

Genetic Changes to ApoE4 Allele that Stop the Progression of or Reverse Alzheimer's Disease Effects

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ABSTRACT

ApoE4 is the major genetic factor in Alzheimer's disease(AD), causing different malignant phenomena such as tau tangles and amyloid plaques. The alleles of the ApoE gene researched in the paper are $\epsilon 2$ and $\epsilon 4$, where the latter one is linked with a higher AD risk. The paper comprises the background research about how the ApoE impacts lipid transport and how the switch from ApoE4 to ApoE2 resolves AD-linked issues.

Main findings include the astrocytes being the target cells for the switch, brain-blood barrier is semi-permeable and the genetic material requires specific technology to pass through it, efficient vectors that can be used for this matter are nanoparticles and viruses equipped with particular receptors, the genetic strategy can be either gene replacement using ApoE2 complementary mRNA or CRISPR/Cas9 modification, effect of $\epsilon 4$ is at the largest extent during the early amyloid development phase.

In conclusion, the model comprises a vector that can pass through the blood-brain barrier and deliver a genetic material to an astrocyte cell with a time consideration. The feasibility of the model derives from the research and the assumptions made about the efficiency of this pathway. Limitations lie in the fact that the penetration of the brain-blood barrier is rarely used in this field of research, and the impact of ApoE protein on internal organs outside of the central nervous system.

INTRODUCTION

Alzheimer's disease(AD) is considered to be one of the most common causes of dementia in the world(60-70% of cases are estimated to be caused by AD). The risks it poses to the work of the central nervous system makes the research of this area an essential part of overall neuroscientific research. While the biochemical phenomena causing the disease, such as amyloid plaques and tau tangles, as well as different risk factors like genetics and environment have been thoroughly studied, the clinical application of them is still in an active discovery phase. Among various risk factors, one that has the biggest impact genetically on the risk of Alzheimer's disease is the apolipoprotein gene, ApoE, with its three alleles that affect biochemical factors of the disorder differently. Alleles are forms of the same gene which have

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slightly different amino acid chains. This allows alleles of the same gene to code for a same trait but in a different way. $\epsilon 4$ has a negative impact on lipid metabolism and protein folding, which are the processes linked to neuronal health and Alzheimer's disease pathology whereas $\epsilon 2$, on the contrary, reduces these risks. Meanwhile, $\epsilon 3$ has a neutral action. Because of these differences, this gene can be effectively used as a framework for a development of a treatment clinically for patients with $\epsilon 4$ allele, which makes up 40-60% of late-onset Alzheimer's disease cases. Modern genetic technologies allow this topic to be seen through the lens of switching alleles as a solution to the disease problem. Various studies utilizing model organisms such as mice have detected a positive change on a biochemical level after different scales of switching $\epsilon 4$ to $\epsilon 2$. Observed amelioration in lipid metabolism and amyloid- β numbers reduction suggests a positive effect of allele switch, which can be utilized for further research. The change in neuronal activity and health was also observed after applying the $\epsilon 2$ particles to $\epsilon 4$ tissues, suggesting a direct effect of the gene on Alzheimer's disease. However, lack of knowledge about how $\epsilon 3$ and $\epsilon 2$ alleles work, compared to what we know about $\epsilon 4$, put constraints on the clarity of this effect. The main insight is that genetic change which leads to the $\epsilon 2$ -like performance is a framework for the potential solution to ApoE-associated Alzheimer's risk. A particular change of expression from $\epsilon 4$ to $\epsilon 2$ is not mandatory though, which opens the doors to explicit considerations of various genetic techniques which lead to the same implication. Another essential consideration for the AD treatment research is the features of gene therapy performed specifically for the brain cells. The blood-brain barrier, which is a border between central nervous system and circulatory system, is a key obstacle to gene therapy in the brain, considering its impermeability to large molecules. In this paper I am taking all described considerations into account to research the ways the ApoE allele switch can be conducted, the benefits and drawbacks of this strategy in comparison to potential other ones, and future clinical applications of the approach.

