Review

Perspectives on the Role of *APOE4* as a Therapeutic Target for Alzheimer's Disease

Kavita Patel[1], Siwangi Srivastava[1], Shikha Kushwah and Ashutosh Mani*
Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, India

Abstract. Alzheimer's disease (AD) is a neurodegenerative disease that is coupled with chronic cognitive dysfunction. AD cases are mostly late onset, and genetic risk factors like the Apolipoprotein E (*APOE*) play a key role in this process. *APOE* ε2, *APOE* ε3, and *APOE* ε4 are three key alleles in the human *APOE* gene. For late onset, *APOE* ε4 has the most potent risk factor while *APOE* ε2 plays a defensive role. Several studies suggests that *APOE* ε4 causes AD via different processes like neurofibrillary tangle formation by amyloid-β accumulation, exacerbated neuroinflammation, cerebrovascular disease, and synaptic loss. But the pathway is still unclear that which actions of *APOE* ε4 lead to AD development. Since *APOE* was found to contribute to many AD pathways, targeting *APOE* ε4 can lead to a hopeful plan of action in development of new drugs to target AD. In this review, we focus on recent studies and perspectives, focusing on *APOE* ε4 as a key molecule in therapeutic strategies.

Keywords: Alzheimer's disease, apolipoprotein, clearance, dementia, insoluble amyloid, regulation, treatment

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease in which neuronal cell death occurs that leads to memory loss and impaired cognitive functions (Fig. 1). It is the most prevalent disease in all brain function disorders that is associated with cognitive decline [1]. AD is identified by extracellular amyloid-β (Aβ) aggregation, intracellular neurofibrillary tangles, with activated glia and neuronal death. The risk of progressing AD is increased by lifestyle

and environmental factors, but genetic risk factors also play a key role in it. Several studies have shown that there are many loci connected with AD which are both susceptible and pathogenic genes. According to the age of onset, AD is defined by two forms, early onset (EOAD) and late onset (LOAD). EOAD occurs in people who are 65 years below and 1–5% cases of AD are early onset AD. LOAD occurs in people who are above 65 years and 95% of AD cases are late onset AD. There are three gene mutations that play a key role in EOAD and Aβ generation, such as amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*), and presenilin-2 (*PSEN2*). On the other note, LOAD is connected with many genes, and it is more complicated. The allele *APOE* ε4 is considered as a preponderating determinant of LOAD via genetics [2].

---

[1]These authors contributed equally to this work.

*Correspondence to: Ashutosh Mani, Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, India-211004. E-mail: amani@mnnit.ac.in.
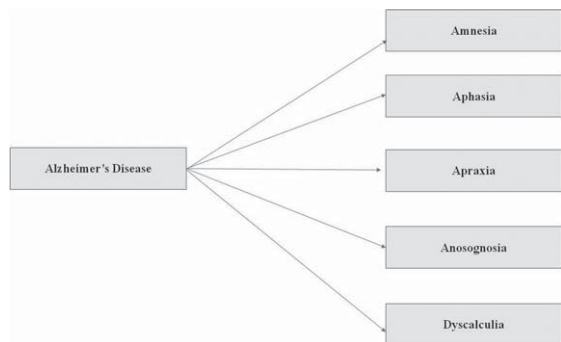
Fig. 1. The symptoms of Alzheimer's disease which are usually seen in a patient include: Amnesia (short term memory loss but later turns into long term memory loss); Aphasia (unable to speak); Apraxia (difficulty with skilled movement); Anosognosia (unaware of difficulties); and Dyscalculia (specific learning disabilities like math and number-based operations).

Single copy of *APOE* ε4 allele inheritance can cause higher risk for AD progression, and the people are at greater risk than those who have inherited two copies of *APOE* ε4 allele for AD progression. It has been found that *APOE* ε4 is also linked to the early onset of memory dysfunction and different symptoms in comparison with people who have AD but do not have the *APOE* ε4 gene. The reason behind the connection of *APOE* ε4 to AD progression is still not clear. Although amyloid plaques are greatly linked with the *APOE* ε4 genes in tissue of AD patient brains, accumulation of amyloid plaques in brain tissue leads to neuronal cell death. It has recently been reported that modifications in tau pathology and neurodegeneration are directly related to *APOE* isoforms [3].

Oxidative stress in AD patients is also linked with the *APOE* ε4 allele, and it has been found to reduce the antioxidant activity of enzymes in the hippocampus of AD brains. Some oxidative stress markers, including increased oxidized proteins, elevated levels of lipid peroxidation, glycosylated products, formation of aldehydes, alcohols, ketones, free carbonyls, and as well as modified RNA and mitochondrial and nuclear DNA due to oxidation, were found in the AD brain tissue and mitochondria isolated from initial phase of AD during postmortem. ApoE has shown to work as an antioxidant in B12 ApoE expressing cell lines against hydrogen peroxide mediated cytotoxicity. It has been found that ApoE-deficient mice have an increased level of plasma peroxide of low-density lipoproteins. The levels of lipid oxidation were found to be increased in the frontal cortex

of *APOE* ε4 homozygous and heterozygous carriers of AD patients in comparison to homozygous *APOE* ε3 carriers. Catalase activity was upregulated in homozygous *APOE* ε4 carriers while superoxide dismutase and glutathione activity was not significantly upregulated in comparison to controls [4, 5].

APOE ε4 was identified to have role in AD development 25 years ago but to date there are no drugs approved for directly targeting it yet. *APOE* isoforms have become a propitious target to understand the progressive pathophysiology of AD patients and adding a novel therapeutic approach towards AD, due to its genetic preponderance. However, there is sufficient clinical research that shows *APOE* ε4 affects various pathways in the brain that lead to AD progression. In this review, we will accentuate *APOE* ε4 targeting studies as a therapeutic strategy to treat AD [6, 7].

AMYLOID-β THEORY OF AD

Amyloid-β is formed by the cleavage of the amyloid-β protein precursor (AβPP) by β-secretase and γ-secretase (see Fig. 2). AβPP is present on chromosome 21. Several alternative splicing isoforms have been observed in humans whose amino acid length ranges from 639–770 amino acids. AβPP has three domains: long extracellular N terminal region, short transmembrane segment, and short C terminal segment.

Amyloid-β 42 starts accumulating and forms clusters known as oligomers, which is the first stage of aggregation. Following this, chains of clusters form fibrils and the final stage is the formation of plaques which are composed of beta-sheet and other substances. These stages of aggregation disrupt cell-to-cell communication and activate immune cells. The activated immune cells stimulate inflammation in the brain, which ultimately leads to the brain cell destruction [9].

