The Pathological Cross Talk Between Apolipoprotein E and Amyloid-β Peptide in Alzheimer’s Disease: Emerging Gene-Based Therapeutic Approaches

Sandra Iurescia\textsuperscript{a,1}, Daniela Fioretti\textsuperscript{a,1}, Francesca Mangialasche\textsuperscript{b,c} and Monica Rinaldi\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Institute of Neurobiology and Molecular Medicine, Department of Medicine, National Research Council (CNR), Rome, Italy
\textsuperscript{b}Department of Clinical and Experimental Medicine, Section of Gerontology and Geriatrics University of Perugia, Perugia, Italy
\textsuperscript{c}Aging Research Center, Karolinska Institutet Stockholm, Sweden

Handling Associate Editor: Patrizia Mecocci

Accepted 28 January 2010

Abstract. Apolipoprotein E (ApoE) plays a key role in lipid transport in the plasma and in the central nervous system through its interaction with members of the low-density lipoprotein receptor family. The three common isoforms of ApoE (ApoE2, ApoE3, and ApoE4) differ in their ability to perform neuronal maintenance and repair functions and differentially affect the risk of developing neurodegenerative disorders. The ApoE4 isoform is a strong genetic risk factor for Alzheimer’s disease. Up-to-date knowledge about the structural and biophysical features of ApoE4 shed light on the molecular basis underlying the isoform-specific pathogenic role of ApoE4 and its contribution to AD pathology through several different mechanisms. ApoE4 impacts on amyloid-β (Aβ) production, Aβ clearance, Aβ fibrillation, and tangle formation as well as on mitochondrial functions leading to neuronal toxicity and synaptic damage. This review summarizes the pathological cross talk between ApoE and Aβ peptide in Alzheimer’s disease. Lastly, we examine emerging gene-based therapeutic approaches encompassing the use of ApoE and their potential opportunities to preventing or treating Alzheimer’s disease.

Keywords: Alzheimer’s disease, amyloid-β, apolipoprotein E, gene-based therapy

INTRODUCTION

Research evidence on the prevalence of age-specific dementia estimated that in 2001, 24 million people lived with Alzheimer’s disease (AD) and other dementia worldwide, with 4.6 million new cases developing annually. The number of people affected will double every 20 years to 42 million by 2020 and 81 million by 2040 \cite{1}.
The updated picture of global incidence and prevalence of AD and other dementia estimates 48.1 million for 2020 and 90.3 million for 2040, which are approximately 10% higher than earlier projections (World Alzheimer Report 2009, Alzheimer’s Disease International) [2].

AD is the most common form of age-related neurodegenerative diseases with key symptoms such as progressive decline in memory, impairments in speech, spatial orientation, and dysfunction in the sensorimotor systems. Such behavioral changes lead to serious social and economic consequences [3,4]. The neuropathological markers are neuronal loss and synaptic damage and deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles [5].

Apolipoprotein E (ApoE) is the main lipid transport protein in the brain and plays a pivotal role in the maintenance and repair of neurons. Of the three common structural isoforms, ApoE4 confers the greatest risk of developing AD [6].

In this review, we discuss current knowledge on biophysical and biochemical features of ApoE, which gives insight into how the ApoE4 variant affects AD pathology. Furthermore, we focus on the pathological cross talk between ApoE4 and the amyloid-β (Aβ) peptide highlighting both Aβ-dependent and Aβ-independent pathways. Finally, we describe potential therapeutic strategies that encompass the use of ApoE to blunt or reduce the AD burden.

We systematically searched the PubMed, the National Library of Medicine journal literature search system, for biomedical articles from MEDLINE and life science journals using MeSH and PubMed search tools. The following keywords were used for the search: “apolipoprotein E AND Alzheimer’s disease”, “apolipoprotein E AND Alzheimer’s disease AND mouse model”, “apolipoprotein E receptors”, “apolipoprotein E AND mitochondrial dysfunction”, “ApoE AND Aβ peptide”, “ApoE gene targeting OR gene delivery OR gene therapy Alzheimer’s disease”. Articles were also identified using the “related articles” function in PubMed. Furthermore, we found additional papers by performing a manual search of the reference lists of relevant retrieved articles. A search on the Alzheimer’s Disease International’s (ADI) web site (http://www.alz.co.uk/) was also done to see the World Alzheimer Report 2009 reporting a global prevalence study of dementia.

