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ORIGINAL PAPER

Understanding the Role of ApoE Fragments in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is one of the most devastating neurodegenerative diseases. It has been known for decades that the *APOE* ε4 allele is the most significant genetic risk factor for late-onset AD and yet its precise role in the disease remains unclear. The *APOE* gene encodes apolipoprotein E (apoE), a 35 kDa glycoprotein highly expressed in the brain. There are three different isoforms: apoE3 is the most common allele in the population, whilst apoE2 decreases, and apoE4 increases AD risk. ApoE has numerous functions that affect neuronal and non-neuronal cells, thus how it contributes to disease onset and progression is hotly debated. The apoE4 isoform has been linked to the accumulation of both of the major pathological hallmarks of AD, amyloid β peptides, and neurofibrillary tangles containing hyperphosphorylated tau protein, as well as other hallmarks of the disease, including inflammation and oxidative stress. Numerous studies have shown that apoE undergoes fragmentation in the human brain, and that the fragmentation pattern varies between isoforms. It was previously shown that apoE4 has neurotoxic functions, however recent data has also identified a neuroprotective role for the apoE N-terminal 25 kDa fragment, which is more prevalent in apoE3 individuals. The ability of the apoE 25 kDa fragment to promote neurite outgrowth was recently demonstrated and this suggests there is a potential loss of neuroprotection in apoE4 individuals in addition to the previously described gain of toxic function for specific apoE4 fragments. Here we review the enzymes proposed to be responsible for apoE fragmentation, the specific functions of different apoE fragments and their possible links with AD.

Keywords Apolipoprotein E · APOE · Alzheimer's disease · Neurodegeneration

Introduction

Apolipoprotein E (apoE) is a 35-kDa glycoprotein widely expressed in the human body that functions as a lipid transporter. Three different alleles of the *APOE* gene are present in the population: ε2, ε3 and ε4, with frequencies of 6.4, 78.3 and 14.5%, respectively [1]. The ε4 allele is the major genetic risk factor of late-onset AD, whilst ε2 protects against the disease [2]. The mechanisms by which *APOE* genotype influence AD onset and progression are incompletely understood. In the brain, apoE is an extracellular protein that is expressed primarily by astrocytes and taken up by neurons; however, under certain circumstances, apoE can also be expressed by microglia and neurons [3–5]. ApoE plays a major role in the transport of cholesterol and other lipids, participating in their redistribution to cells and facilitating their cellular uptake [5]. Moreover, in the brain apoE promotes amyloid β (Aβ) clearance and neuronal signalling [6].

ApoE Structure and Isoforms

The domain structure of apoE is composed of two main domains, with the N- and C-terminus, linked by a hinge region (Fig. 1) [7, 8]. The N-terminal domain (residues 1–167) consists of a four alpha-helix bundle with a region enriched in arginine and lysine residues (135–150) that forms the LDL-receptor binding region. The C-terminal domain (residues 206–299) consists of amphipathic

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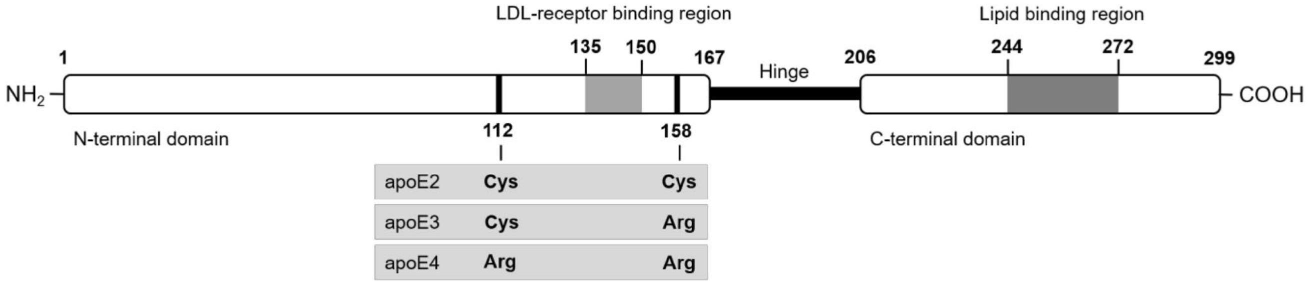