TAU TANGLES PLAY A KEY ROLE IN NEURODEGENERATION

Tau is a microtubule associated protein which maintains cytoskeleton stability and plasticity. It also recruits signaling protein to regulate microtubule mediated axonal transport. Along with these, it has roles in neuronal development, cell signaling, neuroprotection, and apoptosis. To maintain the cytoskeleton plasticity, tau is present as a phosphorylated form and with age plasticity level reduces
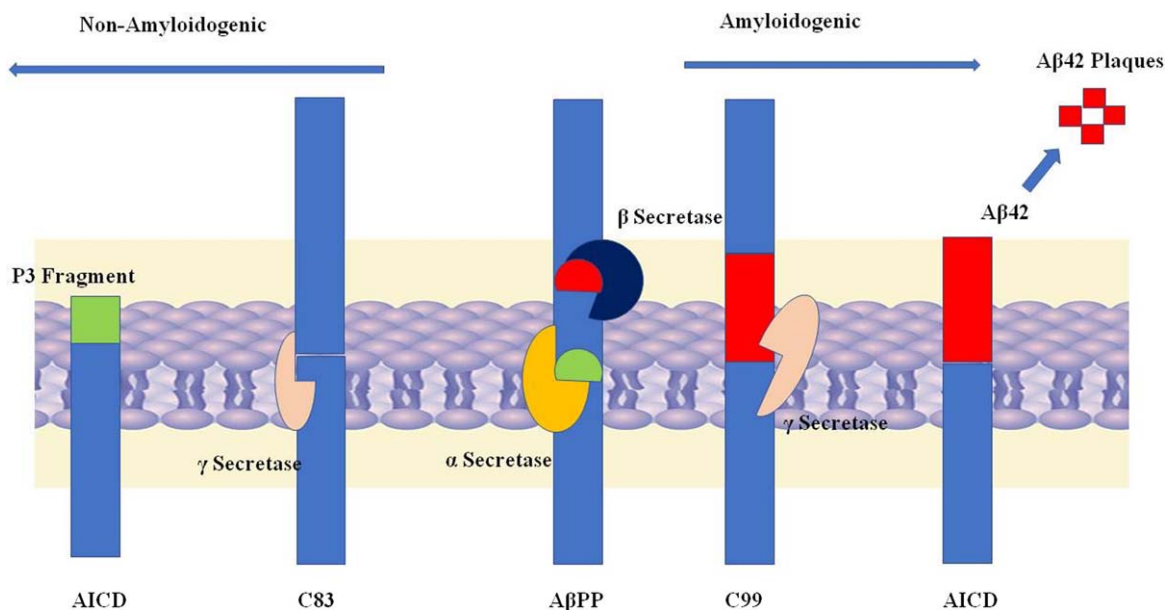
Fig. 2. In most of the cases, AβPP undergoes non-amyloidogenic processing through a consecutive cleavage by α- and γ-secretase. The α-secretase cleaves 639 amino acids longer AβPP into 83 amino acid fragments. γ-secretase further processes those fragments and generates a P3 fragment which has a role in signal transduction [2]. Alternatively, in case of amyloidogenic processing the AβPP is firstly cleaved by β-secretase (BACE 1) and then by γ-secretase. The β-secretase at first generates 99 amino acid long fragments which are cleaved by γ-secretase further. In 90% cases 40 amino acids long, amyloid-β is generated which is harmless, but in rarest condition, 42 amino longer amyloid-β is formed whose accumulation becomes a triggering cause of a neurodegenerative disease, i.e., Alzheimer's disease [8].

[10]. Abnormal or excessive phosphorylation (hyperphosphorylation) transforms the normal adult tau into neurofibrillary tangles (NFT). This consequence of tau hyperphosphorylation may arise due to several factors like interaction of ApoE4-tau, upregulation of kinases, or downregulation of phosphatases. Kinases and phosphatases are key enzymes. The phosphorylated tau binds to microtubules and stimulates their assembly, whereas the hyperphosphorylated ones lose their biological activity. In abnormally phosphorylated tau, tau neither binds to tubulin nor promotes microtubule assembly, instead it inhibits assembly and disrupts the microtubule organization required for maintaining the cytoskeleton plasticity [11]. In a hyperphosphorylated state, tau binds to other tau molecules and forms thread which eventually leads to tangle formation. These tangles block the neuron's transport system and disrupt the synaptic communication between neurons. It was also found that tau hyperphosphorylation induced the over-production of reactive oxygen species which are responsible for cell damage and mitochondrial dysfunctions. Tauopathies dysregulated the balance between reactive oxygen species levels and antioxidant enzyme activity [12].

MUTATION IN PS1/PS2 IS RESPONSIBLE FOR INSOLUBLE AMYLOID AGGREGATION

Presenilin PS1 or PS2 are catalytic units of γ-secretase which is responsible for cleavage of many transmembrane proteins like AβPP. Following the cleavage of AβPP by β-secretase, processing by γ-secretase generates Aβ of varying lengths. $A\beta_{40}$ accounts for 90% of population whereas minor $A\beta_{42}$ is hydrophobic and acts to nucleate amyloid aggregation and plaque formation. Mutation in PS1 or PS2 causes overproduction of $A\beta_{42}$ and acts as one of the responsible factors in causing pathogenic mechanisms in AD [13, 14].

APOLIPOPROTEINS ARE LESSER EXPLORED FROM AD PERSPECTIVE

Apolipoproteins are polar, and thus can travel in polar medium (an aqueous medium) despite being non-polar (lipophilic) in nature. Apolipoproteins interact with receptors and lipid transport proteins to participate in lipid processing and metabolism. They

also serve as cofactors of enzymes participating in lipid metabolism.

There are various classes of apolipoproteins which are present on different types of lipoproteins and regulate their functions. Among them, ApoE has a role in the central nervous system. It plays a role in lipid homeostasis and transports cholesterol to neurons via ApoE receptors. ApoE is produced by microglia and astrocytes, whereas in the peripheral system, it is produced by liver and macrophages. Once ApoE is secreted from cells, the ATP binding cassette transporters (ABCA1 and ABCG1) import cholesterol and phospholipids to nascent ApoE to form lipidated apolipoprotein particles. ApoE acts as a ligand for low density lipoprotein receptors (LDLR) (e.g., ApoE receptor and LRP1) and very low-density lipoprotein receptors (VLDLR). ApoE binds with Aβ. LDLR and VLDLR enable ApoE-Aβ complex to pass through them by interacting with them and participating in the clearance of Aβ from the brain [3, 15].

ApoE has different isoforms which have been classified on the basis of difference in amino-acids at positions 112 and 158. E2 has cysteine at both the position, while E3 has cysteine at 112 and arginine at 158 position. It has a better binding affinity for LDLR to mediate lipolytic processing. E4 has arginine at both the positions due to which repulsion occurs between arginine 61 and arginine 112. This enables the formation of a salt bridge between arginine 61

and glutamate 251. Salt bridge disrupts normal functioning of apolipoproteins. E4 binds with VLDLR with better affinity which internalizes the ApoE-Aβ complex at a slower rate than LRP1. So, E4 was less efficient than E2 and E3 in clearing and promoting Aβ transport across the blood vessel walls [16, 17].

ABUNDANCE OF APOE4 IS RESPONSIBLE FOR SLOW CLEARANCE OF INSOLUBLE AMYLOID AGGREGATES

Apolipoproteins expressed by *APOE ε*4 are low in concentration and also hypolipidated as compared to other isoforms due to which it fails to bind to its receptors and even if it binds to the receptors, it is redirected to VLDLR which internalize the complex at slower rate. (Fig. 3)

APOE4 AFFECTS AND CAUSES THE CONSEQUENCES IN ALZHEIMER'S DISEASE IN NUMEROUS WAYS

ApoE4 is less efficient in clearing the accumulation of Aβ from the brain due to its salt bridge structure and also plays a key role in the over-production of Aβ by affecting the activity of γ-secretase. With respect to clearance, ApoE4 competes with Transcription factor EB to bind with CLEAR (Co-ordinated
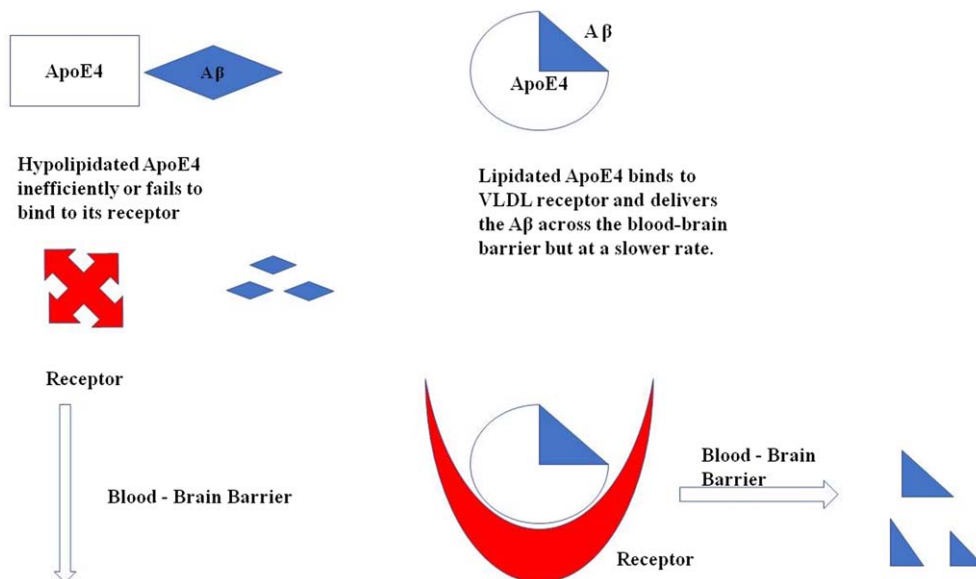

