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# The Exploration of Protein Therapies for the Alzheimers Disease

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**Abstract:**

Alzheimer's disease (AD) is a global puzzle that first appeared in 1901, the number of patients with AD is soaring all over the world these years. While various mechanisms were proposed, such as Aβ cascade hypothesis, the results were still dissatisfactory, and they all seem to be implicated in each other. Until now, only two kinds of medicines are allowed to treat AD by the FDA, but they are not capable of achieving therapeutic effects. New attention is paid to the treatment options for lipids. This paper analyzes references about cholesterol homeostasis and transport in the brain, finding that an excellent curative effect can emerge by intraperitoneal injection of CS-6253 to raise the level of lipidation of APOE 4, which can provide the references on protein therapies and lipids mechanism for AD, promoting the proceeding of AD study. Nevertheless, the topic that make CS-6253 cross the BBB and perform effectively in the human body have not been tackled, further studies can be biased toward this aspect.

**Keywords:** AD; cholesterol; APOE; protein therapy.

## 1. Introduction

As AD develops, the subject of AD has always been a matter of public interest, the population of AD patients has been swelling during the period between 1990 and 2019 [1]. People have been devoted to finding the causes of AD, the well-known mechanism hypotheses are the Amyloid beta (Aβ) cascade hypothesis, Tau protein abnormal phosphorylation hypothesis and etc. However, the mechanism of this disease is still undetermined. At present, only two types of medicines are authorized as temporary medicine, such as inhibitors to cholinesterase enzymes (ChEIs) and antagonists to N-methyl d-aspartate (NMDA). The disappointing fact is that they are not capable to prevent or cure this disease [2]. With the existing research about Apolipoprotein E (APOE), people found the fact that APOE has proven to have a strong relationship with microtubule-associated protein tau and A□ in the central nervous system (CNS), and APOE reduces Aβ aggregation and maintains lipid homeostasis, which thought to be crucial for synaptic plasticity and neural repair processes [3]. Therefore, this article will start with APOE to explore protein therapies for AD.

The characteristics of AD are cognitive impairment, memory loss and irritability. Pathological manifestations are the presence of neurofibrillary tangles and senile plaques in the brain, accompanied by inflammation, etc. The crucial parts in the mechanism of AD include Aβ build-up and neuronal apoptosis [4]. The dominant lipoprotein-competent apolipoprotein in the CNS is APOE, which is chiefly consist of astrocytes. It is indispensable for myeline creation and natural brain utility, such as regulation of Aβ levels, lipid trafficking, and inflammation [2,5].

ATP-binding cassette transporter A1 (ABCA1) is a membrane transporter that efflux cholesterol into the extracellular space, new APOE takes cholesterol and phospholipids by ABCA1 [6], maintaining the Cholesterol homeostasis in the brain.

This assay is mainly aimed at filling a gap in the protein therapy of AD by introducing a novel ABCA1 mimetic peptide (CS-6253), which was created to stimulate ABCA1, bonding the combination of lipids and APOE. It will also discuss the possibility and feasibility of antibody to cure the Alzheimer disease.

## 2. The Role of Cholesterol Homeostasis in the Pathogenesis of AD

Almost 25% cholesterol are distributed in brain, which possesses more lipid than other organs in body. It is also a primary portion of the cellular membrane, regarding to cell signaling pathways and gene transcription [3]. In these cases, cholesterol is considered to be of great importances to CNS. Recent evidence has implicated that cholesterol homeostasis plays an essential role in brain ability and human fitness, and this proposal is proved by several experiments. For instance, hypercholesterolemia has associateship with enhanced brain Aβ immunoreactivity in rabbits, indicating the relevant relation between cholester-

ol and neurodegeneration [7].