Fig. 1 Structure of apolipoprotein E. The N-terminal domain (1–167 aa) is connected by a hinge region with the C-terminal domain (206–299 aa). The LDL-receptor binding (135–150 aa) and the lipid binding (244–272 aa) regions are shown. Positions 112 and 158 show the different amino acids that give rise to each of the apoE isoforms (apoE2, apoE3 and apoE4)

alpha-helices characteristic of the apolipoprotein family [9] and includes the lipid binding region (residues 244–272).

The three major alleles of the *APOE* gene found in the population give rise to six different genotypes: the homozygotes *ε*2/2, *ε*3/3 and *ε*4/4, and the heterozygotes *ε*2/3, *ε*2/4 and *ε*3/4. The difference between the three alleles is only two nucleotides, resulting in a protein change of two amino acids located in positions 112 and 158. As shown in Fig. 1, apoE2 contains Cys^{112} and Cys^{158}, apoE3 Cys^{112} and Arg^{158} and apoE4 Arg^{112} and Arg^{158} [10]. This amino acid change within the isoforms leads to differences in protein stability and interactions: by changing Cys^{112} into Arg^{112} in apoE4, the protein loses the ionic association between Glu^{109} and Arg^{61}, leaving Arg^{61} free to interact with Glu^{255}, and therefore creating an inter-domain interaction that reduces the lipid binding capacity of apoE4. In apoE3 and apoE4 there is a salt-bridge between Arg^{158} and Asparagine (Asp)^{154} that is lost in apoE2 with Cys^{158}. In this case there is a salt-bridge between Asp^{154} and Arg^{150} that removes the positive side chain of Arg^{150} away from the LDL receptor binding region, affecting its binding capacity [11]. Due to these amino acid interactions, the protein stability differs, with the apoE4 isoform the least stable, followed by apoE3, while apoE2 is the most stable [12].

The *APOE* ε4 Allele is the Major Genetic Risk Factor of Alzheimer's Disease

The *ε*4 allele of the *APOE* gene is the major genetic risk factor of late onset AD [2]. Carriers of one copy of the *APOE ε*4 are three times more likely to develop AD, and those that are homozygous for the *APOE ε*4 have a ten times increased risk of developing the disease [13]. Moreover, the age of onset is lower in the *APOE ε*4 AD population [13]. The exact mechanism by which apoE influences AD remains unclear. Two of the major hallmarks of AD are the presence of extracellular deposition of Aβ, forming amyloid plaques, and the intracellular presence of neurofibrillary tangles (NFT), composed of aggregations of hyperphosphorylated tau protein [14–17].

One hypothesis linking apoE and AD involves a direct interaction between apoE and Aβ peptides. ApoE has been identified within Aβ plaques found in AD brains [18, 19], and other studies in vitro have shown that the binding between apoE and Aβ occurs between residues 13–17 of the Aβ peptide with residues 144–148 of the N-terminal region and residues 244–248 of the C-terminal region of apoE [20, 21]. However, another in vitro study detected minimal levels of interaction between apoE and Aβ using the physiological levels found in cerebrospinal fluid (Aβ:apoE molar ratio of 1:50–75), and proposed instead that apoE influences Aβ metabolism via interaction with other transporters and receptors in cells [22]. ApoE knock-out studies in mice have shown that apoE is necessary to initiate and maintain Aβ plaques [23, 24], while another study reported that apoE decreases the initiation of Aβ fibril formation [25]. Moreover, apoE4 has been shown to be less effective in the inhibition of Aβ fibril formation than apoE3 [25, 26]. Even if the exact mechanism by which apoE interacts with Aβ remains to be clarified, microglia could be a central player in mediating this interaction. Microglia upregulate apoE under certain stress conditions, and they have been found surrounding Aβ plaques [27]. Moreover, a recent study showed that apoE deficiency in mice reduced microglial activation around Aβ plaques [28], which could result in a reduced ability to clear Aβ plaques from the brain.

