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Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy

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PREFACE

The vast majority of Alzheimer's disease (AD) cases are late-onset and their development is likely influenced by both genetic and environmental risk factors. A strong genetic risk factor for late-onset AD is the presence of the $\epsilon 4$ allele of the apolipoprotein E (*APOE*) gene, which encodes a protein with crucial roles in cholesterol metabolism. Mounting evidence demonstrates that apoE4 contributes to AD pathogenesis by modulating the metabolism and aggregation of amyloid- β peptide and by directly regulating brain lipid metabolism and synaptic functions through apoE receptors. Emerging knowledge on the contribution of apoE to the pathophysiology of AD presents new opportunities for AD therapy.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Extracellular amyloid plaques and intracellular neurofibrillary tangles are defining lesions in AD^{1,2}. Mounting genetic and biochemical data support the hypothesis that amyloid- β (A β) accumulation and aggregation in the brain is an early and central event in the pathogenesis of AD^{2,3}. A β is derived from sequential proteolytic processing of the amyloid precursor protein (APP) by β - and γ -secretases. Mutations associated with early-onset familial AD (FAD) are dominantly inherited and are found in the *APP* gene itself or in the genes of *presenilin 1* (*PSEN1*) and *PSEN2*, whose products, together with nicastrin, A β -1 and PEN-2, are essential components of a multi-protein complex that is responsible for γ -secretase activity⁴. A common feature of most FAD mutations is that they increase the generation of A β peptides, or increase the proportion of the longer A β 42 form, which has a higher tendency to aggregate and is more toxic than the shorter A β 40³. Because γ -secretase cleavage of an increasingly recognized panel of substrates is important for synaptic function and neuronal survival, a loss-of-function hypothesis for *PSEN* mutations in AD pathogenesis has also been proposed⁵.

FAD genetics and mouse models have shed light on early-onset AD pathogenesis, but the vast majority of AD cases occur late in life. The $\epsilon 4$ allele of the apolipoprotein E (*APOE*) gene is a major risk for late-onset AD (LOAD). This risk allele, discovered in 1993 by Strittmatter, Roses and colleagues^{6,7}, has been validated in numerous genetic association studies (see AlzGene website at <http://www.alzforum.org/res/com/gen/alzgene/default.asp>). Although a gene on chromosome 19 had previously been implicated in LOAD risk, the

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observation that apoE binds to A β in the cerebrospinal fluid (CSF) prompted the testing of *APOE* as a positional candidate⁶. ApoE is a major apolipoprotein and a cholesterol carrier in the brain⁸. In humans, the *APOE* gene exists as three different **polymorphic alleles** (ϵ 2, ϵ 3 and ϵ 4), which engender six different genotypes (ϵ 2/ ϵ 2, ϵ 2/ ϵ 3, ϵ 2/ ϵ 4, ϵ 3/ ϵ 3, ϵ 3/ ϵ 4 and ϵ 4/ ϵ 4). ϵ 3 is the most (77%) and ϵ 2 the least (8%) common allele⁸. The ϵ 4 allele frequency is about 15% in general populations, but is ~40% in AD patients. Individuals with one ϵ 4 allele are 3- to 4-fold more likely to develop AD than those without ϵ 4 alleles^{7,9}. This odds ratio (OR) is much greater than those for other AD-risk alleles, which are typically <1.5⁹. The maximal effects of the ϵ 4 allele on AD risk is between 60 and 70 years of age¹⁰, and the prevalence of the ϵ 4 allele in AD patients is >50%⁷. Interestingly, although rare, the ϵ 2 allele is associated with protection against LOAD compared to the ϵ 3 allele⁷. The ϵ 4 allele is also a risk factor for atherosclerosis⁸, HIV disease progression¹¹ and additional neurological disorders including **cerebral amyloid angiopathy (CAA)** and CAA-associated cerebral hemorrhages¹², tauopathies and Lewy body disease¹³, Parkinson's disease¹⁴ and multiple sclerosis¹⁵. These observations suggest that the ϵ 4 allele may be associated with accelerated neurodegeneration in the development and progression of several neurodegenerative diseases.

The three apoE isoforms (apoE2, apoE3 and apoE4) differ from each other by a single amino acid⁸. In most, if not all, AD putative pathogenic pathways, apoE4 either diminishes protection or augments toxicity when compared to apoE2 and apoE3. In the brain, apoE/lipoprotein particles are produced primarily by astrocytes and deliver cholesterol and other essential lipids to neurons via members of the low-density lipoprotein receptor (LDLR) family^{16,17}. Although the mechanisms underlying the pathogenic nature of apoE4 in AD are still not completely understood, several pathways have been identified *in vitro* and *in vivo*. In this Review, I describe A β -dependent and A β -independent pathways by which apoE isoforms and apoE receptors contribute to AD pathogenesis, with particular attention to the distinct functions of apoE receptors, and the recent advances and challenges in targeting apoE and apoE receptors for AD therapy.

Biochemical properties of apoE and apoE/lipoproteins

ApoE is an ~34-kDa protein that transports cholesterol and other lipids in the plasma and the central nervous system (CNS) by binding to cell surface apoE receptors⁸. Its highest concentration is in the liver and brain. In the periphery, apoE transports liver-synthesized very low-density lipoprotein (VLDL), a subclass of high-density lipoprotein (HDL) and intestinally synthesized chylomicrons. In the brain, apoE is predominantly synthesized by astrocytes and to some extent by microglia^{18,19}. ApoE/lipoprotein particles from human CSF are the size and density of plasma HDL, are spherical and contain a core lipid: esterified cholesterol^{20,21}. Interestingly, nascent apoE/lipoprotein particles secreted by cultured astrocytes are primarily discoid and contain little lipid²¹. It is possible that astrocyte-secreted apoE/lipoprotein particles acquire a core of esterified cholesterol before reaching the CSF. Alternatively, native apoE/lipoprotein particles produced by astrocytes in the brain may differ from those secreted by cultured astrocytes. The stability of apoE in the brain requires its association with lipids, as evidenced by the marked reduction in brain apoE levels when the murine *Abca1* gene, whose product lipidates apoE, is deleted^{22,23}.