STRUCTURAL AND BIOPHYSICAL PROPERTIES OF APOLIPOPROTEIN E

Human ApoE is a member of the family of soluble apolipoproteins. The protein is involved in the efficient hepatic uptake of lipoprotein particles and reverse cholesterol transport in the plasma, maintenance of cholesterol homeostasis in cells, such as macrophages, and preventing foam cell formation in the atherosclerotic lesions [7]. Besides its potent anti-atherogenic action, ApoE plays a key role in lipid transport in the central nervous system (CNS). The brain is second only to the liver in the synthesis of ApoE and, within the CNS, ApoE is expressed and secreted by astrocytes and microglia and delivers cholesterol and other essential lipids to neurons through members of low-density lipoprotein receptor (LDLR family) [8]. ApoE is the major carrier of cholesterol, phospholipids, and sulfatides in the brain and has a critical function in promoting neurite outgrowth, repairing injured neurons, and maintaining synapto-dendritic junctions [8,9].

Human ApoE is a 34-kDa protein polymorphic protein arising from three alleles at a single gene locus (APOE gene) on chromosome 19, each isoform containing 199 amino acids (aa). The polymorphic APOE alleles (ε2, ε3, and ε4) engender six different genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4). The most common allele is ε3, therefore ApoE3 is considered to be the parent form of the protein, while ApoE2 and ApoE4 are variants [9]. The three isoforms ApoE2, ApoE3, and ApoE4 differ from one another only by single amino acid substitutions (positions 112 and 158). Nevertheless, these changes have profound functional consequences at both cellular and molecular levels. The most common isoform ApoE3 contains cysteine (Cys) at position 112 and arginine (Arg) at position 158, while the ApoE2 has cysteine at both positions and ApoE4 has arginine at both 112 and 158 positions (Fig. 1A). ApoE has two independently folded structural domains, an N-terminal domain and a C-terminal domain, which are joined by a flexible protease-sensitive loop (“hinge region”) (Fig. 1B) [10].

The ApoE protein interacts with members of the LDLR family through the N-terminal domain, which is enriched in basic residues [10]. The structure of the carboxy-terminal domain is believed to adopt an amphipathic α-helical conformation and contains high-affinity lipid-binding sites (residues 244-272) [11]. The amino acid differences among the three ApoE isoforms occur in critical regions of the amino terminal portion of the molecule which influences the lipoprotein bind-
Fig. 1. A) Model of the domain structure of human apolipoprotein E. B) Schematic representations of human polymorphic ApoE. The 299 amino acids ApoE protein contains two domains: an NH\textsubscript{2}-terminal domain that includes the LDL receptor-binding region and COOH-terminal domain that contains the lipid-binding region. The residues 112 and 158 that characterize the three ApoE isoforms are indicated. Modified from Hatters et al., Trends Biochem Soc, 2006.

The substitution of residue 158 (cysteine for arginine) in the ApoE2 isoform results in reduced LDLR affinity, leading to less efficient lipids transport, whereas the same amino acid substitution at position 112 does not affect the binding activity of ApoE3 compared with ApoE4 [10]. The ApoE4 isoform shows a preferential binding to large low-density lipoproteins while the ApoE3 prefers smaller high-density lipoproteins such as HDLs. The isoform-dependent differences in binding property are referred to as “domain interaction”. In ApoE4, the presence of Arg at position 112 affects the conformation of the side chain of Arg61, allowing an interaction with glutamic acid 255 (Glu255) in the C-terminal domain and resulting in a compact structure (Fig. 2) [10]. Another key property of ApoE4 isoform is its reduced stability relative to ApoE3 and ApoE2: the N-terminal domain is the least resistant to thermal and chemical denaturation and ApoE4 can assume a stable folding intermediates with features of a “molten globule state” [12] (Fig. 2).

In all species except human, the ApoE gene does not have allelic variants and contains threonine (Thr) at the position equivalent to Arg61. Therefore, with respect to domain interaction, ApoE protein behaves like ApoE3, regardless of whether it has arginine at position...
“Humanizing” mouse ApoE by replacing the Thr codon with an Arg codon introduces domain interaction and allows the mouse “Arg61” ApoE protein to behave like human ApoE4 (the so called “Arg61 mouse ApoE”) [13]. Heterozygous Arg61/wild-type mouse model displays preferential binding to lower density lipoproteins and reduced abundance of Arg61-ApoE in the plasma, reflecting its more rapid catabolism. The lower level of Arg61-ApoE also reflects decreased secretion by astrocytes, the main producer of ApoE in normal brain. Furthermore, mice display age-dependent morphologic and functional synaptic deficits and, as a consequence of changes in synaptic plasticity-related proteins, cognitive and memory deficits [14]. However, this variant of mouse ApoE does not display the instability of ApoE4. Decreased stability can be obtained introducing mutations Gly83Thr and Asn113Gly in the N-terminal domain; the mutant protein remodels phospholipids more quickly than Arg61ApoE supporting the hypothesis that a destabilized conformation promotes ApoE4 lipid binding [15]. Replacement of Glu255 with Ala in the ApoE4 isoform abolishes domain interaction and alters the preference of ApoE4 from VLDL to HDL, demonstrating that Arg61 and Glu255 interact to direct the preference for lipoproteins binding [16].

