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Apolipoprotein E as a Therapeutic Target in Alzheimer's disease: A Review of Basic Research and Clinical Evidence

Yu Yamazaki, Meghan M. Painter, Guojun Bu, and Takahisa Kanekiyo

Department of Neuroscience, Mayo Clinic, Jacksonville, FL. USA

Abstract

Alzheimer's disease (AD) is a devastating neurodegenerative disorder which causes progressive cognitive decline. The majority of AD cases are sporadic and late-onset (> 65 years old) making it the leading cause of dementia in the elderly. While both genetic and environmental factors contribute to the development of late-onset AD (LOAD), *APOE* polymorphism is a major genetic risk determinant for LOAD. In humans, the *APOE* gene has three major allelic variants: $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, of which *APOE* $\epsilon 4$ is the strongest genetic risk factor for LOAD, whereas *APOE* $\epsilon 2$ is protective. Mounting evidence suggests that *APOE* $\epsilon 4$ contributes to AD pathogenesis through multiple pathways including facilitated amyloid- β deposition, increased tangle formation, synaptic dysfunction, exacerbated neuroinflammation and cerebrovascular defects. Since *APOE* modulates multiple biological processes through its corresponding protein apolipoprotein E (apoE), *APOE* gene and apoE properties have been a promising target for therapy and drug development against AD. In this review, we summarize the current evidence regarding how the *APOE* $\epsilon 4$ allele contributes to the pathogenesis of AD and how relevant therapeutic approaches can be developed to target apoE-mediated pathways in AD.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, which is estimated to affect approximately 44 million people worldwide in 2015 [1]. Pathologically, AD is characterized by extracellular amyloid- β ($A\beta$) deposition and intracellular neurofibrillary tangles accompanied by neuronal loss and glial activation [2]. In addition to environmental factors, genetic components are considered to increase the risk of developing this condition. Indeed, studies have identified multiple loci associated with AD, including both causative and susceptibility genes. Among them, *APOE* polymorphism has been identified to be the major genetic determinant of susceptibility in the development of late-onset AD (LOAD); the $\epsilon 4$ allele of the *APOE* gene is the strongest genetic risk factor for LOAD [3–5].

Address correspondence to: Takahisa Kanekiyo, Department of Neuroscience, Mayo Clinic, 4500 San Pablo Road, Jacksonville FL 32224. Tel: 904-953-2483. Fax: 904-953-7370. kanekiyo.takahisa@mayo.edu.

Compliance with Ethical Standards

Conflict of interest

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The causative mutations in *APP*, *PSEN1* or *PSEN2* for AD have been identified only in early-onset or familial cases, which account for <1% of all AD cases. In contrast, *LOAD* accounts for >95% of all AD cases, and numerous susceptibility genes have recently been identified by GWAS and genome sequencing [6–10], albeit at much smaller hazard ratios compared to that of *APOE* (Fig. 1) [11, 12]. Thus, in light of its genetic prevalence and AD risk-determining effect, *APOE* polymorphism has become a promising target to aid in better defining pathophysiologic mechanisms, to identify individuals at risk for AD development, and for development of novel disease treatment strategies. Consistently, mounting evidence from human and animal studies has demonstrated that *APOE* ϵ 4 significantly affects multiple independent biological pathways in the brain, which collectively predispose *APOE* ϵ 4 carriers to AD risk. In this review, we will summarize the current evidence regarding how *APOE* ϵ 4 and its corresponding protein, apolipoprotein E4 (apoE4), contribute to the pathogenesis of AD, and discuss relevant therapeutic approaches targeting apoE.

2. *APOE* genetics and AD

The human *APOE* gene (located on chromosome 19q13.2) is polymorphic, where rs429358 and rs7412 single nucleotide polymorphisms (SNPs) generate three major allelic variants; ϵ 2, ϵ 3 and ϵ 4 [13] (Fig. 2). Among these *APOE* alleles, ϵ 3 is the most common allele with an allele frequency of 67–87%. The *APOE* ϵ 4 allele frequency is around 10% and 14% in Hispanics and Caucasians, respectively [14, 15], while it is around 20% in African-Americans [14]. Of note, the frequency of *APOE* ϵ 4 allele is dramatically increased to 40–65% in patients with AD [14, 16]. AlzGene meta-analysis of all races shows that the odds ratio (OR) of developing AD for *APOE* ϵ 4 carriers is 3.68 (95% CI, 3.30–4.11) when compared to individuals with the *APOE* ϵ 3/ ϵ 3 genotype. In addition, while risk-increasing effect varies across the populations, carrying *APOE* ϵ 4 generally increases the risk of AD onset in an allelic number-dependent manner (Fig. 3). Furthermore, carrying one *APOE* ϵ 4 allele shifts the age of onset an average of 2 to 5 years earlier, whereas the presence of two *APOE* ϵ 4 alleles shifts onset 5 to 10 years earlier [16–19]. In contrast, *APOE* ϵ 2 with an allelic frequency of about 7% exerts a protective effect against AD. When compared to individuals carrying *APOE* ϵ 3/ ϵ 3, carrying an *APOE* ϵ 2 allele reduces the risk of developing AD (OR, 0.621; 95% CI, 0.456–0.85) (<http://www.alzgene.org/meta.asp?geneID=83>). While the allelic number-dependent protective effect of *APOE* ϵ 2 in AD morbidity has not been determined likely due to the relatively small population of *APOE* ϵ 2/ ϵ 2, the risk of cognitive decline during aging tends to be lower in people carrying *APOE* ϵ 2/ ϵ 2 compared to those carrying *APOE* ϵ 2/ ϵ 3 [20].

