

# ApoE and A $\beta$ in Alzheimer's Disease: Accidental Encounters or Partners?

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Among the three human apolipoprotein E (apoE) isoforms, apoE4 increases the risk of Alzheimer's disease (AD). While transporting cholesterol is a primary function, apoE also regulates amyloid- $\beta$  (A $\beta$ ) metabolism, aggregation, and deposition. Although earlier work suggests that different affinities of apoE isoforms to A $\beta$  might account for their effects on A $\beta$  clearance, recent studies indicate that apoE also competes with A $\beta$  for cellular uptake through apoE receptors. Thus, several factors probably determine the variable effects apoE has on A $\beta$ . In this Review, we examine biochemical, structural, and functional studies and propose testable models that address the complex mechanisms underlying apoE-A $\beta$  interaction and how apoE4 may increase AD risk and also serve as a target pathway for therapy.

## Introduction

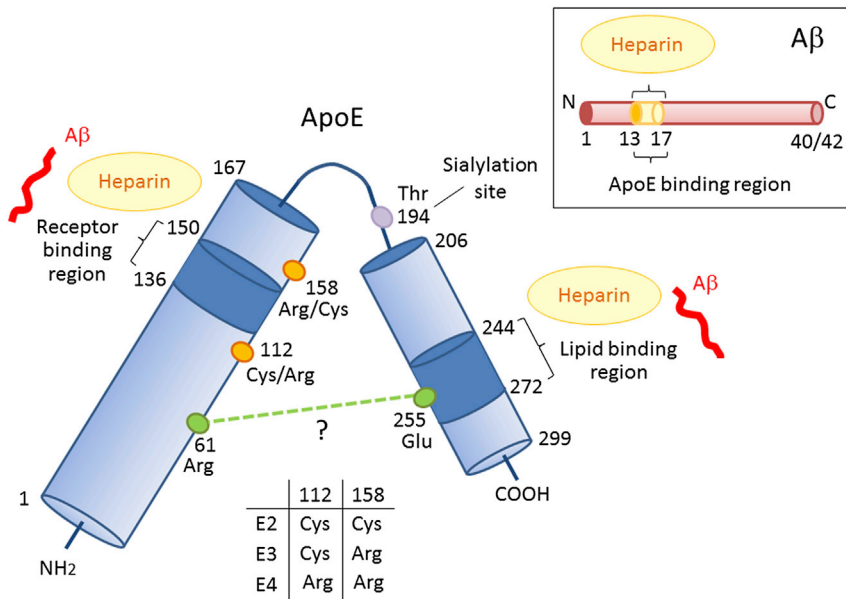
Alzheimer's disease (AD) is the most common form of late-life mental failure in humans, which accounts for an estimated 60%–80% of dementia cases (Thies and Bleiler, 2013). Senile plaques and intracellular neurofibrillary tangles are hallmarks of AD pathology. The generation of amyloid- $\beta$  (A $\beta$ ) peptides of either 40 or 42 amino acids in length, from amyloid precursor protein (APP), and their subsequent accumulation, aggregation, and deposition in brain parenchyma, as senile plaques, and in perivascular regions, as cerebral amyloid angiopathy (CAA), are central and perhaps defining events in the pathogenesis of AD (Hardy and Selkoe, 2002). In addition to A $\beta$  deposits, soluble A $\beta$  oligomers are shown to injure synapses by disrupting normal synaptic functions and triggering downstream toxic pathways leading to eventual neurodegeneration and cognitive deficits (Mucke and Selkoe, 2012). Thus, the “amyloid cascade hypothesis” is strongly supported by evidence of A $\beta$ -related pathology early in the disease process and the specific roles of different forms of A $\beta$  in neurotoxicity.

Less than 1% of AD cases are caused by dominantly inherited genetic mutations in genes including *APP*, *PSEN1*, and *PSEN2* (Thies and Bleiler, 2013). Inheriting any of these genetic mutations accelerates A $\beta$  production, resulting in the development of AD usually before the age of 60, which is commonly referred to as early-onset familial AD (FAD). Late-onset AD (LOAD), or the sporadic occurrence of the disease later in life, represents the majority of AD cases (Hardy and Selkoe, 2002). In LOAD, the disturbance of A $\beta$  clearance machinery appears to be a leading cause of A $\beta$  accumulation in the brain (Mawuenyega et al., 2010). Indisputable evidence showing the  $\epsilon$ 4 allele of the *APOE* gene, which encodes a lipid/cholesterol carrier apolipoprotein E (apoE), is a stronger genetic risk factor for AD than the more common  $\epsilon$ 3 allele, whereas the presence of the  $\epsilon$ 2 allele is protective (Bu, 2009; Liu et al., 2013). The presence of the  $\epsilon$ 4 allele of the *APOE* gene not only dose dependently increases the risk for AD but also lowers the age of onset (Bu, 2009; Corder

et al., 1993; Liu et al., 2013). ApoE4 contributes to the pathogenesis of AD probably by both loss-of-function in neuroprotection and gain-of-function in neurotoxicity compared to apoE3 (Bu, 2009; Huang and Mucke, 2012). Although several pathways underlying the risk associated with apoE4-linked AD have been defined through in vitro and in vivo studies, the exact mechanisms are still not completely understood and those that have been proposed remain controversial. Nonetheless, the differential effects of apoE isoforms on amyloid pathology and A $\beta$  metabolism have been confirmed in humans, animal models, and cellular studies. In this Review, we will discuss how apoE isoforms differentially regulate AD pathogenic pathways with particular focus on A $\beta$ -dependent pathways.

## Back to Basics: Biochemical and Structural Features of apoE

ApoE is a glycoprotein of 299 amino acids with a molecular mass of ~34 kDa. It was originally identified as an apolipoprotein enriched on cholesterol- and triglyceride-rich plasma lipoproteins synthesized by the liver in humans and animals (Mahley, 1988; Mahley and Rall, 2000). ApoE mediates the transport and delivery of cholesterol and other lipids through cell surface apoE receptors (Mahley, 1988; Mahley and Rall, 2000). The mRNA profile has shown that the liver is the major tissue in which apoE is synthesized, accounting for >75% of total apoE, followed by the brain (Elshourbagy et al., 1985). The concentrations of apoE in plasma and cerebrospinal fluid (CSF) are estimated to be ~40–70  $\mu$ g/ml and ~3–5  $\mu$ g/ml, respectively (Mahley et al., 2009; Pitas et al., 1987b). ApoE reporter mice with EGFP insertion into the *ApoE* gene locus exhibit highly expressed apoE in hepatocytes and peritoneal macrophages. In the brain, astrocytes, microglia, vascular smooth muscle cells, and choroid plexus constitutively express apoE, whereas neurons predominantly synthesize apoE under stress conditions (Xu et al., 2006). The multiple isoforms of human apoE were first identified when human apoE polymorphisms were characterized using



**Figure 1. Schematic Illustration of Structural and Functional Regions of apoE and A $\beta$**

Human apoE is a glycosylated protein of 299 amino acids consisting of a receptor-binding region (residues 136–150) in the N-terminal domain (residues 1–167) and a lipid-binding region (residues 244–272) in the C-terminal domain (residues 206–299) (Chen et al., 2011). ApoE also has two heparin-binding sites, each within the N-terminal and the C-terminal domains (Ji et al., 1993; Saito et al., 2003). The residues that distinguish the apoE isoforms are located at residues 112 and 158, where apoE2 has Cys residues at both positions, apoE3 has a Cys residue at 112 and an Arg residue at 158, and apoE4 has Arg residues at both positions (Rall et al., 1982; Weisgraber et al., 1981). The domain interaction between Arg 61 and Glu 255 in apoE4 is also indicated (Mahley and Rall, 2000; Mahley et al., 2009; Wilson et al., 1991), although other structural studies have not confirmed this (Chen et al., 2011). A $\beta$  can interact with both the receptor-binding region and lipid-binding region of apoE, as well as with heparin through its residues 13–17 (Strittmatter et al., 1993; Winkler et al., 1999).

isoelectric focusing with plasma samples from patients with familial lipoprotein disorder type III hyperlipoproteinemia (Utermann et al., 1977). Because of their different charges, human apoE has mainly three patterns of band designated with each isoelectric point as E2 (pH 5.4), E3 (pH 5.55), and E4 (pH 6.1) (Mahley and Rall, 2000; Utermann et al., 1977). The heterogeneity of major isoforms apoE2, apoE3, and apoE4 is due to genetic polymorphisms (Zannis and Breslow, 1981). Complete amino acid sequencing revealed that apoE2 has Cys residues at positions 112 and 158, apoE3 has a Cys residue at 112 and an Arg residue at 158, and apoE4 has Arg residues at both positions (Rall et al., 1982; Weisgraber et al., 1981) (Figure 1). These differences at amino acid residues 112 and 158 among apoE isoforms probably induce significant, perhaps profound, changes in their structures and associated biological functions. The minor isoforms result from variable posttranslational O-linked glycosylation/sialylation of major apoE isoforms at Thr 194, resulting in further negative charges (Figure 1) (Wernette-Hammond et al., 1989; Zannis and Breslow, 1981). Posttranslational modification and subsequent deglycosylation of apoE probably lead to its heterogeneous sialylation. Although newly secreted apoE is highly sialylated (Wernette-Hammond et al., 1989), 80%–85% of human plasma apoE is in the asialo form, with monosialo and disialo isoforms as the minor species (Zannis and Breslow, 1981). Interestingly, apoE in the brain has higher sialylation than that in plasma (Pitas et al., 1987a). Further studies are needed to understand how glycosylation of apoE affects its metabolism and functions in an isoform-dependent and/or tissue-dependent manner.

The nuclear magnetic resonance (NMR) of a monomeric mutant form of apoE3, which has mutations in the C-terminal domain to prevent aggregation, revealed for the first time the full-length structure of apoE in which the N-terminal domain (residues 1–167) and C-terminal domain (residues 206–299) are separated by a hinge region (residues 168–205) (Chen et al.,

2011). The N-terminal domain contains a four-helix bundle, which was detected by both X-ray crystallography (Wilson et al., 1991) and NMR (Sivashanmugam and Wang, 2009). In monomeric mutant apoE3, Arg 61 forms a hydrogen bond with Thr 194 and Glu 255, forming a salt bridge with Lys 95 (Chen et al., 2011). In contrast to this, X-ray crystallographic analyses of the N-terminal domain structure of apoE revealed that Arg 112 forms a salt bridge with Glu 109, resulting in the exposure of Arg 61 away from the four-helix bundle in apoE4, whereas this side chain in apoE3 is buried (Dong et al., 1994). As a result, Arg 61 in apoE4 is predicted to interact with Glu 255, leading to interactions between the N- and C-terminal domains (Mahley and Rall, 2000; Mahley et al., 2009; Wilson et al., 1991). Fluorescence resonance energy transfer (FRET) and electron paramagnetic resonance (EPR) revealed that the distance between Arg 61 and Glu 255 is closer in both lipid-free and phospholipid-bound apoE4 than in apoE3 (Hatters et al., 2005). This domain-domain interaction was also observed in apoE4-expression neuronal cells by live cell imaging (Xu et al., 2004). Although the NMR structure does not support the domain-domain interaction in apoE4, it is likely that the single amino acid difference at position 112 between apoE3 (Cys) and apoE4 (Arg) has an influence on its structure. Future technology that allows for determination of full-length wild-type apoE3 and apoE4, either in lipid-free or lipid-bound state, should teach us how a single amino acid difference can have a profound impact on structure and functions, in particular those related to AD pathways.

The major functional regions of apoE are the receptor-binding site (residues 136–150) in the N-terminal domain and the lipid-binding site (residues 244–272) in the C-terminal domain (Figure 1) (Hatters et al., 2006a). NMR analysis of apoE3 demonstrated that several hydrophilic residues in the C-terminal domain are buried within the domain interface. The exposed hydrophobic residues destabilize the C-terminal domain and form a large exposed hydrophobic surface to attract lipids for initial

binding (Chen et al., 2011). ApoE3 and apoE2 display a preference for high-density lipoproteins (HDL), whereas apoE4 is frequently associated with very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (Dong et al., 1994; Hatters et al., 2006a). In apoE4, replacement of Arg 61 with Thr, or Glu 255 with Ala, leads to apoE3-like HDL preference (Dong et al., 1994; Hatters et al., 2006a). Therefore, Arg 61, Glu 255, or their interaction is probably necessary for VLDL preference in apoE4. One consequence of different lipoprotein preference by apoE4 is the increased plasma cholesterol levels, which in turn increases the risk for cardiovascular diseases, in particular atherosclerosis (Davignon et al., 1988).