Literature Review

Functional differences between isoforms of the APOE are believed to derive from the structural amino acid substitutions, where $\epsilon 2$ possessing two cysteines has a great stability, resistance to thermal and chemical denaturation, and $\epsilon 4$ contains arginines at the same residues, which make the molecule more likely to exhibit domain interactions, hence undergo the misfoldings of the tertiary structure (Raulin et. al., 2022). Thus, the changes in the gene coding for these proteins alter the functions it performs. The amyloid cascade hypothesis argues the underlying imbalance between production and removal of amyloid- β (A- β) in AD pathogenesis is ApoE (Kepp et. al., 2022). It proposes the oligomers of this protein are key in forming amyloid plaques, which are the primary AD biochemical symptom. APOE isoforms in this system facilitate the production and the clearance of the A β , but the extent to which they perform the function is varying. APOE2 is the least effective at the oligomerisation of the A β and shows the best performance on clearing it either by degrading in the cell or transporting to the periphery, while APOE4 shows high rates of forming oligomers and low clearance. At the same time, $\epsilon 2$ and $\epsilon 3$ forms have a high chance of binding to the A β and reducing its neurotoxicity. Another factor believed to be impacting the neurodegeneration is the lipid transport between neurons and glia in the brain (Rahhan et. al., 2026). The regulation of this process is mainly done by apolipoproteins. The accumulation of oxidized lipids in the neurons leads to a ferroptosis, a specific cell death type, which inevitably worsens brain health. Glia are

usually used as containers for the lipids before depositing them, so effective transport of these compounds allows cells to evade ferroptosis. APOE2 isoform shows greater efficiency in effluxing this lipid content out of the cell, whereas $\epsilon 4$ prevents lipid efflux by reducing the function of the lysosomes. At the same time, it was found that APOE2 preferentially effluxes unsaturated lipids, which are the most susceptible to the lipid peroxidation, whereas APOE4 mainly keeps unsaturated lipids in the cell, while releasing less susceptible saturated ones. The connection between two biochemical phenomena can be seen through the ability to delete threatening chemicals from the cell. The APOE2 particles can rescue tissues and cells containing APOE4 from isoform's negative impact, which points to potential medical applications. The effect of APOE isoforms works in a cascade-like style, where the changes in lipid transport lead to the changes in cells' health and electrical activity. Another biochemical factor provoking the disease is the tau pathology, which due to hyperphosphorylation of tau protein creates tangles in the structure, preventing it from working normally (Iqbal et. al., 2004). Normal tau protein stabilizes the microtubules in the neurons, and hence supports the axonal transport, which is essential for maintaining synapses. The hyperphosphorylation can be caused by APOE4 either independently or through the storage of A β particles in the cell. The one way to evade these damaging effects is the introduction of the ApoE2 to the organism with ApoE4 allele. It was shown that ApoE2 reduces the lipid storage in neurons, promotes lipid peroxide efflux, and its particles can rescue ApoE4-mediated processes such as endolysosomal dysfunction and lipid peroxidation (Ralhan et. al., 2026). The problem that arises when thinking of ways to introduce APOE2 to the brain tissues is the blood-brain barrier (Zhao et. al, 2022). The endothelial cells sealed by tight junctions forming this membrane prohibit large molecules including genetic vectors from entering the brain, unless having specifically engineered techniques. Specificity and low permeability of the barrier require molecules to bind particular receptors to pass the membrane. Even when passed through the barrier, efflux pumps actively remove substances back to the blood, so the quantity and concentration of the genetic treatment is to be thoroughly planned.