Another hypothesis linking apoE with AD is the direct interaction between apoE and tau protein. ApoE has been located in NFT mediated by tau [19]. Stable complexes between the microtubule-binding domain of tau and the LDL-receptor binding domain of apoE3, but not apoE4, have been shown in vitro [29]. Overexpression of human apoE4 in neurons of transgenic mice caused an increase in tau hyperphosphorylation mediated by Erk activation [30, 31]. Moreover, apoE-deficient mice show increased hyperphosphorylation of tau than control mice [32], which might indicate that apoE3 protects tau from hyperphosphorylation.

A more recent study using P301S tau mutant transgenic mice on an apoE knock-out or knock-in background showed that apoE4 expression caused more brain atrophy and neurodegeneration than apoE2 or apoE3, while apoE knock-out mice were protected from these changes [33]. This paper also identified a role for microglia in relation to tau, finding that the presence of the apoE4 background promoted microglial reactivity after lipopolysaccharide treatment [33].

ApoE is Proteolytically Cleaved in the Brain

Apolipoprotein E has been shown to be proteolytically cleaved in the human brain generating truncated fragments. Unfortunately, not much is known regarding fragmentation of apoE2, and reports on the mechanism of generation and the function of the fragments are inconsistent in the literature. However it is evident that the apoE fragmentation pattern differs between the apoE3 and apoE4 isoforms [34], therefore understanding the function of the apoE fragments may be critical to understanding the role of apoE in AD. There is a 50% reduction of the 25 kDa N-terminal fragment in apoE4 compared to apoE3 brains [34]. When apoE3 or apoE4 was overexpressed specifically in either neurons or astrocytes, there was no fragmentation of apoE when overexpressed in astrocytes and in neurons there were fewer fragments of apoE4 than apoE3 [35]. This paper also identified that fragmentation was specific to brain regions highly affected in AD, such as the neocortex or hippocampus [35]. Although astrocytes are responsible for high levels of apoE expression, together this data suggests that the enzyme responsible for fragmentation is specifically expressed by neurons and that the fragments may have a role in disease onset or progression.

A number of enzymes have been proposed to be responsible for apoE fragmentation in different studies (Table 1). Enzymes belonging to either the aspartic or the serine protease family have taken most of the attention. The first study by Marques et al. showed that the serine protease, thrombin, generated a 22 kDa N-terminal apoE fragment that was neurotoxic to primary neurons [36]. Moreover, the 22 kDa fragment from the apoE4 isoform showed higher toxicity than the apoE3 fragment [37, 38]. However, the apoE fragmentation pattern generated in vitro by thrombin digestion is different from the pattern observed in the human brain [34]. Cathepsin D, an aspartic protease, was proposed by Zhou et al. to generate a 24 kDa apoE fragment that does correspond to the fragmentation observed in the brain [39]. The apoE fragments and cathepsin D also colocalize with the Aβ plaques and NFTs in post mortem human tissue [39]. A chymotrypsin-like serine protease was proposed by Harris et al., supported by studies using the specific enzyme inhibitor α1-antichymotrypsin [40, 41]. It was also shown that the C-terminal fragment of apoE4 formed with the chymotrypsin-like protease, comprising amino acids 272–299, induces neuropathology and behavioural deficits in transgenic mice overexpressing the fragment [40]. Most recently, high-temperature requirement serine protease A1 (HtrA1) was shown to induce fragmentation of apoE in vitro, generating a fragment of 25 kDa, encompassing amino acids 1–195. HtrA1 was able to cleave apoE4 faster and to a greater extent than apoE3 [42]. Of particular interest, recombinant HtrA1 generated apoE3 fragments, particularly a stable 25 kDa fragment [42], that is very similar to the major apoE fragment detected in the human brain [34]. Moreover, inducing apoE expression in SK-N-SH neuroblastoma cells with all-trans retinoic acid led to the formation of a 25 kDa N-terminal apoE fragment, the formation of which was blocked by either *HTRA1* knock-down or inhibition with the specific HtrA1 boronic acid inhibitor [43]. These last two papers together suggest that HtrA1 expressed by neurons could be responsible for generating the 25 kDa N-terminal apoE fragment.