Human apoE contains 299 amino acid residues and has independently folded N-terminal and C-terminal domains, which are joined together by a flexible hinge region^{24,25} (FIG. 1). The apoE region (residues 136-150) that interacts with apoE receptors is in the N-terminal domain, whereas the major lipid-binding region (residues 244-272) is within the C-terminal domain. The three isoforms, apoE2, apoE3 and apoE4, differ at positions 112 and 158^{8,24,26}. These single amino acid differences among the three apoE isoforms alter the protein structure and influence lipid association and receptor binding²⁴. For example, apoE3 and

apoE4 bind to LDLR with affinities that are ~50-fold greater than the affinity of apoE2. As a result, apoE2 less efficiently transports lipids, and its presence is associated with type III hyperlipoproteinemia. ApoE4 preferentially binds to large lipoprotein particles and is also associated with a modestly increased risk of cardiovascular disease. The preference of apoE4 to larger lipoprotein particles is attributable to the presence of Arg112, which affects the conformation of the side chain of Arg61 resulting in 'domain interaction' between this Arg61 in the N-terminal domain and Glu255 in the C-terminal domain.

In all animal species except human, apoE does not have genetic variants and contains threonine at the position equivalent to Arg61 in human apoE4. Therefore, with respect to domain interaction, apoE in other species behaves functionally like apoE3, regardless of whether it has arginine at position 112²⁴. By introducing an Arg61 residue in murine apoE, murine apoE was converted to a human apoE4-like structure with domain interaction²⁷. This conformation of human apoE4 and mouse Arg61 apoE lowers apoE levels in mouse brains, suggesting that domain interaction directly impacts apoE stability. ApoE isoforms also differ in their conformation and stability, with apoE4 assuming an unstable '**molten globule**' state²⁴. The unique properties of the domain interaction and the molten globule state underlie the pathogenic role of apoE4 in AD^{24,25}.

ApoE receptors, members of the LDLR family

ApoE binds primarily to a group of receptors known as the LDLR family^{16,17}. The prototype of this family is LDLR, which is the main receptor for cholesterol homeostasis^{28,29}. Loss-of-function mutations in the *LDLR* gene cause high plasma LDL levels, which lead to familial hypercholesterolemia and atherosclerosis. LDLR binds both apoB-containing LDL and apoE-containing particles of different densities via electrostatic interactions between the basic amino acid residues on apoB/apoE and the acidic residues on the receptor. In 1988, a 4,544-amino acid protein containing the signature complement-type repeats and epidermal growth factor (EGF) precursor homology domains in LDLR was cloned³⁰ (FIG. 2). This protein, named *LDLR-related protein* (LRP, now commonly known as LRP1 to distinguish it from other LRPs), is highly expressed in the liver and brain, and binds >30 ligands, including apoE, α_2 -macroglobulin (α_2 M), tissue-type plasminogen activator (tPA) and APP. LRP1 is synthesized as a single glycosylated protein (~600 kDa) and then cleaved by furin in the *trans*-Golgi network (TGN) to generate a 515-kDa extracellular subunit and an 85-kDa transmembrane subunit, which remain non-covalently associated with one another³¹. LRP1 is subjected to additional proteolysis that resembles APP/Notch processing (see BOX 1). Like LDLR, LRP1 also undergoes constitutive receptor-mediated endocytosis to transport ligands from the cell surface to intracellular compartments; however, LRP1 has a faster endocytosis rate than LDLR³². LRP1 is essential for early embryonic development as conventional deletion of the *Lrp1* gene in mice results in lethality³³. Brain-specific knockout of the *Lrp1* gene demonstrates that LRP1 plays important roles in synaptic transmission and motor function in the CNS³⁴. The 100-amino acid cytoplasmic tail of LRP1 binds to a variety of adaptor proteins that function in receptor trafficking and signalling¹⁶.

Over ten receptors have now been identified as members of the LDLR family; they all contain characteristic ligand-binding repeats, EGF-like repeats and β -propeller-like structures with YWTD motifs (FIG. 2). These receptors bind a diverse array of ligands, including apoE, to mediate their transport and/or signalling (Supplementary Table). In addition to LRP1, this family also includes two additional ~600-kDa large receptors, megalin/LRP2 and LRP1B. Megalin is expressed abundantly on the apical surface of epithelial cells of proximal tubules in the kidney and functions in ligand resorption³⁵. LRP1B is a putative tumour suppressor that is highly homologous to LRP1 (59% identity at the amino acid level) and binds overlapping ligands with LRP1³⁶. However, because of its

slow rate of endocytosis³⁶, LRP1B antagonizes LRP1 function in ligand uptake^{37,38}. The very low-density lipoprotein receptor (VLDLR) and apoE receptor 2 (apoER2)/LRP8 are structurally similar to LDLR, but instead of functioning as typical endocytic receptors, they primarily serve as reelin signalling receptors³⁹. Multiple EGF-like domain 7 (MEGF7)/LRP4 structurally resembles a portion of LRP1 and functions in limb patterning⁴⁰ and in neuromuscular synapse formation by serving as a receptor for Agrin⁴¹. Sortilin-related receptor with A-type repeats (sorLA)/LR11/SORL1, LRP5 and LRP6 are structurally more diverse than the core members of the LDLR family described above. Whereas sorLA functions primarily in intracellular protein trafficking, LRP5 and LRP6 are co-receptors for canonical Wnt/ β -catenin signalling⁴². Several of these LDLR family members play important roles in neuronal development and synaptic plasticity^{17,43}. All members of the LDLR family bind apoE and receptor-associated protein (RAP), a molecular chaperone that promotes receptor folding and maturation in the secretory pathway⁴⁴. RAP also universally antagonizes ligand-binding to LDLR family members^{44,45}.