3. Biochemical properties of apoE

ApoE is a 299-amino-acid protein with a molecular mass of ~34 kDa. The SNPs in *APOE* induce differences at amino acid residues 112 and 158 in apoE isoforms (apoE2, Cys112/Cys158; apoE3, Cys112/Arg158; apoE4, Arg112/Arg158) (Fig. 2). These single amino acid polymorphisms are considered to profoundly affect the structure and function of apoE including binding to lipids, receptors and A β [21, 22].

ApoE is a secreted, lipid-transporting protein in both the periphery and the central nervous system (CNS) [23, 24]. In the periphery, apoE is mainly secreted by hepatocytes with a plasma concentration of 40–70 µg/ml [25–27]. Lipoprotein preferences of apoE differ significantly depending on isoform; while apoE2 and apoE3 prefer high-density lipoprotein (HDL), apoE4 is usually associated with very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) in plasma [21, 26, 28, 29]. On the other hand, in the CNS, astrocytes are the major source of apoE with a cerebrospinal fluid (CSF) concentration of 3–5 µg/ml [25, 27]. Microglia and vascular smooth muscle cells/pericytes also produce apoE [30–33]. The size of apoE-lipoprotein particles in cerebrospinal fluid (CSF) corresponds to that of plasma HDL [34]. These particles contain core lipid and esterified cholesterol and are spherical in morphology [34].

While the lipid-binding region of apoE is in the C-terminal domain encompassing amino acid residues 244–272, the receptor-binding region of apoE resides within the N-terminal domain comprising amino acid residues 136–150 (Fig. 2) [21, 22, 28, 35]. The LDL receptor (LDLR) family members, including LDLR and LDLR-related protein 1 (LRP1), are major apoE receptors [21, 22, 35]. The binding function of apoE to LDLR is isoform dependent; apoE3 and apoE4 bind similarly to LDLR, while apoE2 has 50–100 times lower binding affinity [21, 26, 35]. In addition, while LDLR binds to naturally secreted or circulating apoE particles, LRP1 preferentially binds to apoE-enriched particles or apoE aggregates [21, 35].

4. Impacts of *APOE* on brain pathophysiology in AD

For subsequent sections, the impacts of *APOE* on AD pathophysiology will be described in two main categories; *APOE* and *Aβ* pathology and *APOE* and *Aβ*-independent pathways in AD pathogenesis. While the concept that accumulation of Aβ triggers pathogenic events associated with AD is a leading hypothesis [36–38], Aβ-independent pathways also significantly contribute to the disease pathogenesis. *APOE* is involved in multiple biological processes related to AD development and progression, thereby highlighting the advantage of targeting *APOE* in AD therapy.

4-1. *APOE* and Aβ pathology

APOE genotype significantly affects Aβ deposition both in humans and in animal models. In humans, having an *APOE* ε4 allele strongly associates with increased amount of Aβ including the more toxic oligomeric form found in post-mortem AD brains [39, 40]. During disease progression, *APOE* ε4 exacerbates intra-neuronal Aβ deposition [41] and plaque deposition [42–44] in the brain parenchyma, and also the formation of cerebral amyloid angiopathy (CAA) in cerebrovasculature [45, 46]. Although *APOE* ε2 is protective against AD, it is likely associated with an increased risk for CAA and CAA-related hemorrhage [47, 48].

As lack of murine *ApoE* dramatically reduces fibrillar Aβ deposition in an amyloid mouse model [53], apoE is shown to regulate brain Aβ aggregation and deposition. In studies using apoE-targeted replacement (TR) mice in which the coding exons 2–4 from murine *ApoE* locus is replaced with the corresponding region of the human *APOE* gene [49], apoE isoforms differentially influence brain Aβ metabolism. When amyloid mouse models were

crossed with apoE-TR mice, the severity of A β deposition is aggravated in the presence of apoE4 compared to apoE2 or apoE3 [50–52].

4-2. APOE and A β -independent pathways in AD pathogenesis

Tau phosphorylation and subsequent formation of neurofibrillary tangles are other pathognomonic processes in AD [2]. The significant association between the presence of neurofibrillary tangles and *APOE* ϵ 4 allele was demonstrated in post-mortem brains [43, 54–56], although data are conflicting in other studies [57–61]. In apoE4 transgenic mice [62] and apoE4-TR mice [63], tau phosphorylation/deposition is increased in murine brain parenchyma. ApoE4-mediated increased neuronal tau accumulation was also reported in transgenic mice carrying three familial AD mutations [64]. ApoE expression is likely induced in neurons under stress conditions [65], where apoE4 is fragmented faster than apoE3 [62, 66]. Thus, the neuron-specific proteolytic cleavage of apoE4 might contribute to the increase in phosphorylated-tau [62, 66], although further studies are needed to clarify the relevance and potential mechanism.