Fig. 3. Illustration of ApoE4 binding with Aβ for its clearance across the blood-brain barrier. In a hypolipidated state ApoE4 fails to transport the Aβ across the cells as it does not bind to its receptor as efficiently as required, whereas the lipidated ApoE4 binds to its receptor VLDL and delivers the Aβ across the cells.

Lysosomal Expression & Regulation) DNA motif to interfere in the expression of three genes (Sequesto-some1, LC3B, and LAMP2) required for the process of autophagy [18]. When Aβ accumulation occurs inside the brain cells, different kinds of proteases are appointed to clear these kinds of aggregation. Neprilysin, a metallo-protease responsible for clearing the Aβ, is usually impaired due to the presence of

allele *APOE* ε4 [12]. NMDAR (N-methyl D aspartate receptor) which is a glutamate receptor and ion channel protein in nerve cells gets activated once glutamate and glycine bind to it. After activation, it allows positive charged ions to flow across the cell membrane. The NMDA receptor is responsible for maintaining synaptic plasticity and regulating the formation of synapse underlying the memory. It also
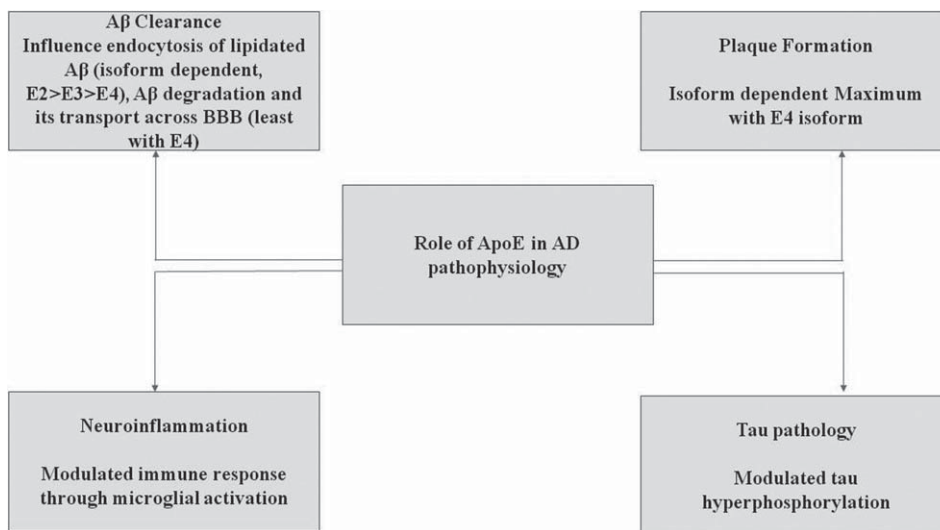

Fig. 4. ApoE4 plays a key role in AD pathophysiology [24]. As mentioned above, ApoE4 is inefficient as compared to other isoforms in clearing Aβ. As ApoE4 fails to clear the accumulation and triggering the over-production of Aβ by increasing the activity of γ-secretase, it eventually leads to the formation of plaques. C) ApoE4 increases the neuroinflammation by elevating the production of cytokines through the microglial activation. D) ApoE4 also plays a significant role in inducing the hyperphosphorylation of tau through ERK activation.
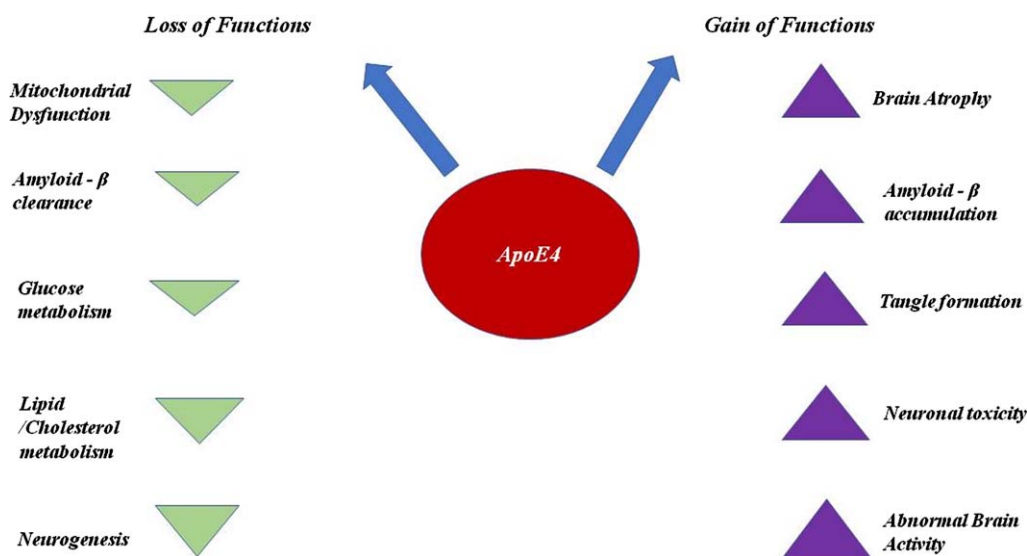

Fig. 5. ApoE4 plays a significant role in increasing the risk of causing AD, as ApoE4 interferes in numerous pathways and downregulates as well as upregulates the phenomena which eventually contributes to the pathological consequences in AD.

leads to the formation of neural networks during developments. ApoE4 blocks this receptor and disrupt the functions carried out by NMDAR [19, 20]. Along with this, ApoE4 blocks certain other receptors like insulin due to which glucose metabolism lowers down. ApoE4 shows the downregulation of brain peroxisome proliferator activated receptor γ (PPARγ) and PPARγ co-activator 1 (PGC1α) signaling, which is involved in maintaining the glucose uptake and metabolism [21]. It also blocks growth factor. ApoE4 is quite prone to proteolytic cleavage due to its unique salt bridge structure. The proteolysis of ApoE4 generates neurotoxic fragments which causes inflammation in the brain [22]. It is also one of the prominent reasons behind the hyperphosphorylation of tau, since it manages to escape the secretory pathways and reaches to cytoplasm where it interacts with zinc and induces the excessive phosphorylation of tau [19]. It acts as an inhibitor of the expression of oxidative respiratory complexes I, IV, and V. This causes mitochondrial dysfunction [14, 19], since Aβ accumulation is high and clearance is low in the carriers of *APOE ε4*, and as a result, they are at greater risk of having cerebral amyloid angiopathy. Cerebral amyloid angiopathy is a condition in which amyloid deposits across the arteries and leads to their shrinkage [23]. ApoE4 is also responsible for other diseases like diabetes, atherosclerosis, and other heart-related diseases as it disturbs lipid homeostasis in cells. See Figs. 4 and 5.