The human brain has a rigorous system to maintain homeostasis, the blood-brain barrier (BBB), exists in brain to prevent any dispersion of large molecules at the level of tight junctional attachments between adjacent capillary endothelial cells [7]. Therefore, the lipids in peripheral cells do not enter the cerebral circulation, contributing to the cholesterol homeostasis in brain. It is obviously that BBB incompleteness pose a hazard to AD, with the research on AD patients showing that the permeability of aging BBB is bigger than intact BBB [8]. A damaged BBB can give rise to the entry of proinflammatory and neurotoxic factors, such as thrombin, immunoglobulins, hemoglobin, bacterial breakdown products, iron, and fibrinogen, hindering the CNS utility, promoting neuroinflammation and neurodegeneration as well [4].

When the cholesterol is excessive, brain will transform it from cholesterol into 24S-hydroxycholesterol, which can get though the BBB. This way is considered as a major excretory mechanism to release extra cholesterol [7]. Another way is that raising the transcription for cholesterol transport proteins to intensify the outflow of lipids [3]. However, when the adjustment is not under control, an accumulated risk of neurodegenerative disorders (NDDs) can be found, such as AD, Huntington's disease (HD), Parkinson's disease (PD) [3]. As the research reveals that impaired microglia function can be a consequence of the overwhelming lipid, including aggravating inflammation and weaken engulf particles, which both are relevant to AD [4]. In addition, if neuronal cell is situated in an environment with high cholesterol settings, the growth of cholesterol in neuronal cell membranes will accelerate producing Aβ. Besides, a vicious cycle will emerge: the damaged microglia will lose the capability of eliminating the Aβ, strengthening the inflammatory signaling and production of reactive oxygen species (ROS) [4]. Next Aβ continues to accumulating, massive plaque generated, so that neurodegeneration happen [4]. The above pathological and physiological mechanisms can confirm the crucial role of cholesterol homeostasis.

27-hydroxycholesterol (27-OHC) is a compound which is Oxidized from cholesterol in the peripheral environment. New research illustrates that 27-OHC can through BBB, resulting in a more severe manifestation of cognitive impairment in memory areas [4]. In contrast, the mechanism of brain transforms it into a substance called 24S-hydroxycholesterol (24S-OHC). It can also be across BBB. Studies shows that the ratio of 27-OHC to 24S-OHC in the brain with AD higher, the AD risk increased as well [4]. This data can be used as a flag signal for the equilibrium of lipid.

In a word, BBB is a guard who controls the access of the human brain, if this barrier is damaged, it is no possible for broken BBB to maintain the homeostasis of cholesterol, and the brain can be attacked by the materials in blood, leading to NDDs.

# 3. The Function of APOE in AD's Mechanism

## 3.1 The Capabilities of APOE

APOE, a secreted glycoprotein composed of 299 amino acids [9], which is composed of two independently folded structural areas: N-terminal domains (residues 1-167) and C-terminal domains (residues 206–299), and they are used to bind to receptors and lipids separately, connected by hinge regions [10].

Because of the existence of BBB, it is unable to arrive at the brain by getting though the BBB, but it can be produced mainly by astrocytes, extent microglia and oligodendrocytes, only a few fractions are made by damaged neurons [3]. APOE is stored in two isolated pools: the CNS and peripheral environment [3]. In peripheral environment, APOE is generated by the liver, regulating the distribution and metabolism of lipids. Lipid metabolism usually includes exogenous and endogenous lipid metabolism. When exogenous cholesterol assesses blood circulation, it will become remnant lipoprotein particles (RLP) after series reaction, and finally transported to the liver by binding to APOE. As the same, APOE carries the exogenous part to the liver [9], while the task of APOE in CNS is delivering the lipid and cholesterol in order to ensure synapse formation and tissue repair [10].

## 3.2 APOE4 Boosts the Damage of AD

APOE has three isoforms (APOE2, APOE3, APOE4), they are differing in amino acid positions 112 and 158, which vary between a cysteine and an arginine (apoE2: Cys112/Cys158; apoE3: Cys112/Arg158; apoE4: Arg112/Arg158) [3]. Among them, APOE2 and APOE4 both have close relationship with the pathogenesis of AD. But what is interesting is that APOE2 can protect the central nervous system, while APOE4 does damage to brain, which is related to AD or other NDDs.

