, liver transplantation changes only the plasma apoE isoform in the recipient to that of the donor but not in the cerebral spinal fluid (CSF) (19). Interestingly, it was recently reported that plasma apoE influences cognitive functions (20). Further investigation is needed to understand whether this effect is apoE isoform-dependent, and to identify the mechanism by which peripheral apoE impacts CNS functions.

The levels of plasma apoE vary with *APOE* genotypes with *APOE* ε2/2 being the highest and *APOE* ε4/4 being the lowest (21, 22). This apoE isoform-dependent difference in apoE levels are also shown in CSF (23) and interstitial fluid (ISF) (24). ApoE4 preferentially forms a molten globule intermediate that makes it less stable than apoE3 (25). The domain interaction of apoE4 through an ionic interaction between arginine-61 and glutamic acid-255 may also increase hepatic clearance and enhance astrocyte degradation of apoE4 (22, 26).

ApoE receptors

The low-density lipoprotein receptor (LDLR) family, including LDLR, LDLR-related protein 1 (LRP1), very-low-density lipoprotein receptor (VLDLR) and apoE receptor 2 (apoER2), are the major apoE receptors which exhibit distinct binding affinity to apoE with different isoforms and lipidation status (6). LRP1 preferentially binds to recombinant apoE or apoE aggregates, whereas LDLR and VLDLR bind to either lipidated apoE particles or lipid-free apoE, respectively (6, 7, 27). In addition to the LDLR family, both lipidated and non-lipidated apoE bind to cell surface heparan sulfate proteoglycans (HSPGs) (28, 29). ApoE2 has a poor binding affinity to the LDLR (1–2% of apoE3 binding) which contributes to an increased risk of a small percent of *APOE2* homozygous individuals for type III hyperlipoproteinemia characterized by increased plasma cholesterol and triglycerides (30, 31). The decreased affinity of apoE2 to LRP1 compared with apoE3 or apoE4 seems to be less severe (40% of apoE3 binding) (31). VLDLR (27)and HSPGs (10) recognize all apoE isoforms with similar affinity. Additionally, LRP1 can form a complex with HSPG in mediating apoE binding, suggesting a cooperative function for these apoE receptors at the cell surface (28).

ApoE in Aβ metabolism and pathology

Effects of apoE on brain Aβ deposition

Using ^{18}F-florbetapir-PET or ^{11}C-Pittsburgh compound B (PiB)-PET imaging, clinical studies showed that cerebral Aβ deposition is positively associated with *APOE4* genotype in cognitively normal subjects, mild cognitive impairment (MCI) cases, and symptomatic AD patients (32–34). Additionally, amyloid imaging positivity appears to begin earlier in cognitively intact *APOE4* carriers (near age 56 years) than *APOE4* non-carriers (at age 76 years) (35). These observations suggest that *APOE4* likely increases the risk of AD by initiating and accelerating Aβ accumulation, aggregation and deposition in the brain. *APOE2* is associated with milder Aβ pathology and slower cognitive decline compared to *APOE3* and *APOE4*, suggesting a neuroprotective effect of *APOE2* in AD (36). Interestingly, in the oldest-old population (90 years and older), the presence of *APOE2* reduces dementia risk but increases AD neuropathology (37), implying that the mechanism by which apoE2 protects the cognitive function is independent of AD pathology. The levels

of oligomeric Aβ, potentially the most neurotoxic Aβ species, are 2.7 times higher in AD brains with *APOE* ε4/4 than those with *APOE* ε3/3, suggesting that apoE4 impacts the metabolism of Aβ oligomers (38). In amyloid mouse models, the presence of human apoE4 is associated with accelerated oligomerization and deposition of Aβ in the brain. By crossing human apoE-targeted replacement (TR) mice to murine apoE(−/−)/APP mice, it was shown that apoE4/APP mice have more severe amyloid deposition compared with apoE3/APP mice (39). Viral-mediated expression of apoE4 in APP/PS1 mouse brain increases oligomeric Aβ within the ISF and exacerbates plaque deposition, whereas expression of apoE2 leads to an opposite effect (40, 41). In transgenic mice expressing five familial AD mutations and human apoE isoforms (EFAD), the levels of plaque deposition and oligomeric Aβ are greater in E4FAD mice in which the plaques are more compact compared to those in E2FAD or E3FAD mice (42). A recent study showed that the effects of apoE on the aggregation of Aβ40 depend on the apoE isoforms, with apoE4 possessing the greatest effects (43), although a conflicting finding was reported previously (44). Whether apoE4 promotes seeding and aggregation of Aβ *in vivo* warrants further investigation.