LDLR and LRP1 are the main apoE/lipoprotein metabolic receptors in the brain

Deletion of the *Ldlr* gene in mice increases apoE levels in brain parenchyma and CSF⁴⁶, suggesting impaired metabolism of apoE. Similarly, conditional deletion of the *Lrp1* gene in mouse forebrain neurons increases apoE levels⁴⁷ and overexpression of a functional LRP1 minireceptor in mouse brain decreases brain apoE levels⁴⁸. Although both LDLR and LRP1 play roles in brain apoE/lipoprotein metabolism, there are important differences between them. First, whereas LRP1 is highly expressed in neurons and to a lesser degree in glia, LDLR is more prominently expressed in glia than neurons^{49,50}. Second, deletion of the *Lrp1* gene in mouse forebrain neurons reduces brain cholesterol levels⁴⁷, whereas cholesterol levels in *Ldlr* knockout mice are unchanged⁴⁶. Third, apoE/lipoprotein particles secreted by astrocytes have higher affinity for LDLR than LRP1⁴⁶, whereas recombinant apoE⁵¹, apoE-enriched lipoprotein particles⁵² and CSF-isolated HDL particles⁵³ bind more avidly to LRP1. The receptor-binding specificity of apoE is probably influenced by its conformation and lipidation state. For example, native apoE-containing β -VLDL is a ligand for LDLR but not LRP1. However, apoE enrichment, with either recombinant apoE or apoE extracted from native β -VLDL, converts these particles into high-affinity ligands for LRP1⁵². Therefore, the inability of apoE on native β -VLDL to bind LRP1 is not due to any permanent modification of apoE but rather to lack of an apoE conformation that is generated at higher concentrations of apoE. It is possible that apoE/lipoprotein particles secreted by astrocytes recruit additional apoE molecules, perhaps bound to heparan sulfate proteoglycan (HSPG), before being transported to the CSF or binding to LRP1 at the neuronal cell surface.

ApoE and apoE receptors in neuronal signalling

In addition to transporting ligands to the cells, apoE receptors also mediate cellular signalling by binding to a variety of extracellular ligands and intracellular adaptor proteins¹⁷. The best-characterized signalling pathway is triggered by reelin and mediated by apoER2 and VLDLR¹⁷. Reelin signalling is crucial for neuronal migration³⁹, dendritic spine development⁵⁴ and synaptic plasticity^{55,56}. Although biochemical evidence suggests that apoE interferes with reelin binding to apoE receptors⁵⁷, the *in vivo* significance of a relationship between reelin and apoE is not clear. In primary neurons, apoE isoforms differentially affect several signalling cascades through apoE receptors, including increased phosphorylation of disabled 1 (Dab1), activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway and inhibition of the c-Jun N-terminal kinase 1/2 (JNK1/2) pathway⁵⁸. ApoE4, but not apoE3, significantly increases resting calcium, calcium response to N-methyl-D-aspartate (NMDA) and neurotoxicity in a manner that depends on the function of LRP1⁵⁹. ApoE3/lipoprotein affords greater protection from apoptosis than apoE4/lipoprotein via LRP1-mediated signalling that involves activation of protein kinase

C δ (PKC δ) and inactivation of glycogen synthase kinase-3 β (GSK3 β)⁶⁰. The isoform-specific signalling function of apoE and the potential *in vivo* relevance to AD pathogenesis require further investigation.

ApoE and apoE receptors in APP trafficking and processing to A β

A β accumulation, oligomerization and deposition in brain are central events in the pathogenesis of AD. The A β level in the brain is the net balance of A β production and clearance. Accordingly, A β accumulation in AD brains could reflect overproduction, inefficient clearance or both. Mounting evidence demonstrates that apoE and apoE receptors play important roles in both processes.

Several apoE receptors interact with APP and modulate its trafficking and processing to A β . Kounnas *et al.*⁶¹ first demonstrated that LRP1 binds soluble APP that contains the Kunitz-type protease inhibitor (KPI) domains (APP751 and APP770) extracellularly, and mediates its cellular catabolism. Further studies demonstrated that LRP1 also interacts with the neuronal isoform of APP lacking the KPI domain (APP695). Two different phosphotyrosine binding (PTB) domains within the adaptor protein FE65 bind to the NPxY motifs within APP and LRP1, thus bridging an interaction between these two membrane proteins intracellularly^{62,63}. The extracellular and intracellular interactions between APP and LRP1 were confirmed in living cells by fluorescence resonance energy transfer (FRET) measurements⁶⁴. The consequence of APP and LRP1 interaction is accelerated APP endocytic trafficking and processing to A β ⁶⁵, due to the fast rate of LRP1 endocytosis compared with that of APP⁶⁶ (FIG. 3). Because the β -secretase BACE1 is abundantly present and active in acidic endosomes⁶⁷, increased distribution of APP in endosomes leads to increased amyloidogenic processing and A β production. The role of LRP1 in facilitating APP processing to A β is also supported by evidence that overexpression of a functional LRP1 minireceptor in neurons in the **PDAPP amyloid mouse model** causes an age-dependent increase in soluble brain A β ⁶⁸. Because LRP1 also has a role in A β clearance (see below), no significant changes in either total A β or plaque burden were detected. ApoE isoforms also differentially regulate APP processing to A β by a mechanism that depends on LRP1 function. In a neuronal cell line overexpressing APP, apoE4 has been shown to increase A β production to a greater extent than apoE3⁶⁹. This difference between the effects of apoE4 and apoE3 was abolished by incubating cells with the receptor antagonist RAP or by reducing LRP1 expression with small interfering RNA.

Three other apoE receptors also interact with APP and regulate its trafficking and processing to A β (FIG. 3). LRP1B, due to its slow rate of endocytosis, contributes to retaining APP at the cell surface and reduces its processing to A β ³⁸. ApoER2 either decreases or increases A β production depending on the experimental conditions. In the presence of F-spondin, which bridges the extracellular interaction between APP and apoER2, the slow endocytosis rate of apoER2 inhibits APP endocytic trafficking and reduces A β production⁷⁰. However, in the absence of common ligands, apoER2 increases the distribution of APP into lipid rafts and APP processing to A β . A third apoE receptor that modulates APP trafficking and processing to A β is sorLA. Expression of sorLA is significantly reduced in AD brains⁷¹ and overexpression of sorLA in neurons shifts APP distribution to the Golgi compartment and decreases its processing to A β ⁷². Importantly, a deletion of the *Sorla* gene in mice increases concentration of A β in the brain⁷². More direct evidence linking sorLA to AD comes from a recent study demonstrating that inherited variants in the *SORL1* gene are associated with LOAD⁷³. Whether apoE isoforms play roles in sorLA regulation of APP trafficking and processing is not known.