Additionally, synapses are one of the earliest sites of AD pathology, where synaptic dysfunctions strongly correlate with cognitive decline in AD patients and in amyloid mouse models [67–71]. Although controversial [72], more severe synaptic damages were observed in AD brains of *APOE* ϵ 4 carriers compared with *APOE* ϵ 3/ ϵ 3 subjects [40, 73]. In animals, reduced excitatory synaptic transmission/dendritic arborisation [74], reduced spine length/density [75, 76], and reduced synaptic markers [63] have been observed in apoE4-TR mice compared to apoE3-TR mice. Enhanced age-dependent cognitive decline in apoE4 accompanied by reduction in postsynaptic density 95 (PSD-95), drebrin and NMDA receptor subunits was also observed in amyloid mouse model when compared to apoE2 or apoE3 [77].

Neuroinflammation is also considered to be one of the key features in AD [78–81]. Interestingly, the profile of plasma inflammatory markers differs depending on *APOE* genotypes; *APOE* ϵ 4 carriers have increased levels of plasma inflammatory markers compared to the non-carriers [82]. In animal studies, in response to lipopolysaccharide (LPS), increased systemic and brain pro-inflammatory cytokines were reported in apoE4-TR mice when compared to apoE3-TR mice [83, 84]. In addition, synaptic protein loss was also more evident in apoE4-TR mice after LPS injection [85]. These studies suggest a differential regulation of neuroinflammatory responses depending on *APOE* genotypes.

Interestingly, neuropathological features of AD and cerebrovascular diseases often overlap [86]. Accumulating evidence suggests that environmental risk factors and overlapping pathogenic mechanisms for both diseases interact synergistically to exacerbate disease progression [86, 87]. The two diseases also share *APOE* ϵ 4 as a strong genetic risk factor [86]. Furthermore, blood-brain barrier (BBB) damage as well as endothelial cell/pericyte degeneration was more evident in the brains of AD patients with *APOE* ϵ 4 than those with *APOE* ϵ 3/ ϵ 3 [88]. In AD brains, *APOE* ϵ 4 is also associated with more severe CAA pathology in a manner dependent on allelic number [44, 89]. While *APOE* ϵ 2 is a protective allele for AD, carrying *APOE* ϵ 2, in particular those with two *APOE* ϵ 2 alleles, is also associated with more severe CAA [90]. In animal models, apoE4-TR mice showed

substantial CAA compared to apoE3-TR mice when crossed with an amyloid mouse model [91]. Reduced cerebral vascularization and cerebral blood flow as well as BBB dysfunction have been reported in apoE4-TR mice compared to apoE2-TR and apoE3-TR mice [92, 93], although some controversies exist regarding whether apoE4 is associated with widespread BBB leakage [94].

5. ApoE-targeted therapy for AD

Increasing evidence supports the concept that *APOE* ϵ 4 genotype significantly shifts multiple biological processes toward susceptible conditions for AD development. While the primary mechanism linking *APOE* ϵ 4 and neurodegeneration still remains to be clarified, it is highly likely that *APOE* modulates numerous biological processes through its corresponding protein apoE. Thus, modulations of *APOE* gene and apoE properties are promising targets for drug development and therapy against AD. In this section, current concepts of apoE-targeted AD therapies will be comprehensively discussed by grouping them into three main categories: 1) regulation of apoE quantity; 2) modification of apoE properties; and 3) indirect therapeutic approaches (Table 1).

5-1. Regulation of apoE quantity

Up-regulation of apoE levels—Recent clinical studies for AD biomarkers have demonstrated conflicting results with respect to whether apoE levels in CSF and plasma are reduced in AD patients compared to normal individuals [95–98]. Nonetheless, there are numerous animal studies evaluating the therapeutic potential of compounds that increase brain apoE levels [99–115]. Transcription of *APOE* is positively regulated by nuclear receptors, retinoid X receptors (RXRs) and liver X receptors (LXRs), which form heterodimers [116]. In fact, oral administration of an RXR agonist, bexarotene, increased brain apoE levels, reduced A β deposition, and improved cognitive functions in amyloid model mice [100]. Since bexarotene is a drug already approved by the U.S. Food and Drug Administration (FDA) to treat cutaneous T-cell lymphoma [117–120], the off-label usage of this drug for AD has attracted the interest of many researchers. Bexarotene significantly slowed cognitive decline in an amyloid mouse model expressing human *APOE* ϵ 3 and *APOE* ϵ 4 [121], and reversed the *APOE* ϵ 4-induced cognitive and neuronal impairments in mice without amyloid background [99]. Age-dependent loss of synaptic proteins is also restored by bexarotene [102]. However, there are conflicting reports regarding the effects of bexarotene treatment on amyloid pathology in mouse models [101, 103, 105, 106, 121]. In addition, bexarotene-induced adverse side effects including hepatic failure have been reported [102, 104]. Furthermore, in a clinical study, four weeks of bexarotene treatment in AD patients did not reduce brain amyloid as measured by PET scans, but the sample size was notably small ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01782742) identifier NCT01782742) [122]. Thus, although bexarotene as a RXR agonist might have beneficial effects on halting AD pathogenesis, further assessments and dosage optimization are required for its potential therapeutic application to treat AD.