Effects of apoE on CSF Aβ

CSF Aβ is one of the promising biomarkers with high accuracy to differentiate patients with AD from control subjects or patients with other neurologic conditions (45). In AD patients, the CSF Aβ42 concentration is decreased about 50% to that of control individuals likely due to amyloid deposition in the brain (45). Although the *APOE4* allele is associated with enhanced cerebral Aβ deposition, how it influences CSF Aβ is still controversial. It was previously shown that *APOE4* is strongly associated with reduced CSF Aβ42 levels in both AD and control populations (46, 47). However, a recent study from 4 centers in different countries revealed that CSF Aβ42 levels are strongly associated with the diagnosis of AD and cerebral Aβ accumulation, but are independent of *APOE* genotype (48). Other studies focusing on the levels of apoE and Aβ42 in CSF revealed that apoE protein levels are positively associated with those of Aβ42 and phosphorylated tau in CSF, making apoE levels a potential biomarker for AD (49, 50).

Mechanisms of apoE and apoE receptors in modulating Aβ metabolism

The Aβ level in the brain represents the net balance of Aβ production and clearance (2); thus, Aβ accumulation in AD brains may reflect overproduction, inefficient clearance, or both. Mounting evidence demonstrates that apoE and apoE receptors play important roles in these processes which will be discussed in more detail below (Figure 2).

ApoE and apoE receptors in Aβ production

APP localizes on the surface of the plasma membrane where it is subjected to proteolytic processing by α-secretase. In the amyloidogenic pathway, APP is internalized into endocytic compartments and cleaved by β-secretase and γ-secretase to generate Aβ peptides (Aβ40 and Aβ42) (51). Several apoE receptors, including LRP1, interact with APP and modulate its trafficking and processing to Aβ (15, 52, 53). For instance, due to the fast endocytosis, LRP1 accelerates APP endocytic trafficking and thus increases the production of Aβ (54, 55). LRP6, an apoE receptor that mediates Wnt signaling, is shown to reduce APP endocytic

trafficking and Aβ production. Consistently, conditional knockout of neuronal LRP6 increases amyloid deposition and associated neuroinflammation (56). In addition, studies show that apoE regulates APP trafficking and Aβ production in an isoform-specific manner. ApoE4 promotes APP internalization which increases Aβ production to a greater extent than apoE3 in a neuronal cell line and this effect is mediated by LRP1 (57). Furthermore, the increase of Aβ production by apoE4 was also reported to be modulated by an apoE-binding protein TMCC2 (58). Most recently, it was reported that apoE isoforms (apoE4>apoE3>apoE2) differentially affect APP transcription and Aβ production through stimulating a non-canonical mitogen-activated protein (MAP) kinase signaling pathway in human neurons (59). Whether the effect of apoE on increasing APP through these signaling pathways contributes to AD risk and its *in vivo* relevance in the brains of apoE-TR mice or humans require further studies.

ApoE and apoE receptors in Aβ clearance

Increasing evidence indicates that impaired Aβ clearance is a major pathogenic event for LOAD (4). Soluble Aβ can be removed from the brain by various clearance pathways (Figure 2) including receptor-mediated cellular clearance (neurons, glial cells and vascular mural cells), cerebrovascular clearance (via the perivascular drainage, BBB and glymphatic system), and proteolytic degradation by Aβ degrading enzymes (6, 60).

LRP1 in Aβ clearance—Among the apoE receptors, LRP1 is the most extensively studied for its role in brain Aβ clearance. An *in vitro* study revealed that overexpression of a functional LRP1 mini-receptor increases Aβ trafficking to lysosomes, whereas knockdown of LRP1 decreases neuronal Aβ uptake (61). Conditional knockout of neuronal *Lrp1* gene in the forebrain of APP/PS1 mice leads to impaired ISF Aβ clearance in the cortex without affecting Aβ production (62). However, reduction of LRP1 in the hippocampus of APP/PS1 mice has little effect on the rate of Aβ accumulation and deposition, indicating that different brain regions may eliminate Aβ by distinct pathways (63).

Aβ can also be cleared locally in the cerebrovasculature by an LRP1-dependent pathway. Conditional deletion of *Lrp1* in vascular smooth muscle cell in APP/PS1 mice accelerates brain Aβ accumulation and exacerbates Aβ deposition without affecting Aβ production (64). Primary mouse brain capillary endothelial cells derived from mice harboring a LRP1 mutation in endocytosis/sorting domain exhibit a reduced Aβ transport from brain-to-blood and blood-to-brain compared with cells with wild-type LRP1, implying that LRP1 might modulate Aβ across the BBB (65). A recent study showed that deletion of LRP1 in brain endothelial cells leads to a reduced brain efflux of injected ^{125}I-Aβ42 and elevated soluble brain Aβ in 5×FAD mice (66). Whether LRP1 has a direct or accelerative role in transporting Aβ across BBB requires further investigation.