POSSIBLE THERAPEUTICS DEVELOPMENT APPROACHES BY TARGETING APOE4

Many studies have suggested that ApoE4 can alter different biological pathways that lead to progression of AD, although the mechanism that links the ApoE to neurodegeneration is still unclear. However, the views to find novel targets for the treatment of AD suggest altering the *APOE* allele and *APOE* structure [25]. Different AD treatment strategies targeting *APOE4* are shown in Fig. 6.

APOE level regulation

APOE level upregulation
Many reports suggest that the quantity of ApoE in cerebrospinal fluid and plasma of AD patients decreased as compared to healthy individuals. Retinoid X receptor (RXRs), nuclear receptors and Liver X Receptors (LXRs) positively control the *APOE* transcription [26–28]. ApoE levels in the brain were increased by taking RXR agonist with bexarotene orally, which also decreased Aβ accumulation. Bexarotene alone was found to reduce cognitive dysfunction, and it also helps with restoration of age dependent synaptic plasticity in mice models [29–36]. Although it has negative effects, such as hepatic failure in mice and also failed to clear amyloid plaques from the brain in human models.
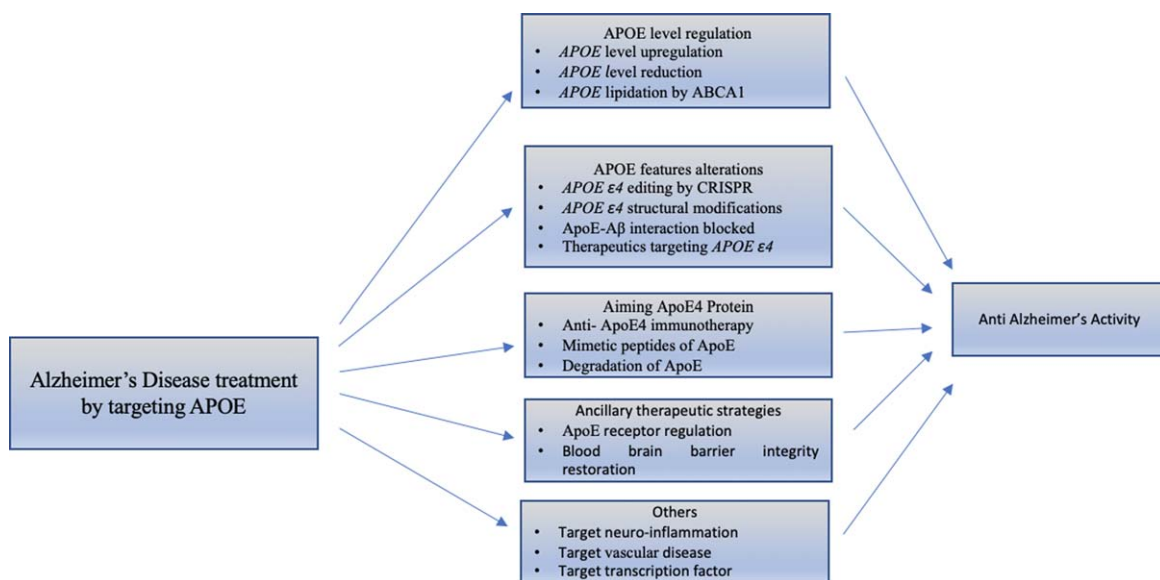

Fig. 6. Classification of strategies that target *APOE* for treatment of Alzheimer's disease. There are different studies that demonstrate the molecular mechanism showing effects of *APOE4*. We emphasized the advanced therapeutic strategies for different *APOE4* characteristics, discussed below.

ApoE level reduction

Recent studies suggest that ApoE haploinsufficiency instead of *APOE* genotypes weakens the Aβ accumulation in targeting *APOE* replacement mice [37–39]. It has also been shown that anti-ApoE antibodies were able to increase spatial learning ability by decreasing ApoE level and Aβ accumulation in animal models [40]. ApoE has bidirectional properties like it increases the AD pathogenesis. So, ApoE removal can help in reducing the toxicity. ApoE isoforms and ApoE fragments deposition can be harmful, so removal of these fragments by immune-depletion like anti monoclonal antibody can be a new therapeutic approach [40–43].

ApoE lipidation by ABCA1

Biologically active ApoE is lipidated and ABCA1 helps in loading of ApoE protein in the brain [44]. Removal of ABCA1 leads to less lipidated ApoE which further leads to amyloidogenic pathogenesis. Moreover, ABCA1 overexpression can reduce the Aβ accumulation in mice models. RXR-LXR agonists can help in transcription of *APOE* and ABCA1, that leads to Aβ clearance and helps in different other functions like synapse regeneration, cholesterol/lipid transport, and modulation of immune cells [45–47]. So, it can be a new therapeutic strategy without increasing the ApoE level.

APOE features alterations

APOE ε4 editing by CRISPR (clustered regularly interspaced short palindromic repeats)

The *APOE ε4* concentration termination and transformation of *APOE ε4* to *APOE ε2* and *APOE ε3* have potential to play an important role in treatment of AD [48, 49]. The DNA coding of *APOE ε4* and *APOE ε3* varies by one nucleotide (112th position of *APOE4* is Arg and its cysteine in *APOE ε3*, and chromosome 19 produces *APOE ε4* allele. CRISPR technology can be employed to modify the *APOE ε4* to *APOE ε3* [50, 51]. Here, CRISPR editing and portable off target gene modification at its initial stage provide promising approaches for AD treatment.

ApoE4 structural modifications

The amino acid residues 112 and 158 at N terminal are markers of ApoE polymorphism, while Glu255 and Arg 61 interaction is the structural characteristic of ApoE4 and ApoE3 respectively [52–54]. An anomalous structure of ApoE4 can be produced by this domain-domain interaction, which leads to neurotoxicity [55, 56]. ApoE4 structure-correctors (PH002 and GIND25) interacts with Arg61 which disrupt this domain-domain (Arg61 at N-terminal and Glu255 at C-terminal) interaction leads to ApoE4 to ApoE3 [53]. Therefore, the pathological confirmation change can provide an alternative therapeutic approach.

ApoE -Aβ interaction inhibition

ApoE and Aβ interaction cause Aβ accumulation in the AD brain. An artificial peptide $Aβ_{12-28P}$, analogous to the ApoE binding site on Aβ, reduced the interaction between ApoE and Aβ, as well as Aβ aggregation in animal models [57–61]. $Aβ_{20–29}$ peptides were also found to reduce the ApoE and Aβ interaction *in vitro*, apart from helping in decreasing fibrillogenesis and cytotoxicity of Aβ [62]. Immunotherapy of ApoE -like antibodies against human ApoE3 and ApoE4 was found to be useful against non-lipidated ApoE, and helps in reducing the Aβ accumulation in mice models [63]. So, inhibiting ApoE and Aβ interaction might be a promising strategy to treat AD.

Therapeutics targeting ApoE2

ApoE2 occurrence in AD brains is two times lower than normal individuals. ApoE2 has been found to decrease cognitive dysfunction and improve neuroprotective activity [64–67]. Viral vectors expressing ApoE2 injection counteract ApoE4 pathological effects in mice models. So, it is a novel approach to upregulate ApoE2 expression in AD patients [68–70].

Aiming ApoE4 protein

Immunotherapy for anti-APOE4

Antibodies against Aβ and tau are introduced into the periphery where it can neutralize the toxic effect of ApoE4 because here ApoE level is 10 times more than brain [71]. It has been found that anti-ApoE antibodies used in the periphery reduce the amyloid deposition even before the amyloid plaque formation. Anti-ApoE monoclonal antibodies have potential to become a promising strategy for anti-ApoE4 immunotherapy [72, 73].