Additionally, the administration of LXR agonists (GW3965 and TO-901317) has been shown to reduce brain A β deposition [107–110, 112] and restore cognitive functions [108,

109, 112, 113] in several amyloid mouse models, although whether these beneficial effects were mediated via increased apoE levels is unclear. The combination of ω -3 fatty acid docosahexaenoic acid (DHA) and bexarotene co-administration further reduced AD pathology and aided in amelioration of cognitive deficits through the activation of LXR-RXR pathway in amyloid mouse model [111].

Moreover, RXRs have been shown to form a complex with other nuclear receptors such as peroxisome proliferator-activated receptor γ (PPAR γ), retinoid acid receptor (RAR), thyroid hormone receptor (TR) or vitamin D receptor (VDR) as transcriptional co-regulators [123–126]. Treatment with a PPAR γ agonist, rosiglitazone, rescued memory deficits and reduced amyloid and tau pathology in amyloid mouse model without increasing brain apoE levels [114]. Another PPAR γ agonist, pioglitazone, increased apoE, decreased A β , and improved cognition in an amyloid mouse model with a synergistic effect observed upon co-treatment with the LXR agonist GW3965 [115]. In addition, all-trans retinoic acid (RA), 9-cis RA and 13-cis RA have been identified as effective modulators of apoE production through RXR and RAR in astrocytes [127]. Thyroid hormones also upregulate *APOE* gene expression by binding to TR/RXR in astrocytes [128]. Together, these pathways mediated by RXR heterodimers are likely central to the regulation of brain apoE levels, which might serve as targets for development of effective AD therapeutics.

Reduction of apoE levels—Recent studies have reported that *APOE* haploinsufficiency in apoE-TR mice attenuates A β deposition regardless of *APOE* genotypes [129, 130]. The intraperitoneal administration of an anti-apoE antibody has also been shown to improve spatial learning performance by reducing brain soluble apoE levels and slowing brain A β plaque formation in an amyloid mouse model without evident side effects [131, 132]. Thus, the reduction of apoE may somehow be beneficial to ameliorate A β pathology; however, this approach needs to be carefully justified for clinical application since apoE deficiency could cause severe dyslipidemia as reported to occur in an apoE-deficient individual homozygous for the c.291del, p.E97fs *APOE* mutation [133].

Of note, *APOE* ϵ 4 is increasingly recognized as a polymorphism which contributes to AD pathogenesis by both *gain-of-functional* and *loss-of-functional* properties when compared to *APOE* ϵ 3 [22, 35]. Thus, to develop effective therapies for AD by targeting apoE, these bi-directional effects of *APOE* ϵ 4 need to be considered. Targeted elimination of apoE4 may be an encouraging strategy to reduce its toxic effects. In fact, the treatment with an anti-apoE4 monoclonal antibody (9D11) likely prevented the apoE4-driven cognitive impairment and brain tau hyperphosphorylation in apoE4-TR mice [134], although follow-up studies are required.

In addition, inactive forms of apoE including apoE aggregates and fragments are predicted to be harmful [135–139] in which apoE4 likely has a higher chance of adopting such conformational changes or proteolysis than other isoforms [140]. Thus, removing apoE aggregates but not the native form of functional apoE by immunodepletion should be an alternative therapeutic concept. Pharmacological approaches to prevent apoE aggregation and fragmentation could also serve as viable therapeutic strategies.

ApoE mimetic peptides—To compensate for the *loss-of-functional* aspect conferred by apoE4, the use of apoE mimetic peptide which mimics the biological features of native apoE may help recover some apoE-mediated homeostatic effects [141]. Treatment with synthetic apoE mimetic peptides composed of 12 to 17 amino acids (COG112 and COG1410) which contain the receptor-binding region in apoE but not lipid- or A β -binding region, significantly reduced neuroinflammation, tau hyper-phosphorylation and defects in adult neurogenesis in AD mouse models [142, 143]. Additionally, another apoE mimetic peptide consisting of tandem repeats of the apoE receptor-binding region (Ac-hE18A-NH₂) reduced A β production in wild-type mice [144] and improved cognition with concomitant suppression of glial activation/A β deposition in amyloid model mice [145]. However, the effects of these peptides on amyloid pathologies in the presence of human apoE isoforms particularly in apoE4 have not been fully evaluated. Further investigations to determine the molecular mechanisms underlying therapeutic effects of apoE mimetic peptides on AD are necessary.