LDLR in Aβ clearance—LDLR is another apoE receptor that is involved in Aβ clearance (67). Deletion of LDLR in astrocytes leads to a decrease in Aβ uptake, whereas increasing LDLR levels significantly enhances the uptake and cellular degradation of Aβ (68). LDLR-deficient mice have accelerated Aβ deposition and Aβ-related neurotoxicity without affecting the APP expression (69, 70). Overexpression of LDLR in mice dramatically

reduces Aβ aggregation and enhances Aβ clearance, as well as attenuates plaque-associated neuroinflammation (71). LDLR overexpression also enhances BBB-mediated clearance of exogenously-administered Aβ and promotes brain-to-blood efflux transport of endogenous Aβ (72).

HSPG in Aβ clearance—HSPG, expressed on all cell types including neurons, has been implicated in several features in the pathogenesis of AD (73–75). HSPG binds to Aβ on the cell surface and functions with LRP1 in a cooperative manner to mediate neuronal Aβ uptake (74). Removal of neuronal heparan sulfate (HS) by conditional deletion of the *Ext1* gene in APP/PS1 mice decreases Aβ oligomers and amyloid deposition, as well as accelerating Aβ clearance rate in the brain ISF. This result suggests that neuronal HSPG either inhibits or represents an inefficient pathway for Aβ clearance (75). It is postulated that binding of Aβ to neuronal HSPG on the cell surface promotes Aβ oligomerization/aggregation, thus depletion of neuronal HS leads to more efficient clearance of Aβ (Figure 2). However, it is still unclear whether HSPG also regulates Aβ clearance in other brain cell types. Additionally, an *in vitro* study showed that apoE particles inhibit HSPG-dependent cellular Aβ uptake without isoform difference (76). Further investigations are required to elucidate the role of apoE isoforms in HSPG-related Aβ metabolism *in vivo*.

ApoE isoform effects on receptor-mediated Aβ clearance—Using *in vivo* microdialysis, a study showed that clearance of Aβ from brain ISF is apoE isoform-dependent (apoE2>apoE3>apoE4) in the amyloid model mice (47). In addressing potential mechanism, early studies have shown that apoE can bind to Aβ and form a complex, which in turns is internalized by the cells through cell surface LRP1 and LDLR (77). The majority of endocytosed apoE is recycled, while the endocytosed Aβ is typically delivered to lysosomes for degradation (7). Since apoE4-Aβ complex is less stable than apoE3-Aβ, apoE4 is less effective in promoting Aβ clearance than apoE3 (78, 79). Other studies have shown that apoE disrupts Aβ clearance across the BBB in an isoform-dependent manner; Aβ binding to apoE4 redirects the rapid clearance of Aβ from LRP1 to the VLDLR, which internalizes apoE4-Aβ complex at the BBB more slowly than LRP1, whereas apoE2-Aβ and apoE3-Aβ complexes are cleared via both VLDLR and LRP1 in a faster speed than apoE4-Aβ complex (80). In addition, apoE may compete with Aβ for binding to common cell surface receptors, LRP1 and LDLR. By infusing apoE to the brain through reverse microdialysis, it was shown that apoE competes with Aβ for LRP1-dependent clearance pathway in astrocytes, with apoE4 exhibiting the strongest blocking effect relative to other apoE isoforms (81).

ApoE isoform effects on protease-mediated Aβ clearance—Aβ can be removed from the brain by proteolytic degradation via Aβ-degrading proteases, including neprilysin (NEP), insulin-degrading enzyme (IDE), endothelin-converting enzyme (ECE), angiotensin-converting enzyme (ACE), plasminogen activators, and the matrix metalloproteinases 2 (MMP2) and MMP9 (82, 83). NEP and IDE are the two most extensively studied Aβ proteases that are expressed in neurons, glia, and vascular mural cells (84–86). ApoE has been shown to facilitate the proteolytic clearance of soluble Aβ from the brain. Interestingly, hippocampal IDE protein is reduced by approximately 50% in *APOE4* AD patients

compared to non-*APOE4* individuals (87), perhaps by down-regulating IDE expression in neurons (88). Studies revealed that apoE enhances both the endolytic degradation of Aβ within microglia (89) and astrocytes by NEP (90), and Aβ degradation extracellularly by IDE (89), with apoE3 functioning more efficiently than apoE4.

ApoE and apoE receptors in modulating synaptic integrity and plasticity

Synaptic failure, including synaptic dysfunction and synapse loss, is an early pathological feature of AD (91–93). It was reported that apoE receptors may be involved in modulating synaptic plasticity. ApoER2 and LRP1 are reported to interact with N-methyl-D-aspartate (NMDA) receptor subunits (94), modulate the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and regulate synaptic plasticity (95). LRP6 and related Wnt signaling are important for the regulation of synaptic integrity and cognition (56). In addition, apoE likely in an isoform-dependent manner modulates synaptic integrity and plasticity (Table 1). ApoE4 suppresses the expression of synaptic proteins and impairs dendritic morphology, synaptic transmission and plasticity in an age-dependent manner; however, the underlying mechanism is not well-established. *In vitro* studies indicate that the protective effects of apoE3 on synaptic loss caused by toxic Aβ are dependent on LRP1 (96, 97). It was also reported that apoE4 reduces cell surface apoER2, leading to impaired Reelin-induced glutamate receptor trafficking and long-term potentiation (LTP) (98). Depending on brain circuits, the strength of LTP differs in mice with different apoE isoforms (Table 1). However, it is still unclear whether long-term depression (LTD), another important synaptic mechanism associated with learning and memory (99), is affected by apoE isoforms.