ApoE and apoE receptors in A β clearance and aggregation

It is widely believed that impaired A β clearance is a major pathogenic event for LOAD. A β has a relatively short half-life in the brain. Using *in vivo* microdialysis and γ -secretase inhibitor, it has been shown that A β has a half-life of ~2 h and ~4 h in young and aged mice, respectively⁷⁴. In human brains, A β clearance rate is 8.3% per hour⁷⁵ indicating that A β is actively and efficiently cleared from the brain.

There are two major pathways by which A β is cleared from the brain: receptor-mediated clearance by cells in brain parenchyma (microglia, astrocytes, neurons), along the interstitial fluid (ISF) drainage pathway or through the blood-brain barrier (BBB); and through **endopeptidase**-mediated proteolytic degradation (FIG. 4). Receptor-mediated clearance of A β in the brain is likely to be mediated by the apoE receptors LRP1, LDLR and VLDLR, which are widely expressed in neurons, astrocytes and microglia of brain parenchyma, as well as in endothelial cells, astrocytes and smooth muscle cells at the BBB and cerebral arteries. ApoE as well as LRP1 and several other LRP1 ligands (e.g., α_2 M and lactoferrin) are present in amyloid plaques⁴⁹. These receptors can bind A β directly⁷⁶ or indirectly via A β chaperones. ApoE is the best-characterized A β chaperone. ApoE immunoreactivity is found in amyloid plaques^{6,49}, suggesting that apoE interacts with A β directly in AD brains. The region of apoE that is responsible for A β binding is in the C-terminal domain, overlapping with the lipid-binding region^{77,78}; this suggests that the lipophilic A β peptide associates with apoE in a process that is analogous to lipid binding. Indeed, A β binding to apoE compromises its lipid-binding function⁷⁸. Furthermore, A β peptides modulate the binding of apoE isoforms differently to apoE receptors^{79,80}. These results demonstrate that A β peptides can interfere with the normal function of apoE in brain lipid metabolism and thus contribute to AD pathogenesis. ApoE3/lipoprotein binds to A β with higher affinity than apoE4/lipoprotein⁸¹. Accordingly, apoE3 clears through cell surface apoE receptors more efficiently than apoE4. Indeed, several studies using different amyloid mouse models expressing either human apoE3 or apoE4 demonstrate that apoE3 mice develop fewer amyloid plaques than apoE4 mice⁸²⁻⁸⁴. Postmortem studies have demonstrated increased amyloid plaque load in the brains of ϵ 4 allele carriers of both sporadic⁸⁵ and genetic AD cases⁸⁶, and this notion is now confirmed by PET imaging studies from 'cognitively normal' controls⁸⁷. Interestingly, a recent study shows that A β binding to apoE4 redirects its clearance from LRP1 to VLDLR, which internalizes A β -apoE4 complexes at the BBB more slowly than LRP1⁸⁸. In contrast, A β -apoE2 and A β -apoE3 complexes are cleared at the BBB via both VLDLR and LRP1 at a substantially faster rate than A β -apoE4 complexes. The important function of apoE receptors in brain A β clearance was demonstrated in the **PDGF-APP^{Sw,Ind} amyloid mouse model** with decreased apoE receptor expression due to a deletion of their chaperone RAP⁸⁹. Recombinant RAP and LRP1 antibody also reduce A β efflux from mouse brain⁹⁰.

The receptor-mediated clearance is, in principle, an efficient way of reducing brain A β because most A β that is internalized by apoE receptors is delivered to lysosomes for degradation or transcytosed into the plasma (FIG. 4). However, it is possible that receptor-mediated clearance of A β into neurons can lead to intraneuronal A β , which is toxic⁹¹. A portion of A β that is internalized by neurons, particularly oligomeric A β 42, accumulates in multivesicular bodies (MVBs)/late endosomes and lysosomes, and contributes to lysosomal dysfunction and neuronal toxicity. By contrast, receptor-mediated internalization of A β by astrocytes⁹² and microglia⁹³ is likely to represent a more functional pathway to clear and eventually degrade A β .

ApoE4 is also associated with increased risk for CAA, which is characterized by A β deposits along the blood vessel walls in the CNS and is associated with cerebral hemorrhage⁹⁴. Interestingly, the A β 40/42 ratio in cerebral vascular A β deposits exceeds that

of senile plaques⁹⁵. Therefore, an increased A β 40/42 ratio can shift A β deposition from brain parenchyma to the vascular wall, perhaps by increasing the solubility of A β to diffuse to the vasculature. ApoE4 alters the A β 40/42 ratio and promotes the formation of CAA in the **Tg2576 amyloid mouse model**⁹⁶. CAA is associated with eventual degeneration of smooth muscle cells, which express high levels of LRP1. It is possible that smooth muscle cells contribute significantly to receptor-mediated A β clearance in normal brains. However, when A β accumulates under pathological conditions such as AD, it can exceed the capacity of this clearance pathway, resulting in local A β aggregation and toxicity to smooth muscle cells. Further studies are needed to test this hypothesis.

A β can also be removed from the brain by proteolytic degradation. Several A β degrading enzymes play important roles in A β clearance^{97,98}. Most of these enzymes are produced by neurons or glia, but some are also expressed in the cerebral vasculature. Neprilysin and insulin-degrading enzyme (IDE) are the two most extensively studied A β proteases that are expressed both in neurons and vascular cells. Reduced expression of these two enzymes in AD has been associated with the ϵ 4 allele. Other A β -degrading enzymes include plasmin, endothelin-converting enzymes (ECE-1 and ECE-2), matrix metalloproteinases (MMP)2, MMP3 and MMP9, and angiotensin-converting enzyme (ACE). The specific roles for each of these enzymes in AD pathogenesis are still unclear. Interestingly, apoE promotes A β degradation by neprilysin and IDE, with apoE3 functioning more efficiently than apoE4⁹⁹. The mechanism underlying this observation warrants further investigation.