**Apolipoprotein E and Alzheimer’s Disease**

Brain ApoE is directly involved in neuronal repair and remodeling pathway: ApoE3 supporting effective repair and remodeling after neuronal injury by harmful agents, and ApoE4 being less effective in these processes [17]. In response to brain lesions, ApoE helps to repair cells by recycling membrane lipidic components and supports synaptic formation and plasticity [18,19]. Several effects for ApoE on nervous system have been proposed (Fig. 3).

The three ApoE structural isoforms differentially affect the risk of developing atherosclerosis and neuropathologies, including AD [20–22]. Recent clinical evidence indicates that APOE polymorphism affects the rate of conversion from mild cognitive impairment (MCI) to probable AD [23]. The ε2 and ε4 alleles have opposite actions in the pathogenesis of AD, with the APOE ε4 conferring the greatest risk and APOE ε2 conferring the lower risk [24]. Hence, APOE ε4 allele represents an important genetic risk factor for familial and sporadic late-onset AD (LOAD) [21,25,26] as well as for autosomal-dominant forms of familial AD (FAD) [27]. Several pathological hallmarks are implicated in development of AD: neuronal loss and synaptic damage, deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles. These neuropathological markers are markedly modulated by the presence of APOE ε4 allele.

APOE ε4 allele is associated with reduction of the cerebral metabolic rate for glucose. Cognitively normal, late-middle-aged carriers of the APOE ε4, MCI patients, and AD patients have abnormally low positron emission tomography (PET) measurements of glucose metabolism in the hippocampus and the cortex [28–30]. Even in young subject showing no signs of dementia and no Aβ accumulation, APOE ε4 is correlated with neuronal glucose hypometabolism [31], probably reflecting dysregulation of brain metabolism that could result in severe oxidative stress during the pathogenesis and progression of dementia, leading to mitochondrial dysfunction and synaptic damage in AD brain tissue [32,33].

![Fig. 2. Influence of domain interaction on the structure of ApoE3 and ApoE4 isoforms. Modified from Hatters et al., Trends Biochem Sci, 2006.](modified from Hatters et al. Trends Biochem Sci 2006)
Fig. 3. Neuroprotective and detrimental effects of ApoE3 and ApoE4 isoforms.

Fig. 4. Role of ApoE in Aβ-dependent and Aβ-independent pathways on AD pathology: the detrimental effects of ApoE4 reflect its unique structural and biophysical properties. Modified from Robert W. Mahley et al., Proc Natl Acad Sci U S A, 2006.
The structural features of ApoE4, “molten globule state” and domain interaction, underlie the pathogenic role of ApoE4 and contribute to AD pathology through both Aβ-dependent and Aβ-independent pathways [10, 34–36] (Fig. 4). Actually, Aβ accumulation, oligomerization, and deposition are central events in AD pathogenesis. Modifications in Aβ clearance and/or its metabolism lead to alteration of Aβ levels in the brain. On the one hand, mitochondrial damage and disruption of cytoskeletal structure caused by ApoE4 fragmentation are a key mechanism in the Aβ-independent neurodegenerative pathway involving ApoE4 (Fig. 4).