ApoE in tauopathy, lipid transport, glucose metabolism, vascular function, and neuroinflammation

see supplemental information.

ApoE is a therapeutic target for AD

ApoE determines therapeutic responses

Several clinical trials in AD therapies demonstrated *APOE* genotype-dependent responses. For example, recent trials revealed that the beneficial effects of intranasal insulin treatment on cognition are modulated by *APOE* genotype status in AD patients (3, 79, 100). Acute treatment of short-acting intranasal insulin leads to cognitive improvement in AD patients who are *APOE4* non-carriers, but not in *APOE4* carriers (78). Long-acting intranasal insulin improves cognition in *APOE4* AD patients but worsens the outcome for *APOE4* non-carriers (79). In addition, a Phase 3 trial using passive immunization of a humanized anti-Aβ antibody, bapineuzumab, prevents the increase of Aβ deposition and reduces CSF phosphorylated tau in AD patients who are *APOE4* carriers, but not in *APOE4* non-carriers, although this antibody did not benefit the clinical outcomes (101). As some therapies are mechanism-based, which potentially impact apoE isoform-specific functions, it will be

crucial to consider *APOE* genotype status to identify clinical trial participants, and determine the therapeutic windows and evaluate treatment efficacy against AD.

Modulation of apoE levels and/or lipidation

The strong association of *APOE4* with AD emphasizes apoE as a critical target for therapy. Here, we briefly discuss several approaches that are currently being explored (Figure 3). There is still debate regarding whether increasing or decreasing apoE is the best strategy in regard to AD therapeutics. Overexpressing ABCA1, which increases the lipidation of apoE, reduces Aβ deposition in an amyloid mouse model (102). Treatment of amyloid model mice with liver X receptor (LXR) agonists (TO901317 and GW3965) or retinoid X receptor (RXR) agonists (bexarotene) improves memory and reduces Aβ burden by increasing apoE level and lipidation (103–105), though conflicting effects on amyloid pathology were reported (106, 107). Bexarotene also restores aging-related synaptic loss mediated by LRP1, and reverses the apoE4-associated cognitive deficits in the absence of amyloid (108, 109). However, the therapeutic application of these agents should be carefully examined in human clinical trials due to their adverse effects in the liver (110). Quercetin, a flavonoid widely distributed in nature, reduces AD-related pathology by stabilizing apoE in an amyloid mouse model (111), suggesting that modulating the stability of apoE might represent an alternative strategy. In addition to pharmacological approaches, nanoparticles for apoE delivery (112) and adeno-associated virus (AAV)-mediated gene delivery of apoE into the brains are also promising strategies to regulate apoE levels (113).

Conversely, decreasing apoE levels by overexpressing LDLR or haploinsufficiency of apoE has also been shown to reduce amyloid pathology (17, 114). ApoE immunotherapy through intraperitoneal administration improves cognitive performance, suppresses Aβ deposition, and enhances Aβ clearance in amyloid mouse models by reducing brain apoE level (115, 116). However, due to the critical functions of apoE in lipid homeostasis and neuronal functions (117), careful assessments and optimization will be required when modulating apoE levels in AD treatment. Importantly, lowering apoE4 expression might be an effective therapy for AD in *APOE4* carriers to reduce the toxic functions associated with apoE4. Although further studies are required, anti-apoE4 immunotherapy and gene silencing approaches against apoE4 (e.g., antisense oligonucleotide) may be therapeutically beneficial.

Modulation of apoE properties

Domain-domain interaction has been suggested to confer apoE4 susceptible to proteolytic cleavage and the generation of neurotoxic fragments (118, 119) though the full-length structure of apoE remains to be determined. Small molecules used as apoE structure correctors to block such interactions have been shown to blunt the effects of apoE4 in neurons (120). In addition, targeting apoE proteolysis to reduce apoE neurotoxic fragments may suppress the detrimental effects of apoE4. With recent advances in CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 technology (121), conversion of apoE4 to apoE3- or apoE2-like structure through genome-editing may also be an attractive approach to increase the normal functions of apoE while reducing the toxicity of apoE4.