Major apoE receptors belong to the LDL receptor (LDLR) family, including LDLR (Innerarity and Mahley, 1978), LDLR-related protein 1 (LRP1) (Beisiegel et al., 1989; Kowal et al., 1989), VLDL receptor (VLDLR), and apoE receptor 2 (apoER2) (Kim et al., 1996). LDLR interacts with both apoB-LDL and apoE-HDL particles (Innerarity et al., 1979); however, apoE-HDL particles have a 10- to 100-fold higher binding affinity to the cell surface receptor than the apoB-LDL particles (Innerarity and Mahley, 1978). LRP1 recognizes more than 30 structurally and functionally distinct ligands, including apoE,  $\alpha$ 2-macroglobulin, tissue-type plasminogen activator, APP, and A $\beta$  (Herz and Strickland, 2001). Although both LRP1 and LDLR are major apoE metabolic receptors, they differ in ligand recognition. While LRP1 prefers apoE-enriched particles or apoE aggregates, LDLR binds naturally secreted or circulating apoE particles (Bu, 2009). Thus, the receptor-binding specificity of apoE is influenced by its lipidation state and the density of apoE epitopes. For binding to LDLR, the positively charged residues within the N-terminal receptor-binding region of apoE are probably buried in a lipid-free state but are exposed upon lipidation (Chen et al., 2011). LRP1 binding, which appears to require either apoE enrichment or aggregation, is less understood but may require simultaneous exposure of multiple receptor-binding epitopes. In addition to the LDLR family, apoE also binds to cell surface heparan sulfate proteoglycans (HSPGs) (Ji et al., 1993), either in lipid-free or lipidated forms (Saito et al., 2003), through both the receptor-binding region in the N-terminal domain and the basic residues around Lys 233 in the C-terminal domain (Saito et al., 2003) (Figure 1). The C-terminal domain of recombinant apoE protein displays a stronger affinity and faster kinetics in terms of binding to heparin. Thus, HSPG binding through basic residues around Lys 233 is likely to be the first step, which makes the receptor-binding region in N-terminal domain available for the second step of HSPG binding (Futamura et al., 2005). Furthermore, LRP1 can form a complex with HSPG (Ji et al., 1993). In the HSPG/LRP1 uptake pathway, either apoE first binds to HSPG and is then transferred to LRP1 for subsequent uptake, or it can bind directly to the HSPG/LRP1 complex (Ji et al., 1993). The receptor-binding function of apoE has been shown to be isoform dependent. While apoE3 and apoE4 bind with similar affinity to LDLR, apoE2 has less binding affinity (Schneider et al., 1981). The unique Cys 158 in apoE2 eliminates a salt bridge between Arg 158 and Asp 154 with the formation of a new salt bridge between Arg 150 and Asp 154, thus reducing its affinity to the LDLR (Dong et al., 1994). Due to this reduced binding of apoE2 to LDLR, apoE2 homozygous individuals are at higher

risk for a genetic disorder termed type III hyperlipoproteinemia, which is characterized by increased plasma levels of cholesterol and triglycerides (Hatters et al., 2006a). The reduced affinity of apoE2 to LRP1 compared to apoE3 and apoE4 appears to be less severe (Kowal et al., 1990) and there seem to be no major differences among the apoE isoforms in their ability to bind to HSPG (Mahley and Rall, 2000).

Although we still do not know the full-length structures of wild-type apoE due to its highly aggregative nature, numerous biochemical, structural, and functional studies in lipid transport have provided important clues as to why subtle changes in sequence among the three apoE isoforms can generate sufficient differences in risk for cardiovascular diseases and AD. As we further explore the mechanisms of apoE isoforms in AD pathogenesis, in particular those related to A $\beta$  pathways, it is important to consider all available knowledge, some of which was generated decades ago (Mahley, 1988), as we design further studies to advance our understanding of apoE physiological and pathophysiological functions.

#### ApoE and Alzheimer's Disease: Amyloid Is the Clue

In humans, the *APOE* gene exists as three polymorphic alleles ( $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4), with the *APOE*  $\epsilon$ 3 allele being the most common (77%) and  $\epsilon$ 2 allele the least common (8%) (Mahley, 1988). Much evidence has confirmed that *APOE4* is the strongest genetic risk factor for AD (Harold et al., 2009; Lambert et al., 2009), as *APOE4* significantly increases the risk for both early-onset AD and late-onset AD (Chartier-Harlin et al., 1994; Houlden et al., 1998). The  $\epsilon$ 4 allele frequency is estimated to be about 15% in the general population but is  $\sim$ 40% in AD patients (Farrer et al., 1997). In addition to increasing the prevalence of AD, the presence of the *APOE4* allele also lowers the age of onset for AD in a gene dose-dependent manner (Corder et al., 1993; Farrer et al., 1997). Among  $\epsilon$ 4 homozygotes, the frequency of AD and mean age at clinical onset are 91% and 68 years of age, respectively, compared to 47% and 76 years of age in  $\epsilon$ 4 heterozygotes and 20% and 84 years in  $\epsilon$ 4 noncarriers (Corder et al., 1993). Importantly, A $\beta$  deposition as senile plaques is more abundant in *APOE4* carriers compared with noncarriers (Kok et al., 2009; Polvikoski et al., 1995), and *APOE4* increases A $\beta$  deposition in the brains of elderly subjects with normal cognitive function, although the association is weaker than that in AD patients (Reiman et al., 2009).

A large-scale study of CSF samples from both normal individuals and AD patients demonstrated that apoE protein levels in CSF positively associated with CSF A $\beta$ 42 levels independent of *APOE4* genotype, although there was no significant difference in CSF apoE levels between AD patients and normal individuals (Cruchaga et al., 2012). Because there is a strong correlation between low CSF A $\beta$ 42 and high A $\beta$  brain deposition detected by Pittsburgh compound B (PiB)-positron emission tomography (PET) imaging, lower CSF apoE levels in effect correlate with increased A $\beta$  deposition in the brain (Fagan et al., 2006). Thus, CSF apoE protein levels appear to hold potential as an endophenotype marker for AD pathology. Interestingly, CSF and plasma apoE protein levels are reduced in *APOE4* carriers (Cruchaga et al., 2012). As there was no association between apoE genotype and apoE mRNA levels (Cruchaga et al., 2012), the effect of apoE isoforms on CSF apoE levels is likely due to the different

conformational stability that affects their proteolysis. The apoE isoforms differ in the stability of their N-terminal domains with apoE4 being the least resistant to thermal and chemical denaturation, apoE2 the most, and apoE3 showing intermediate resistance (Hatters et al., 2006a). These studies suggest that reduced apoE levels in *APOE4* carriers and in AD patients can facilitate the accumulation of A $\beta$  in the brain. Supporting this, when A $\beta$  and apoE levels were analyzed in multiple brain areas of nondemented individuals, apoE levels negatively correlated with A $\beta$  levels both in *APOE4* carriers and noncarriers, indicating that apoE affects region-specific A $\beta$  levels by preventing A $\beta$  accumulation (Shinohara et al., 2013). Taken together, these clinical studies using human samples suggest that apoE isoforms impact AD pathogenesis by driving A $\beta$  pathology. The following sections in this Review discuss preclinical studies highlighting potential pathways by which apoE isoforms affect A $\beta$  metabolism and aggregation.

### ApoE/A $\beta$ Complexes: How We Assess Them Makes a World of Difference

Histological analyses of AD brains reveal that apoE is codeposited with A $\beta$  in amyloid plaques (Namba et al., 1991), indicating a direct association between apoE and A $\beta$  in AD pathogenesis. Several *in vitro* studies have shown that synthetic A $\beta$  peptides can bind to apoE either secreted from cells (LaDu et al., 1994) or purified from human CSF (Wisniewski et al., 1993) and plasma (Strittmatter et al., 1993). Human recombinant apoE binds to immobilized A $\beta$ 40 with a high affinity ( $K_D = \sim 20$  nM). Its interaction is greatly influenced by the conformational state of A $\beta$ , in which apoE shows stronger preference for A $\beta$  peptides with higher  $\beta$  sheet structure (Golabek et al., 1996). Epitope mapping reveals that residues 13–17 in A $\beta$  and residues 144–148 in the apoE N-terminal region, as part of the receptor-binding domain (Winkler et al., 1999), are common sites that interact with each other. In addition, residues 244–272 in the apoE C-terminal region also appear to be critical for the formation of apoE/A $\beta$  complexes (Strittmatter et al., 1993). Interestingly, heparin interacts with both A $\beta$ -binding sites in apoE and an apoE-binding site in A $\beta$  (Figure 1) (Brunden et al., 1993; Saito et al., 2003). Thus, the heparin-binding motifs in both apoE and A $\beta$  are critical for their interaction. HSPG is a major cell surface receptor for both apoE and A $\beta$ ; thus, improved understanding of the interactions between apoE, A $\beta$ , and cell surface HSPG, as discussed later, should provide further clues as to how these interactions influence AD pathogenesis.

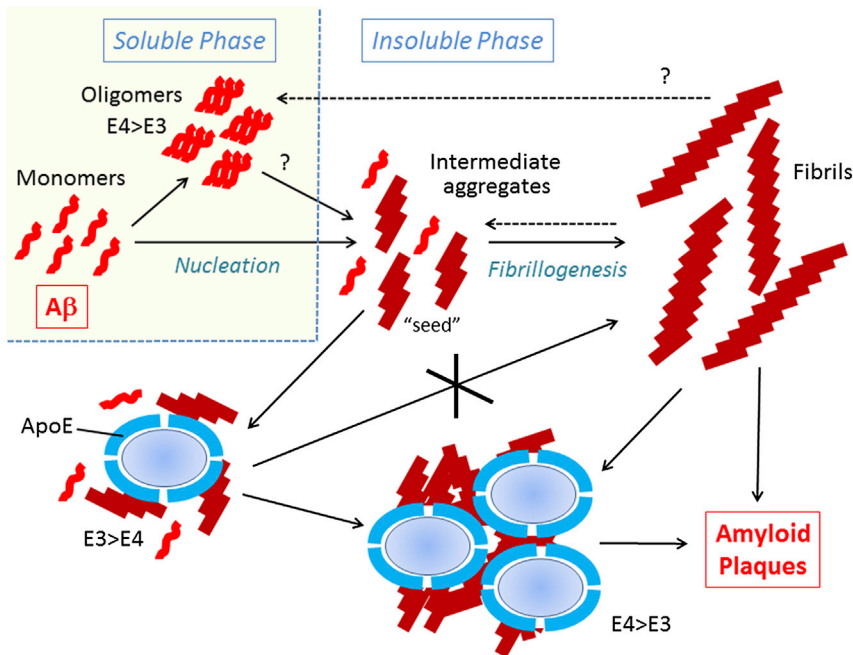
The notion that an A $\beta$ -binding site overlaps with the lipid-binding region within the apoE C-terminal domain suggests that A $\beta$  and lipids might compete with one another for apoE binding (Figure 1). In the brain, apoE is mainly secreted by astrocytes, where a plasma membrane ATP-binding cassette transporter A1 (ABCA1) loads cholesterol and other lipids onto lipoprotein particles (Koldamova et al., 2010). Astrocyte-secreted apoE particles are small and have different lipid components compared with apoE particles in CSF (LaDu et al., 1998), indicating that newly synthesized apoE particles probably undergo modifications before they are transferred to neurons. Indeed, *in vitro* experiments have shown that lipid-free recombinant apoE interacts with immobilized A $\beta$  with a higher affinity than lipidated recombinant apoE particles (Verghese et al., 2013), although conflicting

results have also been reported (Tokuda et al., 2000). Since A $\beta$  can interact with both the lipid-binding site and the receptor-binding site within apoE, the lipidation condition of apoE may dictate both A $\beta$ -binding affinity and the binding site. Further, it has been reported that incubation of recombinant apoE with A $\beta$  oligomers severely impairs its lipid-binding ability (Tamamizu-Kato et al., 2008), suggesting that A $\beta$  oligomers can impair the physiological function of apoE. As such ability of apoE to transport lipids to neurons is critical for synaptic maintenance and repair (Mahley and Rall, 2000), A $\beta$  oligomer-mediated disturbance of lipid binding in AD brains may further compromise synaptic integrity and function.

The interaction between apoE and A $\beta$  appears to depend on the specific apoE isoform being studied, its lipidation status, and the cellular compartment generating it. The methods of choice for evaluating apoE/A $\beta$  complexes may also influence the results. For example, initial studies using purified and delipidated apoE suggest that apoE4 binds to A $\beta$  with faster kinetics than apoE3 when detected by western blotting, and such an interaction is sensitive to both reducing agents and pH (Strittmatter et al., 1993). Subsequent studies using both HEK293 cell-secreted and native plasma apoE particles produced opposite results, with apoE3 forming more abundant SDS-stable complexes with A $\beta$  than apoE4 (LaDu et al., 1994, 1995). By directly comparing purified, delipidated apoE with native apoE, it was shown that apoE purification, which delipidates apoE, changes the behaviors of apoE such that purification itself attenuates isoform-specific binding to A $\beta$  (LaDu et al., 1995). Using EPR to assess apoE binding to A $\beta$  oligomers in solution and surface plasmon resonance (SPR) on solid phase, Petrlova et al. also detected stronger interactions for apoE3 than apoE4 (Petrlova et al., 2011), further supporting the notion that apoE3 might be more proficient in carrying A $\beta$  for cellular clearance and/or preventing A $\beta$  from aggregation or neurotoxicity.