APOE, AβPP TRAFFICKING, AND Aβ PRODUCTION

The intracellular metabolism and distribution of cholesterol governed by ApoE markedly affects amyloid-β protein precursor (AβPP) processing. AβPP inside cholesterol-rich cell membrane lipid rafts is cleaved by β-secretase BACE-1, whereas AβPP outside rafts undergoes cleavage by α-secretase promoting secretion of soluble AβPP (sAβPP) and decreased production of Aβ peptide [37]. Several ApoE receptors interact with AβPP and modulate its trafficking and processing to Aβ. The LDLR-related protein 1 (LRP-1), a multifunctional endocytic and signaling receptor, binds and internalizes some forms of sAβPP as well as Aβ-ApoE complex [38,39]. The rapid internalization of LRP-1 enhances AβPP endocytosis and proteolytic processing to generate highly toxic Aβ peptide, due to the sequential action of β-secretase BACE-1 and γ-secretase [40]. During its biogenesis, the LDLR-1 itself is subjected to several proteolytic events that resemble AβPP/Notch processing: LRP-1 associates with β-secretase BACE-1 in lipid rafts where LRP-1 is a BACE-1 substrate [41]. ApoE4 promotes AβPP amyloidogenic processing in an LRP-1 dependent fashion in cultured rat neuronal cells overexpressing AβPP, thus enhancing Aβ production to a greater extent than ApoE3 isoform [42]. The difference in Aβ production was abolished by receptor-associated protein, which blocks the LRP pathway, or by reducing LRP expression by small interference RNA. Replacing Arg61 with threonine in ApoE4 also attenuated the differences. Thus, ApoE4 appears to modulate AβPP processing and Aβ production through both the LRP pathway and intramolecular domain interaction. LRP also binds ApoE-enriched lipoproteins, linking in a single metabolic pathway two molecules strongly implicated in the pathophysiology of AD [43].

APOE, Aβ AGGREGATION, AND CLEARANCE

According to the amyloid hypothesis, accumulation of Aβ in the brain is the primary factor driving AD pathogenesis, resulting from an imbalance between Aβ production and Aβ clearance [44]. Both in vitro and in vivo studies suggested potential mechanisms to explain the ApoE4 contribution to AD development [34]. ApoE/Aβ complexes are major components of AD brain amyloid deposits and, among AD patients, homozgyosity for APOE ε4 correlates with increased amyloid plaque deposits [6,45]. Using the PDAPP amyloid mouse model overexpressing a mutated V717F human AβPP, together with various combinations of the murine and human ApoE isoforms, the role of ApoE on amyloid deposition has been demonstrated [46,47]. ApoE4 is more effective than ApoE3 in promoting Aβ deposition and its conversion to a fibrillar form, which could trigger Aβ nucleation and plaque formation [46–48]. ApoE may also be involved in Aβ clearance, as the binding of ApoE to Aβ actually reduces Aβ toxicity in cell cultures. The protection from Aβ-induced neurotoxicity in primary cultures of rat hippocampal pyramidal neurons afforded by ApoE3 treatment may result from clearance of the peptide by ApoE3/Aβ complex formation and uptake by ApoE receptors [34,49]. Major Aβ clearance pathways include receptor-mediated clearance by cells in the brain parenchyma (neurons and glia), along the interstitial fluid drainage pathway or through the blood-brain barrier (BBB), and proteolytic degradation by endopeptidases [43].