ApoE in A β -induced neurotoxicity

A β assemblies, particularly A β oligomers, are highly toxic to neurons. Synthetic A β 42 oligomers inhibit neuronal viability of cultured neurons 10-fold more than A β 42 fibrils and 40-fold more than unaggregated peptides¹⁰⁰. ApoE4 augments A β 42 oligomer-induced neurotoxicity to a larger extent than apoE3¹⁰¹. A β dimers from human AD brains¹⁰² and A β dodecamer, also known as A β *56, from Tg2576 mouse brains¹⁰³ inhibit long-term potentiation (LTP) and memory in animal models, but it is not known if these toxicities are further regulated by apoE. Recently, apoE4 and A β aggregates, induced by inhibiting A β -degrading neprilysin, were found to act synergistically to induce neurodegeneration in mouse brain¹⁰⁴. Because neurodegeneration was not reported in several amyloid mouse models expressing human apoE4, it is not clear why A β accumulation induced by inhibiting neprilysin caused neurodegeneration, whereas A β accumulation induced by over-production from mutant APP did not. Studies of the potential roles of soluble APPs and APP intracellular domain (AICD)¹⁰⁵, which are more abundant in APP-overexpressing mice, might explain this difference.

ApoE fragmentation, tau phosphorylation and mitochondrial dysfunction

Hyperphosphorylated microtubule-associated protein tau is toxic to neurons and is the major component of neurofibrillary tangles. Tau is essential for A β - and excitotoxin-induced neuronal dysfunction; reducing endogenous tau blocks A β -induced cognitive impairments in the PDGF-APP_{Sw,Ind} amyloid model mice¹⁰⁶. Transgenic over-expression of apoE4 in neurons, but not in astrocytes, increases tau phosphorylation in mice^{107,108}, suggesting a neuron-specific effect of apoE4 on tau phosphorylation. The pathophysiological significance of this pathway is not clear, as apoE is generally produced by astrocytes and microglia, not neurons. However, apoE expression in neurons has been reported after injury¹⁰⁹. Although apoE is not expressed in hippocampal neurons under normal conditions, neuronal injury induced by kainic acid upregulates apoE expression in neurons¹¹⁰. Therefore, it is possible that in stressed AD brains, abnormal apoE expression in neurons facilitates tau hyperphosphorylation.

Another question that remains to be solved is how apoE and tau, which are normally separated by the plasma or organelle membrane, come in physical contact. One hypothesis is that the C-terminal-truncated fragments of apoE, detected in both human AD brains and in a neuronal apoE4 transgenic mouse model, enter the cytosol and interact directly with tau¹⁰⁸. Alternatively, apoE isoforms might differentially regulate apoE receptor-mediated signalling cascades that in turn modify the function of tau kinases and phosphatases.

The receptor- and lipid-binding regions of apoE4 fragments act together to cause mitochondrial dysfunction and neurotoxicity¹¹¹. Using a cultured neuronal cell line, it was shown that the receptor-binding region of apoE4 C-terminal truncated fragments is required for its escape from the secretory pathway and the lipid-binding region mediates interaction with mitochondria leading to mitochondrial dysfunction. The *in vivo* relevance of this apoE4-mediated toxic pathway requires further investigation¹¹².

ApoE and apoE receptors in brain cholesterol and lipid transport

The primary function of apoE in the brain is to transport cholesterol, mainly from astrocytes to neurons. Cholesterol is an essential component of membranes and myelin sheaths and is crucial for synaptic integrity and neuronal function¹¹³. The reduced synthesis and greater need for cholesterol by neurons in adult brains require active cholesterol transport to support synaptic functions and repair. Cholesterol associated with apoE/lipoprotein particles secreted by astrocytes is essential for the formation of mature synapses *in vitro* through a mechanism that requires functional apoE receptors¹¹⁴ (FIG. 5). Interestingly, an association between cholesterol metabolism and the risk of AD has been proposed¹¹⁵. In humans, early studies indicated that the use of statins, which inhibit cholesterol synthesis, is associated with a significant decrease in AD prevalence; however, several recent retrospective studies do not support such a conclusion¹¹⁵. Because statins also exert other pleiotrophic effects including regulation of gene expression and **protein prenylation and isoprenylation**, the specific effects of statins on AD pathology in different model systems are likely to be influenced by various biological and pathological processes such as the efficiency of blood flow to the brain and the presence of other disease states including hypertension, diabetes and hypercholesterolaemia. At the cellular level, the effect of cholesterol on the amyloidogenic processing of APP to A β remains controversial^{116,117}. Cholesterol and statins clearly modulate APP processing *in vitro* and *in vivo*^{118,119}. Much of the effects of cholesterol on APP processing relates to the important role of cholesterol in lipid rafts, where APP processing by β - and γ -secretase cleavage is favoured^{119,120}.

A reduction of brain cholesterol levels is observed in AD brains^{121,122}. Interestingly, apoE4 knock-in mice have decreased brain cholesterol concentrations even though the peripheral cholesterol levels are increased¹²³. This finding confirms that brain apoE/lipoprotein metabolism is distinct from that in plasma. Although the exact functional differences among the three apoE isoforms in brain cholesterol metabolism are still not clear, there is some evidence that apoE4 might be less efficient than apoE3 in transporting brain cholesterol⁵⁰. ApoE4 is also less efficient than apoE3 in promoting cholesterol efflux from both neurons and astrocytes¹²⁴. Using cultured astrocytes from apoE-TR mice, it was shown that apoE3 has the ability to generate similarly sized lipid particles with less number of apoE molecules than apoE4, suggesting that apoE3-expressing astrocytes can supply more cholesterol to neurons than apoE4-expressing astrocytes¹²⁵. Despite these interesting observations, the roles of apoE isoforms in brain cholesterol metabolism require further investigation. The structural differences among apoE isoforms that determine their lipid and receptor binding specificities in the brain environment may account for their differences in modulating brain cholesterol metabolism. It is important to note that there are likely to be LRP1-mediated cholesterol transport mechanisms in the brain that are independent of apoE because LRP1-deficiency in the brain, but not apoE-deficiency, leads to decreased brain cholesterol levels.