Our recent collaborative study led by the LaDu group has shown that apoE4/A $\beta$  complexes are less stable than those formed with apoE2 and apoE3 (Tai et al., 2013). Using a novel ELISA quantifying apoE/A $\beta$  complexes, it was shown that despite similar amounts of apoE/A $\beta$  complexes formed between A $\beta$  and apoE2, apoE3, and apoE4, apoE4/A $\beta$  complexes are less stable in the presence of a denaturing agent or lower pH. Interestingly, the amounts of apoE/A $\beta$  complexes are fewer in *APOE4* carriers and in CSF from AD patients, which also have higher levels of A $\beta$  oligomers. These results indicate the apoE isoform-dependent function of apoE/A $\beta$  complexes and suggest that the amounts of both apoE/A $\beta$  complexes and A $\beta$  oligomers can serve as potential biomarkers for AD. One possible mechanism responsible for the less stable nature of apoE4/A $\beta$  complexes is the poorer lipidation status of apoE4 lipoprotein particles. However, the pathophysiological factors that influence the stability of apoE/A $\beta$  complexes and how A $\beta$  binding to apoE impacts both the amounts and toxic functions of A $\beta$  oligomers require further investigation.

Despite abundant evidence that apoE exhibits isoform-specific binding to A $\beta$  and that apoE/A $\beta$  complexes might modulate A $\beta$  metabolism, the significance of apoE/A $\beta$  complexes was called into question by a recent study led by the Holtzman group (Verghese et al., 2013). In this study, when astrocyte-secreted or



**Figure 2. A $\beta$  Aggregation: Role of apoE**

During the A $\beta$  aggregation process, A $\beta$  monomers change their conformation to a  $\beta$  sheet-rich structure and form soluble oligomers or insoluble intermediate aggregates. Such nuclei further accelerate the fibrillogenesis to form large insoluble fibrils as “seeds” (Harper and Lansbury, 1997). The association of apoE with an A $\beta$  nucleus is likely to block its seeding effect, which accelerates A $\beta$  fibrillogenesis. Under certain conditions, apoE and A $\beta$  may form large coaggregates. Newly generated A $\beta$  fibrils can bind to existing aggregates, resulting in the formation of even larger coaggregates, with or without additional apoE (Wood et al., 1996a). Finally, these aggregates may deposit as amyloid plaques in the brain.

genesis appear to depend on the origin of apoE. In this respect, it is interesting to note that expression of human apoE3 and apoE4 by astrocytes in the *ApoE*-KO background decreased early A $\beta$  deposition in PDAPP mice (Holtzman et al., 1999). Human apoE-targeted replacement (TR) mice, in which the mouse *ApoE* gene was replaced with those encoding human

in-vitro-reconstituted apoE particles were mixed with cell-derived A $\beta$  using concentrations resembling physiological conditions, i.e., apoE:A $\beta$  ratio at 50–150:1 (Hesse et al., 2000; Wahrle et al., 2007), the authors detected minimal amounts of apoE/A $\beta$  complexes, accounting for ~5% of total A $\beta$  (Verghese et al., 2013). Interestingly, in astrocytes, apoE particles compete with, rather than facilitate, cellular A $\beta$  uptake. These results further highlight the importance of the source of apoE and A $\beta$ , as well as experimental conditions, in addressing the roles of apoE/A $\beta$  complexes in AD-related pathways. It is important to note that despite significantly higher concentrations of apoE than A $\beta$  in CSF, the exact concentrations of these molecules in brain parenchyma, in particular around the synapses where A $\beta$  is probably produced and exhibits neurotoxicity, is unclear. Future studies should focus on developing technology, for example, real-time in vivo microdialysis, to assess apoE-A $\beta$  interactions and how a perturbation of their interaction affects A $\beta$  metabolism, oligomerization, and toxicity.

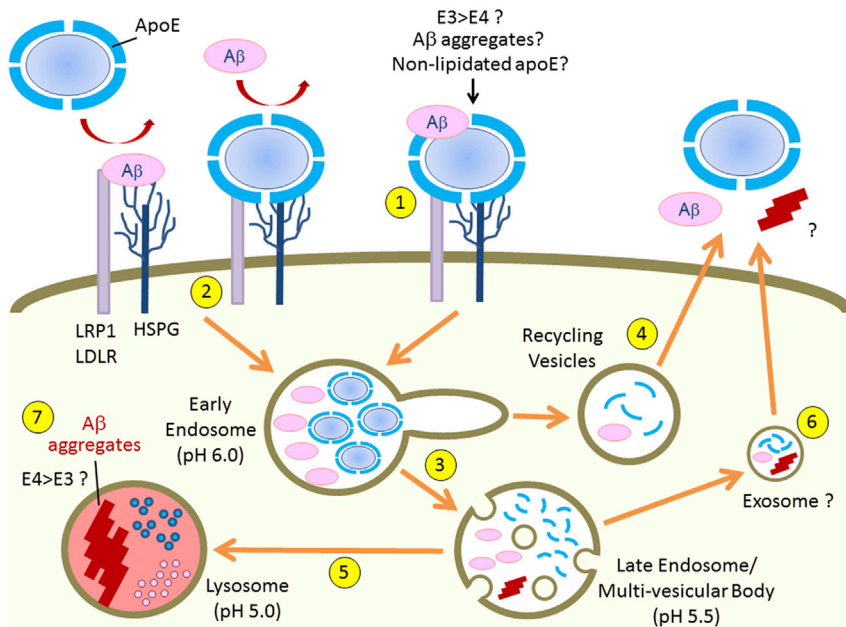
### ApoE and A $\beta$ Aggregation: ApoE Is Needed to Seed Amyloid

Several in vivo studies have clearly shown that apoE is essential for A $\beta$  deposition in APP transgenic amyloid model mice. When *ApoE* knockout (KO) mice were crossed with amyloid model PDAPP or Tg2576 mice, A $\beta$  deposition in the form of amyloid plaques and cerebral amyloid angiopathy (CAA) was dramatically reduced (Bales et al., 1997; Irizarry et al., 2000). In particular, although there is still significant, sometimes increased, A $\beta$  deposition as diffused plaques, thioflavin S-positive fibril plaques were virtually absent. These results clearly demonstrate the essential role of mouse apoE in A $\beta$  fibrillogenesis, stabilization of fibrillar A $\beta$ , and/or maturation of amyloid plaques (Bales et al., 1997; Irizarry et al., 2000). However, the effects of apoE on A $\beta$  fibrillo-

apoE isoforms, had less A $\beta$  deposition in the background of Tg2576 mice compared with the control mice expressing mouse apoE (Fryer et al., 2005b). Thus, human apoE isoforms probably determine the amounts, morphology, localization, and fibrillogenesis of A $\beta$  deposits depending on whether mouse apoE is present or not.

During the A $\beta$  aggregation process, soluble A $\beta$  peptides are known to change their conformation into a  $\beta$  sheet structure and form nuclei (lag phase), which further accelerates the process of fibrillogenesis to form insoluble fibrils with enriched  $\beta$  sheet structures as “seed” (elongation phase) (Harper and Lansbury, 1997). There are numerous studies analyzing the effects of apoE on A $\beta$  aggregation in vitro. However, the conclusions are somewhat controversial as to whether apoE facilitates or inhibits A $\beta$  aggregation. It has been shown that high concentrations of apoE form high molecular weight coaggregates with A $\beta$  (Chan et al., 1996), where apoE4 is likely to promote A $\beta$  aggregation more than apoE3 (Castano et al., 1995; Ma et al., 1994). Moreover, it was shown that apoE increases the level of A $\beta$  oligomers in an isoform-dependent manner (apoE4 > apoE3 > apoE2) (Hashimoto et al., 2012). Furthermore, apoE4 stabilizes A $\beta$  oligomers more than apoE3 (Cerf et al., 2011). These findings imply that apoE4 harmfully accelerates A $\beta$  aggregation in AD.

In contrast, other studies have reported that apoE decreases A $\beta$  fibrillogenesis. ApoE strongly inhibits the initiation of A $\beta$  fibril formation, when analyzed either with or without the addition of preformed A $\beta$  aggregates as seeds (Naiki et al., 1997; Wood et al., 1996b). Because apoE prefers to interact with A $\beta$  peptides that are in  $\beta$  sheet structure (Golabek et al., 1996), apoE probably captures A $\beta$  nuclei and prevents its seeding effects (Figure 2) (Wood et al., 1996a). ApoE3 appears to interact with A $\beta$  more than apoE4 as described; therefore, it is possible that apoE4 is less effective in the inhibition of A $\beta$  fibril formation. In this case,



**Figure 3. Cell Surface Binding and Endocytic Trafficking of apoE and Aβ**

ApoE probably binds to Aβ in an isoform-dependent manner with apoE3 forming more stable apoE/Aβ complexes than apoE4 (LaDu et al., 1994, 1995). LRP1, LDLR, and HSPG are major cell surface receptors that bind apoE, Aβ, and apoE/Aβ complexes. In addition to forming a stable complex with Aβ (1), apoE probably competes with Aβ to common cell surface receptors (2) (Verghese et al., 2013). Endocytosed apoE either dissociates from lipid components within the early endosomes due to lower pH (3) and recycles (4) or is transported to lysosomes for degradation (5). Endocytosed Aβ is typically delivered to lysosomes for degradation (5), although a small amount of Aβ can be recycled (4) (Li et al., 2012). In some conditions, apoE and Aβ may be transferred through exosomes from the late endosomes/multivesicular body (6). When Aβ accumulation overwhelms the capacity of lysosomes for degradation, the low pH in the lysosomes provides a suitable environment to initiate Aβ aggregation (7) (Hu et al., 2009), which could injure lysosomes and also provide seeding for further Aβ aggregation.

apoE4 may be less effective in supporting the beneficial effects of apoE in preventing Aβ fibrillation in AD. If the amount of apoE/Aβ complex increases as the sole product of the reaction, it may form large coaggregates (Figure 2) (Wood et al., 1996a). ApoE is also known to aggregate with irregular protofilament-like morphology, where the aggregates form at substantially different rates depending on the isoform (apoE4 > apoE3 > apoE2) (Hatters et al., 2006b). Thus, apoE4 may be able to produce more coaggregates with Aβ through its self-aggregating propensity. Consistent with these findings, in vivo experiments have also shown that apoE4-TR mice had more Aβ deposition than apoE3-TR mice in the background of amyloid model mice (Bales et al., 2009; Fryer et al., 2005b). In yet a more aggressive amyloid model mice referred to as 5xFAD (Oakley et al., 2006), amyloid plaque deposition was in general greater in E4FAD mice; E2/E3FAD mice have significantly more diffuse plaques with E4FAD exhibiting more compact plaques (Youmans et al., 2012). Collectively, these studies indicate that compared to apoE2 or apoE3, apoE4 is either more likely to promote Aβ fibrillogenesis or less effective in preventing Aβ aggregation, or both. Again, the specific outcomes can be influenced by apoE isoform, lipidation status, aggregation states, and the time and location of its presence during the disease process. Further studies, perhaps by time-lapsed recording in living mice with exogenously introduced apoE isoforms with different lipidation statuses, might allow us to generate a clearer picture regarding the specific effects of apoE on Aβ and deposition. The effects of diffuse and fibrillar plaques on synaptic functions and behaviors, in the presence of different apoE isoforms, also warrant further investigation.

#### ApoE and Aβ in Endocytic Trafficking: Common Receptors and Trafficking to Lysosomes

The major apoE receptors, LDLR, LRP1, and HSPG, are abundantly expressed in the brain. These receptors mediate cellular

uptake of Aβ as well as apoE (Figure 3). The mRNAs of LDLR and LRP1 are detected in several types of brain cells including neurons, astrocytes, microglia, and oligodendrocytes (Fan et al., 2001). While LDLR deficiency in mice leads to apoE accumulation in the brain (Fryer et al., 2005a), overexpression of LDLR decreases apoE levels by 50%–90% (Kim et al., 2009), demonstrating the important role of LDLR in brain catabolism of apoE. Since apoE2 has a lower affinity to the LDLR (Dong et al., 1994), apoE2 catabolism is probably slower compared to apoE3 or apoE4. Consistent with this notion, apoE levels in CSF and plasma are higher than those of apoE3 or apoE4 both in human (Cruchaga et al., 2012) and in apoE-TR mice (Bales et al., 2009). The increased absolute levels of apoE in APOE2 individuals might help with preventing Aβ accumulation in the brain and decrease the risk for developing AD. In vitro experiments have revealed that Aβ can bind directly to LDLR (Basak et al., 2012). Overexpression of LDLR significantly increases cellular uptake of Aβ in astrocytes, whereas deletion of LDLR has the opposite effect (Basak et al., 2012). Consistent with these findings, overexpression of LDLR in the brain decreases Aβ deposition in amyloid model mice (Kim et al., 2009); however, the effects of LDLR deletion on Aβ deposition is less clear (Cao et al., 2006; Fryer et al., 2005a), indicating that although LDLR is capable of metabolizing Aβ in the brain, its pathophysiological role requires further investigation.