Gene therapy targeting *APOE*—In addition to pharmacological approaches using compounds and peptides, *APOE* gene delivery is a promising strategy to regulate brain apoE levels. Adeno-associated virus (AAV)-mediated gene delivery of *APOE* into the brains of an amyloid mouse model clearly demonstrated *APOE* isoform-dependent effects on A β pathology. Specifically, expressing apoE2 through AAV even at the relatively low level (~15% of the endogenous murine apoE) could reduce brain A β levels compared to controls, while expressing apoE4 (~10% of the endogenous murine apoE) exacerbated synaptic loss and the accumulation/deposition of A β in amyloid mouse models with murine apoE [146]. Consistently, similar opposing effects of AAV-mediated *APOE* gene delivery on mouse endogenous A β metabolism were reported in apoE4-TR mice without amyloid background [147]. Furthermore, a significant decrease of A β 42 levels and A β deposition was observed when apoE2 was expressed using a lentiviral vector in amyloid model mice [148]. Collectively, these results indicate that gene therapy which increases apoE2, but not apoE4, might be beneficial irrespective of A β pathology in AD pathogenesis. For future clinical application of apoE-targeted gene therapy, the therapeutic effect of viral mediated *APOE* gene delivery needs to be experimentally determined in amyloid mouse models carrying different human apoE isoforms. For example, targeted, stereotactic injection to astrocytes within the pathology-prone areas of the frontal lobe or hippocampus of amyloid model mice via AAV vector expressing *APOE* under the control of the glial fibrillary acidic protein promoter [147] would provide appropriate *in vivo* pre-clinical assessment. In the case of *APOE* ϵ 4 carriers, gene silencing approaches using RNA interference and antisense oligonucleotide for *APOE* may exhibit better therapeutic efficacy than overexpression of the gene, although further studies are needed.

5-2. Modification of apoE properties

Structural modification of apoE—While the amino acid residues 112 and 158 in the N-terminus are the determinants of apoE isoforms, the interaction between residues Arg61 and Glu255 is likely an additional feature that structurally distinguishes apoE4 from apoE3. This domain-domain interaction likely generates abnormal conformation in apoE4, leading to neurotoxicity [149], although the structure of the full-length, native apoE has not been determined to date. Whilst expressing apoE4 induces mitochondrial dysfunction in neuronal

N2A cells, this effect is abolished in an apoE4 mutant lacking the domain interaction (apoE4-R61T) [150]. Further, several small molecular compounds (GIND25 and PH002) have been reported to reverse detrimental effects of apoE4 by blocking the domain-domain interaction in neurons [151]. Thus, modifying the pathological structure of apoE4, such as the domain-domain interaction, may be a potential therapeutic approach for AD. However, the efficacy of these compounds on AD-related pathways has not yet been tested *in vivo*.

In addition, advancements in genome-editing technologies [152–155] also highlight the potential of using CRISPR/Cas9 system to convert *APOE* ϵ 4 to *APOE* ϵ 2 or ϵ 3 [156]. Thus, apoE structural and functional changes from apoE4 to apoE2 or apoE3 through the CRISPR/Cas9-mediated gene editing may be an interesting approach to treat AD patients carrying *APOE* ϵ 4, although further studies are needed.

Increase of apoE lipidation—Since the biologically-active form of apoE protein is associated with lipids [26, 157], lipidation status of apoE significantly affects its function [21]. In the brain, ATP binding cassette subfamily A member 1 (ABCA1) plays an important role in loading lipid particles on apoE proteins [158]. Deletion of ABCA1 diminishes the generation of properly lipidated apoE particles resulting in highly amyloidogenic pathologies in amyloid model mice [159]. On the other hand, overexpression of ABCA1 reduces A β deposition in an amyloid mouse model in a manner dependent on apoE [160], which suggests a beneficial role of ABCA1-mediated lipidation of apoE in halting AD pathogenesis. As described above, agonists for LXRs and RXRs can reduce A β deposition and ameliorate cognition in amyloid model mice. Moreover, these pathways control the transcription of *ABCA1* as well as *APOE* [100, 108, 109]. Thus, these studies support the concept that increasing apoE lipidation might be a beneficial approach to treat AD, although it may be difficult to manipulate the lipidation status without altering apoE level.

Of note, apoE4 is less lipidated than apoE2 and apoE3 both in humans [161] and in apoE-TR mice [52, 162]. Increasing evidence suggests that lipid-mediated mechanisms may play a role in the detrimental properties of apoE4 [163]. Consistent with this, the treatment of apoE4-TR mice with the RXR agonist, bexarotene, reversed apoE4-induced cognitive and neuronal deficits presumably by increasing lipidation of apoE4 [99]. Thus, modulating the lipidation status of apoE4 appears to be an attractive therapeutic approach [164]; however, the activation of the LXRs/RXRs-ABCA1 axis could potentially increase the levels of apoE4, thereby amplifying *gain-of-toxic functions* of apoE4 [22].

Interestingly, the ablation of Acyl-CoA:cholesterol acyltransferase 1 (ACAT1) enzymatic activity resulted in a reduction of A β deposition as well as improved cognitive functions in an amyloid mouse model [165]. Since ACAT converts free cholesterol to cholesteryl esters [166], it is possible that the deletion of ACAT influences apoE lipidation status. Thus, ACAT1 inhibitors may serve as anti-AD drugs by targeting upstream of apoE lipidation. In fact, an ACAT1 inhibitor, K604, can promote A β 42 clearance through autophagy-mediated lysosomal proteolysis in microglia [167], although it remains unknown if apoE lipidation is involved in this mechanism. In addition, a functional polymorphism in *CETP*, encoding the cholesteryl ester transfer protein (CETP), has been shown to be associated with increased AD risk [168]. Thus, it is important to determine how the metabolic pathways for lipids and

cholesterol regulate apoE lipidation and its function. These answers might provide novel insights into the development of apoE-targeted therapy for AD.

