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Transcriptional regulation of APP by apoE: To boldly go where no isoform has gone before:

ApoE, APP transcription, and AD: Hypothesized mechanisms and existing knowledge gaps

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Abstract

Alzheimer's disease (AD) is the most common form of dementia, gradually disrupting the brain network to impair memory, language, and cognition. While the amyloid hypothesis remains the leading proposed mechanism to explain AD pathophysiology, anti-amyloid therapeutic strategies have yet to translate into useful therapies, suggesting that amyloid β -protein and its precursor, the amyloid precursor protein (APP) are but a part of the disease cascade. Further, risk of AD can be modulated by a number of factors, the most impactful being the $\epsilon 4$ isoform of apolipoprotein E (apoE). A recent study reported a novel isoform-dependent transcriptional regulation of APP by apoE. These interesting new results add to the myriad of mechanisms that have been proposed to explain how apoE4 enhances AD risk, highlighting the complexities of not only apoE and AD pathophysiology, but also of disease itself.

Keywords

Alzheimer's disease; APP; A β ; ApoE; transcription

Introduction

The neuropathological hallmarks of Alzheimer's disease (AD), the most common age-associated neurodegenerative disorder, include synapse and neuronal loss, extracellular deposits of β -amyloid (A β) peptides derived by proteolysis of the amyloid precursor protein (APP), and formation of intraneuronal neurofibrillary tangles (NFTs). Aside from the rare autosomal dominant mutations in the *PSEN1*, *PSEN2*, and *APP* genes causative of the hereditary forms of AD, the causes of sporadic AD cases remain unclear. The amyloid cascade hypothesis has held a dominant position as the leading mechanism proposed for AD

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pathophysiology for decades, but this knowledge has unfortunately not translated to any effective treatments to date [1, 2]. This hypothesis posits that the accumulation and progressive deposition of A β in brain initiates a cascade of events that eventually leads to dementia. Among these downstream events is the abnormal phosphorylation of the microtubule associated protein, tau, and subsequent formation of NFTs in neuronal perikarya and neurites, which destabilises microtubules and disrupts the cytoskeleton to induce neuronal dysfunction and death [3]. Recent evidence supports the view that both of these mechanisms may not only co-exist in a synergistic manner, but are also not mutually exclusive [4]. Indeed, both synapse loss and presence of neurofibrillary pathology are better correlated to severity of cognitive impairment and dementia than A β burden in brain [5], suggesting that A β may initiate the events but require other cellular alterations to result in the full manifestations of AD [6]. The amyloid cascade can be modulated by a number of factors, such as age, diabetes, head trauma, etc. but the largest and most consistent genetic risk factor for AD is presence of the ϵ 4 allele of apolipoprotein E (*apoE*) gene. ApoE4 dose-dependently increases the risk of AD and lowers the average age of disease onset in non-familial AD individuals. Yet to date, almost 25 years after discovery of this most significant risk factor, the cellular mechanisms by which apoE4 contributes to AD pathophysiology remain unresolved. In part, this is because apoE4 may contribute to AD risk via multiple mechanisms, both amyloid-dependent and amyloid-independent, thus pointing to a much more complex picture than initially thought. In this context, a recent publication from the Südhof lab [7] adds new intrigue to the apoE story by implicating a direct action of apoE4 on APP transcription, a notion not previously examined in the field. In this commentary, we will briefly review the results of this new study and discuss how it fits within our current knowledge on the contribution of apoE4 to AD risk.