LRP1 is an important apoE metabolic receptor in the brain and also among the most studied Aβ receptors. In vivo experiments show that overexpression of a functional LRP1 minireceptor (mLRP2) in mouse brain significantly decreases apoE levels (Zerbinatti et al., 2006), whereas neuronal-specific LRP1 deletion leads to an increase of apoE levels (Liu et al., 2007). LRP1 has been shown to mediate cellular Aβ uptake in neurons (Kanekiyo et al., 2011), astrocytes (Koistinaho et al., 2004), microglia (Laporte et al., 2004), vascular smooth muscle cells (Bell et al.,

2009; Kanekiyo et al., 2012), and endothelial cells (Deane et al., 2004; Yamada et al., 2008). The in vivo experiments have shown that exogenous A $\beta$  application through intracerebral microinjections is rapidly removed from the brain, whereas an LRP1-specific antibody slows this elimination process (Shibata et al., 2000). Consistent with these results, conditional knockout of the *Lrp1* gene in mouse forebrain neurons (Kanekiyo et al., 2013) or vascular smooth muscle cells (Kanekiyo et al., 2012) exacerbated amyloid pathology in amyloid model mice by suppressing cellular A $\beta$  uptake and lysosomal degradation. A $\beta$  binds directly to immobilized LRP1 receptor fragments with high affinity (Deane et al., 2004), although an opposing result was also reported (Yamada et al., 2008). LRP1 also indirectly interacts with A $\beta$  through its ligands, including apoE,  $\alpha$ 2-macroglobulin (Narita et al., 1997), and prion proteins (Rushworth et al., 2013). It was shown that cellular prion protein (PrP<sup>C</sup>) mediates A $\beta$  oligomer binding to the cell surface (Laurén et al., 2009; Wang et al., 2013), where LRP1 functions as a coreceptor of PrP<sup>C</sup> (Rushworth et al., 2013). Furthermore, it is important to note that LRP1 and HSPG can form immunoprecipitable complexes at the cell surface, which might further regulate the metabolism of apoE and A $\beta$  (Wilsie and Orlando, 2003). Our work has shown that A $\beta$  appears to initially bind to cell surface HSPG, followed by endocytosis through either LRP1-independent or -dependent manners (Kanekiyo et al., 2011). HSPG deficiency significantly decreases cellular A $\beta$  binding and uptake (Kanekiyo et al., 2011). The fact that several types of HSPG are found to be colocalized with senile plaques and CAA in the brain of AD patients (van Horsen et al., 2003) further supports the important role HSPG plays in apoE and A $\beta$  cellular metabolism. Interestingly, peripheral treatment with a low-molecular-weight heparin inhibits the binding of A $\beta$  to HSPG and significantly decreases A $\beta$  concentration and deposition in the brain of amyloid model mice (Bergamaschini et al., 2004). The effects observed with heparin treatment are different to the phenotype displayed with LRP1 or LDLR deletion, where A $\beta$  deposition is exacerbated. These observations suggest that the pathways of cellular A $\beta$  and apoE uptake are differently regulated depending on their binding to specific apoE receptor LDLR, LRP1, and/or HSPG. Conditional deletion of HSPG in the brain can help to address the in vivo roles of this sometimes promiscuous molecule in brain A $\beta$  metabolism. In this regard, it is important to note that HSPG also plays important roles in cellular uptake of tau and  $\alpha$ -synuclein (Holmes et al., 2013), a critical step for propagation of disease-related pathology (Guo et al., 2013). Thus, further studies to dissect how HSPG regulates the endocytosis and trafficking of a variety of pathogenic proteins including A $\beta$ , apoE, tau, and  $\alpha$ -synuclein may provide new insights into the pathogenesis of multiple neurodegenerative diseases.

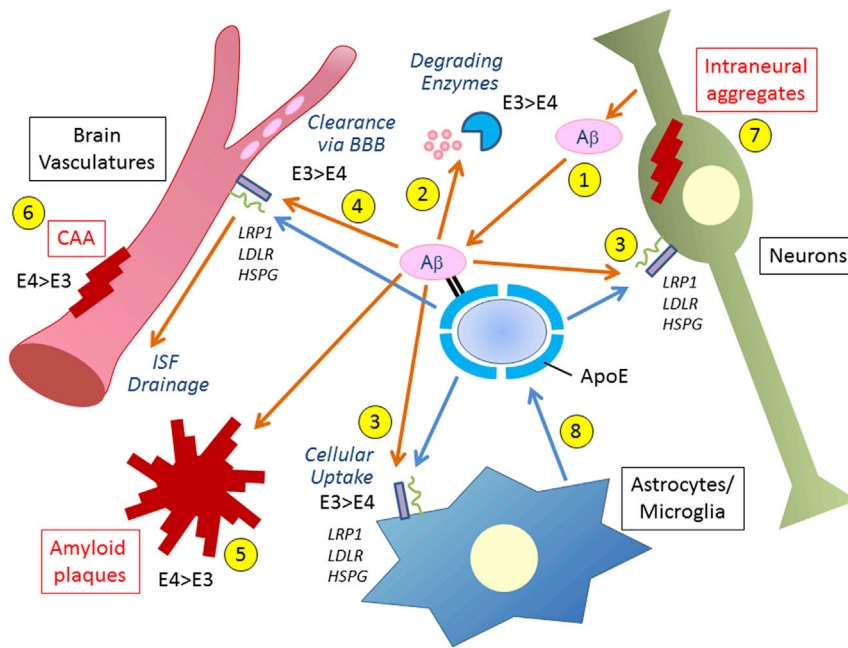
Several studies have demonstrated that apoE enhances cellular A $\beta$  uptake through its ability to form a complex. Soluble A $\beta$  and recombinant apoE form SDS-stable complexes, which enhance A $\beta$  internalization in primary neurons (Gyls et al., 2003). Recombinant apoE accelerates neuronal A $\beta$  uptake in an isoform-dependent manner, with apoE3 more efficiently facilitating A $\beta$  binding to the cell surface than apoE4 (Li et al., 2012). However, other studies have shown that apoE might compete with A $\beta$  for receptor binding and subsequent cellular uptake.

Recombinant apoE was shown to reduce the uptake of A $\beta$  oligomers, but not fibrils, in astrocytes (Nielsen et al., 2010). ApoE particles interfere with the cellular uptake of soluble A $\beta$  through an LRP1-dependent pathway in astrocytes (Verghese et al., 2013). These seemingly conflicting results may be induced by specific experimental conditions, including the source and the amounts of apoE and A $\beta$ , the specific cell types tested, and/or the specific detection methods. Because A $\beta$  and heparin are capable of binding to both the lipid-binding region and the receptor-binding region in apoE (Figure 1) and the complex interacting network of apoE receptors, apoE might either facilitate or compete with A $\beta$  for cellular binding and uptake depending on their concentrations, A $\beta$  aggregation state, apoE isoform, apoE lipidation state, and expression pattern of the receptors on the cell surface (Figure 3). Further studies, in particular those designed in vivo, are necessary to clarify apoE isoform-dependent functions in cellular A $\beta$  uptake and metabolism.

It is clear that the majority of cell-internalized A $\beta$  traffics through the early and late endosomes en route to lysosomes for degradation (Hu et al., 2009; Li et al., 2012), although a small portion of cell-internalized A $\beta$  does recycle through Rab11-positive recycling endosomes (Li et al., 2012). Thus, cellular A $\beta$  uptake is in general a beneficial pathway for A $\beta$  clearance. However, when lysosomal degradation is impaired (Li et al., 2012) or when A $\beta$  concentration overwhelms this compartment in AD brains (Hu et al., 2009; Knauer et al., 1992), trafficking to lysosomes can be detrimental as it could lead to A $\beta$  aggregation (Hu et al., 2009), which is highly favorable in the acidic environment found in lysosomes (Perálvarez-Marín et al., 2008) (Figure 3). A $\beta$  aggregates in turn likely induce lysosomal dysfunction and cellular toxicity. More importantly, these lysosomal-initiated A $\beta$  aggregates can further accelerate A $\beta$  aggregation, which could eventually contribute to the genesis of extracellular A $\beta$  oligomers and amyloid plaque deposition. Interestingly, apoE also aggregates faster under acidic pH conditions found in lysosomes with apoE4 aggregating more than apoE3 (Garai et al., 2011). Thus, the aggregative nature of both A $\beta$  and apoE4 in the acidic lysosomes demonstrates that these AD pathogenic molecules might coaggregate in the lysosomes as accidental encounters and/or partners.

#### ApoE in A $\beta$ Clearance: Distinct Roles and Isoform-Specific Effects

While A $\beta$  is continuously generated in the brain, it is efficiently eliminated under physiological conditions. In human brains, the A $\beta$  clearance rate is calculated to be 8.3% per hour (Bateman et al., 2006). There are three major pathways by which A $\beta$  is cleared from the brain: (1) through proteolytic degradation, (2) by cellular clearance through lysosomal degradation in brain parenchyma cells (microglia, astrocytes, neurons), and (3) by cerebrovascular system-mediated clearance including the interstitial fluid (ISF) drainage pathway, local cellular clearance, and blood-brain barrier (BBB) (Figure 4). In vivo experiments have shown that A $\beta$  clearance is slower in apoE4-TR mice compared with apoE3-TR mice (Castellano et al., 2011). However, the pathways contributing to apoE-regulated A $\beta$  clearance can be complex as discussed above as the clearance of soluble A $\beta$  in brain ISF is increased in apoE-KO mice (DeMattos et al., 2004).



**Figure 4. Major Aβ Clearance Pathways and Effects of apoE Isoforms**

Aβ is predominantly generated in neurons (1) and eliminated through three major clearance pathways including proteolytic degradation by (2) endopeptidases (e.g., NEP, IDE) (Saido and Leissring, 2012), (3) cellular clearance by cells in the brain parenchyma (neurons, astrocytes, and microglia) (Kanekiyo et al., 2013; Koistinaho et al., 2004), and (4) ISF drainage, where it is degraded by vascular cells (Bell et al., 2009; Kanekiyo et al., 2012) or transported out of the brain through the BBB (Ito et al., 2013). Disturbance of these pathways induces Aβ accumulation and deposition in the brain parenchyma as amyloid plaques (5), in the perivascular region as CAA (6), and sometimes also inside neurons (7). ApoE is generated mainly by the glial cells (8) and encounters Aβ in all these pathways. ApoE probably facilitates Aβ clearance by activating enzymatic degradation (2) and phagocytosis (3) in an isoform-dependent manner (apoE3 > apoE4). However, apoE might also suppress Aβ clearance (apoE4 > apoE3) by either competing with Aβ for receptor binding or by retaining Aβ from its clearance through the BBB (4). ApoE4 exacerbates Aβ deposition as amyloid plaques and CAA formation when compared with apoE3 (5 and 6) (Fryer et al., 2005b). LRP1, LDLR, and HSPG, which are expressed in all major cellular Aβ clearance pathways, regulate Aβ clearance either directly or through apoE.

Aβ is degraded by a large set of proteases including neprilysin (NEP) and insulin-degrading enzyme (IDE) in both intracellular and extracellular compartments (Figure 4) (Saido and Leissring, 2012). Deletion of NEP or treatment with an NEP inhibitor leads to increased levels of Aβ (Farris et al., 2007; Iwata et al., 2000). The IDE-KO mice show increased accumulation of endogenous Aβ in the brain (Farris et al., 2003). Consistent with these findings, overexpression of NEP and/or IDE lowers Aβ levels by around 90% and reduces amyloid pathology (Leissring et al., 2003). Soluble apoE/Aβ complexes isolated from human brains were more susceptible to proteolytic degradation than apoE-free Aβ (Russo et al., 1998). Similarly, NEP-mediated Aβ degradation in microglia is enhanced by exogenous apoE, in an isoform-dependent manner (apoE2 > apoE3 > apoE4). Extracellular Aβ degradation by IDE and NEP is also facilitated by apoE. Interestingly, lipidated apoE shows stronger effects on the capacities of NEP and IDE to degrade Aβ than nonlipidated apoE (Jiang et al., 2008). Although further studies are needed, it is tempting to speculate that apoE facilitates Aβ degradation by converting the Aβ structure into one that is more recognizable by NEP and IDE.

Cellular uptake of Aβ by astrocytes and microglia is likely to represent a more functional pathway for Aβ clearance (Figure 4). When brain sections bearing Aβ plaques from amyloid model mice were cultured with adult mouse astrocytes, the astrocytes internalized and degraded Aβ in both an apoE- and LRP1-dependent manner (Koistinaho et al., 2004). In microglia, soluble Aβ is probably internalized by fluid-phase macropinocytosis into lysosomes for degradation (Mandrekar et al., 2009), whereas Aβ aggregates interact with a multicomponent cell surface receptor complex and are internalized through phagocytosis (Bamberger et al., 2003). Microglia and macrophage are known to have two activation statuses: classically activated (M1) and alternatively

activated (M2) stages. M1 cells produce proinflammatory cytokines, which damage neurons, whereas M2 cells trigger anti-inflammatory/neurotrophic pathways and clear Aβ by phagocytosis in AD (Aguzzi et al., 2013). Microglial activation is probably modulated by apoE, as apoE has been shown to convert macrophages from the proinflammatory M1 to the anti-inflammatory M2 phenotype (Baitsch et al., 2011). ApoE4 is less effective in anti-inflammatory functions than apoE3 in microglia (Zhu et al., 2012). Furthermore, apoE isoforms differently regulate microglia migration in response to activation, with microglia from apoE4-TR mice exhibiting slower migration than those from apoE3-TR mice (Cudaback et al., 2011). Thus, apoE promotes Aβ clearance by activating phagocytosis and migration in microglia, where apoE4 has a reduced capacity to induce these phenotypes than apoE3. Recent genetic studies have also identified several new AD risk genes that are potentially involved in regulating neuroinflammation-related functions including *TREM2*, *CLU*, *CR1*, *CD33*, and *ABCA7* (Guerreiro et al., 2013; Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011). Although the functional relationships between apoE and proteins encoded by these genes are not fully understood, the discovery of these new AD risk genes further implies a critical relationship between neuroinflammation and AD pathogenesis. As *Clu* and *ABCA7* are also related to lipid metabolism, further studies may demonstrate their cooperative roles with apoE in Aβ clearance.