Receptor-mediated uptake and degradation of Aβ in the brain is likely to be promoted by ApoE receptors, which can bind Aβ directly or indirectly through chaperons: ApoE is the best-characterized Aβ chaperon. ApoE acts as an active scavenger of Aβ in the extracellular space. The protein domain involved in Aβ binding is localized in the C-terminal domain, which also harbors high-affinity lipoprotein binding region. ApoE3 binds to Aβ with greater avidity than ApoE4, suggesting that the lipidation status of ApoE modifies its ability to interact with Aβ peptides and further underscore the importance of subtle differences in ApoE conformation to its biological activity. The avid binding of ApoE3 to the Aβ peptide might enhance clearance of the complex, preventing the conversion of Aβ into a neurotoxic species. In contrast to ApoE4, ApoE3 might protect against Aβ-induced cell death and apoptosis [49–51]. When the isolated ApoE C-terminal domain is present in a lipid-bound state, Aβ appears to be localized within the lipid milieu of the lipoprotein par-
ticle. Accordingly, interaction with Aβ peptide could severely impair the lipid binding ability of ApoE, which may have implications not only in terms of amyloid buildup but also in terms of the accumulation of cholesterol at extracellular sites. Indeed, the isolated ApoE C-terminal domain, which does not contain the LDL receptor binding region, cannot mediate cellular particle clearance via cell surface-lipoprotein receptors [52]. Furthermore, Aβ peptide differentially modulates the binding of different ApoE isoforms to ApoE receptors, interfering with cellular lipoprotein or cholesterol metabolism. This also suggests that Aβ may also compromise ApoE function in the AD affected brain [37,53]. Most transport of circulating Aβ occurs via LRP-1-mediated uptake that is influenced by binding of ligands such as α2-macroglobulin and/or ApoE, and may be impaired in AD [37,54,55]. As compared to adult wild-type mice, Aβ efflux is significantly reduced in young and old ApoE knockout mice, and in old wild-type mice [54]. Accordingly, plasma Aβ levels in mice lacking ApoE are significantly higher when compared to mice with a complete complement of mouse ApoE [6]. ApoE disrupts Aβ clearance across the BBB in an isoform-specific manner. Aβ binding to ApoE4 redirected its clearance from LRP1 to very-low density lipoprotein receptor (VLDLR), which internalized ApoE4 and Aβ-ApoE4 complexes at the BBB more slowly than LRP1. In contrast, ApoE2 and ApoE3 as well as Aβ-ApoE2 and Aβ-ApoE3 complexes were cleared at the BBB via both VLDLR and LRP1 at a substantially faster rate than Aβ-ApoE4 complexes [56]. Another ApoE receptor that modulates AβPP trafficking and processing to amyloid peptide is sortilin-related receptor with A-type repeats (SORLA, also known as SORL-1). Inherited mutations in the SORL-1 gene are associated with late-onset AD [57] and expression of SORLA is reduced in the brain of patients with AD [58]. SORLA is involved in trafficking of AβPP into recycling pathways and shift distribution of AβPP to the Golgi compartment, decreasing processing to Aβ. Thus, SORLA acts as a sorting receptor that protects AβPP from processing into Aβ and thereby reduces the burden of amyloidogenic peptide formation [57,58]. Most Aβ that is internalized by ApoE receptors is delivered to lysosomes for degradation or transferred into the plasma by transcytosis. However, oligomeric Aβ42 internalized by neurons can aggregate and accumulate in late endosomes/lysosomes, contributing to lysosomal damage and neuronal toxicity [59]. ApoE4 potentiates Aβ-induced lysosomal leakage and apoptosis in cultured Neuro-2a cells: low pH of lysosomes accentuates the conversion of ApoE4 to a molten globule, inducing reactive intermediates that avidly binds phospholipids and destabilizes lysosomal membranes [60]. Furthermore, ApoE4 remodels and disrupts the phospholipid vesicles to a greater extent than ApoE3 or ApoE2. Neutralization of lysosomal pH abolishes the ApoE4 enhancement of lysosomal damage and apoptosis in response to Aβ42 [61]. Aβ peptide affect neuronal viability in an “aggregation state”-dependent manner, Aβ1-42 oligomers inhibiting the cells viability more than fibrils and unaggregated peptide [62]. Aβ oligomers isolated from AD brains and from animal models brains are highly neurotoxic and potently impair synapse plasticity and memory [63,64]. Dose-dependent neurotoxicity induced by oligomeric Aβ1-42 is affected by ApoE isoforms with a ranking order of ApoE4 > ApoE3 demonstrating a gain of negative function for ApoE4, synergistic with oligomeric Aβ1-42, in mediating neurotoxicity [65]. The nucleation and aggregation of Aβ were specifically enhanced in the ApoE4 mice brain where inhibition of the Aβ-degrading enzyme neprilysin is induced. ApoE4 and Aβ1-42 aggregates may cooperate to induce lysosomal activation and neuronal degeneration leading to cognitive deficits in the ApoE4 mouse [66]. Recently, ApoE4-dependent Aβ-mediated neurodegeneration has been associated with brain area specific inflammatory activation. In ApoE transgenic mouse model, the activation of the amyloid cascade results in the activation of microgliosis and astrogliosis in the hippocampus of ApoE4, but not in ApoE3 transgenic mice [67]. Aβ clearance in the mammalian brain also takes place through endopeptidases-mediated proteolytic degradation. The Aβ-cleaving proteases neprilysin, a membrane metalloprotease, and the insulin-degrading enzyme (IDE) are expressed in neurons, glia, and cerebral vasculature cells and play a pivotal role in the regulation of cerebral Aβ levels [68]. ApoE facilitates the proteolytic degradation of soluble Aβ, both within microglia and in the extracellular milieu, through the action of both neprilysin and IDE. The capacity of ApoE to promote Aβ degradation is dependent upon the ApoE isoform and its lipidation status. The transfer of lipids to ApoE is accomplished principally by ATP-binding cassette transporter ABCA1. Liver X receptors, which are ligand-activated transcription factors, regulate the expression of both ABCA1 and ApoE, and their activation results in increased levels of lipidated ApoE. Notably, the ApoE4 isoform exhibits an impaired ability to promote Aβ proteolysis compared to the ApoE2 and ApoE3 isoforms [69].
**Aβ-INDEPENDENT APOE PATHWAYS**

Destabilized conformation of ApoE4 isoform underlies potential physiological implications.