Blocking the apoE-A β interaction—While the apoE-A β interaction may be minimal under physiological conditions [169], apoE and A β co-localize in amyloid plaques in human brains [170]. It is increasingly recognized that the interaction of apoE with A β influences aggregation and deposition of A β in AD brains [21]. Indeed, inhibition of the apoE-A β interaction by a synthetic peptide (A β 12-28P: homologous to the apoE binding site on the full-length A β) resulted in reduced A β deposition and intra-neuronal A β accumulation, and ameliorated memory deficits in amyloid mouse models [171, 172]. In 3xTg AD mouse model which harbors both A β and tau pathology, blocking the apoE-A β interaction by A β 12-28P reduced A β deposition and insoluble tau accumulation in the brain [173]. Further, the treatment with A β 12-28P resulted in reduced A β oligomers and A β plaque load, and ameliorated neuritic degeneration in an amyloid mouse model with apoE2-TR or apoE4-TR mouse background [174]. Thus, inhibition of the apoE-A β interaction appears to be beneficial for preventing aggregation and deposition of A β regardless of the apoE isoforms, albeit therapeutic efficacy of synthetic peptides may differ depending on the apoE isoform being targeted. A β 20–29 peptides may also block apoE/A β interaction thereby reducing A β fibrillogenesis and cytotoxicity *in vitro* [175]. Interestingly, apoE immunotherapy dramatically decreases amyloid deposition in amyloid mouse models as described above [131, 132]. Whilst approaches using anti-apoE antibodies likely impact A β metabolism through multiple mechanisms [131, 132], it is also possible that these antibodies block the apoE-A β interaction thereby facilitating A β clearance.

5-3. Indirect therapeutic approaches

Regulation of apoE receptors—Endocytosis of apoE is mediated by members of the LDLR family, including LDLR and LRP1 [24]. ApoE likely facilitates either the cellular uptake of A β through these receptors by generating apoE/A β complexes or by suppressing the interaction of A β through competition for receptor binding. These events are further complicated by apoE isoform-dependent, concentration-dependent and lipidation-status-dependent mechanisms [21, 24]. Interestingly, accumulating evidence has highlighted the critical roles of LRP1 and LDLR in brain A β clearance as well as lipid metabolism [176–179]; however, whether these phenotypes are mediated by apoE remains to be elucidated. Thus, increasing LRP1 and LDLR could serve as a therapeutic approach to activate A β clearance pathways in AD. Indeed, several compounds including rifampicin, caffeine, and fluvastatin are likely to have protective effects against AD by increasing LRP1 levels [180, 181]. In addition, LRP1 and another apoE receptor, APOER2, play critical roles in maintaining synaptic functions [24, 182]. While Reelin, a large extracellular matrix glycoprotein, enhances synaptic glutamate receptor activity through APOER2, apoE4 disrupts this pathway by impairing APOER2 recycling [183]. APOER2 levels in hippocampus are also reduced specifically in apoE4-TR mice [184]. Thus, regulating APOER2 levels may be considered as an apoE-mediated anti-AD therapy, although further studies are needed.

Restore blood-brain barrier (BBB) integrity—In an animal study, apoE4 expression in astrocytes, but not apoE2 or apoE3 expression, has been shown to activate the proinflammatory cyclophilin A (CyA)–nuclear factor κ B–matrix metalloproteinase 9 (MMP-9) pathway in brain pericytes thereby causing tight junction protein degradation and BBB breakdown [93]. Importantly, these BBB pathologies in apoE4-TR mice were reversed by cyclosporine A treatment [93]. Age-dependent BBB breakdown as measured by Q_{Alb} index as well as elevated CyA and active MMP-9 levels were also detected in CSF from cognitively normal *APOE* ϵ 4 carriers [185]. Thus, cyclosporine A can be considered as an anti-AD drug, while it is still controversial whether apoE4-mediated BBB disruption is severe enough to alter the global homeostatic capacity of the BBB contributing to AD pathogenesis [92, 94, 186].