A recent study identifies *APOE3-V236E* variant to be protective against AD with a population carrier frequency of 0.3% (Figure 1) (122). It is the first LOAD-associated apoE variant that locates within the C-terminal region that is critical for apoE oligomerization and lipidation (122, 123). ApoE4 was shown to be easily self-aggregated (124) which might reduce its lipidation capacity and promote amyloid deposition (125). Thus, inhibition of apoE aggregation will facilitate apoE-mediated functions and suppress amyloidosis which holds therapeutic potential for AD treatment. In addition, studies show that blocking apoE-Aβ interaction by a synthetic peptide (Aβ12–28P) decreases amyloid deposition in amyloid mouse models (126). Aβ12–28P treatment also reduces brain Aβ accumulation, neuritic degeneration, and prevents memory deficit in amyloid model mice expressing apoE2 and apoE4 (127), suggesting that targeting the apoE/Aβ interaction might serve as an alternative therapeutic approach for AD.

Other apoE-related therapeutic approaches

LRP1, LDLR, and HSPG are cell surface receptors for both apoE and Aβ in the brain and have been shown to regulate lipid metabolism and Aβ clearance (6, 72, 75). Identifying small molecules to promote the functions of LRP1 and LDLR or to specifically block HSPG-Aβ interaction could serve as therapeutic approaches for AD treatment. Restoring the intracellular trafficking of apoER2 may ameliorate apoE4-mediated synaptic deficits (98). Moreover, treatment of amyloid model mice with apoE-mimetic peptides (apoE133–149) displays anti-inflammatory and neuroprotective activities (128), indicating that restoring the normal apoE functions may be beneficial. In addition, AAV-mediated delivery of apoE2 increases apoE-associated cholesterol and decreases Aβ pathology, emphasizing the therapeutic potential of apoE2-based treatment (40, 113). Finally, improving vascular health (i.e., physical exercise, healthy diet, and lifestyle) could be beneficial in reducing the risk of AD and cognitive decline, particularly in *APOE4* carriers.

Although apoE-targeted therapies remain in early phase of development they hold great promises in the fight against AD. It is likely that up-regulation of apoE3 benefits synapses and other apoE-related functions, whereas down-regulation of apoE4 reduces its toxic effects and should reduce amyloid deposition when treated early. Due to the differential roles of apoE isoforms in AD pathogenesis, isoform-specific targeting approach will be an encouraging strategy for treating AD. With recent advances in the technology of induced-pluripotent stem cell (iPSC), patient-derived iPSC with different apoE isoforms can be differentiated to various brain cell types for disease modeling, drug screening, and potentially replacement therapy (129). Testing therapeutic efficacy with better preclinical study design in animal models with apoE isoforms would be an instrumental tool to improve the translational potential of AD therapeutics targeting apoE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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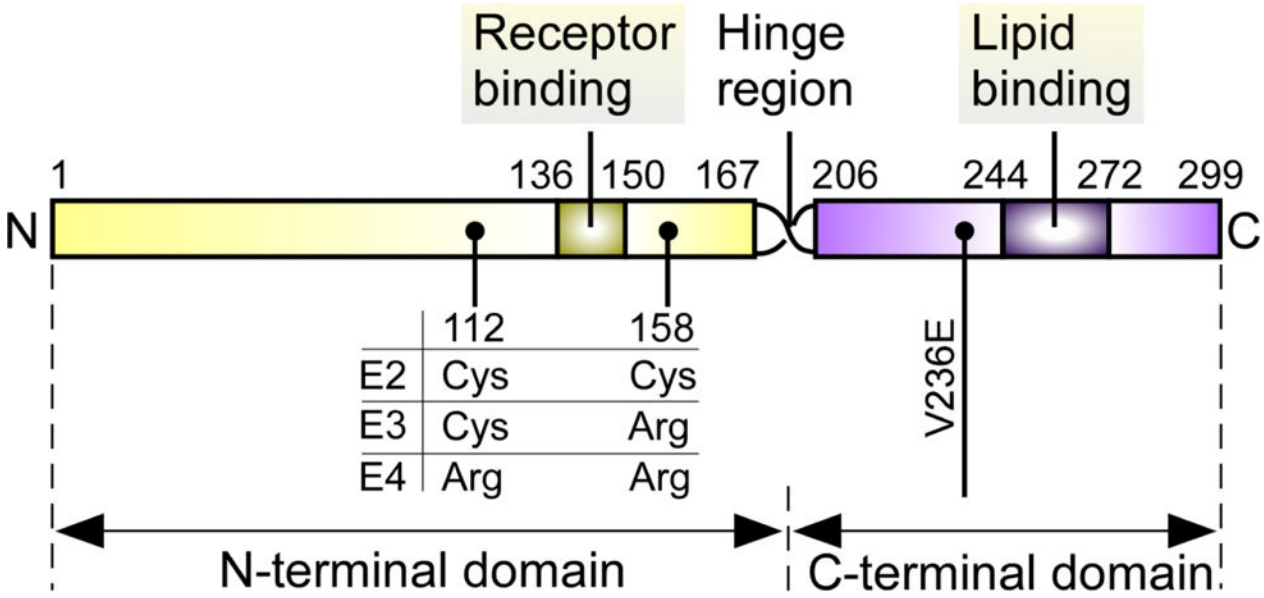