Some studies have shown a brain homogenate pattern composed of one fragment detected at ~ 23–25 kDa and a smaller fragment detected at ~ 10 kDa [44]. The hinge region of apoE (that connects the N-terminal and C-terminal regions) is highly exposed, thus it is a likely potential target of enzyme cleavage. Previous studies have shown that apoE4 is degraded more rapidly than apoE3 and this may be due to the inherently unstable structure of the protein, i.e. previous data indicates that apoE4 forms a molten globule-like state in which the four-helix bundle is partially opened and elongated thereby exposing the hydrophobic core of the protein [45]. This may result in increased proteolytic enzyme access to the hinge region and to hydrophobic regions of

Table 1 Enzymes proposed to cleave apolipoprotein E

	Enzyme proposed	Fragment generated
Marques et al. [36]	Thrombin	ApoE 22 kDa N-terminal
Zhou et al. [39]	Cathepsin D	ApoE 24 kDa N-terminal
Harris et al. [40] Tamboli et al. [41]	Chymotrypsin-like protease	ApoE 272–299 C-terminal
Chu et al. [42] Muñoz et al. [43]	HtrA1	ApoE 25 kDa N-terminal

the apoE4 isoform of the protein. Predicted cleavage sites for cathepsin D, chymotrypsin-like protease and HtrA1 are located throughout the amino acid sequence of the protein, and specifically in the hinge region. Interestingly, recombinant Htra1, which cleaves apoE after Val^{195} degrades apoE4 more rapidly than apoE3 [42]. As we have recently discussed [43], binding of substrate internal hydrophobic stretches of amino acids to HtrA1 may modulate its protease activity, and this could contribute to the apoE isoform specific degradation of apoE by HtrA1. Overall this is consistent with the pattern seen in the human brain, where apoE4 is present at lower levels than apoE3 and at least the initial cleavage occurs in the hinge region (as the predicted molecular weight of the N-terminal domain is ~ 24 kDa and the C-terminal domain is ~ 10 kDa). It is worth noting that variations in apoE molecular weight in different studies could be due to O-linked glycosylation at Thr^{194} [45].

Understanding the Function of ApoE Fragments

The difference in the apoE fragmentation pattern observed in apoE3 and apoE4 brains leads us to question the biological roles of the different apoE fragments and whether they have a relationship with AD pathogenesis (Table 2). Specific apoE fragments have been directly linked with the formation of Aβ plaques, tau hyperphosphorylation/NFT formation, and the initiation of neurodegeneration [35, 40, 46–48]. An 18 kDa N-terminal fragment (1–172 aa) of apoE4 was found within NFT of AD patients [49], however the presence of this fragment in cerebrospinal fluid or plasma did not correlate with AD or *APOE* genotype [50]. Huang et al. found a different fragment, 1–271 aa of apoE4, to be part of NFT-like inclusions in neuronal cells [47]. Moreover, there was reduced truncation of apoE3 compared to apoE4 and the inclusions did not occur in non-neuronal cells [47]. The same group generated a transgenic mouse overexpressing the fragment 1–271 of apoE4. The mice that expressed high levels of the fragment died at 2–4 months of age, showing AD-like neurodegeneration including tau pathology in the cortex and hippocampus [40]. Meanwhile, the mice that expressed lower levels of the fragment showed impaired learning and memory at 6–7 months of age [40]. Transgenic mice hAPP$_{\mathrm{FAD}}$ [51] overexpressing apoE3 or apoE4 showed a decrease in Aβ deposition and increase in Aβ clearance compared to mice that did not overexpress human apoE. However, the overexpression of the apoE4 1–271 fragment resulted in lower binding to Aβ peptides than apoE3 or apoE4, suggesting there was a decrease in Aβ clearance and an increase in Aβ deposition [52]. A similar fragment size was also tested in Neuro-2a mouse neuroblastoma cells, showing that the 1–272 apoE4 fragment (including both LDL-receptor binding and lipid binding regions) induced neurotoxicity and mitochondrial dysfunction, whereas the 1–240 fragment of apoE4 (containing only the LDL-receptor biding region) or full-length apoE4 did not [53]. However, the 214–272 fragment of apoE4 (containing only the lipid-binding region) did not induce neurotoxicity; this data therefore suggests a requirement for both regions of apoE4 to cause mitochondrial dysfunction and neurotoxicity [53].