Genetic polymorphisms of apoE and AD risk

There are three polymorphic alleles of the *apoE* gene, ϵ 2, ϵ 3, and ϵ 4, which encodes for the three apoE isoforms and they differ at residues 112 or 158 (Fig. 1). *ApoE* encodes a protein of 299 amino acids and while expressed by many cells types, the highest expression is found in liver and brain [8]. Initial hints of a connection between apoE and AD came from the detection of apoE immunoreactivity in amyloid deposits in brain [9, 10], but the discovery of a genetic association with AD was the first concrete evidence of this disease link [11, 12]. While ϵ 3 of *apoE* is the most common allele, only ϵ 2 and ϵ 4 are linked to AD, with the former conveying, surprisingly, a protective effect. The increased risk of AD in apoE4 individuals has been confirmed worldwide, and to date remains the strongest risk factor known [13, 14]. Importantly, the apoE4 isoform decreases the age of AD onset in a dose dependent manner and also increases the amount of A β deposits in brain [15].

A β -dependent mechanisms of apoE in AD

Isoform-specific effects of apoE on A β aggregation and clearance

As mentioned, the presence of amyloid deposits in senile plaques is a pathological hallmark of AD such that there has been considerable focus on how A β accumulates in brain. Because the toxicity of A β appears to be related, in part, to its oligomeric rather than monomeric

state and A β tends to be deposited in brain in fibrillary form, it has been hypothesized that the process of aggregation is pivotal to A β induced pathophysiology. Consequently, one factor where apoE4 may influence AD pathogenesis is via aggregation. Further, because the level of A β in brain is a balance between production and removal, it is reasonable to ask whether apoE4 influences these pathways in brain. It was recognized early on that apoE deficiency resulted in a dramatic reduction in amyloid deposits in brains of APP overexpressing transgenic mice [16]. Subsequent studies convincingly confirmed the predicted apoE isoform-specific effects in brain such that amyloid burden is highest in mice expressing human apoE4 isoform, followed by apoE3 and lowest in apoE2 [17]. These observations have been interpreted to indicate that apoE4 promotes A β aggregation while reducing clearance to promote greater amyloid deposition in brain.

More specifically, initial evidence supporting apoE4 in A β aggregation came from in vitro studies in which there was isoform-specific increased binding of apoE4 to A β peptides as well increased rate of polymerization of A β 42 into amyloid filaments [18, 19]. While this mechanism is dependent on factors such as lipidation state of apoE, subsequent studies have since confirmed the early observations that apoE enhances the formation and stabilization of fibrillar A β [20, 21]. ApoE4, in particular, increases and stabilizes A β oligomerization more strongly compared to apoE3 [22, 23]. In transgenic mice overexpressing human APP crossed to apoE4 expressing transgenic mice, not only were the levels of amyloid elevated in brain, but also the plaque deposits were also more compact than the diffuse deposits observed in E2/E3 expressing mice [24], consistent with the view that apoE impacts A β aggregation/fibrillization and subsequent deposition in an isoform-dependent manner.

The turnover of A β in brain is rapid and the majority of extracellular A β appears to be cleared by transport across the BBB, interstitial fluid (ISF) bulk flow [25, 26], and local degradation [27]. A β clearance is mediated in part via interactions with apoE receptors, especially the low-density lipoprotein (LDL) receptor related protein 1 (LRP1), one of the largest in the LDL family, as well as other receptors such as LDLR and VLDLR [17]. This concept is supported in vivo by targeted deletion of LRP1 in brain endothelial cells where there is less efflux of A β out of the brain and a concomitant elevation of A β levels in brain [28]. A β can also be cleared from the brain via proteolytic degradation and cellular uptake, and absorption into cerebrospinal fluid (CSF) [29]. For example, measurement of A β in interstitial fluid in brains of APP transgenic mice using micro-dialysis showed that A β half-life in apoE4 mice is significantly longer than E3 or E2 mice, indicating that the attenuation of A β clearance in E4 expressing animals [30].