***APOE* as a determinant of therapeutic response in AD**—*APOE* ϵ 4 carriers show a differential response to several therapies for AD in the clinical settings [187]. Indeed, phase III trials of a passive immunization of bapineuzumab, a humanized anti-A β monoclonal antibody, for mild-to-moderate AD patients showed a significant difference in key biomarker responses through treatment between *APOE* ϵ 4 carriers and non-carriers, although bapineuzumab did not improve clinical outcomes in the patients [188]. In *APOE* ϵ 4 carriers, the bapineuzumab treatment (0.5 mg/kg) prevented the increase of A β deposition detected by positron-emission tomographic amyloid imaging with Pittsburgh compound B (PIB-PET) from baseline to week 71. On the other hand, *APOE* ϵ 4 non-carriers did not show the increase in A β deposition in both groups at the same time point, and the significant differences were also not observed between the placebo group and the bapineuzumab treatment groups (0.5 mg/kg and 1.0 mg/kg). In addition, the CSF phospho-tau concentration was significantly reduced by 0.5 mg/kg bapineuzumab treatment at week 71 in *APOE* ϵ 4 carriers, but not *APOE* ϵ 4 non-carriers, although the treatment with increased dosage of bapineuzumab (1.0 mg/kg) induced significant reduction on the marker in *APOE* ϵ 4 non-carriers. Furthermore, higher rates of amyloid-related imaging abnormalities with effusion or edema (ARIA-E) were observed in *APOE* ϵ 4 carriers compared to non-carriers. Overall, these results indicate that differential bapineuzumab treatment responses with regard to A β metabolism and downstream pathways in AD patients might depend on *APOE* genotypes.

Insulin administration may serve as a promising therapeutic intervention for AD; however, differential outcomes following treatment appear to be influenced by *APOE* genotype [189]. When a condition of hyperinsulinemia (~ 80 μ U/ml in plasma) was induced in AD patients by continuous intravenous administration of insulin and 20% dextrose solution, significant improvements in cognition were observed in *APOE* ϵ 4 non-carriers, but not in the ϵ 4 carriers [190]. Beneficial effects of intranasal insulin administration on cognition are further affected by the presence of *APOE* ϵ 4 allele in AD patients [191, 192].

Additionally, the Cardiovascular Health Cognition Study revealed that the usage of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of developing AD in a cohort over 65 years of age; however, the benefit of NSAIDs was evident only in individuals carrying an *APOE* ϵ 4 allele [193].

Of note, the association of *APOE* $\epsilon 4$ with an increased risk for AD incident was significantly stronger in women than men [194]. While the benefit of hormone replacement therapy in female AD patients is controversial likely depending on the timing of hormone initiation, *APOE* genotype also modulates the effect of estrogen on neuroprotection as the therapy is associated with worse rates of decline in general cognition among *APOE* $\epsilon 4$ carriers [195].

Collectively, *APOE*-dependent differences have been observed in the outcomes of several AD-related therapies. Thus, whenever clinical trials are designed to evaluate therapeutic efficacy against AD, the potential impact of *APOE* genotype needs to be carefully considered. In addition, *APOE* $\epsilon 4$ allelic number-dependent increase in AD risk is likely different depending on race/ethnicity [196]. Therefore, heterogeneity of population such as different racial/ethnic distribution might be a confounding factor when considering *APOE* as a therapeutic response predictor in the analysis of clinical trials [187, 197]. These approaches may also provide an opportunity for better understanding of apoE biology in humans, which should ultimately lead to the establishment of novel apoE-targeted therapies for AD.

6. Summary and Perspective

Despite intense research efforts in the field of neurodegeneration since the 1993 discovery of *APOE* $\epsilon 4$ as a strong genetic risk factor for LOAD [16, 198], to date, no therapeutic interventions targeting *APOE* gene and apoE properties have been successfully established. In addition, we are still at an early stage to determine therapeutic window and patient stratification for introducing apoE-targeted therapy for AD. Since the mechanisms responsible for apoE isoform-specific differences on brain homeostasis are complicated and the risk-increasing effect of *APOE* $\epsilon 4$ on AD development is likely driven by the synergy of 'gain-of-toxic functional' and 'loss-of-functional' profiles of apoE4 in different cell populations, it has been difficult to determine/prioritize which apoE-mediated mechanisms and therapeutic concepts are relevant *in vivo* and translatable to the clinical setting. Therefore, more precise understanding of apoE through the diverse approaches might be essential for developing effective apoE-targeted therapy for AD.

To this end, mouse models carrying human apoE isoforms would help researchers to define *APOE* $\epsilon 4$ -driven pathophysiology and validate apoE-targeted anti-AD therapy in a more standardized manner. Our recent study using apoE-TR mice could recapitulate the observations in a large human cohort from the National Alzheimer's Coordinating Center (NACC) showing age-related cognitive decline in an *APOE* genotype-dependent manner [20]. Therefore, apoE-TR mice may serve as an appropriate model for apoE-targeted therapy when cognitive function is used as an important outcome. In addition to genetically engineered mice, advances in viral-mediated transgene delivery *in vivo* to individual CNS cell types [199] would further tease apart the impact of *APOE* expression on brain homeostasis and disease progression in a cell-autonomous manner. For example, use of two recently engineered AAV serotypes that are able to differentially transduce astrocyte (AAV-PHP.A) [199] or microglial (rAAV6 with F4/80 or CD68 promoter) [200] populations may allow for rapid *in vivo* assessment of cellular *APOE* effects especially regarding the