Although the exact mechanisms by which apoE fragments may regulate AD pathology remain to be fully defined, there are indications that at least in the case of tau pathology, activation of specific kinases may be involved. In mice that express neuron-specific apoE4 (i.e. akin to those reporting apoE fragmentation [35]), extracellular signal-related kinase (Erk) was shown to be selectively upregulated [30]. In light of recent studies showing apoE4 markedly exacerbates tau pathology in a mouse model of tauopathy [33], the pathways by which toxic apoE4 fragments may regulate kinase activity is an area that appears to warrant further study.

Other research groups have used mimetic peptides to address the function of specific apoE fragments. The apoE fragment 141–155 was shown to cause specific degeneration of neurites in embryonic chick sympathetic neurons [54], whereas a shorter fragment, apoE 141–149, showed inhibition in proliferation and cytotoxicity in IL2-dependent T lymphocytes [55]. Another peptide, apoE 133–149, corresponding with the LDL-receptor binding region, was shown to be responsible for reducing inflammation after injury. The peptide was able to mimic the effects of the full-length protein, suppressing microglial activation and release of tumor necrosis factor-α and nitric oxide after lipopolysachharide administration in BV2 mouse microglial cells [56, 57]. The same 133–149 apoE peptide showed a neuroprotective function after exposure to *N*-methyl-ᴅ-aspartate, suppressing cell death and calcium influx in primary neuronal-glia mixed cultures from brain cortices of fetal rats [58]. From the same group, the peptide was studied in mouse models of other disease models, including multiple sclerosis and post-ischemia necrosis and also led to an improvement after administration of the 133–149 apoE peptide [59, 60]. Another group used the same peptide apoE 133–149 to understand its interaction with neuronal nicotinic acetylcholine receptors (nAChRs) [61–63]. There was a complete inhibition of acetylcholine-evoked responses in rat hippocampal slices when the peptide was added. Furthermore, the same effect was seen when using the shorter peptide apoE 141–148, but not with 133–140, demonstrating that the interaction with nAChR is mediated by the arginine-rich domain [63]. Further studies in *Xenopus laevis* oocytes showed that the interaction of the apoE 133–149 peptide, or the shorter 141–148, disrupted nAChR signaling by blocking the α7 nAChRs [61, 62].

Isoform-specific proteolysis has been identified in a number of different studies, showing higher levels of

Table 2 Proposed functions of apolipoprotein E fragments in cell and animal models related to AD and differences in apoE isoforms