Several hypotheses regarding the influence of apoE in A β aggregation and clearance also stem from their proposed binding. The stability of the apoE/A β complex has been found to be isoform-specific: apoE4-containing lipoproteins are less lipidated compared to apoE2 and E3 [31], which affect its A β -binding properties. This results in decreased formation of the E4 complex and increased A β accumulation, particularly the oligomeric A β species [32]. However, others reported that apoE and A β do not significantly bind under physiological conditions, and proposed instead that apoE isoforms and soluble A β (sA β) compete for receptor-mediated clearance. For example, immortalized astrocytes cultured from apoE-knockout (KO) mice showed significantly higher A β clearance compared to that from

apoE2/E3/E4 knock-in mice, indicating that apoE is not required for astrocyte sA β clearance. Competition assays with lipidated apoE isoforms and sA β in apoE-KO astrocytes showed that only about half of the total A β cellular uptake was blocked by apoE isoforms, suggesting that apoE-independent clearance mechanisms were also involved [33]. In summary, there is considerable evidence to support the view that apoE mediates A β aggregation and clearance from brain in an isoform-dependent manner, where E4 is significantly poorer compared to E2 and E3 [17, 34].

A β -independent mechanisms of apoE in AD

ApoE is involved in the regulation of brain insulin signalling

Diabetic patients, who either lack sufficient insulin or desensitized to insulin that is necessary for normal glucose metabolism, are more vulnerable to AD, suggesting a link between the two diseases [35]. Whether diabetes increases AD risk by promoting AD pathology, or causes metabolic or other vascular dysfunction that worsens brain function in the setting of AD, is unresolved. Nonetheless, it has been reported that the association of diabetes and AD is particularly strong among carriers of *apoE* ϵ 4 allele [36 – 38] and in these individuals, AD pathology (neurofibrillary tangles, amyloid deposits, and amyloid angiopathy) is more severe than non-E4 individuals [36]. Insulin and insulin-like growth factor (IGF-1), as well as their receptors, IR and IGF-1R, are differentially expressed in various parts of the brain, including the hippocampus and cortex [39]. While studies still ponder whether brain insulin is produced locally [40] or crosses the blood-brain-barrier (BBB) from the periphery [41, 42], insulin and IGF-1 signalling via phosphoinositide 3-kinase (PI3K) and MAPK pathways are known to be involved in neuronal survival, synaptic maintenance and plasticity, and learning and memory [39, 43, 44]. Further, insulin is in part metabolized by insulin-degrading enzyme (IDE), which also degrades amylin [45] and A β , especially the monomeric species of A β [46, 47]. Therefore, hyperinsulinemia seen in type II diabetes may elevate A β levels secondary to competition between insulin and A β for IDE. Concurrently, the metabolic alterations accompanying diabetes frequently result in hyperlipidaemia and vascular complications, especially cardiovascular, cerebrovascular and microvascular changes, which are more common in apoE4 individuals. For example, apoE4 subjects show increased LDL levels as compared to other apoE genotypes [48] and oxidised LDL impairs insulin signaling in vitro by activating serine/threonine kinases, resulting in degradation of the insulin receptor substrate-1 (IRS-1) and decreased GLUT4 activity [49]. ApoE3 and apoE4 mice with induced hyperinsulinemia also displayed significantly decreased tau phosphorylation in the former but not latter strain upon treatment with pioglitazone, a peripheral insulin sensitizer, but not in non-treated mice [50]. Thus, perturbations in neuronal insulin signalling in diabetics also present in AD individuals provide potential mechanistic links between the two disorders that are exacerbated by apoE4 [43, 51].

Cerebrovascular disruption is associated with apoE4

Increasing evidence support the view that vascular dysfunction in brain can result in or exacerbate cognitive impairment. Cerebrovascular dysfunction can originate from large to small infarcts and even microinfarcts and microhaemorrhages. These vascular changes can

be associated with not only diabetes, hypertension, and hyperlipidaemia, but also with *apoE* $\epsilon 4$ allele. For example, both the AGES-Reykjavik and ongoing Rotterdam population study reported that apoE4 was found to be associated with cerebral microhaemorrhages or microbleeds, indicative of arteriosclerotic angiopathy [52, 53]. In turn, microbleeds are highly correlated with cognitive impairment [54, 55].