Mimetic peptides of APOE

These tiny peptides have ApoE domains which includes the amphipathic helix domain. It helps in reducing the neurodegeneration after brain insult and reduces the Aβ and tau pathogenetic effects in mice models [74–76]. Some studies have revealed that

these peptides have anti- inflammatory effect and are found to play defensive role in ApoE3 and ApoE4 mice after brain insult [77–79].

Degradation of APOE4

As described earlier, ApoE3 and ApoE4 domain-domain interaction makes it more vulnerable to proteases and contributes to enhanced neurotoxicity through fragments of ApoE4 [80]. ApoE4 fragment generation leads to stressful neurotoxic actions of ApoE4. So, the inhibitor against these ApoE4 fragments could lead to a neutralizing strategy against ApoE4 [81, 82].

Ancillary therapeutic strategies

ApoE receptor regulation

Endocytosis of ApoE is mediated by LDLR and LRP1 (low density lipoprotein receptor related protein 1). These receptors are responsible for the uptake of Aβ either by making ApoE-Aβ complex or by Aβ interaction inhibition to these receptors by competitive inhibition [83–87]. Hence, it is clear that LDLR and LRP1 helps in clearing the Aβ plaques [87–89].

Blood-brain barrier integrity restoration

Blood-brain barrier breaks down in AD by activated astrocytes (that depends on ApoE4 expression on astrocytes) which activates the cyclophilin A-(CyA-) pro-inflammatory molecule, and nuclear factor κB-matrix metalloproteinase 9 (MMP-9) and this further disrupts the tight junctions [90, 91]. Cyclosporin-A was found to be effective in the restoration of blood-brain barrier (BBB) in ApoE4 mice. CyA- and MP-9 was found to be increased in age dependent AD cerebrospinal fluid. A prospective approach including Cyclosporin-A can provide promising strategy to treat AD [92, 93].

Others approaches

Targeting neuro-inflammation

Activated microglia is mostly responsible for the inflammation and the microglia activation varies for different gene expressions. This microglia activation can be controlled [94, 95]. The neuro-inflammation plays protective role at initial stage but causes increased pathogenesis at later stage of AD. So, the therapeutic strategies targeting this inflammation may be helpful in relieving the patient from AD symptoms [96, 97].

Targeting vascular disease

It has been reported that AD risk increases due to vascular atherosclerosis, diabetes and hypertension as ApoE4 is related to vascular atherosclerosis that lead to dementia and disrupted BBB [98–100]. So, the therapeutic strategies that aim to find molecules related to ApoE4 and vascular disease may be helpful in AD treatment also [100–102].

Target transcription factors

ApoE has recently been discovered to translocate to the nucleus and bind with DNA at different locations, some of which are present on the promoter, implying that it functions as a transcription factor that suppresses promoters of genes implicated to AD [103–106]. Though the complete mechanism of its escape from the endoplasmic reticulum and translocation into nucleus is still unknown but it provides a hope for transcriptional regulation-based approach for AD treatment.

CONCLUSION

Recent advancements in technology have helped in understanding AD in a better manner. Studies suggest that multiple factors may be involved in neurodegenerative diseases like AD, and it may be difficult to target a single factor for therapeutic purposes. Diagnosis and therapeutic strategies require clear understanding of the factors involved in disease development and progression at molecular level. Recently APOE ε4 has emerged as a promising therapeutic target for treatment of AD as it plays key role in triggering the disease [107]. Studies suggest that AD progression can be avoided by targeting APOE ε4 gene and gene products as well as its functions with the aid of the above mentioned approaches [108]. Immunomodulation with ApoE4 monoclonal antibodies, mimesis through peptides of ApoE and ApoE4 degradation may be helpful as a promising approach for delaying the onset of disease.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

[1] Weller J, Budson A (2018) Current understanding of Alzheimer's disease diagnosis and treatment. *F1000 Research* **7**, F1000 Faculty Rev-1161.

[2] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* **1**, a006189.

[3] Uddin MS, Kabir MT, Al Mamun A, Abdel-Daim MM, Barreto GE, Ashraf GM (2019) APOE and Alzheimer's disease: evidence mounts that targeting APOE4 may combat Alzheimer's pathogenesis. *Mol Neurobiol* **56**, 2450–2465.

[4] Uddin MS, Kabir MT, Jeandet P, Mathew B, Ashraf GM, Perveen A, Bin-Jumah MN, Mousa SA, Abdel-Daim MM (2020) Novel anti-Alzheimer's therapeutic molecules targeting amyloid precursor protein processing. *Oxid Med Cell Longev* **2020**, 7039138.

[5] Rahman MA, Rahman MR, Zaman T, Uddin MS, Islam R, Abdel-Daim MM, Rhim H (2020) Emerging potential of naturally occurring autophagy modulators against neurodegeneration. *Curr Pharm Des* **26**, 772–779.

[6] Sultana R, Mecocci P, Mangialasche F, Cecchetti R, Baglioni M, Butterfield DA (2011) Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer's disease: insights into the role of oxidative stress in Alzheimer's disease and initial investigations into a potential biomarker for this dementing disorder. *J Alzheimers Dis* **24**, 77–84.

[7] Miyata M, Smith JD (1996) Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet* **14**, 55–61.

[8] Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K, Xu HE (2017) Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol Sin* **38**, 1205–1235.

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

[10] Noble W, Hanger DP, Miller CCJ, Lovestone S (2013) The importance of tau phosphorylation for neurodegenerative diseases. *Front Neurol* **4**, 83.

[11] Kolarova M, García-Sierra F, Bartos A, Ricny J, Ripova D (2012) Structure and pathology of tau protein in Alzheimer disease. *Int J Alzheimers Dis* **2012**, 731526.

[12] Alavi Naini SM, Soussi-Yanicostas N (2015) Tau hyperphosphorylation and oxidative stress, a critical vicious circle in neurodegenerative tauopathies? *Oxid Med Cell Longev* **2015**, 151979.

[13] Wang X, Wang W, Li L, Perry G, Lee H gon, Zhu X (2014) Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochim Biophys Acta* **1842**, 1240–1247.

[14] Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, Holtzman DM, Zlokovic BV (2008) ApoE isoform–specific disruption of amyloid β peptide clearance from mouse brain. *J Clin Invest* **118**, 4002–4013.

[15] Goyal M, Grover S, Dhanjal JK, Goyal S, Tyagi C, Chacko S, Grover A (2013) Novel natural structure corrector of apoe4 for checking Alzheimer's disease: Benefits from high throughput screening and molecular dynamics simulations. *Biomed Res Int* **2013**, 620793.

[16] Luo J, Maréchal JD, Wärmländer S, Gräslund A, Perálvarez-Marín A (2010) In silico analysis of the apolipoprotein E and the amyloid β peptide interaction: misfolding induced by frustration of the salt bridge network. *PLoS Comput Biol* **6**, e1000663.

[17] Parcon PA, Balasubramaniam M, Ayyadevara S, Jones RA, Liu L, Shmookler Reis RJ, Barger SW, Mrak RE, Griffin WST (2018) Apolipoprotein E4 inhibits autophagy gene products through direct, specific binding to CLEAR motifs. *Alzheimers Dement* **14**, 230–242.

[18] Safieh M, Korczyn AD, Michaelson DM (2019) ApoE4: an emerging therapeutic target for Alzheimer's disease. *BMC Med* **17**, 64.

[19] Chen Y, Durakoglugil MS, Xian X, Herz J (2010) ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A* **107**, 12011–12016.

[20] Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G (2019) Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat Rev Neurol* **15**, 501.