*In vitro* partially folded conformations of ApoE are more sensitive to proteolysis and could be more vulnerable to degradation pathways [10]. In normal conditions, ApoE is expressed by astrocytes and glia. In response of brain injury and stressful conditions (i.e., Aβ neurotoxicity), neuronal ApoE synthesis may be induced or enhanced for repair or remodeling [34,70]. However, in the context of ApoE4, these events trigger proteolytic processing and fragment generation, which are detrimental. ApoE4 is much more susceptible to carboxyl-terminal truncation than ApoE3. ApoE4 preferentially undergoes proteolytic cleavage in AD brains and in cultured neuronal cells, resulting in the accumulation of bioactive carboxyl-terminal-truncated fragments of ApoE4(1-272) that are neurotoxic [71,72]. Aβ12 treatment of neuronal cells enhances the generation of truncated ApoE4 and increases the number of cells with the intracellular neurofibrillary tangles (NFT)-like inclusions (compared with ApoE3 plus Aβ12 treatment) [71]. Carboxyl-terminal-cleaved products of ApoE4, which occur in AD brains mice, are sufficient to elicit AD-like neurodegeneration and behavioral deficits *in vivo* [35,72]. Neuron-specific proteolytic cleavage of ApoE4 is associated with increased phosphorylation of tau in transgenic ApoE mice and plays a key role in axonal degeneration and gliosis [73,74]. In contrast, transgenic mice expressing human ApoE4 in astrocytes remained normal throughout life and do not show any AD-related neuronal deficits [75]. The amino-terminal domain appears to modulate the ability of the C-terminal-truncated ApoE fragments to induce intracellular NFT-like inclusions. Deletion of N-terminal portion (81 aa) that includes Arg61 in the ApoE4(1-272) does not affect ability to form NFT-like inclusions, whereas deletion of the first 126 amino acids including the residue 112 which distinguishes ApoE3 and ApoE4 markedly reduces ability of ApoE4 to induce inclusions. Thus, domain interaction affecting this biological activity appears to involve interactions other than Arg61 [71]. Notably, mutation of Arg61 to Thr or Glu255 to Ala demonstrates that domain interaction is responsible for the susceptibility of ApoE4 to proteolysis [35]. C-terminal-truncated ApoE4 probably escapes the secretory or the endosomal-lysosomal internalization pathway, enters the cytosol, interacts with cytoskeleton components and induces NFT-like inclusions containing phosphorylated tau and phosphorylated neurofilaments of high molecular weight to a greater extent than similarly truncated ApoE3 [71]. The ApoE4(1-272) fragment can also interact with mitochondria and disrupt mitochondrial energy balance leading to neuronal death [35,76]. Positively charged-receptor binding region is required for escape from the secretory pathway and play a key role in translocation whereas the lipid-binding region mediates mitochondrial interaction. Thus, the lipid- and receptor-binding regions in ApoE4 fragments act together to cause mitochondrial dysfunction and neurotoxicity, which may be important in AD pathogenesis [76]. Mitochondrial dysfunction and oxidative damage in AD patients correlates with the ApoE4 genotype and suggests an intimate and early association between these features in AD [77,78]. A deficient or altered energy metabolism able to change the oxidative microenvironment for neurons during the AD pathogenesis could lead to mitochondrial enzymes impairment and brain glucose hypometabolism [30,32]. In this perspective, hypometabolism, oxidative stress, and energy shortage may largely contribute to the initiation of neurodegenerative cascade and progression of synaptic pathology in the AD [30,33]. The ApoE4(1-272) truncated protein associated with the mitochondria may also induce the mitochondrial-apoptotic pathway: in Neuro-2a cells, ApoE4 enhances H2O2- or staurosporine-induced cell death and apoptosis [60]. While the lipid-binding region of ApoE is responsible for the interaction with Aβ peptides [79], the receptor-binding region is responsible for binding with tau *in vitro* [80]. Hence, ApoE4 fragments might also interact with Aβ or tau or both via two different domains and cause dysfunction of both the cytoskeleton and the mitochondria, leading to neuronal and behavioral deficits [72,76]. Accordingly, ApoE4 expression in mice results in a significant loss of synapto-dendritic connections in the neocortex and in the hippocampus [81]. The ApoE fragment interaction with the cytoskeleton could also disrupt mitochondrial trafficking, resulting in failure to transport these organelles to locations with high metabolic requirement and impairing calcium homeostasis in neurons [82,83]. Hence, abnormal mitochondrial dynamics plays a key role in causing the dysfunction of mitochondria and damaged mitochondria might not satisfy the high energy demands required at synapses, which may lead to impaired neurotransmission and, ultimately, to cognitive failure [84]. Although ApoE4(1-272) has been shown to be translocated to mitochondria, it still remain unclear how the ApoE fragments associates with mitochondria and induces mitochondrial dysfunction.
A recent study shows that ApoE4 binds to ubiquinol cytochrome c reductase core protein 2 (UQRC2) and cytochrome C1, both of which are components of mitochondrial respiratory complex III, and cytochrome c oxidase subunit 4 isoform 1 (COX IV 1), which is a component of complex IV, in Neuro-2a cells. Complexes III and IV are related to ATP synthesis and maintenance of mitochondrial membrane potential. Interestingly, these proteins associate with ApoE4(1–272) more strongly than intact ApoE4(1-299) [85].