In addition, breakage of the BBB could injure neurons or white matter or cause neuronal dysfunction. In a notable study, the plasma albumin quotient (QA1b) was found to be larger in cognitively normal older apoE3/4 carriers as compared to non-apoE4 carriers, suggestive of a BBB dysfunction [56]. Data from apoE4 knock-in mice also supported the apoE4-induced BBB changes observed in humans. Magnetic resonance imaging (MRI) showed that apoE4 mice had decreased cerebral blood flow and exhibited vascular disruption, measured by manganese contrast, in the temporal lobe at seven months of age [57]. Mice deficient in *apoE*, mimicking the loss-of-function effect of apoE4, appeared to have increased BBB permeability as measured by Evan blue dye extravasation after induction of brain injury and also in an age-dependent manner [58, 59]. Loss of capillary-pericyte coverage, reduction in brain vascularization and microvascular length, and decreased collagen IV and laminin levels were also observed in apoE4 mice [60 – 62], all indicative of severe vascular defects associated with the *apoE* $\epsilon 4$ genotype.

Although BBB dysfunction in aged apoE4 knock-in and apoE-deficient mice were observed in earlier studies, the exact mechanism by which apoE contributes to vascular integrity remains unclear. A recent study, however, suggested that the immunophilin, cyclophilin A (CypA), mediates the apoE effect on cerebrovascular integrity. Disruption of the BBB in apoE4 and apoE-KO was rescued either by the genetic ablation of CypA or inhibiting CypA's action with cyclosporine A treatment [60]. It was also observed that the vascular alterations in these mice precede neuronal dysfunction, and astrocyte-secreted apoE4 was proposed to activate the CypA-nuclear factor- κ B-matrix-metalloproteinase-9 pathway in pericytes, eventually leading to capillary breakdown. Taken together, the collective findings indicate that apoE4 impairs cerebral function via its effects on cerebral atherosclerotic changes as well as BBB integrity (Figure 3).

Does apoE directly stimulate APP transcription?

The increased risk of AD in *apoE* $\epsilon 4$ allele carriers is well established and appears to be due in part to reduced A β clearance, together with other proposed mechanisms, some of which are described above. In this context, a welcomed new entry into potential mechanisms was reported just recently by Huang and colleagues, but this new finding came about almost serendipitously.

The authors first set out to identify what astrocyte-secreted proteins modulate A β production in neurons, by co-culturing embryonic stem cell (ESC)-derived human excitatory neurons (iN) with mouse embryonic fibroblasts (MEFs). Interestingly, neurons grown on MEFs, which eliminated the confounding influence of other glial-secreted proteins, produce less A β 40 and A β 42 than neurons grown on glia. Of the 24 abundantly-produced glial proteins, they identified three which significantly increased A β production in neurons co-cultured

with MEFs. In particular, addition of HEK-produced or recombinant cholesterol-free apoE led to a three- to five-fold increase in APP mRNA levels, and stimulated A β 40 and A β 42 secretion. These effects were isoform-dependent—the increase in A β 42 concentrations was highest in apoE4-treated neurons, compared to apoE3 and apoE2 treatment. Interestingly, the apoE-induced increase in APP and A β levels was not observed in neurons cultured with glial, possibly due to the interaction among other secreted proteins, and this may account for why this effect was not noted previously. Specificity for APP was demonstrated by the lack of changes in APLP1 or APLP2, the other two members of the APP gene family.

Besides its canonical role in mediating lipoprotein uptake, apoE also activates a mitogen-activated protein (MAP) kinase signalling pathway in primary neuron cultures [63]. The MAP kinase kinase kinase, dual leucine-zipper kinase (DLK), had been associated with axonal growth [64] and neuronal degeneration [65] but the potential connection between DLK and apoE has been largely unexplored, until now. Probing for a link between apoE and APP, the authors found that apoE's interaction with its receptors blocked proteasomal degradation of DLK. Phosphorylation of MAP kinase kinase MKK7 by DLK was found to trigger ERK 1/2 phosphorylation, increasing APP transcription and ultimately A β levels. Pre-treatment with a lipoprotein receptor antagonist, RAP, which blocks all apoE known receptors [66, 67], abolished apoE-induced ERK 1/2 phosphorylation, indicating that the changes observed were dependent on apoE binding to its receptor.