The alternative way of delivering the vector, which is injecting it in the cerebrospinal fluid, is not efficient enough (Haley, McCormick, 1956). The drugs from the fluid usually diffuse short distances into the brain tissue (around 0.2 mm.), thus limiting the efficiency of this method. Therefore, the focus of this paper will be on receptor-mediated transport, which leverages the use of BBB's specific systems. Molecular Trojan Horses are the fusion proteins that can bind the BBB receptors and the genetic vector. The entire complex can only then be transported through the barrier, reinforcing the idea that substances should undergo a modification to pass through it.

METHODS

In the paper I use the method of literary review for the collection and analysis of data. The topics of paper analysis included the background of Alzheimer's disease and particularly of ApoE gene's impact and part, biochemical phenomena causing AD and their genetic regulation, information on the brain-blood barrier and specific vectors, as well as the hand-on research studies on the genetic switch from $\epsilon 2$ to $\epsilon 4$, different vectors' action, effect of different types of genetic sequences, and time-associated features of

ApoE4 work.

The source of the review is reviews, experimental studies and theoretical papers from credible academic databases such as Google Scholar and JSTOR. To make use of individual papers, I screened their results and methodology, extracted key information from the credible and relevant ones, and identified topics and patterns. I was focusing on the arguments specifically about the means of genetic switch and its impact. To draw conclusions I used deductive reasoning. The conclusions were then merged into common topics, forming the findings.

RESULTS

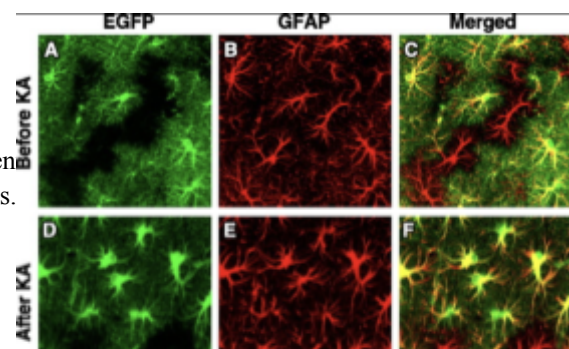
I found the following four key findings, which contribute to answering the identified research question: the target cells are primarily astrocytes; blood-brain barrier requires specifically designed transporters for ApoE2 material to pass through it; Content of the vectors affects the impact treatment would have on the cells; ApoE4 effect is prevalent in early amyloid development phase.

1. The target cells for this gene therapy are primarily astrocytes

In order to make the gene therapy more efficient, the directionality should be planned in the most productive way. In the comparative study of switching ApoE4 allele to ApoE2 in (Golden et. al., 2025) mice with a mix of human ApoE4 and hidden ApoE2 were triggered to overexpress the latter allele. The research group tested the whole-body switch as well as an astrocyte-specific switch. Astrocytes are shown to be the dominant ApoE producers in the CNS which makes them an obvious target for the therapy. Study has shown that both whole-body switch and astrocyte-based one had nearly the same effect. They both introduced an 'ApoE2-like' lipid metabolism, reducing amyloid plaques and maintaining synapses' health. When the astrocyte based pathway was performed, non-astrocyte cells such as microglia or oligodendrocytes had their expression changed. At the same time, astrocyte-based methods require less dosage and therefore are more cost effective for clinical treatment use. However, in a study of ApoE expression in CNS of mice (Xu et. al., 2006) it was shown that a specific subclass of astrocytes(25% of the general amount in the experiment) did not express the ApoE (Figure 1). This suggests that practical assays of treatment's effect on the astrocytes should be done prior to introducing the treatment. That way the efficiency of a therapy can be maximised by reducing unnecessary effort.