Other LRP1 ligands such as lipoprotein lipase might also play a role. Alternatively, LRP1 may serve as a cholesterol sensor that influences cholesterol synthesis and/or intracellular transport.

In addition to cholesterol, apoE also mediates the transport of other brain lipids, some of which are not produced in astrocytes. For example, sulfatide, an oligodendrocyte-synthesized lipid that is crucial for neuronal spine and myelin sheath integrity, is actively transported by an apoE- and LRP1-dependent mechanism¹²⁶. It is possible that apoE/lipoprotein particles are modified by myelin-associated lipids prior to being transported into neurons. Interestingly, sulfatide is a potential biomarker for AD diagnosis as its levels are decreased in AD brains¹²⁶.

ApoE and apoE receptors in synaptic plasticity, repair and dendritic spine integrity

Synaptic failure is an early pathological feature of AD¹²⁷. ApoE/apoE receptor-mediated lipid redistribution and signalling play important roles in synaptic integrity and plasticity. Increasing evidence demonstrates that apoE isoforms differentially regulate synaptic plasticity and repair. Human apoE4-targeted replacement (**apoE4-TR**) mice display synaptic deficits in the absence of neuropathology¹²⁸. LTP in the hippocampus of apoE4-TR mice is also significantly impaired compared with apoE3-TR mice and WT mice¹²⁹.

ApoE expression is up-regulated in rat brains after ischemia¹³⁰ and in injured neurons upon kainic acid treatment¹¹⁰. The primary function of injury-induced apoE is probably to redistribute lipids and strengthen apoE-mediated signalling for neuronal and synaptic repairs. Using β -VLDL enriched with apoE isoforms or apoE-containing HDL isolated from CSF, it was shown that apoE3 increases neurite outgrowth, whereas apoE4 either decreases outgrowth or has no effect^{53,131,132}. Similarly, apoE3-containing lipoprotein particles are more effective than apoE4 particles in protecting CNS neurons from apoptosis⁶⁰. Collectively, these studies indicate that apoE4 is less effective than apoE3 in maintaining and repairing synapses and neurons, which may explain why under certain neuronal injury conditions, such as severe head trauma and stroke, individuals with apoE4 tend to have poorer outcome. It is possible that LOAD is a result of cumulative injury from A β and other neuronal stresses, such as oxidation and inflammation, and that apoE4 is less effective in maintaining and repairing injured synapses and neurons.

ApoE isoforms also differentially regulate dendritic spines during aging. ApoE3 and WT mice have a higher density of dendritic spines than apoE4 and apoE-KO mice at 1-2 years of age but not at 3 weeks of age¹³³. This age-dependent difference suggests that the effects of apoE isoforms on neuronal integrity might relate to the increased risk of dementia in aged individuals with apoE4. In human brains, apoE4 dose correlates inversely with dendritic spine density in both AD and aged normal controls¹³³. Interestingly, apoE2 overexpression ameliorates dendritic spine loss in Tg2576 amyloid model mice, suggesting that apoE isoforms functionally interact with A β to regulate neuronal integrity¹³⁴. Moreover, a recent *in vitro* study showed that rosiglitazone, a peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonist, increases dendritic spine density and rescues spine loss caused by apoE4 in primary neurons¹³⁵. Whether the different roles of apoE isoforms in dendritic spine density relate to apoE/apoE receptor-mediated lipid metabolism, signalling and/or synaptic plasticity and repair is not currently clear.

ApoE and apoE receptors as therapeutic targets for AD

Most AD therapeutic approaches have been designed to reduce A β production or aggregation, or promote its clearance. Because apoE and apoE receptors play critical roles in both A β -dependent and A β -independent AD pathogenic pathways, it is logical to also

consider strategies to regulate their expression and functions (TABLE 1). As apoE4 is a strong risk factor for AD compared with apoE3, one attractive approach is to convert an apoE4 to an apoE3-like molecule. The unique domain interaction in apoE4 but not apoE3 seems to be responsible for most of apoE4-associated neuropathology and provides a target for agents to disrupt this interaction. Indeed, several molecules that can disrupt apoE4 domain interaction have been identified. Among these, GIND-25, a disulfonate, and GIND-105, a monosulfoalkyl, decrease A β production induced by apoE4 to a similar level to that induced by apoE3¹¹². Whether or not these compounds can diminish apoE4 toxic functions in animals or humans *in vivo* is currently unknown.

Another potential strategy is to regulate apoE expression levels in the brain. However, this strategy obligates careful consideration as to whether apoE4 effects in AD brains are caused by loss-of-protection, gain-of-toxicity or both. It has been shown that the promoter polymorphisms of the *APOE* gene may lead to changes in apoE expression levels by altering transcription of the *APOE* gene¹³⁶. Therefore, regulating *APOE* promoter function might be one strategy to alter apoE expression. Compounds that alter brain apoE expression might also be identified through screening. Using an apoE4-specific immunoassay, a recent study demonstrated that apoE4 has a faster turnover and lower steady-state concentration in apoE4-TR mice than in apoE3-TR mice¹³⁷, suggesting that low levels of total apoE4 might directly contribute to AD pathogenesis, perhaps by reducing apoE function in lipid metabolism, synaptic repair and A β clearance. Therefore, increasing the expression of apoE in all apoE genotypes might prevent or slow the progression of AD. However, increasing the expression of apoE, particularly apoE4, may also generate deleterious effects such as slowing A β clearance through the BBB⁸⁸ and augmenting A β neurotoxicity¹⁰¹. The specific effects of altering apoE expression on AD pathogenesis will likely depend on apoE isoforms, stage of the disease and the presence of other pathophysiological conditions.