A novel role for ApoE in the CNS may contribute to gain new insights into the relationship of ApoE with AD. ApoE display a marked effect on the sulfatide content in both the brain and CSF. Sulfatides, a subclass of myelin sphingolipids synthesized by oligodendrocytes, play a pivotal role in the neuronal spine and myelin sheath structural stability. They are specifically associated with ApoE-containing high-density lipoproteins, suggesting that sulfatide levels in the CNS are likely to be modulated by the same metabolic pathways that regulate levels of ApoE-containing CNS lipoproteins. The sulfatide content in brain tissues from human ApoE4-expressing mice was approximately 60% less than those found in wild type mice of the same age [86]. Furthermore, a specific depletion of sulfatide has been found in postmortem brains from subjects at the very mild stage of AD. Thus, ApoE-mediated sulfatide trafficking can lead to sulfatide depletion in the brain and the sulfatide content is significantly dependent on the APOE genotypes of the subjects [87].

A reduction in sulfatide levels and a concomitant increase in ceramide, an apoptotic lipid second messenger thought to be a degradation product of sulfatide, also occur very early in AD. The concomitant activation of neutral sphingomyelinase, an enzyme that cleaves myelin sphingomyelins to ceramides, by Aβ42 could lead to increased amount of ceramides in the brain regions of AD patients [88]. The sulfatides carried by ApoE particles are probably transported to lysosomes through trafficking of vesicles containing ApoE [87]. Accordingly, abnormal sulfatide metabolism trafficking can induce neuronal cell apoptosis through endosome-generated ceramide accumulation and/or lysosome swelling and sulfatide toxicity [89].

NEW FRONTIERS FOR GENE-BASED THERAPEUTIC APPROACHES AND TARGETS

Currently, there is no known etiology or cure for sporadic AD and, as the current population ages, it remains an enormous public health concern. New biological insights suggest that ApoE may be a possible candidate for the ongoing research on gene-based neuroprotective therapy.

ApoE-based therapeutic perspectives

Emerging knowledge of the contribution of ApoE to the pathophysiology of AD presents new opportunities for AD therapy [90]. In humans, low levels of ApoE characterize APOE ε4 allele carriers and the poor ability of the ε4 genetic variant to respond to physiologic inducers of expression, which is actually problematic in aging subjects. In this model, it has been postulated that the restoration of ApoE concentrations in the brains of APOE ε4 carriers to levels found normally in APOE ε3 subjects (or APOE ε2 subjects) would either delay disease onset or slow down the rate of progression. Multipotent mesenchymal stromal cells (mMSCs) have been evaluated as promising vector for the administration of ApoE3 in humans [91]. Mouse mesenchymal stem cells were implanted into the brains of ApoE null mice, resulting in production of ApoE in the brain and attenuation of cognitive deficits. Expression of ApoE in astrocytes by the adeno-associated virus (AAV) vector should provide a helpful tool for gene therapy of AD. By stereotactically injecting AAV vector, Feng and co-workers demonstrated high level ApoE expressions in the brain of AD mouse, lasting for 12 months, and specifically expressed in astrocytes [92]. ApoE2 and ApoE4 appear, respectively, most and least effective forms of the protein facilitating the metabolism of pathogenic Aβ forms. This observation suggests that enhancing the activity of ApoE2 might diminish Aβ levels. Dodart and co-workers firstly demonstrated that intracerebral gene delivery of the lentivirus encoding ApoE constructs results in efficient and sustained expression of human ApoE in the hippocampus. Furthermore, the authors investigated if direct intracerebral administration of lentiviral vectors expressing the three common human ApoE isoforms could differentially alters hippocampal Aβ and amyloid burden in a mouse model of AD. While expression of ApoE4 in the absence of mouse ApoE increased hippocampal Aβ42 levels and amyloid burden, by contrast, expression of ApoE2, even in the presence of mouse ApoE, markedly reduced hippocampal Aβ burden, suggesting that gene delivery of ApoE2 may prevent or reduce brain Aβ burden and the subsequent development of neuritic plaques [93]. At present we do not know whether ApoE2 gene transfer will mitigate the action of an endogenous APOE ε4 allele, without any evidence that
gene transfer of ApoE2 can counteract the negative effect of endogenous APOE ε4 alleles. Moreover, it is not clear whether increased or supraphysiologic levels of ApoE2 can provide benefit in ApoE2 subjects and whether increased levels will have deleterious consequences. However, these important observations pertaining to ApoE functioning in the adult CNS further underscore that apolipoprotein E represents a potent target for the treatment of sporadic AD.