2. Blood-brain barrier requires specifically designed transporters for ApoE2 material to pass through it.

For an ApoE2 treatment to affect the neurons an efficient and harmless delivery to the CNS is required. Even though the direct intracerebral injections are most commonly used in scientific research because of their relative simplicity, their presence in real treatments can pose a range of negative consequences. The study of the effects of different stimulants, such as adrenalin, acetylcholine, atropine and others, injected intracerebrally into mice has shown lots of the harmful downsides(Pardridge, 2020). This included depression, hyperexcitability, tail posture changes,



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slowed breathing, depression, seizures. They had a finite effect on the organism, which varied in duration, and did not induce any residual consequences. Regardless, some of the high doses were lethal. Therefore, prolonged usage of the drugs can be risky if injected intracerebrally. Intravenous injection of the vector, hence, seems to be a more plausible variant. However, the transport of vectors from blood to CNS requires the passing through or bypassing of a semi-permeable blood-brain barrier.

The mature blood–brain barrier (BBB) is composed of capillary endothelial cells (ECs) tightly connected with tight junctions (TJs) and adherens junctions (AJs)(Bors, Erdo, 2019). This structure is also impacted by astrocytes, pericytes and neuroglia, which regulate the proliferation, migration, and vascular branching of the brain endothelial cells(Bors, Erdo, 2019). Lack of fenestrations in ECs as well as tight junctions between cells restricts the transcellular and paracellular transport of chemicals. Hence, there is a need for specifically designed ways to pass through the ECs. The control of the BBB is dynamic and depends on the function of some cells. Accessory cells regulate various features of the barrier: pericytes are involved in TJs formation, astrocytes in releasing permeability factors and protective factors(including ApoE), microglia in disruption of TJs and increasing permeability(Zhao et. al., 2022). The high efflux rate in the BBB is supported by transporter proteins, which allows for efficient extrusion of metabolic waste(which can be generated by the vectors carrying genetic material) (Figure 2). Small, non-polar chemicals can passively diffuse through the membranes of the ECs, but molecules with opposite features require facilitated movement. Presence of tight junctions makes transcellular transport more plausible for chemicals. Thus, molecules used in brain gene therapies can pass through the barrier through either carriers, receptors or adsorption, with adsorption being the least efficient way. Therefore, specifically engineered decisions are required for the genetic treatment to pass through the BBB.

One of the vectors encountered in general gene therapy is viral vectors, which involve engineering of naturally occurring viruses. One group is adeno-associated viruses(AAVs). It consists of various serotypes

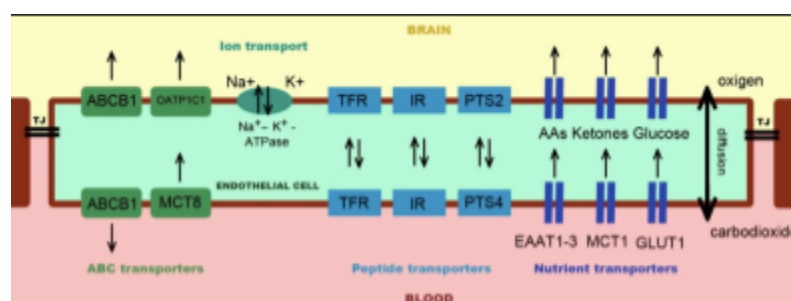


Figure 2: The schematic representation of the most important transport systems at the blood–brain barrier endothelial cells(Bors, Erdo, 2019)

with different abilities of passing through the BBB, based on capsid structure(Bors, Erdo, 2019). Proteins of capsids bind to receptors on the ECs, which allow the virus to undergo transcytosis. This suggests that direct engineering of the capsids, involving mixing samples from different serotypes and insertion of accessory peptides can enhance the ability and efficiency of passing through the BBB. The key action is to research specific abilities of different serotypes and peptides,

such as transcytosis capabilities, receptor binding to ECs and resistance to efflux. Then these species with different valuable properties are mixed together to create the most effective carrier.

Another vector that has an alternative route of transferring the genetic material is a herpes simplex virus(HSV-1). Instead of passing through the BBB, the virus travels to CNS through axonal transport entering the path on peripheral nerve terminals. This is an alternative way, which does not require additional engineering for more efficient binding to ECs' receptors. However, this virus naturally shows more cytotoxicity to the surrounding cells than AAVs, therefore requires additional effort spent on the purification(Ho et. al., 1995).