Liver X receptors (LXRs) are oxysterol receptors that serve as transcription factors to regulate cholesterol homeostasis. In brain, LXRs up-regulate apoE and ABCA1, thereby promoting cholesterol efflux in neurons and glia¹³⁸. In Tg2576 amyloid mouse model, LXR agonists facilitate the clearance of A β 42 and reverse the contextual memory deficit in these mice¹³⁹. Thus, LXR agonists may represent an important therapeutic approach to AD. Another related approach is to identify apoE peptides that perform apoE-related functions and cross the BBB¹⁴⁰. In this regard, apoE-mimetic peptides suppress glial inflammation and protect neurons from injury^{141,142}. Because these apoE peptides do not contain lipid-binding domains, their exact mechanism of function is currently unknown. Nonetheless, their promising effects make them strong candidates for AD therapy.

Because apoE is essential for amyloid deposition in mice¹⁴³, probably by promoting A β fibrillization, disrupting apoE and A β interaction could be another therapeutic approach. An A β 12-28P synthetic peptide, which is homologous to the apoE binding site on the full-length A β BBB-permeable and nontoxic, reduced the total brain A β levels, A β plaques, CAA burden and memory performance in two amyloid mouse models¹⁴⁴. This suggests that inhibiting apoE and A β interaction *in vivo* might decrease total brain A β .

ApoE receptors can also be potential targets for AD therapy. LRP1 and LDLR play critical roles in brain apoE/lipoprotein metabolism and in A β clearance. Because LRP1 level is decreased in AD brains¹⁴⁵, it will be interesting to test whether restoring LRP1 expression in AD brains augments A β clearance and reduces AD pathology. ApoER2 and VLDLR are both implicated in reelin/apoE signalling, which is important for synaptic repair and plasticity. Therefore, these apoE signalling receptors might be novel targets for compounds that up-regulate their expression and/or function. One caveat, however, is that up-regulating apoE receptor expression/function might increase the risk of other diseases such as cancer.

Other apoE/apoE receptor-related strategies include reducing apoE fragmentation, which might block interactions between apoE fragments and tangles or mitochondria, preserving brain apoE levels by facilitating apoE recycling efficiency, and blocking the interaction between APP and certain apoE receptors (such as LRP1) that promotes APP processing. Strategic combinations of modulating A β and apoE pathways are highly promising avenues of investigations for combating AD.

Perspectives

ApoE4 is a strong risk factor for LOAD. An emerging body of data has identified multiple pathways that could explain the pathogenic nature of apoE4. These include A β production, A β clearance, A β fibrillization, tangle formation, cholesterol homeostasis, synaptic plasticity and repair, and neuronal toxicity (FIG. 6). These multiple pathways regulated by apoE isoforms and apoE receptors present opportunities to identify the pathways that are most relevant to AD pathogenesis and novel targets with tractable efficacy for AD therapy. Greater understanding of the differential expression and function of apoE isoforms has been facilitated by novel animal models including human apoE isoform-TR mice and humanized mouse apoE expressed in the apoE-KO background. Inducible expression of apoE isoforms in aged animals should further clarify the roles of apoE isoforms in aging brains. Furthermore, the relationship between brain apoE-mediated cholesterol/lipid metabolism and apoE-mediated A β /tau pathology should be studied *in vivo*. Finally, the functional relationship between apoE isoforms and multiple apoE receptors requires more in-depth investigation. Because apoE4 affects a much greater AD population than early-onset AD, therapies based on apoE/apoE receptors as targets might benefit more AD patients and offer hope to those reaching old age.

Box 1. LRP1 undergoes APP/Notch-like proteolytic processing and shedding

In addition to furin cleavage in the *trans*-Golgi network (TGN) during its biogenesis, mature LRP1 is also subjected to several sequential proteolytic events that resemble APP/Notch processing (see FIG). First, LRP1 undergoes ectodomain shedding, which is mediated by metalloproteases (MMPs), from a region close to the membrane, releasing a soluble form of LRP1 (sLRP1) that is capable of binding ligands (ligand-binding domains shown in pink)^{146,147}. LRP1 shedding occurs under physiological conditions, as evidenced by abundant sLRP1 in human plasma^{146,148}. Second, LRP1 associates with BACE1 in lipid rafts and is a substrate for BACE1¹⁴⁹. Third, LRP1 can be cleaved at an intramembrane site by the γ -secretase¹⁵⁰, releasing a LRP1 intracellular domain (LICD), which is capable of regulating gene transcription^{151,152}. The major enzyme(s) responsible for LRP1 shedding and the extent, if any, of altered LRP1 processing in AD brains remain unknown. Furthermore, how these processing events and their cleavage products affect LRP1 cellular function is not clear. Other LDLR family members, such as apoER2¹⁵³, also undergo similar proteolytic processing, but their mechanisms and significance are also poorly understood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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GLOSSARY

Polymorphic alleles	Natural variants in a gene that occur with fairly high frequency (>1%) in the general population.
Cerebral amyloid angiopathy (CAA)	A neurological condition in which amyloid protein is deposited onto the walls of the arteries of the brain. CAA increases the risk of bleeding into the brain causing haemorrhagic stroke.
Molten globule	A stable, partially folded protein state found in mildly denaturing conditions such as low pH, mild denaturant or high temperature. It is also used to refer to certain protein folding intermediates.
PDAPP amyloid mouse model	Mouse model of AD in which a human APP transgene bearing the amyloidogenic V717F mutation is overexpressed under the control of the human platelet derived growth factor β (PDGF- β) chain gene promoter.
Endopeptidases	A group of enzymes that catalyze the hydrolysis of peptide bonds in the interior of a polypeptide chain or protein molecule.

PDGF-APP _{Sw,Ind} amyloid mouse model	Mouse model of AD in which a human APP transgene bearing the amyloidogenic V717F and K670N/M671L mutations is overexpressed under the control of the human platelet derived growth factor β (PDGF- β) chain gene promoter.
Tg2576 amyloid mouse model	Mouse model of AD in which a human APP transgene bearing the amyloidogenic K670N/M671L mutations is overexpressed under the control of the hamster prion protein gene promoter.
Protein prenylation and isoprenylation	Covalent attachment of hydrophobic prenyl or isoprenyl groups to a protein. Play roles in protein attachment to cell membranes and protein-protein interactions.
ApoE4-TR mice	Mouse model of human apoE4 in which human <i>APOE-ϵ4</i> gene is inserted into the mouse <i>ApoE</i> gene locus. Expression of human apoE4 in these mice temporally and spatially resembles that of mouse endogenous apoE.