Neurons also have an ability to eliminate Aβ through its uptake and lysosomal degradation (Figure 4) (Li et al., 2012). It should be noted that neurons have the highest risk of encountering Aβ in the brain, because Aβ is predominantly produced in neurons. The functional role of neurons in brain Aβ clearance is clearly shown by our recent work demonstrating that when LRP1 is deleted exclusively in neurons in adult mouse brain, the



half-life of ISF A $\beta$  increases, which leads to more abundant A $\beta$  accumulation and pathology (Kanekiyo et al., 2013).

Cerebrovascular systems also play critical roles in A $\beta$  clearance (Figure 4). Pathological studies from AD patients show that A $\beta$  deposits in the vascular smooth muscle cell layer of cerebral blood vessels and capillaries in the form of CAA as well as senile plaques (Rensink et al., 2003; Revesz et al., 2003). ISF flows along periarterial spaces in the brain and joins the CSF to drain into the cervical lymph nodes and/or venous blood flow. Part of ISF can directly enter the arterial blood flow through the BBB (de Boer and Gaillard, 2007; Marques et al., 2013). In the ISF drainage pathway, A $\beta$  is thought to be eliminated through cellular degradation by vascular cells (smooth muscle cells, pericytes, endothelial cells, and astrocytes), proteolytic degradation, and clearance through the BBB. Remaining A $\beta$  in ISF may be eventually drained into lymph flow and venous blood flow. Thus, the disturbance of ISF drainage pathway probably causes A $\beta$  accumulation in the brain. In fact, the water channel aquaporin-4 has been shown to be a crucial component in this process. Aquaporin-4 null mice exhibit an ~70% reduction in ISF clearance rate and a suppressed clearance of soluble A $\beta$  through reduction of ISF drainage (Iliiff et al., 2012), supporting an importance role of this pathway in A $\beta$  clearance. Epidemiological studies have also shown that practically all well-described risk factors for AD, including diabetes mellitus, atherosclerosis, stroke, hypertension, transient ischemic attacks, microvessel pathology, and smoking, have a vascular component that disturbs cerebral vascular functions (de la Torre, 2002). Pathological evidence indicates that LRP1 levels are significantly decreased during aging and AD, in particular in the brain vasculature (Bell et al., 2009; Deane et al., 2004). LRP1 in vascular smooth muscle cells regulates A $\beta$  clearance in the ISF drainage pathway. Deletion of LRP1 in vascular mural cells enhances A $\beta$  deposition both in brain parenchyma as amyloid plaques and in vasculature as CAA (Kanekiyo et al., 2012). BBB efflux of A $\beta$  also represents a major pathway for brain A $\beta$  clearance. The overall clearance rate constant of A $\beta$ 40 in mouse cerebral cortex is  $3.21 \times 10^{-2}/\text{min}$ , while the elimination rate for brain-blood clearance is  $1.48 \times 10^{-2}/\text{min}$  (Ito et al., 2013). Due to technical limitations, it is difficult to precisely distinguish A $\beta$  clearance through the BBB and simultaneous clearance through other pathways. Current research implicates apoE and apoE receptors as major regulators in A $\beta$  clearance through the BBB. One such study demonstrates that A $\beta$  is cleared rapidly across the BBB through LRP1, where apoE significantly disturbs this process (Bell et al., 2007). ApoE2/A $\beta$  and apoE3/A $\beta$  complexes are cleared at the BBB via LRP1 and VLDLR at a substantially faster rate than apoE4/A $\beta$  complexes (Figure 4) (Deane et al., 2008). In addition, LDLR has also been shown to mediate brain-blood A $\beta$  clearance (Castellano et al., 2012). Moreover, apoE4, but not apoE2 or apoE3, leads to the breakdown of the BBB (Bell et al., 2012; Nishitsuji et al., 2011), which causes a reduction in cerebral blood flow (Bell et al., 2012). This harmful effect of apoE4 on BBB integrity might further compromise A $\beta$  clearance. Consistent with these findings, *APOE4* carriers have more severe CAA pathology, in particular capillary CAA when compared with *APOE4* noncarriers (Richard et al., 2010; Thal et al., 2002). Taken together, apoE and apoE receptors are

clearly involved in A $\beta$  clearance through the cerebrovascular systems, probably in ways depending on the presence of specific apoE isoforms and apoE receptors. Future studies should focus on addressing whether apoE isoforms produced in brain parenchyma differ from those by vascular cells in regulating brain A $\beta$  clearance and how apoE receptors expressed in specific vascular cell types regulate these events.

#### ApoE-Targeted Therapy for AD: The Dilemma when Modulating apoE

Because of the importance of apoE in AD pathogenesis, several therapeutic strategies that target apoE for AD have been proposed. Among them, retinoid X receptor (RXR) and liver X receptor (LXR) agonists are promising candidates. RXR forms heterodimers with LXR, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), or retinoic acid receptor (RAR) to regulate gene networks that control multiple metabolic systems. In particular, these nuclear receptors are known to control the transcription of ABCA1 and apoE (Pérez et al., 2012). Oral administration of an RXR agonist, Bexarotene, suppressed A $\beta$  deposition and improved cognitive function in an apoE-dependent manner in amyloid model mice (Cramer et al., 2012), although these results are likely somewhat disputed by several follow-up studies. The LXR agonist TO901317 is also shown to increase apoE levels in the brain, facilitate A $\beta$  clearance, and reverse the memory deficit in amyloid model mice (Riddell et al., 2007; Terwel et al., 2011). Thus, treatments that result in increased levels of apoE in the brain are probably beneficial in AD therapy. However, this notion has to be carefully dissected as apoE4 clearly has harmful effects (Bu, 2009). Under stress conditions, neuronally expressed apoE often becomes fragmented, which damages mitochondria and cytoskeleton (Huang and Mucke, 2012). In addition, in vitro experiments have shown that apoE4 facilitates A $\beta$  production by promoting amyloidogenic processing of APP as a functional consequence of its interaction with APP (Vincent and Smith, 2001). Thus, it might be particularly detrimental to upregulate apoE4 production in AD. Rather than focusing on increasing apoE, it has been proposed that increasing apoE lipidation might be the key for apoE-based therapy. ApoE lipidation is mediated by ABCA1 (Koldamova et al., 2010). Importantly, deletion of ABCA1 increases A $\beta$  deposition in amyloid model mice (Wahrle et al., 2005), whereas its overexpression suppresses A $\beta$  deposition (Wahrle et al., 2008). Furthermore, the haploinsufficiency of ABCA1 significantly decreases A $\beta$  clearance in apoE4-TR mice, but not in apoE3-TR mice (Fitz et al., 2012), suggesting that the less efficient lipid transport phenotype associated with apoE4 might allow for greater manipulation at the level of apoE lipidation.

The argument to decrease apoE expression to treat AD is championed by the fact that apoE is essential for A $\beta$  deposition as discussed above. Recent studies have also shown that decreased apoE expression under haploinsufficiency of human apoE also results in less A $\beta$  deposition in amyloid mouse models, which is independent of apoE isoforms (Bien-Ly et al., 2012; Kim et al., 2011). Further, immunotherapy for apoE also reduces A $\beta$  accumulation. When amyloid model mice were intraperitoneally administered with anti-mouse apoE-specific antibody for 14 weeks, amyloid deposition was dramatically reduced by

60%–80% and insoluble A $\beta$  levels were significantly decreased (Kim et al., 2012). These results suggest that decreasing apoE levels has beneficial effects and that anti-apoE immunization can be explored as a novel therapeutic tool, at least from the perspective of A $\beta$  deposition. However, further studies are needed to determine how decreased apoE levels affect A $\beta$  oligomerization, neurotoxicity, synaptic integrity, and cognitive function. It is also an undisputed fact that apoE mediates cholesterol homeostasis, particularly in the brain; thus, these approaches to solely decrease apoE levels over extended periods of time may induce harmful, perhaps unforeseen, side effects.

The interaction between apoE and A $\beta$  can also be targeted for therapy, although the outcome is less clear. Despite evidence supporting a beneficial role of apoE/A $\beta$  complexes, the presence of these proteins in an environment that favors A $\beta$  aggregation, such as lysosomes and amyloid plaques, could be harmful. Supporting this, a synthetic peptide mimicking the apoE-binding sequence of A $\beta$ , A $\beta$ 12–28P, reduces A $\beta$  deposition and ameliorates memory deficits in amyloid model mice (Sadowski et al., 2004). Other methods that target apoE for therapy include apoE4 structural correctors (Chen et al., 2012) and increasing the functions of apoE receptors (Shinohara et al., 2010).

Taken together, the choice of increasing or decreasing apoE is an unavoidable dilemma in our efforts to develop apoE-based AD therapies. Despite this dilemma, apoE remains a promising target. Both pharmacological manipulations of apoE expression and lipidation and potentially new genetic mouse models that allow for testing the effects of increasing or reducing apoE should help to clarify our path to new AD therapy.

### Summary and Perspective

APOE4 is the strongest genetic risk factor for late-onset AD. ApoE4 appears to drive amyloid pathology in humans and in animal models; however, it is clear that apoE4 also contributes to AD pathogenesis in a manner that is independent of A $\beta$ . ApoE and A $\beta$  interact with each other and share common receptors including LRP1, LDLR, and HSPG. Importantly, the interactions among apoE, A $\beta$ , and their receptors probably vary depending on their concentrations, the apoE isoform involved, lipidation status, A $\beta$  aggregation status, and receptor distribution patterns. Thus, apoE isoforms probably have different, sometimes seemingly conflicting, roles in A $\beta$  aggregation and clearance. First, apoE reduces A $\beta$  oligomerization and fibril formation but is also essential for amyloid deposition. Second, apoE competes with A $\beta$  for their receptor binding but can also facilitate cellular A $\beta$  uptake by forming apoE/A $\beta$  complexes. Third, apoE facilitates the enzymatic degradation and phagocytosis of A $\beta$  by glial cells, but apoE compromises A $\beta$  elimination through the BBB. As a result, it has been difficult to elucidate the exact pathological mechanism by which apoE regulates A $\beta$  clearance. Nonetheless, it is clear that multiple pathways are involved and apoE and A $\beta$  appear to share several properties, suggesting that their interaction goes beyond accidental encounters. In fact, apoE-targeted therapy aimed at ameliorating amyloid pathology and improving cognitive function via RXR/LXR agonists or increasing apoE lipidation hold promise and warrant further investigation.

In summary, apoE has multiple functions in regulating A $\beta$  clearance, A $\beta$  aggregation, and A $\beta$ -independent pathways in

AD pathogenesis. A critical challenge is to determine whether increasing or decreasing apoE, or simply its lipidation, is beneficial, and how the presence of apoE4 affects the outcomes. Future studies should also be focused on addressing why a single amino acid change from apoE3 to apoE4 causes profound differences in their properties and functions and how we can utilize our knowledge to design new therapies for AD targeting apoE pathway.

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### REFERENCES

- Aguzzi, A., Barres, B.A., and Bennett, M.L. (2013). Microglia: scapegoat, saboteur, or something else? *Science* 339, 156–161.
- Baitsch, D., Bock, H.H., Engel, T., Telgmann, R., Müller-Tidow, C., Varga, G., Bot, M., Herz, J., Robenek, H., von Eckardstein, A., and Nofer, J.R. (2011). Apolipoprotein E induces antiinflammatory phenotype in macrophages. *Arterioscler. Thromb. Vasc. Biol.* 31, 1160–1168.
- Bales, K.R., Verina, T., Dodel, R.C., Du, Y., Altstiel, L., Bender, M., Hyslop, P., Johnstone, E.M., Little, S.P., Cummins, D.J., et al. (1997). Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Genet.* 17, 263–264.
- Bales, K.R., Liu, F., Wu, S., Lin, S., Koger, D., DeLong, C., Hansen, J.C., Sullivan, P.M., and Paul, S.M. (2009). Human APOE isoform-dependent effects on brain beta-amyloid levels in PDAPP transgenic mice. *J. Neurosci.* 29, 6771–6779.
- Bamberger, M.E., Harris, M.E., McDonald, D.R., Husemann, J., and Landreth, G.E. (2003). A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. *J. Neurosci.* 23, 2665–2674.
- Basak, J.M., Verghese, P.B., Yoon, H., Kim, J., and Holtzman, D.M. (2012). Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of A $\beta$  uptake and degradation by astrocytes. *J. Biol. Chem.* 287, 13959–13971.
- Bateman, R.J., Munsell, L.Y., Morris, J.C., Swann, R., Yarasheski, K.E., and Holtzman, D.M. (2006). Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. *Nat. Med.* 12, 856–861.
- Beisiegel, U., Weber, W., Ihrke, G., Herz, J., and Stanley, K.K. (1989). The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 341, 162–164.
- Bell, R.D., Sagare, A.P., Friedman, A.E., Bedi, G.S., Holtzman, D.M., Deane, R., and Zlokovic, B.V. (2007). Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. *J. Cereb. Blood Flow Metab.* 27, 909–918.
- Bell, R.D., Deane, R., Chow, N., Long, X., Sagare, A., Singh, I., Streb, J.W., Guo, H., Rubio, A., Van Nostrand, W., et al. (2009). SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. *Nat. Cell Biol.* 11, 143–153.
- Bell, R.D., Winkler, E.A., Singh, I., Sagare, A.P., Deane, R., Wu, Z., Holtzman, D.M., Betsholtz, C., Armulik, A., Sallstrom, J., et al. (2012). Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* 485, 512–516.
- Bergamaschini, L., Rossi, E., Storini, C., Pizzimenti, S., Distaso, M., Perego, C., De Luigi, A., Vergani, C., and De Simoni, M.G. (2004). Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. *J. Neurosci.* 24, 4181–4186.
- Bien-Ly, N., Gillespie, A.K., Walker, D., Yoon, S.Y., and Huang, Y. (2012). Reducing human apolipoprotein E levels attenuates age-dependent A $\beta$