	ApoE fragment studied	Function of the peptide	Model of study	Differences within apoE isoforms
Crutcher et al. [54]	ApoE 141–155	Causes neurite degeneration	Embryonic chick sympathetic ganglia in vitro	N/A
Clay et al. [55]	ApoE 141–149	Exhibits cytotoxicity	IL2-dependent T lymphocytes	N/A
Marques et al. [36]	ApoE 22 kDa N-terminal	Exhibits cytotoxicity, increased in apoE4 background compared to apoE3	Primary neurons	ApoE4 fragment has higher toxicity than apoE3
Tolar et al. [37]	ApoE 22 kDa N-terminal	The neurotoxic function of the fragment is receptor-mediated	Embryonic chick sympathetic ganglia in vitro and embryonic rat hippocampal tissue	ApoE4 fragment showed higher toxicity than apoE3
Tolar et al. [38]	ApoE 22 kDa N-terminal	Induces calcium influx and neurotoxicity involving cell surface receptors	Embryonic chick sympathetic ganglia in vitro and embryonic rat hippocampal tissue	Only apoE4 studied
Huang et al., 2001 [47]	ApoE 1–271 N-terminal	Induces NFT-like inclusion in neuronal cells	Neuro-2a mouse neuroblastoma cells	ApoE4 showed more inclusions than apoE3
Laskowitz et al., 2001 [56]	ApoE 133–149	Suppresses microglial activation and release of TNFα and NO	BV2 mouse microglia cells	N/A
Aono et al. [58]	ApoE 133–149	Suppresses neuronal cell death and calcium influx associated with N-methyl-D-aspartate	Primary neuronal-glia cells	N/A
Harris et al. [40]	ApoE 1–271 N-terminal	Causes AD-like tau pathology and behavioural deficits	Transgenic mice C57BL6/J Overexpressing ApoE 1–271	Only apoE4 studied
Lynch et al. [57]	ApoE 133–149	Supresses inflammatory response after LPS administration	Wild-type mice C57BL6/J	N/A
Klein and Yakel, 2004 [63]	ApoE 133–149 ApoE 141–148	Inhibit Ach-evoked responses in a dose-dependent manner	Rat hippocampal slices	N/A
Chang et al. [53]	ApoE 1–272 N-terminal	Exhibits neurotoxicity via mitochondrial dysfunction	Neuro-2a mouse neuroblastoma cells	Only apoE4 studied
Wellnitz et al. [64]	ApoE 13 kDa C-terminal	Inhibits Aβ fibril formation and induces formation and stabilization of hexameric species of Aβ	Neuro-2a mouse neuroblastoma cells	ApoE4 generates more 13 kDa fragment than apoE2 or 3
Gay et al. [61, 62]	ApoE 133–149 ApoE 141–148	Block α7 nAChRs disrupting nAChR signalling	*Xenopus laevis* oocytes	N/A
Dafnis et al. [65, 66]	ApoE 1–165 19 kDa and apoE 1–185 21 kDa N-terminal	ApoE 1–165 stimulates the intracellular accumulation of Aβ42 that generates ROS, but apoE 1–185 do not	SK-N-SH human neuroblastoma	Effects not seen in apoE3
Bien-Ly et al. [52]	ApoE 1–271 N-terminal	Decreases Aβ clearance and increases Aβ deposition	Transgenic mice J20 line of hAPP$_{FAD}$ and overexpressing apoE 1–271	Full-length apoE3 and apoE4 expressing mice were able to stimulate Aβ clearance
Dafnis et al. [67]	ApoE 1–185 21 kDa N-terminal	Promotes MMP9/TIMP1 imbalance by inducing IL-1β and reducing IL-10 expression	SK-N-SH human neuroblastoma and SW-1783 human astrocytoma cells	Only apoE4 studied

Table 2 (continued)

	ApoE fragment studied	Function of the peptide	Model of study	Differences within apoE isoforms
Love et al. [48]	ApoE 1–151 17 kDa N-terminal	Trafficking to the nucleus and increase in cell death	BV2 mouse microglia cells	Effects not seen in apoE3
Muñoz et al. [43]	ApoE 1–195 25 kDa N-terminal	Promotes neuritogenesis	SK-N-SH/SH-SY5Y human neuroblastoma cells	Only apoE3 studied