[21] Bano S, Rasheed MA, Jamil F, Ibrahim M, Kanwal S (2019) In silico identification of novel apolipoprotein E4 inhibitor for Alzheimer's disease therapy. *Curr Comput Aided Drug Des* **15**, 97–103.

[22] Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol* **9**, 106–118.

[23] Dhiman K, Blennow K, Zetterberg H, Martins RN, Gupta VB (2019) Cerebrospinal fluid biomarkers for understanding multiple aspects of Alzheimer's disease pathogenesis. *Cell Mol Life Sci* **76**, 1833–1863.

[24] Boehm-Cagan A, Bar R, Liraz O, Bielicki JK, Johansson JO, Michaelson DM (2016) ABCA1 agonist reverses the ApoE4-driven cognitive and brain pathologies. *J Alzheimers Dis* **54**, 1219–1233.

[25] Boehm-Cagan A, Michaelson DM (2014) Reversal of apoE4-driven brain pathology and behavioral deficits by bexarotene. *J Neurosci* **34**, 7293–7301.

[26] Veeraraghavalu K, Zhang C, Miller S, Hefendehl JK, Rajapaksha TW, Ulrich J, Jucker M, Holtzman DM, Tanzi RE, Vassar R, Sisodia SS (2013) Comment on "ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models." *Science* **340**, 924.

[27] Cramer PE, Cirrito JR, Wesson DW, Lee CYD, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel WD, James MJ, Brunden KR, Wilson DA, Landreth GE (2012) ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models. *Science* **335**, 1503–1506.

[28] Tachibana M, Shinohara M, Yamazaki Y, Liu CC, Rogers J, Bu G, Kanekiyo T (2016) Rescuing effects of RXR agonist bexarotene on aging-related synapse loss depend on neuronal LRP1. *Exp Neurol* **277**, 1–9.

[29] Laclair KD, Manaye KF, Lee DL, Allard JS, Savonenko A V., Troncoso JC, Wong PC (2013) Treatment with bexarotene, a compound that increases apolipoprotein-E, provides no cognitive benefit in mutant APP/PS1 mice. *Mol Neurodegener* **8**, 18.

[30] Tai LM, Koster KP, Luo J, Lee SH, Wang YT, Collins NC, Aissa M Ben, Thatcher GRJ, LaDu MJ (2014) Amyloid-β

pathology and APOE genotype modulate retinoid X receptor agonist activity *in vivo*. *J Biol Chem* **289**, 30538–30555.

[31] Tesseur I, Lo AC, Roberfroid A, Dietvorst S, Broeck B Van, Borgers M, Gijsen H, Moechars D, Mercken M, Kemp J, Hooge RD', De Strooper B (2012) Comment on "ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models." *Science* **340**, 924-e

[32] Burns MP, Vardanian L, Pajoohesh-Ganji A, Wang L, Cooper M, Harris DC, Duff K, Rebeck GW (2006) The effects of ABCA1 on cholesterol efflux and Abeta levels *in vitro* and *in vivo*. *J Neurochem* **98**, 792–800.

[33] Koldamova RP, Lefterov IM, Staufenbiel M, Wolfe D, Huang S, Glorioso JC, Walter M, Roth MG, Lazo JS (2005) The liver X receptor ligand T0901317 decreases amyloid beta production *in vitro* and in a mouse model of Alzheimer's disease. *J Biol Chem* **280**, 4079–4088.

[34] Casali BT, Corona AW, Mariani MM, Karlo JC, Ghosal K, Landreth GE (2015) Omega-3 fatty acids augment the actions of nuclear receptor agonists in a mouse model of Alzheimer's disease. *J Neurosci* **35**, 9173–9181.

[35] Riddell DR, Zhou H, Comery TA, Kouranova E, Lo CF, Warwick HK, Ring RH, Kirksey Y, Aschmies S, Xu J, Kubek K, Hirst WD, Gonzales C, Chen Y, Murphy E, Leonard S, Vasylyev D, Oganesian A, Martone RL, Pangalos MN, Reinhart PH, Jacobsen JS (2007) The LXR agonist TO901317 selectively lowers hippocampal Abeta42 and improves memory in the Tg2576 mouse model of Alzheimer's disease. *Mol Cell Neurosci* **34**, 621–628.

[36] Vanmierlo T, Rutten K, Dederen J, Bloks VW, van Vark-van der Zee LC, Kuipers F, Kiliaan A, Blokland A, Sijbrands EJG, Steinbusch H, Prickaerts J, Lütjohann D, Mulder M (2011) Liver X receptor activation restores memory in aged AD mice without reducing amyloid. *Neurobiol Aging* **32**, 1262–1272.

[37] Suidan GL, Ramaswamy G (2019) Targeting Apolipoprotein E for Alzheimer's disease: an industry perspective. *Int J Mol Sci* **20**, 2161.

[38] Bien-Ly N, Gillespie AK, Walker D, Yoon SY, Huang Y (2012) Reducing human apolipoprotein E levels attenuates age-dependent Aβ accumulation in mutant human amyloid precursor protein transgenic mice. *J Neurosci* **32**, 4803–4811.

[39] Kim J, Jiang H, Park S, Eltorai AEM, Stewart FR, Yoon H, Basak JM, Finn MB, Holtzman DM (2011) Haploinsufficiency of human APOE reduces amyloid deposition in a mouse model of amyloid-β amyloidosis. *J Neurosci* **31**, 18007–18012.

[40] Kim J, Eltorai AEM, Jiang H, Liao F, Verghese PB, Kim J, Stewart FR, Basak JM, Holtzman DM (2012) Anti-apoE immunotherapy inhibits amyloid accumulation in a transgenic mouse model of Aβ amyloidosis. *J Exp Med* **209**, 2149–2156.

[41] Mak ACY, Pullinger CR, Tang LF, Wong JS, Deo RC, Schwarz JM, Gugliucci A, Movsesyan I, Ishida BY, Chu C, Poon A, Kim P, Stock EO, Schaefer EJ, Asztalos BF, Castellano JM, Wyss-Coray T, Duncan JL, Miller BL, Kane JP, Kwok PY, Malloy MJ (2014) Effects of the absence of apolipoprotein E on lipoproteins, neurocognitive function, and retinal function. *JAMA Neurol* **71**, 1228.

[42] Bu G (2009) Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci* **10**, 333–344.

[43] Al Mamun A, Sahab Uddin M, Fahim Bin Bashar M, Zaman S, Begum Y, Bulbul IJ, Siddiqul Islam M, Shahid Sarwar M, Mathew B, Shah Amran M, Md Ashraf G, Bin-Jumah MN, Mousa SA, Abdel-Daim MM (2020) Molecular insight into the therapeutic promise of targeting APOE4 for Alzheimer's disease. *Oxid Med Cell Longev* **2020**, 5086250.

[44] Hauser PS, Narayanaswami V, Ryan RO (2011) Apolipoprotein E: from lipid transport to neurobiology. *Prog Lipid Res* **50**, 62–74.

[45] Wahrle SE, Jiang H, Parsadanian M, Legleiter J, Han X, Fryer JD, Kowalewski T, Holtzman DM (2004) ABCA1 is required for normal central nervous system ApoE levels and for lipidation of astrocyte-secreted apoE. *J Biol Chem* **279**, 40987–40993.

[46] Wahrle SE, Jiang H, Parsadanian M, Hartman RE, Bales KR, Paul SM, Holtzman DM (2005) Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. *J Biol Chem* **280**, 43236–43242.

[47] Wahrle SE, Jiang H, Parsadanian M, Kim J, Li A, Knoten A, Jain S, Hirsch-Reinshagen V, Wellington CL, Bales KR, Paul SM, Holtzman DM (2008) Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease. *J Clin Invest* **118**, 671.