As APOE ε4 is a strong risk factor for AD, one attractive approach is to convert ApoE4 to an ApoE3-like molecule. Targeting the APOE ε4 gene in brains of patients with AD to express ApoE3 (or ApoE2) could prove beneficial and an attractive therapeutic possibility, which could be explored first in transgenic mice expressing human ApoE4 [94,95]. Given the enormous potential of oligonucleotide-directed gene repair, it is hoped that such developments will allow progress to treat disease caused by point mutations, including neurodegenerative disorders associated with the ε4 allele. Synthetic RNA/DNA oligonucleotides (Chimeraplasts) have been used to repair point mutations in episomal and genomic DNA [96,97]. These have successfully converted the dysfunctional APOE ε2 gene to wild-type APOE ε3, both in vitro and in vivo [98]. Because ApoE has crucial roles in both A/β-dependent and A/β-independent AD pathogenic pathways, it is logical to also consider strategies that regulate its expression in the brain. Since it has been shown that the promoter polymorphisms of the APOE gene may lead to changes in ApoE expression levels by altering transcription of the APOE gene [99], regulating APOE promoter function might be one strategy to alter ApoE expression. The human APOE promoter DNA contains putative sites for AP-1, AP-2, and NF-κB transcription factors. The promoter region also contains inflammatory response transcription factor sites, interleukin-6 (IL-6), interleukin-6 responsive element-binding protein (IL6RE-BP), multiple start site element downstream 1 (MED1), signal transducer and activator of transcription 1 (STAT1), and signal transducer and activator of transcription 2 (STAT2). Thus, the characterization of APOE regulatory elements, including their interactions with different transcription factors, is important for understanding the regulation of APOE gene expression. Before exciting new directions can offer new opportunities for AD therapy, greater understanding of the differential expression and function of the ApoE isoforms is needed and will be facilitated by new animal models, including mice that express humanized ApoE in an ApoE-knockout background. Inducible expression of the ApoE isoforms in aged animals should further clarify the roles of the ApoE isoforms in aging brains.

CONCLUSION

In the CNS, ApoE is expressed and secreted by astrocytes and microglia and deliver cholesterol phospholipids and sulfatides in the brain. Hence, it has a critical function in promoting neurite outgrowth, repairing injured neurons and maintaining synapto-dendritic junctions. Human polymorphic APOE alleles (ε2, ε3, and ε4) encode for the three isoforms ApoE2, ApoE3, and ApoE4. Even though the ApoE variants differ from one another only by single amino acid substitutions, these changes induce functional consequences at both cellular and molecular levels. The ApoE4 isoform is a strong genetic risk/susceptibility factor for developing AD. The neuropathological markers involved in AD development such as deposition of extracellular amyloid plaques, intracellular neurofibrillary tangles, and synaptic damage/neuronal loss are markedly modulated by the presence of APOE ε4 allele. The deleterious effects of ApoE4 mirror its unique structural and biophysical properties. Actually, “molten globule state” and domain interaction support the pathogenic role of ApoE4 and contribute to AD pathology through both Aβ-dependent and Aβ-independent pathways. While Aβ-dependent pathway mediates Aβ production and Aβ clearance as well as Aβ fibrillation and tangle formation, Aβ-independent pathway impact mainly on mitochondria function and trafficking. Impaired mitochondrial metabolism, oxidative stress and glucose hypometabolism play a pivotal role in the initiation and progression of synaptic pathology in AD. New potential areas for therapeutics are focused on gene delivery of ApoE isoforms or regulation of ApoE protein levels. The combined use of gene-based therapeutic approaches targeting both the ApoE protein and the ApoE pathways might raise alternative and safer immunotherapeutic strategies to reduce the burden of this disease in the future.

DISCLOSURE STATEMENT


REFERENCES