protective effect of the *APOE* ϵ 2 allele, and more importantly, the therapeutic potential of expressing *APOE* ϵ 2 in a specific CNS cell type independent of inherent *APOE* genotype. Additionally, inducible expression of *APOE* provided by technologies such as these may define the therapeutic time window during brain aging and disease course. However, it should always be taken into consideration that mice are never humans [201–203]. Thus, efficacy in anti-AD therapies in animals does not necessarily translate into clinical efficacy in AD patients. More complex models may be required to improve the translational potential of *APOE* research. In this regard, results from *in vivo* animal studies would be complemented with recent advances in *in vitro* experimental technologies [204], including induced pluripotent stem cell (iPSC)-based models [205, 206] and three-dimensional culture systems [207], recapitulating true human brain biology in a dish. In addition, the translational potential of AD therapeutics targeting apoE may be enhanced via more careful preclinical study design in animal models such as the utilization of heterogeneous animal cohorts with variations in age, sex, strain and comorbidities such as hyperglycemia, hypertension and chronic cerebral hypo-perfusion. Because AD is a chronic-progressive disorder that develops over decades, there might not be ‘single-bullet-fits-all’ approach for the disease modeling. Future studies utilizing these comprehensive approaches and careful consideration of lessons learned from previous anti-AD approaches [37, 208] might allow for successful design of AD therapies by targeting the *APOE* gene and apoE properties.

Lastly, *APOE* genotype likely modulates therapeutic responses to several potential AD therapies even though apoE is not directly targeted. Thus, patient stratification for both treatment regimens and outcome measures by *APOE* genotype should be considered in developing preventative and curative AD therapies.

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Key Points

- The $\epsilon 4$ allele of the *APOE* gene is the strongest and most validated genetic risk factor for late-onset Alzheimer's disease (AD).
- Human and animal studies suggest that carrying *APOE* $\epsilon 4$ not only affects amyloid- β ($A\beta$) metabolism but also contributes to AD pathogenesis by $A\beta$ -independent mechanisms that involve tau metabolism, synaptic plasticity, neuroinflammation and cerebrovascular functions.
- Anti-AD therapies targeting *APOE* and its corresponding protein apolipoprotein E (apoE) can be categorized into 1) regulation of apoE quantity; 2) modification of apoE isoforms and lipidation status; and 3) indirect therapeutic approaches related to apoE.

Figure 1. Genetic causative/risk factors for AD

Risk-increasing effects (vertical axis) were plotted against prevalence in general population (horizontal axis) between either causative or susceptible genes/loci. The figure is updated and modified with permission from Karch et al [12].

Figure 2. Schematic representation of human *APOE* genotype and apoE isoform
The human *APOE* gene is located on the long arm of Chromosome 19. Two non-synonymous single nucleotide polymorphisms (SNPs) in exon 4 of chromosome 19 (rs429358 and rs7412) generate three major allelic variants (ε2, ε3 and ε4). The resulting apoE2, apoE3 and apoE4 isoforms differ from one another at amino acid residues 112 or 158. There are two domains in apoE, where receptor-binding region is located in the N-terminus and lipid-binding region is located in the C-terminus.

Figure 3. The relative odds ratios for AD development according to the allelic number of *APOE* $\epsilon 4$ in worldwide populations

Data from the studies in Nigeria [209], Tunisia [210], Iran [211], Korea [212], Japan [213], France [214], Norway [17], US Caucasians (clinical) [14], US Caucasians [14], US Africans [14], US Hispanics [51], US Japanese [51], Brazil [215], and Chile [216] were shown. The figure was adapted and reformatted with permission from Raichlen et al [196].

Table 1

ApoE-targeted therapy for AD

Strategy	Concept	Therapeutic candidates	<i>in vivo</i> species tested	<i>APOE</i> isoforms tested	References
Regulation of <i>apoE</i> quantity					
Up-regulation of apoE levels	Promotes A β clearance, lipid homeostasis and synaptic function	LXR, RXR, PPAR γ agonists etc.	Mouse	Yes	[99–115, 122]
Reduction of apoE levels	Decreases apoE-associated toxic effects and A β aggregation/deposition	anti-apoE4 (9D11) monoclonal antibody, immune-depletion of apoE	–	Yes?	[129–132, 134]
ApoE mimetic peptide	Decreases neurotoxicity and inflammation, increases apoE3-associated protective functions	small peptides containing the receptor-binding region in apoE	Mouse	No	[141–145]
Gene therapy	Increases apoE-associated neuroprotective effects	viral-mediated apoE2 expression	Mouse	Yes	[146–148]
Modification of <i>apoE</i> properties					
Structural modification of apoE	Interferes domain-domain interaction in apoE4 thereby decreasing its toxic effects	small-molecule structure correctors (GIND25 and PH002)	–	No	[149–151]
Increase apoE lipidation	Promotes A β clearance, lipid homeostasis and synaptic function	LXR and RXR agonists, ACAT1 inhibitors?	Mouse	Yes	[99, 100, 108, 109, 122, 167]
Block apoE-A β interaction	Reduces A β aggregation and deposition	A β 12-28P, A β 20-29 peptide, small molecule inhibitors	Mouse	No	[171–175]
Others					
Increase apoE receptor levels	Enhances A β clearance, cholesterol transport and apoE-signaling	small molecules	Mouse	No	[180, 181]
Restore blood-brain barrier (BBB) integrity	Decreases leakage of blood-derived toxic molecules in <i>APOE</i> ϵ 4-carrying brain	Cyclosporine A	Mouse	Yes	[93]