While all three apoE isoforms activated each step of the pathway, apoE4 was the most robust, followed by apoE3 and apoE2. Employing clustered regularly interspaced short palindromic repeats interference (CRISPRi), blockage of the AP-1 transcription factor binding site was shown to abolish apoE-stimulated APP transcription. Knocking down DLK in mouse neurons reduced MKK7 and ERK 1/2 phosphorylation and blocked apoE-induced increase in APP protein and A β levels. This mechanism was directly tested in mice by blocking the AP-1 binding site with CRISPRi, and the predicted reduction in APP mRNA and protein levels was observed. Hence, the authors proposed that the apoE isoform-specific differential activation of the AP-1 signalling pathway to stimulate APP transcription is an additional mechanism by which apoE4 confers risk for developing AD.

Relevance of the novel model: Strengths and limitations

It is well recognized that individuals homozygous for the ϵ 4 allele are at higher risk and exhibit earlier onset of AD than heterozygous ϵ 4 carriers, who in turn, show similar increased risk and lower onset age than non- ϵ 4 carriers. Put another way, apoE's risk for AD is gene dosage dependent. In addition, at autopsy, ϵ 4 carriers have higher amyloid burden in brain, findings that are also seen in rodent models. Accordingly, any mechanism proposed to address the apoE4 risk must adequately address these well-established phenotypes. The recent study by Huang and colleagues, suggesting an apoE isoform-dependent modulation of APP transcription/production via the DLK-MKK7-ERK1/2 pathway and in turn A β production, would indeed provide an adequate explanation for the observed effects of apoE4 in humans when this increase in APP synthesis may occur over a lifetime. It is also consistent with findings from individuals with trisomy 21 (Down's syndrome) where the *APP* gene is located, and those with autosomal dominant early-onset AD secondary to *APP*

gene locus duplication [68 – 70]; in which increased *APP* gene dosage leads to elevated APP expression, A β generation, and early onset of AD pathology, one of the key underpinnings of the amyloid hypothesis. In this way, this new observation is very attractive. However, despite the elegance of this study, it will be critical to obtain more definitive evidence to support this pathway in human brain.

Prior to this study, there was one earlier report showing that despite a slight increase in cortical APP mRNA levels in AD brains, apoE genotype did not influence mRNA levels of APP and APLP2, suggesting that changes in APP levels were not associated with the isoforms [71]. Other in vitro studies did not find a significant apoE isoform-dependent effect on APP processing or A β production, which would be a predicted downstream outcome of elevated APP transcription. In fact, it has even been reported that all three isoforms of apoE treatment decreased A β 40 and A β 42 levels in cultured neurons and non-neural cells overexpressing APP [72]. On the other hand, a recent report by Theenkara and colleagues demonstrated apoE-directed transcriptional activity in cultured cells, although APP was not noted to be one of its targets [73]. However, it is possible that the use of transformed cells in the Theenkara study instead of induced neurons may account for an absence of effect on APP transcription. In addition, unlike previous studies demonstrating the nuclear localization of apoE or its fragments [74, 75], which further argues for a role of apoE in transcription, Huang and colleagues failed to detect the presence of RAP-dependent internalised apoE in the neuronal nucleus. Nonetheless, when taken together, it seems reasonable to conclude that there is now sufficient evidence to point to a role of apoE in transcription.