fragmentation of apoE4 compared to apoE3. A study in Neuro2a mouse neuroblastoma cells identified an intracellular apoE C-terminal 13 kDa fragment following transfection with the human apoE3 or apoE4. This fragment, that was highly expressed in apoE4-transfected cells, inhibited the formation of Aβ fibrils and stabilized the formation of Aβ hexamers in vitro [64]. Other studies have focused on the role of different N-terminal apoE4 fragments ranging from 17 to 22 kDa. Love et al. suggested the extracellular proteases collagenase and matrix metalloprotease-9 produce a 17 kDa apoE 1–151 fragment and in the apoE4 background the fragment traffics to the nucleus of BV2 mouse microglial cells increasing cell death [48]. Dafnis et al. showed in the SK-N-SH human neuroblastoma cell line that the 19 kDa N-terminal fragment of apoE4 (apoE 1–165) stimulated the intracellular accumulation of Aβ42, generating ROS, but this effect was absent with apoE3 or with the 21 kDa fragment (apoE 1–185) [65, 66]. However, the 1–185 fragment of apoE4 promotes matrix metalloprotease 9/tissue inhibitor of metalloprotease 1 (MMP-9/TIMP1), induced by expression of IL-1β in human neuroblastoma SK-N-SH cells and astrocytoma cells SW-1783 cells [67]. The 22 kDa fragment proposed to be generated by thrombin cleavage exhibited neurotoxicity from apoE4 but not when generated from apoE3 in primary neurons [36]. The same fragment was studied in embryonic chick sympathetic ganglia and embryonic rat hippocampus tissue showing that the fragment generated an increase in intracellular calcium and the neurotoxic function of the apoE4 fragment was mediated through the heparan sulfate proteoglycan-LDL receptor-related protein complex [37, 38]. We recently identified that the 25 kDa fragment generated by HtrA1 cleavage in the apoE3 background of SK-N-SH human neuroblastoma cells was stable and induced neuritogenesis [43]. This suggests that the 25 kDa apoE fragment may also promote neurite outgrowth in vivo. Thus, the reduction of this 25 kDa N-terminal fragment in apoE4 subjects could contribute to a reduction in neuritogenesis, in addition to the potential neurotoxic effects previously proposed for the apoE4 C-terminal fragments along with other apoE peptides (Table 2).

Conclusions

Since the *APOE ε*4 allele is the major genetic risk factor for AD, understanding its role in the development of AD is paramount. A direct link between apoE and the two major pathological hallmarks of the disease, Aβ plaques and NFTs, has been identified but the precise roles that apoE plays in AD are still debated. ApoE is expressed in the brain mainly by astrocytes, however the evidence suggests that neurons rather than astrocytes express the enzymes leading to apoE fragmentation. A number of enzymes have been proposed