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Figure 1. Schematic representation of human apoE

The 299-amino acid human apoE contains two independently folded domains: an N-terminal domain that includes the receptor-binding region and a C-terminal domain that contains the major lipid-binding region. The residues that distinguish the apoE isoforms (112 and 158) are marked. ApoE2 has cysteines at both positions, apoE4 has arginines at both positions, and apoE3 has Cys at position 112 and Arg at position 158. Domain interaction between Arg61 and Glu255 in apoE4 is also indicated.

Figure 2. ApoE receptors, members of the LDLR family

Structural organization of the LDLR family members. All receptors are type I receptors, each containing a single membrane-spanning domain and a relatively short cytoplasmic tail. The extracellular regions of these receptors contain three characteristic modules: ligand-binding repeats (also called complement-type repeats), EGF repeats and YWTD-containing β -propeller domains. The furin cleavage sites in LRP1 and LRP1B are indicated with *purple arrow heads*. The four clusters of ligand-binding repeats in LRP1 are labelled (I-IV). Highlighted in bright green are the two extra sequences in LRP1B encoded by two extra exons (compared to LRP1): a ligand-binding repeat in the fourth ligand-binding domain and a 33-amino acid insert in the cytoplasmic tail. LRP5/6 and sorLA/LR11 are distant members of the family with atypical structural arrangements. Several other LDLR family members, including LRP3, LRP9 and LRP12, whose functions are poorly defined, are not depicted here.

Figure 3. APP processing pathways regulated by LDLR family members and apoE
 Newly synthesized APP traffics through the secretory pathway to the plasma membrane, where it is primarily cleaved by the α -secretase in the non-amyloidogenic pathway, producing a minimally toxic peptide P3. When APP is internalized through the clathrin-mediated pathway, it is delivered to endosomes, where it is cleaved first by the β -secretase (BACE1), and then by the γ -secretase to generate highly toxic A β . Although most A β peptides are secreted to extracellular space, some A β peptides may aggregate in the late endosomes/lysosomes contributing to intraneuronal A β accumulation. The fast endocytosis of LRP1 enhances APP endocytosis and processing to A β , whereas the slow endocytosis of LRP1B and apoER2 contributes to retaining APP at the cell surface and promotes non-amyloidogenic processing. SorLA/LR11 likely shuttles APP to the Golgi compartments and reduces its processing by β -secretase in the early endosomes, thus decreasing A β production. ApoE4 promotes APP amyloidogenic processing in a manner that depends on LRP1 function. APP's intracellular domain (AICD), a product of both non-amyloidogenic and amyloidogenic processing, interacts with FE65 and Tip60, and regulates gene transcription in the nucleus.

Figure 4. Major Aβ clearance pathways in the brain: role of apoE isoforms

Aβ accumulation in the brain parenchyma leads to formation of Aβ oligomers and amyloid plaques, which are toxic to neurons, whereas its accumulation in the perivascular region leads to the formation of CAA, which disrupts vessel function and is associated with cerebral haemorrhage. Aβ has a relatively short half-life in the brain, ~4 h in older mice and ~6 h in humans. Major Aβ clearance pathways include receptor-mediated clearance by cells in brain parenchyma (by neurons and glia), along the interstitial fluid (ISF) drainage pathway, through the blood-brain barrier (BBB) and proteolytic degradation by endopeptidases. The comparative effects of apoE3 and apoE4 are indicated. In all major cellular Aβ clearance pathways, LRP1 is involved and is likely to clear Aβ either directly or when Aβ binds to its chaperones that are also LRP1 ligands (e.g. apoE, α₂M). LDLR function in Aβ clearance is likely to involve Aβ-apoE complexes. VLDLR also has a role in the clearance of Aβ-apoE complexes at the BBB.

Figure 5. Synapse formation and repair depend on cholesterol transport from astrocytes to neurons via the apoE/apoE receptor pathway

ApoE, secreted by astrocytes, assembles cholesterol and other lipids into lipoprotein particles. The ATP-binding cassette transporter subfamily A member 1 (ABCA1) at the plasma membrane transports and loads lipids to apoE. Liver X receptors (LXRs) increase the expression of both ABCA1 and apoE. ApoE/lipoprotein particles may undergo modifications such as recruiting oligodendrocyte-specific lipids and additional apoE molecules prior to binding to neuronal apoE receptors LRP1/LDLR or being transported to the cerebrospinal fluid (CSF). Cholesterol and other lipids transported to neurons play important roles in synaptic formation and repair (see main text for details). The inset shows the main components of apoE/lipoprotein particles: cholesterol, cholesterol esters and phospholipids.

Figure 6. Roles of apoE isoforms in the healthy brain and AD pathogenesis
 Summary of apoE functions in normal brain function and the pathogenic processes of AD.
 Differential regulations by apoE3 and apoE4 are indicated.

Table 1
Strategies of apoE/apoE receptor-based AD therapy

Strategy	Method	Benefit
Convert apoE4 to apoE3	Disrupt apoE4 'domain interaction' with pharmacological approach	Increase apoE3-related function, decrease apoE4-related function
Increase apoE level	LXR agonists, pharmacological approach	Increase A β clearance, lipid homeostasis, synaptic function
Pseudo apoE	ApoE-mimetic peptides	Decrease neurotoxicity, inflammation
Block apoE4/A β interaction	A β 12-28P, pharmacological approach	Decrease A β aggregation
Block apoE fragmentation	Pharmacological approach	Decrease tau phosphorylation, mitochondria toxicity
Increase apoE lipidation	Increase ABCA1 expression with LXR agonists, other pharmacological approach	Increase apoE half-life, decrease amyloid deposition
Increase LRP1/LDLR level	Pharmacological approach	Increase A β clearance, cholesterol transport, synaptic plasticity
Increase apoER2/VLDLR level	Pharmacological approach	Increase apoE signalling, synaptic plasticity