An advantage of this study is the use of induced human neurons, thus providing the opportunity to examine neurons in an isolated state, away from the normal complement of glia and other brain cells, and without transgene-mediated APP overexpression. These iNs derived from human stem cells or induced pluripotent cells may behave differently from cultured rodent neurons, especially with regards to the receptor that mediates this apoE effect or the downstream DLK activation. Could different receptors or binding affinities account for the differential potencies of apoE2, E3 and E4 in inducing APP transcription or even the lack of effects on APLP1 or APLP2? For example, VLDL-packaged or lipid-free recombinant apoE particles bind preferentially to LRP1, [76, 77] while astrocyte-secreted apoE-lipoprotein particles have a higher affinity for low-density lipoprotein receptor (LDLR) [78, 79]. Moreover, RAP suppression of apoE-induced DLK MAP kinase cascade observed by the authors hinted at the involvement of canonical apoE receptors. While both LDLR and LRP1 are mostly involved in cholesterol metabolism, LRP1 has been reported to undergo an APP/Notch-like proteolytic processing, releasing an LRP1 intracellular domain (LICD) that can regulate transcription [80]. Thus, characterizing the receptor involved could possibly resolve some of the pathological pathways related the biological activities of apoE and its isoforms. Lastly, the binding of apoE to its receptors also appears to depend on its lipidation status and conformation state. ApoE-VLDL enriched with recombinant apoE increased its affinity for LRP1 over LDLR [76]. However, the apoE particles secreted by HEK cells used in this study contain little to no cholesterol [81] and it will be important to assess the effects of highly-lipidated apoE on APP expression as well.

In summary, this newly described effect of apoE4 on APP levels and A β secretion not only deserves confirmation and further study, but it also requires direct in vivo validation. The latter presents new challenges because astrocytes are the major source of apoE in the brain and glial-derived factors could neutralize the reported effects on neurons, resulting in no net increase in APP transcription at basal conditions. Alternatively, it is possible that however modest this apoE transcriptional effect is in brain, the cumulative increase in APP transcription over a lifetime results in a significant increase in A β generation in apoE4 as compared to apoE3 individuals. If true, searching for such minor changes will be challenging. It is also quite plausible that the balance between the glial and neuronal responses can be perturbed from the basal state to favour the “neuronal” pathway, hence resulting in more APP being transcribed and translated. Resolving these potential mechanisms, amongst other proposed pathways, will be difficult but certainly worthy to investigate, given the major contribution of apoE4 to AD risk.

Conclusion

Despite our best efforts, how AD pathophysiology is modified by apoE4 remain to be clarified at the cellular level. Many plausible mechanisms (Figure 4) have been proposed, pointing to both amyloid-dependent and amyloid-independent pathways. In the former, apoE4 has been implicated in A β aggregation and clearance (Figure 2) while effects on production have heretofore not been rigorously studied. This recent paper by Huang and colleagues described an apoE-isoform dependent regulation of APP transcription and proposed that this finding represents an additional contributor to higher amyloid burden in ϵ 4 carriers, thus adding enhanced A β production to the mix of mechanisms whereby apoE4 confers added risk to AD in affected carriers. These results will almost certainly generate new enthusiasm to investigate apoE-directed transcriptional regulation of not only APP but also other gene targets that might be relevant to its roles in normal and pathological states. As the community waits with interest to see how this surprising story unfolds, the results from Huang and colleagues nonetheless shed new light on apoE and AD pathogenesis, adding yet further twists to the already complex nature of this disease, reminding us that we are far from a comprehensive understanding of AD and that the linear pathways we have come to learn, teach, or preach are simply far too simplistic [82, 83].

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Abbreviations

AD Alzheimer's disease

APP	amyloid precursor protein
BBB	blood-brain-barrier
LDL	low-density lipoprotein

Figure 1.

Schematic representation of apoE. The three 34kDA isoforms containing 299 amino acids differ by just arginine to cysteine changes in apoE2 (Cys112, cys158) and apoE3 (Cys112, arg158); apoE4 (arg112, arg158) contains arginine at both sites [84, 85]. These critical differences affect the structure and subsequent functions of apoE, which consists of a receptor binding region (amino acids 134-150 and Arg172) in the N-terminal domain (amino acids 1-191), and a lipid binding region (amino acids ~244-272) in the C-terminal domain (amino acids ~ 225-299), separated by a hinge region [86].