[48] Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. *Science* **346**, 1258096.

[49] Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **533**, 420-424.

[50] Rabinowitz R, Kadair A, Ben-Zur T, Michaelson D, Offen D (2019) ApoE4 allele specific knockout using a synthetic Cas9 variant as a potential gene therapy approach for Alzheimer's disease. *Cytotherapy* **21**, e7.

[51] Zhong N, Scearce-Levie K, Ramaswamy G, Weisgraber KH (2008) Apolipoprotein E4 domain interaction: synaptic and cognitive deficits in mice. *Alzheimers Dement* **4**, 179–192.

[52] Chen HK, Ji ZS, Dodson SE, Miranda RD, Rosenblum CI, Reynolds IJ, Freedman SB, Weisgraber KH, Huang Y, Mahley RW (2011) Apolipoprotein E4 domain interaction mediates detrimental effects on mitochondria and is a potential therapeutic target for Alzheimer disease. *J Biol Chem* **286**, 5215–5221.

[53] Brodbeck J, McGuire J, Liu Z, Meyer-Franke A, Balestra ME, Jeong DE, Pleiss M, McComas C, Hess F, Witter D, Peterson S, Childers M, Goulet M, Liverton N, Hargreaves R, Freedman S, Weisgraber KH, Mahley RW, Huang Y (2011) Structure-dependent impairment of intracellular apolipoprotein E4 trafficking and its detrimental effects are rescued by small-molecule structure correctors. *J Biol Chem* **286**, 17217–17226.

[54] Cacabelos R, Torrellas C (2015) Epigenetics of aging and Alzheimer's disease: implications for pharmacogenomics and drug response. *Int J Mol Sci* **16**, 30483–30543.

[55] Smith JA, Zagel AL, Sun Y V, Dolinoy DC, Bielak LF, Peyser PA, Turner ST, Mosley TH, Kardia SL (2014) Epigenomic indicators of age in African Americans. *Hered Genet* **3**, 137.

[56] Ma Y, Smith CE, Lai CQ, Irvin MR, Parnell LD, Lee YC, Pham L, Aslibekyan S, Claas SA, Tsai MY, Borecki IB, Kabagambe EK, Berciano S, Ordovás JM, Absher DM, Arnett DK (2015) Genetic variants modify the effect of age

on APOE methylation in the Genetics of Lipid Lowering Drugs and Diet Network study. *Aging Cell* **14**, 49–59.

[57] Verghese PB, Castellano JM, Garai K, Wang Y, Jiang H, Shah A, Bu G, Frieden C, Holtzman DM (2013) ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions. *Proc Natl Acad Sci U S A* **110**, E1807-E1816.

[58] Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K (1991) Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res* **541**, 163–166.

[59] Kuszczyk MA, Sanchez S, Pankiewicz J, Kim J, Duszczyk M, Guridi M, Asuni AA, Sullivan PM, Holtzman DM, Sadowski MJ (2013) Blocking the interaction between apolipoprotein E and Aβ reduces intraneuronal accumulation of Aβ and inhibits synaptic degeneration. *Am J Pathol* **182**, 1750–1768.

[60] Sadowski MJ, Pankiewicz J, Scholtzova H, Mehta PD, Prelli F, Quartermain D, Wisniewski T (2006) Blocking the apolipoprotein E/amyloid-β interaction as a potential therapeutic approach for Alzheimer's disease. *Proc Natl Acad Sci U S A* **103**, 18787–18792.

[61] Liu S, Breitbart A, Sun Y, Mehta PD, Boutajangout A, Scholtzova H, Wisniewski T (2014) Blocking the apolipoprotein E/amyloid β interaction in triple transgenic mice ameliorates Alzheimer's disease related amyloid β and tau pathology. *J Neurochem* **128**, 577–591.

[62] Hao J, Zhang W, Zhang P, Liu R, Liu L, Lei G, Su C, Miao J, Li Z (2010) Aβ20–29 peptide blocking apoE/Aβ interaction reduces full-length Aβ42/40 fibril formation and cytotoxicity *in vitro*. *Neuropeptides* **44**, 305–313.

[63] Liao F, Li A, Xiong M, Bien-Ly N, Jiang H, Zhang Y, Finn MB, Hoyle R, Keyser J, Lefton KB, Robinson GO, Serrano JR, Silverman AP, Guo JL, Getz J, Henne K, Leyns CEG, Gallardo G, Ulrich JD, Sullivan PM, Lerner EP, Hudry E, Sweeney ZK, Dennis MS, Hyman BT, Watts RJ, Holtzman DM (2018) Targeting of nonlipidated, aggregated apoE with antibodies inhibits amyloid accumulation. *J Clin Invest* **128**, 2144–2155.

[64] Hyman BT, Gomez-Isla T, Briggs M, Chung H, Nichols S, Kohout F, Wallace R (1996) Apolipoprotein E and cognitive change in an elderly population. *Ann Neurol* **40**, 55–66.

[65] Staehelin HB, Perrig-Chiello P, Mitrache C, Miserez AR, Perrig WJ (1999) Apolipoprotein E genotypes and cognitive functions in healthy elderly persons. *Acta Neurol Scand* **100**, 53–60.

[66] Wilson RS, Bienias JL, Berry-Kravis E, Evans DA, Bennett DA (2002) The apolipoprotein E ε2 allele and decline in episodic memory. *J Neurol Neurosurg Psychiatry* **73**, 672–677.

[67] Conejero-Goldberg C, Gomar JJ, Bobes-Bascaran T, Hyde TM, Kleinman JE, Herman MM, Chen S, Davies P, Goldberg TE (2014) APOE2 enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. *Mol Psychiatry* **19**, 1243–1250.

[68] Dodart JC, Marr RA, Koistinaho M, Gregersen BM, Malkani S, Verma IM, Paul SM (2005) Gene delivery of human apolipoprotein E alters brain Aβ burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **102**, 1211–1216.

[69] Suri S, Heise V, Trachtenberg AJ, Mackay CE (2013) The forgotten APOE allele: a review of the evidence and

suggested mechanisms for the protective effect of APOE ε2. *Neurosci Biobehav Rev* **37**, 2878–2886.

[70] Hu J, Liu CC, Chen XF, Zhang YW, Xu H, Bu G (2015) Opposing effects of viral mediated brain expression of apolipoprotein E2 (apoE2) and apoE4 on apoE lipidation and Aβ metabolism in apoE4-targeted replacement mice. *Mol Neurodegener* **10**, 6.

[71] Bittar A, Sengupta U, Kayed R (2018) Prospects for strain-specific immunotherapy in Alzheimer's disease and tauopathies. *NPJ Vaccines* **3**, 9.

[72] Mahley RW (2016) Central nervous system lipoproteins: ApoE and regulation of cholesterol metabolism. *Arterioscler Thromb Vasc Biol* **36**, 1305–1315.

[73] Luz I, Liraz O, M. Michaelson D (2016) An anti-apoE4 specific monoclonal antibody counteracts the pathological effects of apoE4 *in vivo*. *Curr Alzheimer Res* **13**, 918–929.

[74] Ghosal K, Stathopoulos A, Thomas D, Phenis D, Vitek MP, Pimplikar SW (2013) The apolipoprotein-E-mimetic COG112 protects amyloid precursor protein intracellular domain-overexpressing animals from Alzheimer's disease-like pathological features. *Neurodegener Dis* **12**, 51–58.

[75] Vitek MP, Christensen DJ, Wilcock D, Davis J, Van Nostrand WE, Li FQ, Colton CA (2012) APOE-mimetic peptides reduce behavioral deficits, plaques and tangles in Alzheimer's disease transgenics. *Neurodegener Dis* **10**, 122–126.