Figure 1. Schematic illustration of the structure of human apoE

ApoE can be subdivided into two major domains (6–8). The amino-terminal domain includes the receptor-binding region (residues 136–150), which is responsible for the interaction of apoE and its receptors. The carboxyl-terminal domain contains the major lipid-binding region (residues 244–272), which is responsible for lipid binding. The middle region of apoE (residues 167–206) is a hinge region that joins the two domains together. The apoE2, apoE3, and apoE4 isoforms differ from one another at amino acid residues 112 and/or 158 (11). ApoE2 has Cys at both positions, apoE3 has Cys at position 112 and Arg at position 158, and apoE4 has Arg residues at both positions. The V236E variance just outside the lipid-binding region, which reduces the risk of late-onset of Alzheimer's disease (LOAD) (122), is marked.

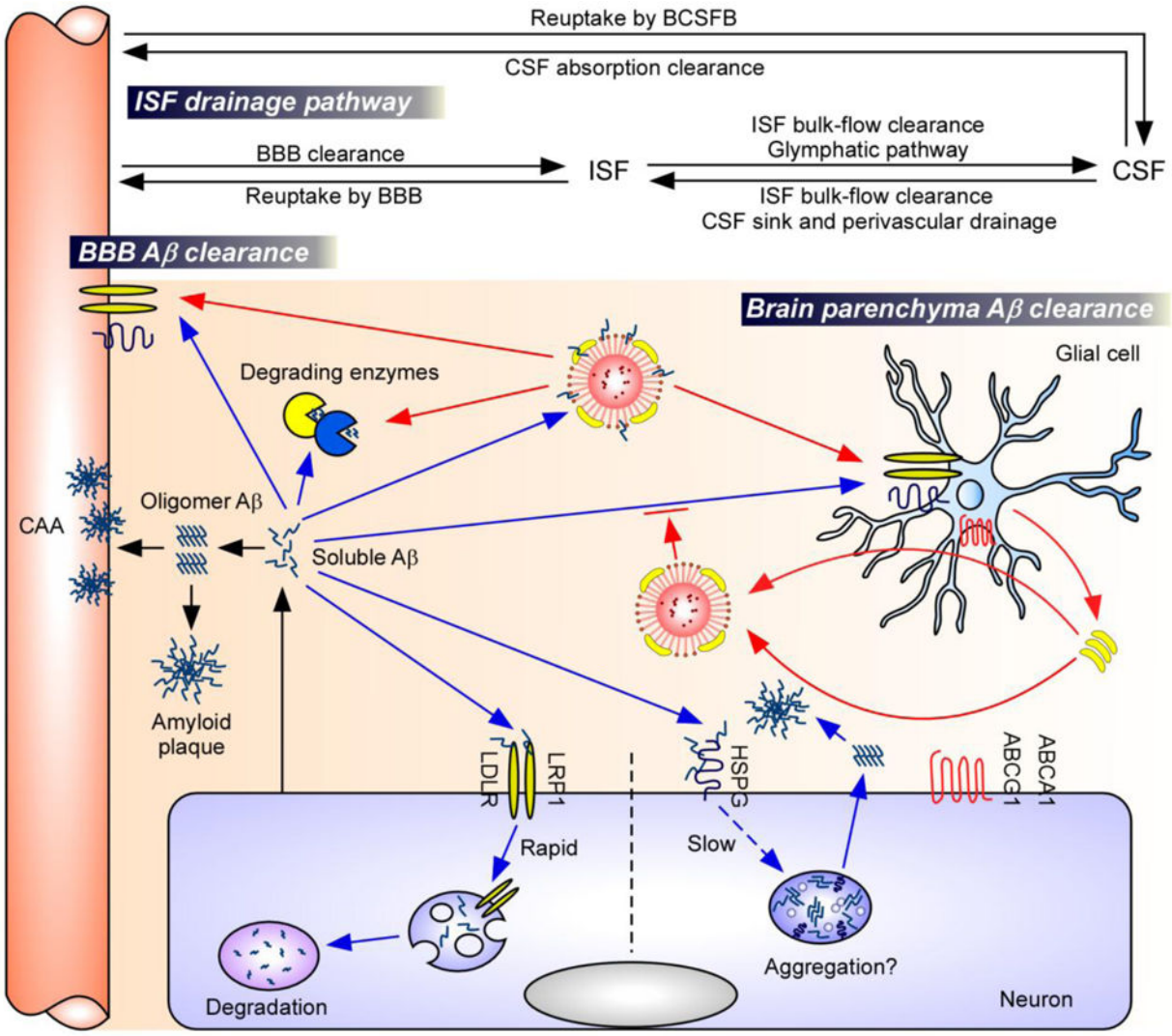

Figure 2. Major Aβ clearance pathways and the roles of apoE and apoE receptors

Aβ is predominantly produced by neurons (6–8). The accumulation of soluble Aβ in the brain parenchyma leads to the formation of Aβ oligomers and amyloid plaques, whereas its accumulation in the perivascular region leads to CAA (6–8). Soluble Aβ can be removed from the brain by various clearance pathways closely linked to each other: soluble Aβ in ISF can be cleared in brain parenchyma, transported to the blood through BBB, or is degraded by vascular mural cells. Aβ can also traffic along the ISF bulk flow into CSF sink where it could be absorbed into the circulatory and lymphatic systems (6, 60). Major Aβ clearance pathways include receptor-mediated clearance (e.g., LRP1, LDLR) by cells in the brain parenchyma (neurons and glia), uptake from the perivascular space by vascular mural cells, or proteolytic degradation by endopeptidases (e.g., NEP, IDE) (6–8). Binding of Aβ to neuronal HSPGs on the cell surface hinders proteolytic degradation of Aβ, and promotes Aβ oligomerization and aggregation (75). ApoE is generated mainly by the glial cells and lipidated by ABCA1 and ABCG1 transporters, forming lipoprotein particles (15). Lipidated apoE could either bind to Aβ and facilitates Aβ clearance through cell-surface receptors

(e.g., LRP1, LDLR) (apoE2 > apoE3 > apoE4) (77–80). Alternatively, apoE might also suppress Aβ clearance by competing with Aβ for receptor binding in astrocytes (apoE4 > apoE3) (81).

Abbreviations: Aβ, amyloid-β; BBB, blood-brain barrier; BCSFB, blood-CSF barrier; CSF, cerebrospinal fluid; ISF, interstitial fluid; CAA, cerebral amyloid angiopathy; NEP, neprilysin; IDE, insulin-degrading enzyme; LRP1, low-density lipoprotein receptor-related protein 1; LDLR, low-density lipoprotein receptor; HSPG, heparan sulfate proteoglycan; ABCA1, ATP-binding cassette A1; ABCG1, ATP-binding cassette G1.