- accumulation in mutant human amyloid precursor protein transgenic mice. *J. Neurosci.* 32, 4803–4811.
- Brunden, K.R., Richter-Cook, N.J., Chaturvedi, N., and Frederickson, R.C. (1993). pH-dependent binding of synthetic beta-amyloid peptides to glycosaminoglycans. *J. Neurochem.* 61, 2147–2154.
- Bu, G. (2009). Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat. Rev. Neurosci.* 10, 333–344.
- Cao, D., Fukuchi, K., Wan, H., Kim, H., and Li, L. (2006). Lack of LDL receptor aggravates learning deficits and amyloid deposits in Alzheimer transgenic mice. *Neurobiol. Aging* 27, 1632–1643.
- Castano, E.M., Prelli, F., Wisniewski, T., Golabek, A., Kumar, R.A., Soto, C., and Frangione, B. (1995). Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E. *Biochem. J.* 306, 599–604.
- Castellano, J.M., Kim, J., Stewart, F.R., Jiang, H., DeMattos, R.B., Patterson, B.W., Fagan, A.M., Morris, J.C., Mawuenyega, K.G., Cruchaga, C., et al. (2011). Human apoE isoforms differentially regulate brain amyloid- $\beta$  peptide clearance. *Sci. Transl. Med.* 3, 89ra57.
- Castellano, J.M., Deane, R., Gottesdiener, A.J., Verghese, P.B., Stewart, F.R., West, T., Paoletti, A.C., Kasper, T.R., DeMattos, R.B., Zlokovic, B.V., and Holtzman, D.M. (2012). Low-density lipoprotein receptor overexpression enhances the rate of brain-to-blood A $\beta$  clearance in a mouse model of  $\beta$ -amyloidosis. *Proc. Natl. Acad. Sci. USA* 109, 15502–15507.
- Cerf, E., Gustot, A., Goormaghtigh, E., Ruyschaert, J.M., and Raussens, V. (2011). High ability of apolipoprotein E4 to stabilize amyloid- $\beta$  peptide oligomers, the pathological entities responsible for Alzheimer's disease. *FASEB J.* 25, 1585–1595.
- Chan, W., Fornwald, J., Brawner, M., and Wetzel, R. (1996). Native complex formation between apolipoprotein E isoforms and the Alzheimer's disease peptide A beta. *Biochemistry* 35, 7123–7130.
- Chartier-Harlin, M.C., Parfitt, M., Legrain, S., Pérez-Tur, J., Brousseau, T., Evans, A., Berr, C., Vidal, O., Roques, P., Gourlet, V., et al. (1994). Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. *Hum. Mol. Genet.* 3, 569–574.
- Chen, J., Li, Q., and Wang, J. (2011). Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. *Proc. Natl. Acad. Sci. USA* 108, 14813–14818.
- Chen, H.K., Liu, Z., Meyer-Franke, A., Brodbeck, J., Miranda, R.D., McGuire, J.G., Pleiss, M.A., Ji, Z.S., Balestra, M.E., Walker, D.W., et al. (2012). Small molecule structure correctors abolish detrimental effects of apolipoprotein E4 in cultured neurons. *J. Biol. Chem.* 287, 5253–5266.
- Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., and Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921–923.
- Cramer, P.E., Cirrito, J.R., Wesson, D.W., Lee, C.Y., Karlo, J.C., Zinn, A.E., Casali, B.T., Restivo, J.L., Goebel, W.D., James, M.J., et al. (2012). ApoE-directed therapeutics rapidly clear  $\beta$ -amyloid and reverse deficits in AD mouse models. *Science* 335, 1503–1506.
- Cruchaga, C., Kauwe, J.S., Nowotny, P., Bales, K., Pickering, E.H., Mayo, K., Bertelsen, S., Hinrichs, A., Fagan, A.M., Holtzman, D.M., et al.; Alzheimer's Disease Neuroimaging Initiative (2012). Cerebrospinal fluid APOE levels: an endophenotype for genetic studies for Alzheimer's disease. *Hum. Mol. Genet.* 21, 4558–4571.
- Cudaback, E., Li, X., Montine, K.S., Montine, T.J., and Keene, C.D. (2011). Apolipoprotein E isoform-dependent microglia migration. *FASEB J.* 25, 2082–2091.
- Davignon, J., Gregg, R.E., and Sing, C.F. (1988). Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 8, 1–21.
- de Boer, A.G., and Gaillard, P.J. (2007). Drug targeting to the brain. *Annu. Rev. Pharmacol. Toxicol.* 47, 323–355.
- de la Torre, J.C. (2002). Alzheimer disease as a vascular disorder: nosological evidence. *Stroke* 33, 1152–1162.
- Deane, R., Wu, Z., Sagare, A., Davis, J., Du Yan, S., Hamm, K., Xu, F., Parisi, M., LaRue, B., Hu, H.W., et al. (2004). LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron* 43, 333–344.
- Deane, R., Sagare, A., Hamm, K., Parisi, M., Lane, S., Finn, M.B., Holtzman, D.M., and Zlokovic, B.V. (2008). apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. *J. Clin. Invest.* 118, 4002–4013.
- DeMattos, R.B., Cirrito, J.R., Parsadanian, M., May, P.C., O'Dell, M.A., Taylor, J.W., Harmony, J.A., Aronow, B.J., Bales, K.R., Paul, S.M., and Holtzman, D.M. (2004). ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism in vivo. *Neuron* 41, 193–202.
- Dong, L.M., Wilson, C., Wardell, M.R., Simmons, T., Mahley, R.W., Weisgraber, K.H., and Agard, D.A. (1994). Human apolipoprotein E. Role of arginine 61 in mediating the lipoprotein preferences of the E3 and E4 isoforms. *J. Biol. Chem.* 269, 22358–22365.
- Eishourbagy, N.A., Liao, W.S., Mahley, R.W., and Taylor, J.M. (1985). Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc. Natl. Acad. Sci. USA* 82, 203–207.
- Fagan, A.M., Mintun, M.A., Mach, R.H., Lee, S.Y., Dence, C.S., Shah, A.R., LaRossa, G.N., Spinner, M.L., Klunk, W.E., Mathis, C.A., et al. (2006). Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. *Ann. Neurol.* 59, 512–519.
- Fan, Q.W., losbe, I., Asou, H., Yanagisawa, K., and Michikawa, M. (2001). Expression and regulation of apolipoprotein E receptors in the cells of the central nervous system in culture: A review. *J. Am. Aging Assoc.* 24, 1–10.
- Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., Pericak-Vance, M.A., Risch, N., and van Duijn, C.M.; APOE and Alzheimer Disease Meta Analysis Consortium (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. *JAMA* 278, 1349–1356.
- Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E.A., Frosch, M.P., Myers, C.B., Tanzi, R.E., Selkoe, D.J., and Guenette, S. (2003). Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc. Natl. Acad. Sci. USA* 100, 4162–4167.
- Farris, W., Schütz, S.G., Cirrito, J.R., Shankar, G.M., Sun, X., George, A., Leissring, M.A., Walsh, D.M., Qiu, W.Q., Holtzman, D.M., and Selkoe, D.J. (2007). Loss of neprilysin function promotes amyloid plaque formation and causes cerebral amyloid angiopathy. *Am. J. Pathol.* 171, 241–251.
- Fitz, N.F., Cronican, A.A., Saleem, M., Fauq, A.H., Chapman, R., Lefterov, I., and Koldamova, R. (2012). Abca1 deficiency affects Alzheimer's disease-like phenotype in human ApoE4 but not in ApoE3-targeted replacement mice. *J. Neurosci.* 32, 13125–13136.
- Fryer, J.D., DeMattos, R.B., McCormick, L.M., O'Dell, M.A., Spinner, M.L., Bales, K.R., Paul, S.M., Sullivan, P.M., Parsadanian, M., Bu, G., and Holtzman, D.M. (2005a). The low density lipoprotein receptor regulates the level of central nervous system human and murine apolipoprotein E but does not modify amyloid plaque pathology in PDAPP mice. *J. Biol. Chem.* 280, 25754–25759.
- Fryer, J.D., Simmons, K., Parsadanian, M., Bales, K.R., Paul, S.M., Sullivan, P.M., and Holtzman, D.M. (2005b). Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. *J. Neurosci.* 25, 2803–2810.
- Futamura, M., Dhanasekaran, P., Handa, T., Phillips, M.C., Lund-Katz, S., and Saito, H. (2005). Two-step mechanism of binding of apolipoprotein E to heparin: implications for the kinetics of apolipoprotein E-heparan sulfate proteoglycan complex formation on cell surfaces. *J. Biol. Chem.* 280, 5414–5422.
- Garai, K., Baban, B., and Frieden, C. (2011). Self-association and stability of the ApoE isoforms at low pH: implications for ApoE-lipid interactions. *Biochemistry* 50, 6356–6364.
- Golabek, A.A., Soto, C., Vogel, T., and Wisniewski, T. (1996). The interaction between apolipoprotein E and Alzheimer's amyloid beta-peptide is dependent on beta-peptide conformation. *J. Biol. Chem.* 271, 10602–10606.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogava, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J.S., Younkin, S., et al. (2013). TREM2 variants in Alzheimer's disease. *N. Engl. J. Med.* 368, 117–127.

Guo, J.L., Covell, D.J., Daniels, J.P., Iba, M., Stieber, A., Zhang, B., Riddle, D.M., Kwong, L.K., Xu, Y., Trojanowski, J.Q., and Lee, V.M. (2013). Distinct  $\alpha$ -synuclein strains differentially promote tau inclusions in neurons. *Cell* 154, 103–117.

Gylys, K.H., Fein, J.A., Tan, A.M., and Cole, G.M. (2003). Apolipoprotein E enhances uptake of soluble but not aggregated amyloid-beta protein into synaptic terminals. *J. Neurochem.* 84, 1442–1451.

Hardy, J., and Selkoe, D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–356.

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M.L., Pahwa, J.S., Moskva, V., Dowzell, K., Williams, A., et al. (2009). Genome-wide association study identifies variants at *CLU* and *PICALM* associated with Alzheimer's disease. *Nat. Genet.* 41, 1088–1093.

Harper, J.D., and Lansbury, P.T., Jr. (1997). Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu. Rev. Biochem.* 66, 385–407.

Hashimoto, T., Serrano-Pozo, A., Hori, Y., Adams, K.W., Takeda, S., Banerji, A.O., Mitani, A., Joyner, D., Thyssen, D.H., Bacskai, B.J., et al. (2012). Apolipoprotein E, especially apolipoprotein E4, increases the oligomerization of amyloid  $\beta$  peptide. *J. Neurosci.* 32, 15181–15192.

Hatters, D.M., Budamagunta, M.S., Voss, J.C., and Weisgraber, K.H. (2005). Modulation of apolipoprotein E structure by domain interaction: differences in lipid-bound and lipid-free forms. *J. Biol. Chem.* 280, 34288–34295.

Hatters, D.M., Peters-Libe, C.A., and Weisgraber, K.H. (2006a). Apolipoprotein E structure: insights into function. *Trends Biochem. Sci.* 31, 445–454.

Hatters, D.M., Zhong, N., Rutenber, E., and Weisgraber, K.H. (2006b). Amino-terminal domain stability mediates apolipoprotein E aggregation into neurotoxic fibrils. *J. Mol. Biol.* 361, 932–944.

Herz, J., and Strickland, D.K. (2001). LRP: a multifunctional scavenger and signaling receptor. *J. Clin. Invest.* 108, 779–784.

Hesse, C., Larsson, H., Fredman, P., Minthon, L., Andreasen, N., Davidsson, P., and Blennow, K. (2000). Measurement of apolipoprotein E (apoE) in cerebrospinal fluid. *Neurochem. Res.* 25, 511–517.

Hollingworth, P., Harold, D., Sims, R., Gerrish, A., Lambert, J.C., Carrasquillo, M.M., Abraham, R., Hamshere, M.L., Pahwa, J.S., Moskva, V., et al. (2011). Alzheimer's Disease Neuroimaging Initiative; CHARGE consortium; EADI1 consortium (2011). Common variants at *ABCA7*, *MS4A6A/MS4A4E*, *EPHA1*, *CD33* and *CD2AP* are associated with Alzheimer's disease. *Nat. Genet.* 43, 429–435.