### 3.3.1 Allele

Alleles have six combination types: three homozygous (APOE2/2, APOE3/3, and APOE4/4) and three heterozygous (APOE2/3, APOE2/4, and APOE3/4) genotypes [3]. Alleles have influences on the risk of AD differently, and the three alleles (ε2, ε3, ε4) have distinctive distributions with geography and ethnicity. The APOE ε4 carriers of the USA reached the point of one in four, and ε3 is considered a neutral allele that has no effect on AD [3]. However, a meta-analysis shows that although ε4 has a higher venture,

ε3 can dimmish the progress in the Chinese group [11]. The existence of APOE4 aggravates the risk developing of AD by quadruple (one allele) to 14 times (two alleles) for Americans [3].

#### 3.2.2 Lipids

Due to that tiny discernibility, APOE4 is the most tricky and hereditary isoform. In terms of lipids, people still do not understand the principle of how it leads to AD clearly, but the researches on it reveal the mystery gradually.

APOE4 is a high risky factor of AD. Initially, APOE4 forms smaller lipid complexes in both wild-type mouse brains, and mouse brains transfected with viral-expressing different APOE isoforms compared with APOE2 [3]. Next, evidences reveal the fact that APOE4 promotes the spread and aggregation of Aβ in oligomers and fibrils, less cholesterol efflux and stronger toxicity to neuronal cells. [12]. Besides, APOE4 can't maintain the integrity of the BBB, as proved by experiments [13], which could be another potential cause to AD [14]. What matters most is that the instability of microglial lipid homeostasis fails to remain monitoring the neural networks, as a result of less lipidated APOE4 [15]. An investigation the prefrontal cortex (PFC) from APOE4-carriers (APOE3/4 or APOE4/4) and non-carriers (APOE3/3) shows that the cholesterol biosynthesis, trafficking, and localization in human and mouse oligodendrocytes is changed by APOE4, which is potentially linked to descending myelination.

To sum up, ApoE4 has a weaker lipidation than ApoE2 or ApoE3 and suffers more rapid degradation in the CNS [11]. It may be unable to ship enough cholesterol out of the brain, leading to the metabolism abnormalities of cholesterol, which is associated with neurodegenerative diseases [12].

## 4. Exploitation of protein therapy

### 4.1 ABCA1 Maintains the Cholesterol Homeostasis

ABCA1 is an integral membrane protein, whose key feature is dominating cholesterol efflux. For instance, ABCA1 can help new APOE to take cholesterol and phospholipids, then excreted into the brain parenchyma as discoidal lipoproteins [6]. Similarly, it has the ability to transform cholesterol and phospholipids to apolipoprotein A-I (APOA-I), producing high-density lipoprotein (nHDL) [16], so that cholesterol can be removed from vascular walls and peripheral tissues via reverse cholesterol transport (RCT), avoiding the produce of noxious oxidized lipids [6]. At the same, HDL is known to prevent transporters from degradation, sustaining proper cholesterol levels. All in all, ABCA1 overexpression exhibit to be inversely correlated with the risk of AD because of lipids facilitate lipidation of APOE and clearance of Aβ [17]. As shown in Figure 1, the difference APOE allele, APOE4 results in minor lipoproteins with less cholesterol. A therapeutic strategy is that enhancing the expression of ABCA1 to keep the balance of lipids, which is highly effective in improving APOE lipidation, especially the amelioration of APOE4 [6].