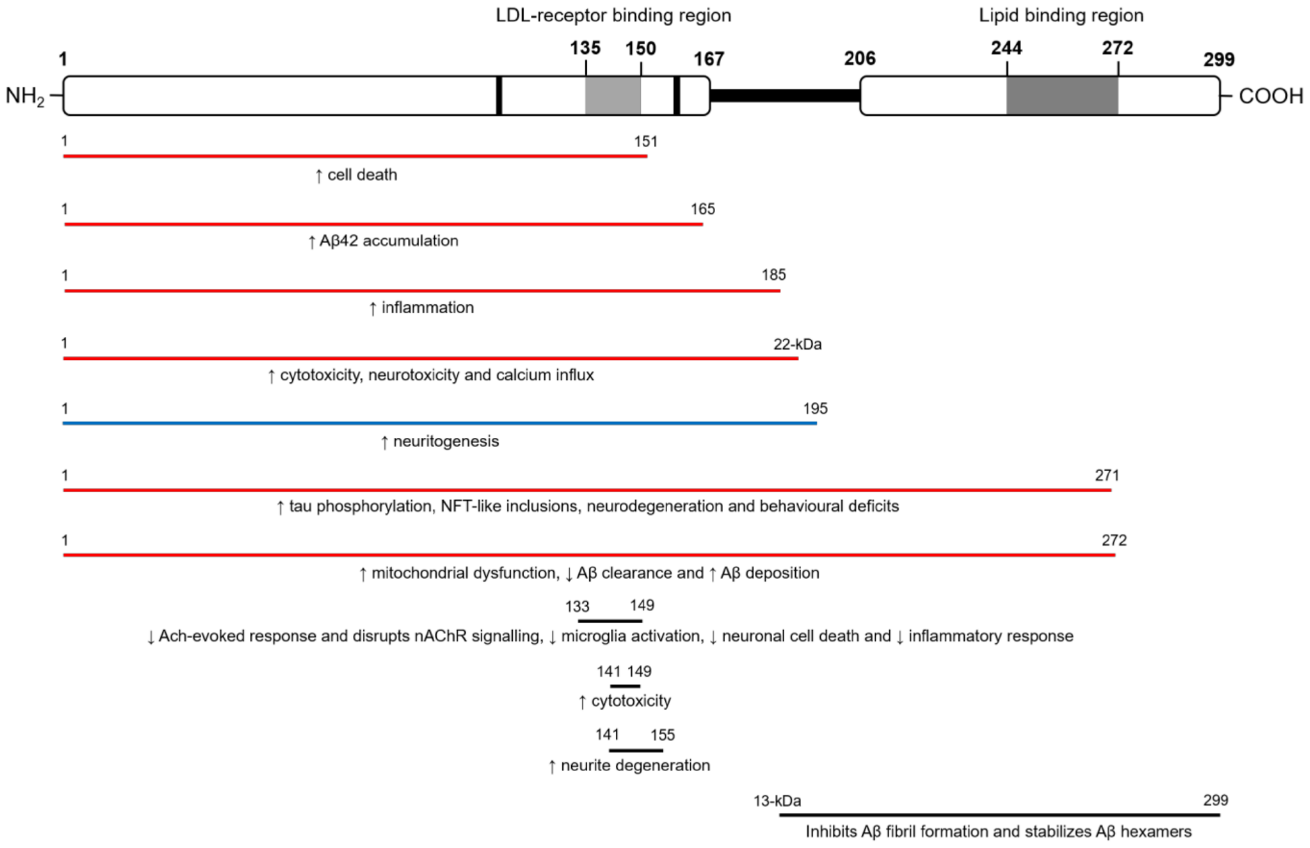

Fig. 2 Overview of apoE structure and the function assigned to each of the fragments studied. Red fragment, apoE4; blue fragment, apoE3; black fragment, N/A. (Color figure online)

to cleave apoE, whilst different fragments have been shown to have diverse effects on cells. The majority of studies have focused on the toxic impact of apoE4 (reviewed [68]) promoting neurotoxicity [36], mitochondrial dysfunction [53], tau phosphorylation and NFT-like inclusions [47], neurodegeneration [40], cell death [48] or suppressing microglial activation [56] (Fig. 2). However, our recent study has found a protective function of the apoE 25 kDa fragment that is more prevalent in apoE3 than apoE4 brains [43]. This suggests that in addition to the neurotoxic effects of apoE4 fragments, a loss of neuroprotective apoE3 fragments may also contribute to neurodegeneration in AD. Future studies need to address the role of apoE fragments in astrocytes, microglia and other non-neuronal cell types and their precise role in AD pathogenesis.. Understanding the differences between apoE isoforms in different cell types will undoubtedly involve the use of induced pluripotent stem cells (iPSCs) that express physiological levels of endogenous genes, rather than relying on overexpression models. In particular the use of genome-edited isogenic iPSC lines has highlighted the role of apoE4 in the neuropathology of AD [69, 70], and shown higher levels of apoE fragments (between 12 and 20 kDa) in the apoE4 background [70] that will require further analysis to understand the function. Some studies have started to look at the effect of external addition of the different isoforms in iPSC-derived neurons including apoE2 [71], but very little is known about its fragmentation. The generation of different iPSC-derived cell types with apoE2, apoE3 and apoE4 background can delineate the specific effects of endogenous levels of apoE fragments from each genotype and their role in AD pathogenesis.

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