Holmes, B.B., DeVos, S.L., Kfoury, N., Li, M., Jacks, R., Yanamandra, K., Ouidja, M.O., Brodsky, F.M., Marasa, J., Bagchi, D.P., et al. (2013). Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc. Natl. Acad. Sci. USA* 110, E3138–E3147.

Holtzman, D.M., Bales, K.R., Wu, S., Bhat, P., Parsadanian, M., Fagan, A.M., Chang, L.K., Sun, Y., and Paul, S.M. (1999). Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* 103, R15–R21.

Houlden, H., Crook, R., Backhovens, H., Prihar, G., Baker, M., Hutton, M., Rossor, M., Martin, J.J., Van Broeckhoven, C., and Hardy, J. (1998). ApoE genotype is a risk factor in nonpresenilin early-onset Alzheimer's disease families. *Am. J. Med. Genet.* 81, 117–121.

Hu, X., Crick, S.L., Bu, G., Frieden, C., Pappu, R.V., and Lee, J.M. (2009). Amyloid seeds formed by cellular uptake, concentration, and aggregation of the amyloid-beta peptide. *Proc. Natl. Acad. Sci. USA* 106, 20324–20329.

Huang, Y., and Mucke, L. (2012). Alzheimer mechanisms and therapeutic strategies. *Cell* 148, 1204–1222.

Iliff, J.J., Wang, M., Liao, Y., Plogg, B.A., Peng, W., Gundersen, G.A., Benveniste, H., Vates, G.E., Deane, R., Goldman, S.A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Sci. Transl. Med.* 4, 147ra111.

Innerarity, T.L., and Mahley, R.W. (1978). Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoproteins. *Biochemistry* 17, 1440–1447.

Innerarity, T.L., Pitas, R.E., and Mahley, R.W. (1979). Binding of arginine-rich (E) apoprotein after recombination with phospholipid vesicles to the low density lipoprotein receptors of fibroblasts. *J. Biol. Chem.* 254, 4186–4190.

Irizarry, M.C., Rebeck, G.W., Cheung, B., Bales, K., Paul, S.M., Holtzman, D., and Hyman, B.T. (2000). Modulation of A beta deposition in APP transgenic mice by an apolipoprotein E null background. *Ann. N Y Acad. Sci.* 920, 171–178.

Ito, S., Matsumiya, K., Ohtsuki, S., Kamiie, J., and Terasaki, T. (2013). Contributions of degradation and brain-to-blood elimination across the blood-brain barrier to cerebral clearance of human amyloid-beta peptide(1–40) in mouse brain. *J. Cereb. Blood Flow Metab.* 33, 1770–1777.

Iwata, N., Tsubuki, S., Takaki, Y., Watanabe, K., Sekiguchi, M., Hosoki, E., Kawashima-Morishima, M., Lee, H.J., Hama, E., Sekine-Aizawa, Y., and Saito, T.C. (2000). Identification of the major Abeta1–42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat. Med.* 6, 143–150.

Ji, Z.S., Brecht, W.J., Miranda, R.D., Hussain, M.M., Innerarity, T.L., and Mahley, R.W. (1993). Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *J. Biol. Chem.* 268, 10160–10167.

Jiang, Q., Lee, C.Y., Mandrekar, S., Wilkinson, B., Cramer, P., Zelcer, N., Mann, K., Lamb, B., Willson, T.M., Collins, J.L., et al. (2008). ApoE promotes the proteolytic degradation of Abeta. *Neuron* 58, 681–693.

Kanekiyo, T., Zhang, J., Liu, Q., Liu, C.C., Zhang, L., and Bu, G. (2011). Heparan sulphate proteoglycan and the low-density lipoprotein receptor-related protein 1 constitute major pathways for neuronal amyloid-beta uptake. *J. Neurosci.* 31, 1644–1651.

Kanekiyo, T., Liu, C.C., Shinohara, M., Li, J., and Bu, G. (2012). LRP1 in brain vascular smooth muscle cells mediates local clearance of Alzheimer's amyloid- $\beta$ . *J. Neurosci.* 32, 16458–16465.

Kanekiyo, T., Cirrito, J.R., Liu, C.C., Shinohara, M., Li, J., Schuler, D.R., Shinohara, M., Holtzman, D.M., and Bu, G. (2013). Neuronal clearance of amyloid- $\beta$  by endocytic receptor LRP1. *J. Neurosci.* 33, 19276–19283.

Kim, D.H., Iijima, H., Goto, K., Sakai, J., Ishii, H., Kim, H.J., Suzuki, H., Kondo, H., Saeki, S., and Yamamoto, T. (1996). Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. *J. Biol. Chem.* 271, 8373–8380.

Kim, J., Castellano, J.M., Jiang, H., Basak, J.M., Parsadanian, M., Pham, V., Mason, S.M., Paul, S.M., and Holtzman, D.M. (2009). Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular A beta clearance. *Neuron* 64, 632–644.

Kim, J., Jiang, H., Park, S., Eltorai, A.E., Stewart, F.R., Yoon, H., Basak, J.M., Finn, M.B., and Holtzman, D.M. (2011). Haploinsufficiency of human APOE reduces amyloid deposition in a mouse model of amyloid- $\beta$  amyloidosis. *J. Neurosci.* 31, 18007–18012.

Kim, J., Eltorai, A.E., Jiang, H., Liao, F., Verghese, P.B., Kim, J., Stewart, F.R., Basak, J.M., and Holtzman, D.M. (2012). Anti-apoE immunotherapy inhibits amyloid accumulation in a transgenic mouse model of A $\beta$  amyloidosis. *J. Exp. Med.* 209, 2149–2156.

Knauper, M.F., Soreghan, B., Burdick, D., Kosmoski, J., and Glabe, C.G. (1992). Intracellular accumulation and resistance to degradation of the Alzheimer amyloid A4/beta protein. *Proc. Natl. Acad. Sci. USA* 89, 7437–7441.

Koistinaho, M., Lin, S., Wu, X., Esterman, M., Koger, D., Hanson, J., Higgs, R., Liu, F., Malkani, S., Bales, K.R., and Paul, S.M. (2004). Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. *Nat. Med.* 10, 719–726.

- Kok, E., Haikonen, S., Luoto, T., Huhtala, H., Goebeler, S., Haapasalo, H., and Karhunen, P.J. (2009). Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. *Ann. Neurol.* 65, 650–657.
- Koldamova, R., Fitz, N.F., and Lefterov, I. (2010). The role of ATP-binding cassette transporter A1 in Alzheimer's disease and neurodegeneration. *Biochim. Biophys. Acta* 1801, 824–830.
- Kowal, R.C., Herz, J., Goldstein, J.L., Esser, V., and Brown, M.S. (1989). Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA* 86, 5810–5814.
- Kowal, R.C., Herz, J., Weisgraber, K.H., Mahley, R.W., Brown, M.S., and Goldstein, J.L. (1990). Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. *J. Biol. Chem.* 265, 10771–10779.
- LaDu, M.J., Falduto, M.T., Manelli, A.M., Reardon, C.A., Getz, G.S., and Frail, D.E. (1994). Isoform-specific binding of apolipoprotein E to beta-amyloid. *J. Biol. Chem.* 269, 23403–23406.
- LaDu, M.J., Pederson, T.M., Frail, D.E., Reardon, C.A., Getz, G.S., and Falduto, M.T. (1995). Purification of apolipoprotein E attenuates isoform-specific binding to beta-amyloid. *J. Biol. Chem.* 270, 9039–9042.
- LaDu, M.J., Gilligan, S.M., Lukens, J.R., Cabana, V.G., Reardon, C.A., Van Eldik, L.J., and Holtzman, D.M. (1998). Nascent astrocyte particles differ from lipoproteins in CSF. *J. Neurochem.* 70, 2070–2081.
- Lambert, J.C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., Combarros, O., Zelenika, D., Bullido, M.J., Tavernier, B., et al.; European Alzheimer's Disease Initiative Investigators (2009). Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat. Genet.* 41, 1094–1099.
- Laporte, V., Lombard, Y., Levy-Benezra, R., Tranchant, C., Poindron, P., and Warter, J.M. (2004). Uptake of Abeta 1-40- and Abeta 1-42-coated yeast by microglial cells: a role for LRP. *J. Leukoc. Biol.* 76, 451–461.
- Laurén, J., Gimbel, D.A., Nygaard, H.B., Gilbert, J.W., and Strittmatter, S.M. (2009). Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457, 1128–1132.
- Leissring, M.A., Farris, W., Chang, A.Y., Walsh, D.M., Wu, X., Sun, X., Frosch, M.P., and Selkoe, D.J. (2003). Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron* 40, 1087–1093.
- Li, J., Kanekiyo, T., Shinohara, M., Zhang, Y., LaDu, M.J., Xu, H., and Bu, G. (2012). Differential regulation of amyloid- $\beta$  endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms. *J. Biol. Chem.* 287, 44593–44601.
- Liu, Q., Zerbinatti, C.V., Zhang, J., Hoe, H.S., Wang, B., Cole, S.L., Herz, J., Muglia, L., and Bu, G. (2007). Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1. *Neuron* 56, 66–78.
- Liu, C.C., Kanekiyo, T., Xu, H., and Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat. Rev. Neurol.* 9, 106–118.
- Ma, J., Yee, A., Brewer, H.B., Jr., Das, S., and Potter, H. (1994). Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 372, 92–94.
- Mahley, R.W. (1988). Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622–630.
- Mahley, R.W., and Rall, S.C., Jr. (2000). Apolipoprotein E: far more than a lipid transport protein. *Annu. Rev. Genomics Hum. Genet.* 1, 507–537.
- Mahley, R.W., Weisgraber, K.H., and Huang, Y. (2009). Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J. Lipid Res.* 50 (Suppl.), S183–S188.
- Mandrekar, S., Jiang, Q., Lee, C.Y., Koenigsnecht-Talboo, J., Holtzman, D.M., and Landreth, G.E. (2009). Microglia mediate the clearance of soluble Abeta through fluid phase macropinocytosis. *J. Neurosci.* 29, 4252–4262.
- Marques, F., Sousa, J.C., Sousa, N., and Palha, J.A. (2013). Blood-brain-barriers in aging and in Alzheimer's disease. *Mol. Neurodegener.* 8, 38.
- Mawuenyega, K.G., Sigurdson, W., Ovod, V., Munsell, L., Kasten, T., Morris, J.C., Yarasheski, K.E., and Bateman, R.J. (2010). Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 330, 1774.
- Mucke, L., and Selkoe, D.J. (2012). Neurotoxicity of amyloid  $\beta$ -protein: synaptic and network dysfunction. *Cold Spring Harb. Perspect. Med.* 2, a006338.
- Naiki, H., Gejyo, F., and Nakakuki, K. (1997). Concentration-dependent inhibitory effects of apolipoprotein E on Alzheimer's beta-amyloid fibril formation in vitro. *Biochemistry* 36, 6243–6250.
- Naj, A.C., Jun, G., Beecham, G.W., Wang, L.S., Vardarajan, B.N., Buross, J., Gallins, P.J., Buxbaum, J.D., Jarvik, G.P., Crane, P.K., et al. (2011). Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat. Genet.* 43, 436–441.
- Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E., and Ikeda, K. (1991). Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res.* 541, 163–166.
- Narita, M., Holtzman, D.M., Schwartz, A.L., and Bu, G. (1997). Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein. *J. Neurochem.* 69, 1904–1911.
- Nielsen, H.M., Mulder, S.D., Beliën, J.A., Musters, R.J., Eikelenboom, P., and Veerhuis, R. (2010). Astrocytic A beta 1-42 uptake is determined by A beta-aggregation state and the presence of amyloid-associated proteins. *Glia* 58, 1235–1246.
- Nishitsuji, K., Hosono, T., Nakamura, T., Bu, G., and Michikawa, M. (2011). Apolipoprotein E regulates the integrity of tight junctions in an isoform-dependent manner in an in vitro blood-brain barrier model. *J. Biol. Chem.* 286, 17536–17542.
- Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., et al. (2006). Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J. Neurosci.* 26, 10129–10140.
- Perálvarez-Marín, A., Barth, A., and Gräslund, A. (2008). Time-resolved infrared spectroscopy of pH-induced aggregation of the Alzheimer Abeta(1-28) peptide. *J. Mol. Biol.* 379, 589–596.
- Pérez, E., Bourguet, W., Gronemeyer, H., and de Lera, A.R. (2012). Modulation of RXR function through ligand design. *Biochim. Biophys. Acta* 1821, 57–69.
- Petrova, J., Hong, H.S., Bricarello, D.A., Harishchandra, G., Lorigan, G.A., Jin, L.W., and Voss, J.C. (2011). A differential association of Apolipoprotein E isoforms with the amyloid- $\beta$  oligomer in solution. *Proteins* 79, 402–416.
- Pitas, R.E., Boyles, J.K., Lee, S.H., Foss, D., and Mahley, R.W. (1987a). Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochim. Biophys. Acta* 917, 148–161.
- Pitas, R.E., Boyles, J.K., Lee, S.H., Hui, D., and Weisgraber, K.H. (1987b). Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B,E(LDL) receptors in the brain. *J. Biol. Chem.* 262, 14352–14360.
- Polvikoski, T., Sulkava, R., Haltia, M., Kainulainen, K., Vuorio, A., Verkkoniemi, A., Niinistö, L., Halonen, P., and Kontula, K. (1995). Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N. Engl. J. Med.* 333, 1242–1247.
- Rall, S.C., Jr., Weisgraber, K.H., and Mahley, R.W. (1982). Human apolipoprotein E. The complete amino acid sequence. *J. Biol. Chem.* 257, 4171–4178.
- Reiman, E.M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., Ayutyanont, N., Keppler, J., Reeder, S.A., Langbaum, J.B.S., et al. (2009). Fibrillar amyloid- $\beta$  burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 106, 6820–6825.
- Rensink, A.A., de Waal, R.M., Kremer, B., and Verbeek, M.M. (2003). Pathogenesis of cerebral amyloid angiopathy. *Brain Res. Brain Res. Rev.* 43, 207–223.