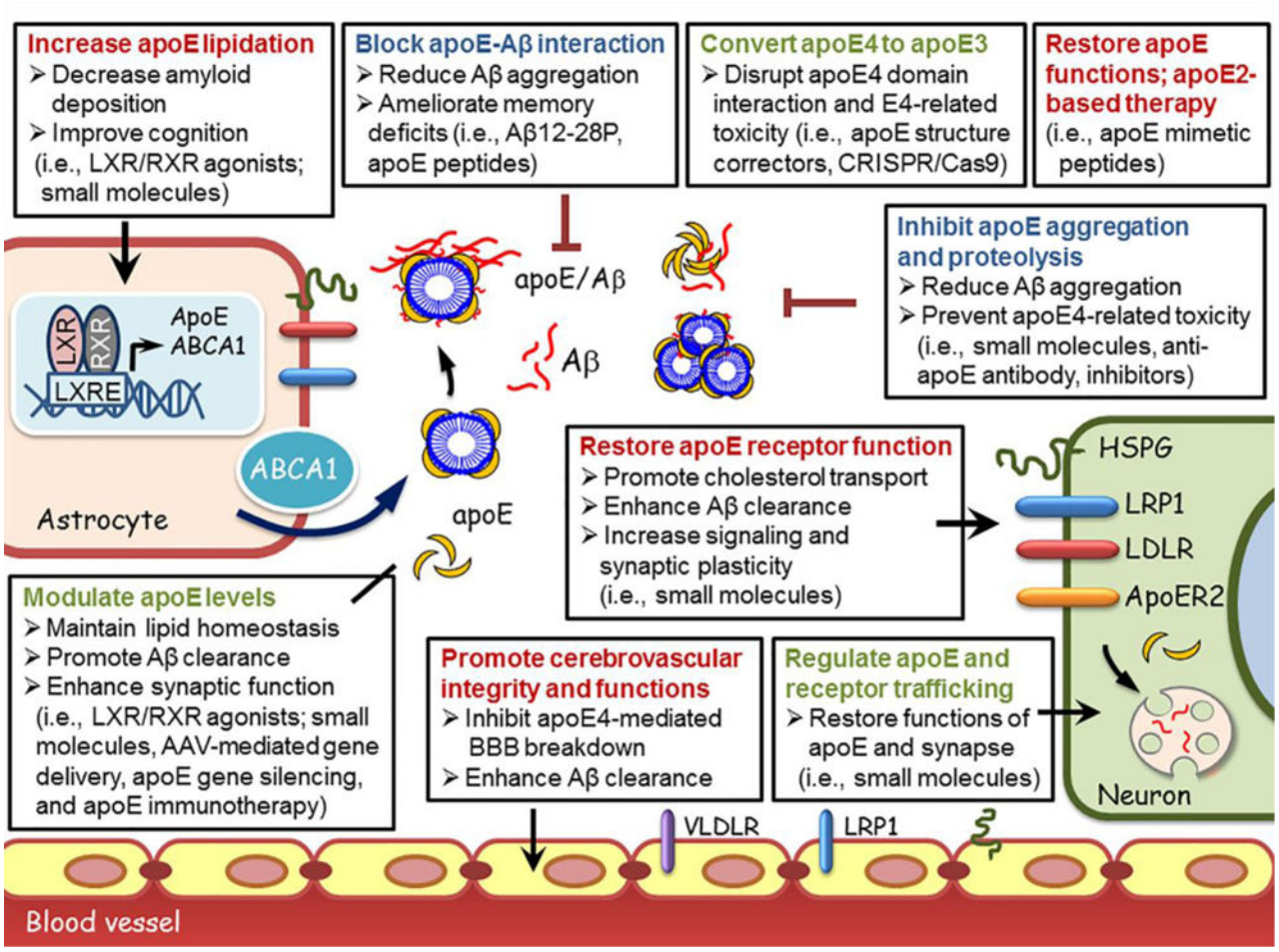

Figure 3. ApoE-targeting strategies for treatment of Alzheimer's disease

ApoE is primarily synthesized by astrocytes, and is lipidated by the ABCA1 transporter, forming apoE lipoprotein particles. Lipidated apoE binds soluble Aβ and facilitates Aβ uptake through cell surface receptors including LRP1, LDLR, VLDLR, and HSPG (6). ApoE and apoE receptors play critical roles in both Aβ-dependent and Aβ-independent AD pathogenic pathways (8). ApoE-directed approaches that are currently explored are categorized as below: 1) Modulating apoE level, stability and lipidation (ie., LXR/RXR agonists (103–105, 108), apoE stabilizing compounds (111), nanoparticles or AAV-mediated gene delivery of apoE (40, 113), gene silencing approaches and anti-apoE immunotherapy (115, 116); 2) Modulating apoE properties by converting apoE4 to apoE3 (i.e., structure correctors, CRISPR/Cas9 genome-editing) (118–121), suppressing apoE aggregation (122) and proteolysis (118, 119) and blocking apoE-Aβ interaction (126, 127); 3) Regulating the levels, intracellular trafficking and functions of apoE and apoE receptors (6, 72, 75, 98); 4) Restoring normal apoE functions (i.e., apoE-mimetic peptides) (128); 5) Restoring apoE4-mediated defects by apoE2-based treatment (40, 113), and 6) Promoting cerebrovascular functions (i.e., physical exercise, healthy diet and lifestyle), particularly in *APOE*4 carriers (142).

Abbreviations: Aβ, amyloid-β; apoE, apolipoprotein E; LXR, liver X receptor; RXR, retinoid X receptor; ABCA1, ATP-binding cassette A1; HSPG, heparan sulphate