Another group of vectors that are able to pass through the BBB are polymeric nanoparticles, which are solid colloids of different sizes and origins. Polymeric nanoparticles are a promising choice as drug delivery platform for CNS targeting, due to their tunable architecture(10 to 1000 nm), non-toxicity, biocompatibility, and controllable drug release (Zhang et. al., 2021). The ligands can be added to these nanoparticles to bind to receptors on ECs or perform other functions which lead to enhancing the transcytosis. Such ligands include surfactants, peptides and antibodies, and they were shown to increase the possibility of the BBB crossing. For instance, PS80 surfactant (Zhang et. al., 2021) has allowed the nanoparticle to adsorb the apolipoprotein, allowing it to pass through the BBB (Figure 3).

Different features of the nanoparticles decide the pathways they can take and their efficiency. Positively charged nanoparticles undergo adsorptive-mediated transcytosis, which is a slower approach with a lower capacity and higher risk of toxicity. This pathway is based on the strong electrostatic interactions, it can disrupt cell membranes and inner chemistry. Negatively charged nanoparticles have an increased chance of interacting with BBB receptors, but longer circulation times. Hence, neutralizing of the charge is often needed for using the advantages of both pathways. Size of particles is essential for them to pass through

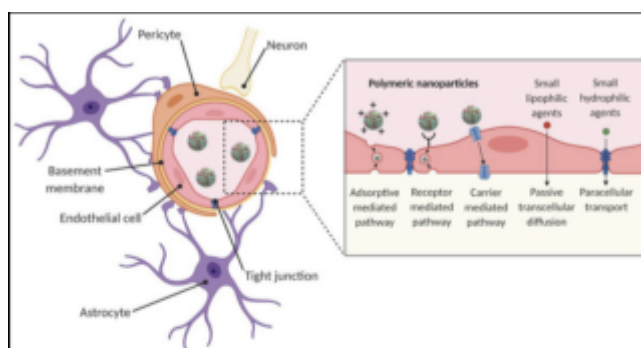


Figure 3. The neurovascular unit. Cross-section schematic of a brain capillary depicting endothelial cells interconnected via tight junctions. On the brain side of the endothelial cells, the basement membrane surrounds the endothelial cells and embeds pericytes that span several endothelial cells. Astrocytic end-feet are in contact with the endothelial cells. Neurons are present in the tissue of the brain. Polymeric nanoparticles are transported across the endothelium carrier via carrier-mediated, receptor-mediated, and adsorptive-mediated pathways (Zhang et. al., 2021)

the barrier and not be rapidly cleared out. Size of 5-10 nm and less makes particles cleared by the kidneys, whereas a size over 200 nm slows the BBB transport down. At the same time, the optimal size can vary depending on the ligand and nanoparticle used in the pathway. For instance, PEG-coated PLGA nanoparticle (Zhang et. al., 2021) showed the highest efficiency when applied to the particle with a diameter of 100 nm, whereas PEG-vitamin E applied with the same conditions showed the highest efficiency in the BBB penetration at 20 nm. In both cases the smaller size allows more effective BBB crossing.

Shape plays a big role in nanoparticles' interactions with the endothelial membrane and intracellular transport. The nature of the effect of the shape is not well-defined as of the charge and size, but is still an essential consideration. The shape defines the interaction of particles with ligands, and therefore can alter the efficiency of ligand's work. Hence, observations on the work of different shapes of the particles have

suggested the elongated rod-shaped particles have less affinity with the ECs compared to spherical ones, but show higher BBB transport if the association is fixed.

An essential group of nanoparticles apart from polymeric are inorganic particles, which use metals or non-metals as the covering material for the treatment. They all have a very low toxicity, and can pursue different pathways based on their features: magnetic particles, mostly those with an iron core, can adsorb to the ECs due to magnetic properties; gold nanoparticles improve biocompatibility and targeting, carbon nanotubes can have varying flexibilities based on the amount of graphene layers, which is important in transcytosis.