- Revesz, T., Ghiso, J., Lashley, T., Plant, G., Rostagno, A., Frangione, B., and Holton, J.L. (2003). Cerebral amyloid angiopathies: a pathologic, biochemical, and genetic view. *J. Neuropathol. Exp. Neurol.* *62*, 885–898.
- Richard, E., Carrano, A., Hoozemans, J.J., van Horsen, J., van Haastert, E.S., Eurelings, L.S., de Vries, H.E., Thal, D.R., Eikelenboom, P., van Gool, W.A., and Rozemuller, A.J. (2010). Characteristics of dyschoric capillary cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol.* *69*, 1158–1167.
- Riddell, D.R., Zhou, H., Comery, T.A., Kouranova, E., Lo, C.F., Warwick, H.K., Ring, R.H., Kirksey, Y., Aschmies, S., Xu, J., et al. (2007). The LXR agonist TO901317 selectively lowers hippocampal Abeta42 and improves memory in the Tg2576 mouse model of Alzheimer's disease. *Mol. Cell. Neurosci.* *34*, 621–628.
- Rushworth, J.V., Griffiths, H.H., Watt, N.T., and Hooper, N.M. (2013). Prion protein-mediated toxicity of amyloid- $\beta$  oligomers requires lipid rafts and the transmembrane LRP1. *J. Biol. Chem.* *288*, 8935–8951.
- Russo, C., Angelini, G., Dapino, D., Piccini, A., Piombo, G., Schettini, G., Chen, S., Teller, J.K., Zaccaro, D., Gambetti, P., and Tabaton, M. (1998). Opposite roles of apolipoprotein E in normal brains and in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* *95*, 15598–15602.
- Sadowski, M., Pankiewicz, J., Scholtzova, H., Ripellino, J.A., Li, Y., Schmidt, S.D., Mathews, P.M., Fryer, J.D., Holtzman, D.M., Sigurdsson, E.M., and Wisniewski, T. (2004). A synthetic peptide blocking the apolipoprotein E/ beta-amyloid binding mitigates beta-amyloid toxicity and fibril formation in vitro and reduces beta-amyloid plaques in transgenic mice. *Am. J. Pathol.* *165*, 937–948.
- Saido, T., and Leissring, M.A. (2012). Proteolytic degradation of amyloid  $\beta$ -protein. *Cold Spring Harb. Perspect. Med.* *2*, a006379.
- Saito, H., Dhanasekaran, P., Nguyen, D., Baldwin, F., Weisgraber, K.H., Wehrli, S., Phillips, M.C., and Lund-Katz, S. (2003). Characterization of the heparin binding sites in human apolipoprotein E. *J. Biol. Chem.* *278*, 14782–14787.
- Schneider, W.J., Kovanen, P.T., Brown, M.S., Goldstein, J.L., Utermann, G., Weber, W., Havel, R.J., Kotite, L., Kane, J.P., Innerarity, T.L., and Mahley, R.W. (1981). Familial dysbetalipoproteinemia. Abnormal binding of mutant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits, and cows. *J. Clin. Invest.* *68*, 1075–1085.
- Shibata, M., Yamada, S., Kumar, S.R., Calero, M., Bading, J., Frangione, B., Holtzman, D.M., Miller, C.A., Strickland, D.K., Ghiso, J., and Zlokovic, B.V. (2000). Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J. Clin. Invest.* *106*, 1489–1499.
- Shinohara, M., Sato, N., Kurinami, H., Takeuchi, D., Takeda, S., Shimamura, M., Yamashita, T., Uchiyama, Y., Rakugi, H., and Morishita, R. (2010). Reduction of brain beta-amyloid (Abeta) by fluvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, through increase in degradation of amyloid precursor protein C-terminal fragments (APP-CTFs) and Abeta clearance. *J. Biol. Chem.* *285*, 22091–22102.
- Shinohara, M., Petersen, R.C., Dickson, D.W., and Bu, G. (2013). Brain regional correlation of amyloid- $\beta$  with synapses and apolipoprotein E in non-demented individuals: potential mechanisms underlying regional vulnerability to amyloid- $\beta$  accumulation. *Acta Neuropathol.* *125*, 535–547.
- Sivashanmugam, A., and Wang, J. (2009). A unified scheme for initiation and conformational adaptation of human apolipoprotein E N-terminal domain upon lipoprotein binding and for receptor binding activity. *J. Biol. Chem.* *284*, 14657–14666.
- Strittmatter, W.J., Weisgraber, K.H., Huang, D.Y., Dong, L.M., Salvesen, G.S., Pericak-Vance, M., Schmechel, D., Saunders, A.M., Goldgaber, D., and Roses, A.D. (1993). Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA* *90*, 8098–8102.
- Tai, L.M., Bilousova, T., Jungbauer, L., Roeske, S.K., Youmans, K.L., Yu, C., Poon, W.W., Cornwell, L.B., Miller, C.A., Vinters, H.V., et al. (2013). Levels of soluble apolipoprotein E/amyloid- $\beta$  (A $\beta$ ) complex are reduced and oligomeric A $\beta$  increased with APOE4 and Alzheimer disease in a transgenic mouse model and human samples. *J. Biol. Chem.* *288*, 5914–5926.
- Tamamizu-Kato, S., Cohen, J.K., Drake, C.B., Kosaraju, M.G., Drury, J., and Narayanaswami, V. (2008). Interaction with amyloid beta peptide compromises the lipid binding function of apolipoprotein E. *Biochemistry* *47*, 5225–5234.
- Terwel, D., Steffensen, K.R., Verghese, P.B., Kummer, M.P., Gustafsson, J.A., Holtzman, D.M., and Heneka, M.T. (2011). Critical role of astroglial apolipoprotein E and liver X receptor- $\alpha$  expression for microglial A $\beta$  phagocytosis. *J. Neurosci.* *31*, 7049–7059.
- Thal, D.R., Ghebremedhin, E., Rüb, U., Yamaguchi, H., Del Tredici, K., and Braak, H. (2002). Two types of sporadic cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol.* *61*, 282–293.
- Thies, W., and Bleiler, L.; Alzheimer's Association (2013). 2013 Alzheimer's disease facts and figures. *Alzheimers Dement.* *9*, 208–245.
- Tokuda, T., Calero, M., Matsubara, E., Vidal, R., Kumar, A., Permanne, B., Zlokovic, B., Smith, J.D., Ladu, M.J., Rostagno, A., et al. (2000). Lipidation of apolipoprotein E influences its isoform-specific interaction with Alzheimer's amyloid beta peptides. *Biochem. J.* *348*, 359–365.
- Utermann, G., Hees, M., and Steinmetz, A. (1977). Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. *Nature* *269*, 604–607.
- van Horsen, J., Wesseling, P., van den Heuvel, L.P., de Waal, R.M., and Verbeek, M.M. (2003). Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. *Lancet Neurol.* *2*, 482–492.
- Verghese, P.B., Castellano, J.M., Garai, K., Wang, Y., Jiang, H., Shah, A., Bu, G., Frieden, C., and Holtzman, D.M. (2013). ApoE influences amyloid- $\beta$  (A $\beta$ ) clearance despite minimal apoE/A $\beta$  association in physiological conditions. *Proc. Natl. Acad. Sci. USA* *110*, E1807–E1816.
- Vincent, B., and Smith, J.D. (2001). Astrocytes down-regulate neuronal beta-amyloid precursor protein expression and modify its processing in an apolipoprotein E isoform-specific manner. *Eur. J. Neurosci.* *14*, 256–266.
- Wahrle, S.E., Jiang, H., Parsadanian, M., Hartman, R.E., Bales, K.R., Paul, S.M., and Holtzman, D.M. (2005). Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. *J. Biol. Chem.* *280*, 43236–43242.
- Wahrle, S.E., Shah, A.R., Fagan, A.M., Smemo, S., Kauwe, J.S., Grupe, A., Hinrichs, A., Mayo, K., Jiang, H., Thal, L.J., et al. (2007). Apolipoprotein E levels in cerebrospinal fluid and the effects of ABCA1 polymorphisms. *Mol. Neurodegener.* *2*, 7.
- Wahrle, S.E., Jiang, H., Parsadanian, M., Kim, J., Li, A., Knoten, A., Jain, S., Hirsch-Reinshagen, V., Wellington, C.L., Bales, K.R., et al. (2008). Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease. *J. Clin. Invest.* *118*, 671–682.
- Wang, H., Ren, C.H., Gunawardana, C.G., and Schmitt-Ulms, G. (2013). Overcoming barriers and thresholds - signaling of oligomeric A $\beta$  through the prion protein to Fyn. *Mol. Neurodegener.* *8*, 24.
- Weisgraber, K.H., Rall, S.C., Jr., and Mahley, R.W. (1981). Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J. Biol. Chem.* *256*, 9077–9083.
- Wernette-Hammond, M.E., Lauer, S.J., Corsini, A., Walker, D., Taylor, J.M., and Rall, S.C., Jr. (1989). Glycosylation of human apolipoprotein E. The carbohydrate attachment site is threonine 194. *J. Biol. Chem.* *264*, 9094–9101.
- Wilsie, L.C., and Orlando, R.A. (2003). The low density lipoprotein receptor-related protein complexes with cell surface heparan sulfate proteoglycans to regulate proteoglycan-mediated lipoprotein catabolism. *J. Biol. Chem.* *278*, 15758–15764.
- Wilson, C., Wardell, M.R., Weisgraber, K.H., Mahley, R.W., and Agard, D.A. (1991). Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. *Science* *252*, 1817–1822.
- Winkler, K., Schamagl, H., Tisjar, U., Hoschützky, H., Friedrich, I., Hoffmann, M.M., Hüttinger, M., Wieland, H., and März, W. (1999). Competition of Abeta amyloid peptide and apolipoprotein E for receptor-mediated endocytosis. *J. Lipid Res.* *40*, 447–455.

- Wisniewski, T., Golabek, A., Matsubara, E., Ghiso, J., and Frangione, B. (1993). Apolipoprotein E: binding to soluble Alzheimer's beta-amyloid. *Biochem. Biophys. Res. Commun.* *192*, 359–365.
- Wood, S.J., Chan, W., and Wetzel, R. (1996a). An ApoE-Abeta inhibition complex in Abeta fibril extension. *Chem. Biol.* *3*, 949–956.
- Wood, S.J., Chan, W., and Wetzel, R. (1996b). Seeding of A beta fibril formation is inhibited by all three isoforms of apolipoprotein E. *Biochemistry* *35*, 12623–12628.
- Xu, Q., Brecht, W.J., Weisgraber, K.H., Mahley, R.W., and Huang, Y. (2004). Apolipoprotein E4 domain interaction occurs in living neuronal cells as determined by fluorescence resonance energy transfer. *J. Biol. Chem.* *279*, 25511–25516.
- Xu, Q., Bernardo, A., Walker, D., Kanegawa, T., Mahley, R.W., and Huang, Y. (2006). Profile and regulation of apolipoprotein E (ApoE) expression in the CNS in mice with targeting of green fluorescent protein gene to the ApoE locus. *J. Neurosci.* *26*, 4985–4994.
- Yamada, K., Hashimoto, T., Yabuki, C., Nagae, Y., Tachikawa, M., Strickland, D.K., Liu, Q., Bu, G., Basak, J.M., Holtzman, D.M., et al. (2008). The low density lipoprotein receptor-related protein 1 mediates uptake of amyloid beta peptides in an in vitro model of the blood-brain barrier cells. *J. Biol. Chem.* *283*, 34554–34562.
- Youmans, K.L., Tai, L.M., Nwabuisi-Heath, E., Jungbauer, L., Kanekiyo, T., Gan, M., Kim, J., Eimer, W.A., Estus, S., Rebeck, G.W., et al. (2012). APOE4-specific changes in A $\beta$  accumulation in a new transgenic mouse model of Alzheimer disease. *J. Biol. Chem.* *287*, 41774–41786.
- Zannis, V.I., and Breslow, J.L. (1981). Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and posttranslational modification. *Biochemistry* *20*, 1033–1041.
- Zerbinatti, C.V., Wahrle, S.E., Kim, H., Cam, J.A., Bales, K., Paul, S.M., Holtzman, D.M., and Bu, G. (2006). Apolipoprotein E and low density lipoprotein receptor-related protein facilitate intraneuronal Abeta42 accumulation in amyloid model mice. *J. Biol. Chem.* *281*, 36180–36186.
- Zhu, Y., Nwabuisi-Heath, E., Dumanis, S.B., Tai, L.M., Yu, C., Rebeck, G.W., and LaDu, M.J. (2012). APOE genotype alters glial activation and loss of synaptic markers in mice. *Glia* *60*, 559–569.