Figure 2.

Apolipoprotein E and Aβ metabolism in the brain. ApoE is synthesized by the astrocytes, and lipidated to form lipoprotein particles. Lipo-apoE binds to soluble Aβ and facilitates its clearance across the blood-brain barrier into the bloodstream. Impaired Aβ clearance due to improper binding of apoE4 or competition for the lipoprotein receptors can result in accumulation in the brain, and triggering the formation of Aβ oligomers and amyloid plaques. ApoE4 can also interact with Aβ to form insoluble plaques.

Figure 3.

Apolipoprotein E and cerebrovascular dysfunction. Astrocyte-secreted apoE4, but not apoE3, activates the matrix metalloproteinase (MMP) pathway, which then breaks down the basement membrane and tight junctions. The impairment of the blood-brain barrier may lead to accumulation of toxic substances in the brain, eventually resulting in neuronal injury and death.

“Gain-of-function” effects“Loss-of-function” effects **Figure 4.**

Apolipoprotein E4 as a risk factor in Alzheimer's disease. ApoE4 is thought to contribute to the pathophysiology of AD through several Aβ-dependent and independent pathways.

Table 1
A summary of the proposed roles of apoE in AD pathogenesis

Target of influence	Evidence for apoE's involvement	Reference	Outstanding questions/ Knowledge gaps
A β aggregation and clearance	Decreased amyloid deposition in apoE-deficient APP overexpressing transgenic mice	[16]	(i) To what extent is apoE regulation of A β aggregation and clearance mediated by their binding?
	Amyloid burden in apoE-expressing mice: E4>E3>E2	[17]	
	ApoE enhances the formation and stabilization of fibrillar A β	[19]	
		[18]	
		[20]	
		[21]	
		[22]	
		[23]	
	A β metabolism is modulated by LRP1, LDLR and VLDLR	[17] [28]	
	ApoE mediates A β clearance (E2 > E3 > E4)	[24] [30]	
Brain insulin signalling	Increased association of diabetes and AD in apoE e4 carriers	[36]	(i) Does apoE impact insulin signalling directly or indirectly via regulation of A β , LDL or tau?
		[37]	
		[38]	
	IDE can metabolize insulin, amylin and A β	[45]	
		[46]	
	ApoE4+ patients have increased LDL load	[47]	
		[48]	
Vascular dysfunction	oxidised LDL impairs insulin signalling	[49]	(i) What pathways are involved in apoE contribution to vascular integrity?
	ApoE isoform-dependent tau phosphorylation in pioglitazone-treated hyperinsulinemic mice	[50]	
	ApoE4 is associated with defects linked to arteriosclerotic angiopathy	[52]	
		[53]	
	Cognitively normal aged apoE3/4 carriers show signs of BBB defects	[56]	
	ApoE4 mice display vascular disruption and reduced cerebral blood flow at 7m/o	[57]	
	ApoE-deficient mice display age-dependent increase in BBB permeability	[58]	
		[59]	
		[60]	
		[61]	
	ApoE4 mice display various defects in vascular integrity	[62]	

Target of influence	Evidence for apoE's involvement	Reference	Outstanding questions/ Knowledge gaps
APP transcription and A β production	Isoform-dependent <i>in vitro</i> increase in APP mRNA and A β 40 and A β 42 secretion following apoE treatment	[7]	(i) How relevant is this mechanism in other physiologically-relevant models? (ii) What is the receptor involved in apoE-mediated DLK activation?
	ApoE-induced ERK1/2 phosphorylation leads to increased levels of APP transcription and A β		
	CRISPRi inhibition of AP-1 abolished apoE-stimulated APP transcription.		