[76] Wang W, Zhu X (2018) HDL mimetic peptides affect apolipoprotein E metabolism: equal supplement or functional enhancer? *J Neurochem* **147**, 580–583.

[77] Laskowitz DT, Song P, Wang H, MacE B, Sullivan PM, Vitek MP, Dawson HN (2010) Traumatic brain injury exacerbates neurodegenerative pathology: improvement with an apolipoprotein E-based therapeutic. *J Neurotrauma* **27**, 1983–1995.

[78] Yao X, Vitek MP, Remaley AT, Levine SJ (2012) Apolipoprotein mimetic peptides: A new approach for the treatment of asthma. *Front Pharmacol* **3**, 37.

[79] Wang H, Anderson LG, Lascola CD, James ML, Venkatraman TN, Bennett ER, Acheson SK, Vitek MP, Laskowitz DT (2013) ApolipoproteinE mimetic peptides improve outcome after focal ischemia. *Exp Neurol* **241**, 67–74.

[80] Brecht WJ, Harris FM, Chang S, Tesseur I, Yu GQ, Xu Q, Fish JD, Wyss-Coray T, Buttini M, Mucke L, Mahley RW, Huang Y (2004) Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. *J Neurosci* **24**, 2527–2534.

[81] Huang Y, Liu XQ, Wyss-Coray T, Brecht WJ, Sanan DA, Mahley RW (2001) Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proc Natl Acad Sci U S A* **98**, 8838–8843.

[82] Harris FM, Brecht WJ, Xu Q, Tesseur I, Kekonius L, Wyss-Coray T, Fish JD, Masliah E, Hopkins PC, Scearce-Levie K, Weisgraber KH, Mucke L, Mahley RW, Huang Y (2003) Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proc Natl Acad Sci U S A* **100**, 10966–10971.

[83] Holtzman DM, Herz J, Bu G (2012) Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. *Cold Spring Harb Perspect Med* **2**, a006312.

[84] Kanekiyo T, Zhang J, Liu Q, Liu CC, Zhang L, Bu G (2011) Heparan sulphate proteoglycan and the low-density

lipoprotein receptor-related protein 1 constitute major pathways for neuronal amyloid-beta uptake. *J Neurosci* **31**, 1644–1651.

[85] Liu Q, Trotter J, Zhang J, Peters MM, Cheng H, Bao J, Han X, Weeber EJ, Bu G (2010) Neuronal LRP1 knockout in adult mice leads to impaired brain lipid metabolism and progressive, age-dependent synapse loss and neurodegeneration. *J Neurosci* **30**, 17068–17078.

[86] Kim J, Castellano JM, Jiang H, Basak JM, Parsadanian M, Pham V, Mason SM, Paul SM, Holtzman DM (2009) Overexpression of low density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular Aβ clearance. *Neuron* **64**, 632.

[87] Kanekiyo T, Cirrito JR, Liu CC, Shinohara M, Li J, Schuler DR, Shinohara M, Holtzman DM, Bu G (2013) Neuronal clearance of amyloid-β by endocytic receptor LRP1. *J Neurosci* **33**, 19276–19283.

[88] Shinohara M, Sato N, Kurinami H, Takeuchi D, Takeda S, Shimamura M, Yamashita T, Uchiyama Y, Rakugi H, Morishita R (2010) Reduction of brain β-amyloid (Aβ) by fluvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, through increase in degradation of amyloid precursor protein C-terminal fragments (APP-CTFs) and Aβ clearance. *J Biol Chem* **285**, 22091.

[89] Qosa H, Abuznait AH, Hill RA, Kaddoumi A (2012) Enhanced brain amyloid-β clearance by rifampicin and caffeine as a possible protective mechanism against Alzheimer's disease. *J Alzheimers Dis* **31**, 151–165.

[90] Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, Berk BC, Zlokovic BV (2012) Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* **485**, 512–516.

[91] Alata W, Ye Y, St-Amour I, Vandal M, Calon F (2015) Human apolipoprotein E ε4 expression impairs cerebral-vascularization and blood–brain barrier function in mice. *J Cereb Blood Flow Metab* **35**, 86.

[92] Bien-Ly N, Boswell CA, Jeet S, Beach TG, Hoyte K, Luk W, Shihadeh V, Ulufatu S, Foreman O, Lu Y, DeVoss J, van der Brug M, Watts RJ (2015) Lack of widespread BBB disruption in Alzheimer's disease models: focus on therapeutic antibodies. *Neuron* **88**, 289–297.

[93] Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I (2017) A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* **169**, 1276-1290.e17.

[94] Brown GC, St George-Hyslop PH (2017) Deciphering microglial diversity in Alzheimer's disease. *Science* **356**, 1123–1124.

[95] Wattananit S, Tornero D, Graubardt N, Memanishvili T, Monni E, Tatarishvili J, Miskinyte G, Ge R, Ahlenius H, Lindvall O, Schwartz M, Kokaia Z (2016) Monocyte-derived macrophages contribute to spontaneous long-term functional recovery after stroke in mice. *J Neurosci* **36**, 4182–4195.

[96] Uddin MS, Kabir MT, Mamun A Al, Barreto GE, Rashid M, Perveen A, Ashraf GM (2020) Pharmacological approaches to mitigate neuroinflammation in Alzheimer's disease. *Int Immunopharmacol* **84**, 106479.

[97] Bell RD (2012) The imbalance of vascular molecules in Alzheimer's disease. *J Alzheimers Dis* **32**, 699–709.

[98] Altman R, Rutledge JC (2010) The vascular contribution to Alzheimer's disease. *Clin Sci* **119**, 407–421.

[99] Rohn TT (2014) Is apolipoprotein E4 an important risk factor for vascular dementia? *Int J Clin Exp Pathol* **7**, 3504.

[100] Mahley RW, Weisgraber KH, Huang Y (2009) Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res* **50 Suppl**, S183–S188.

[101] Davey DA (2014) Alzheimer's disease and vascular dementia: one potentially preventable and modifiable disease? Part II: Management, prevention and future perspective. *Neurodegener Dis Manag* **4**, 261–270.

[102] Theendakara V, Peters-Libeu CA, Spilman P, Poksay KS, Bredesen DE, Rao RV (2016) Direct transcriptional effects of apolipoprotein E. *J Neurosci* **36**, 685–700.

[103] Anne UB, Urfer R (2017) Identification of a nuclear respiratory factor 1 recognition motif in the apolipoprotein E variant APOE4 linked to Alzheimer's disease. *Sci Rep* **7**, 40668.

[104] Rohn TT (2017) Nuclear localization of apolipoprotein E4: a new trick for an old protein. *Int J Neurol Neurother* **4**, 067.

[105] Theendakara V, Peters-Libeu CA, Bredesen DE, Rao R V. (2018) Transcriptional effects of ApoE4: relevance to Alzheimer's disease. *Mol Neurobiol* **55**, 5243–5254.

[106] Huang HJ, Chen HY, Lee CC, Chen CYC (2014) Computational design of apolipoprotein e4 inhibitors for Alzheimer's disease therapy from traditional Chinese medicine. *Biomed Res Int* **2014**, 452625.

[107] Tun MKM, Herzon SB (2012) The pharmacology and therapeutic potential of (–)-huperzine A. *J Exp Pharmacol* **4**, 113.

[108] Bossu P, Salani F, Ciaramella A, Sacchinelli E, Mosca A, Banaj N, Assogna F, Orfei MD, Caltagirone C, Gianni W, Spalletta G (2018) Anti-inflammatory effects of homotaurine in patients with amnestic mild cognitive impairment. *Front Aging Neurosci* **10**, 285.