proteoglycan; LDLR, low-density lipoprotein receptor; LRP1, low-density lipoprotein receptor-related protein 1; BBB, blood-brain barrier; ApoER2, apolipoprotein E receptor 2; AAV, adeno-associated virus.

Table 1

The effects of apoE isoforms on synaptic integrity and plasticity

Synaptic protein	
Synaptophysin Sytaxin1 PSD95	Specific synaptic proteins are altered in the superior temporal cortex of normal aged human brains in an apoE isoform-specific manner (apoE2>apoE3 >apoE4) (130).
Glutamate receptors	Glutamate receptors are decreased in *APOE4* carriers of human AD brains (131) and aged female apoE4-TR mice (132).
Dendritic morphology	
Dendritic spine density	*APOE4* dose inversely correlates with dendritic spine density of DG neurons in both AD patients and aged normal controls (133). ApoE4-TR mice showed age-dependent decrease of spine density in DG and cortex (apoE4<apoE3) (133, 134).
Dendrite complexity	ApoE4-TR mice show progressive dendritic spine deficits with aging (apoE4<apoE3) (135, 136).
EI balance (transmission)	
EPSC vs IPSC	Young apoE4-TR mice exhibit deficits in EPSC (apoE4<apoE3) (135, 136), whereas EI balance in aged apoE4-TR mice shows a bias toward inhibition (137).
Synaptic plasticity	
LTP	Young apoE4-TR mice exhibit higher LTP strength in the Schaffer collateral pathway of hippocampus (apoE4>apoE3) (98, 138, 139) but lower strength in the perforant pathway than apoE3 (apoE4<apoE3) (140). Under Aβ stress, perforant LTP was severely impaired in apoE4 mice compared with apoE2-TR mice (141).
LTD	Unknown

Abbreviations: DG, dental gyrus; EPSC, excitatory postsynaptic potential; IPSC, inhibitory postsynaptic potential; LTP, long-term potentiation; LTD, long-term depression; Aβ, amyloid-β.