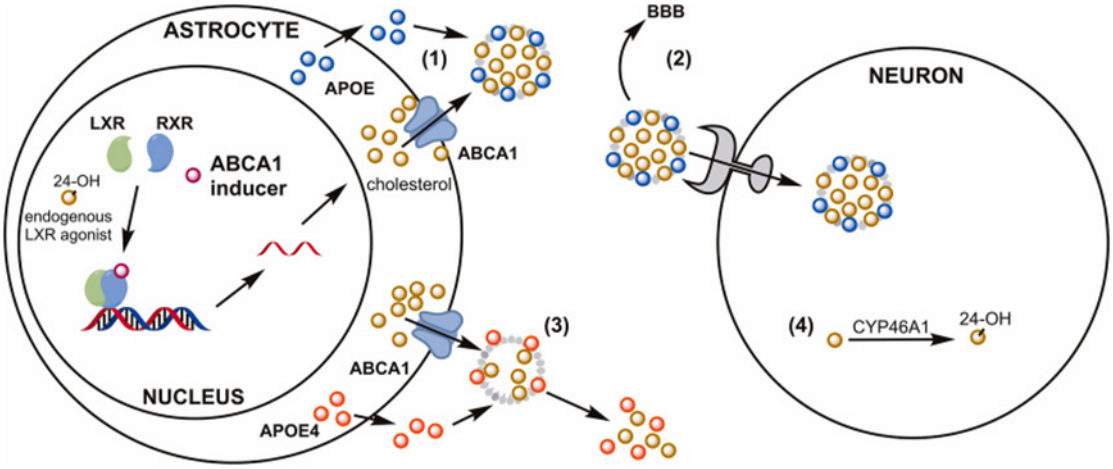


**Figure 1 Reprinted with permission from references [6].**

### 4.2 Make ABCA1 Agonist Mimetic Peptide to Increase Lipid Level

CS-6253, a small α-helical peptide, designed by the C-terminal domain of APOE, was previously developed for the treatment of atherosclerosis by Artery Therapeutics [18]. However, it didn't happen overnight. CS-6253 is a modified version of ATI-6251, which is effective in acute coronary syndrome and atherosclerosis. However, ATI-6251 was found that muscle toxicity can end in wild-type C57Bl/6 mice when in an environment with high doses because of two amino acids that are responsible for toxicity (phenylalanine residues and interfacial arginine resi-

dues) [17]. Besides, it will raise CPK, ALT activities, and plasma Tg levels [19]. To change this situation, ATI-6251 was reformed: after two replacements were manipulated on those harmful amino acids, which prominently eliminate the toxicity and 90% of the elevating effect of TG, CS-6253 has all the anti-atherosclerotic properties of the mother [17].

Initially, CS-6253 decreased Aβ level. According to the research, CS-6253 can be combined with ABCA1 better than APOE, stimulating the ABCA1 to remove the Aβ and deliver lipids [20], which give rise to the alleviation of cognitive impairment. Next, ABCA1 is crucial to the lipids transport. CS-6253 can not only stimulate the ABCA1 but also deter the gathering of APOE4 and ABCA1 in hippocampal homogenates of ApoE4-TR mice [20]. In terms of lipid efflux, the interaction between lipid free CS-6253 and ABCA1 can generate HDL-CS-6253 particles, which can transport cholesterol into liver cells by SR-BI in-vitro [19]. Besides, as the discussion analyzed in section 4.1, HDL is also convenient for the outflow of lipids.

### 4.3 Other Approaches-antibody Therapy

Because of the high fatalness of APOE4, APOE antibodies can target it to decline the gathering rate of Aβ. 7-month-old APP/PS1 mice were treated with a kind of monoclonal antibody called HJ 6.3 for 21 weeks, it was found that plaques dropped by 20%, and functional connectivity improved well [21]. However, a dullness of actions can be a result of HJ6.3 while it is beneficial to spatial learning ability compared to PBS group [21]. In another way, peripheral Aβ antibodies is a promising treatment that can assist the efflux of Aβ [20].

## 5. Conclusion

By reading this paper, a clearer insight into the relationship between APOE, lipids and AD can be displayed to the scholars which can promote the development of the possibility of a cure for AD. CS-6253 can be an emerging therapy to cure or avoid being sickened effectively, which can improve APOE4, eliminate plaques and etc. However, it will lose functions under high level of Aβ, which is not discussed in this paper. Also due to the principle of BBB remains a mystery, there are limitations in carrying CS-6253 through BBB to make a difference in CSF, which has a momentous significance in the treatment strategy. Maybe CS-6253 will be modified in the future. Based on the analysis above, CS-6253 may be regarded as a light star in the field of AD, which brings new ideas to create a hopeful medicine for humans to prevent and cure AD

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