While all instruments for transporting genetic material through the blood-brain barrier have various ways of improving and development, polymeric nanoparticles can be considered as the best option. With a high rate of control and biocompatibility, and low toxicity, they provide more space for amelioration. The ability to apply ligands allows to extend the BBB crossing, giving the advantage to the polymeric nanoparticles. Nanoparticles are more flexible towards medical modifications. Recombinant AAVs or those with added receptors can provoke innate or adaptive immune responses. The instance is the TLR9 recognising CpG motifs in AAV genomes, which triggers interferon and cytokine release (Arjomandnejad et. al., 2023). Therefore, nanoparticles can be more favourable for modifications of transporting the treatment.

3. Content of the vectors affects the impact treatment would have on the cells.

The nature of a gene therapy can take various forms, amongst which the most important ones in the study of ApoE genes are methods of replacement of genes by transporting mRNA plasmids and modification of the mutation which is done by specific nucleases and proteins. These two pathways have different processes involved in their execution, and, therefore, different advantages and disadvantages when

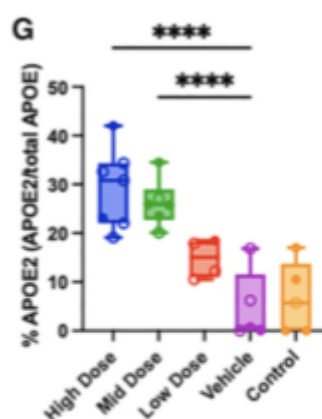


Figure 4: Mass spectrometry-determined CSF APOE2 concentration as percentage of total APOE ($F_{4,23} = 14.72$, $p < 0.0001$; post hoc Dunnett's test to vehicle) (Jackson et. al., 2024)

considering their usage for the APOE gene therapy. The gene replacement pathway consists in the idea of inducing the expression of the ApoE2 allele to reduce the effect of the ApoE4 allele. The vector also carries the promoter which would start the expression, upstream intron which would elevate the rate of expression and a growth hormone. The usage of an ependymal-specific promoter, namely VWA3(Jackson et. al., 2024), allows expression of the gene in the ependymal cells, which release ApoE2 protein in cerebrospinal fluid(CSF). Therefore, the isoform can impact lipid metabolism of all neurons in the brain. Study of ApoE2 replacement therapy on mice models(Jackson et. al., 2024) has shown that through this model, around 30% of total CSF ApoE becomes ApoE2 (Figure 4). The expression of ApoE4, however, remains unchanged, but the effect of it reduces due to the presence of recently introduced ApoE2. The model is proven to decrease the number and size of plaques as well as A β 42 levels. Synaptic integrity, which is the main factor in AD-driven

dementia, is also shown to be improved. In the same study, mice with a high dose of the treatment show almost normal synapse levels, which implies a high level of reduction of the ApoE4's effect. Overall, this methodology provides a relatively easy pathway for reducing the amyloid deposition and decreasing the rate of neurodegeneration.

An alternative pathway in changing the nature of ApoE isoforms is the modification of malignant ApoE4 by CRISPR/Cas9. Based on the research of the mice brain models, this method works through engineering the dual RNA structure, which comprises CRISPR RNA and trans-activating CRISPR RNA, both of which have distinguished roles(providing the guide sequence and stabilizing complex. The structure is then fused into single-guide RNA(Kim et. al., 2025). This allows all sequences to be condensed in the one strand. RNA also directs Cas9 to a complementary DNA sequence for altering the expression. This instrument allows modification of the genes expressed, and therefore, a controlled therapy. One of the CRISPR/Cas9 models relies on the observation of miR-33(a precursor of microRNA) being upregulated in ApoE4 cells compared to ApoE3 controls. The CRISPR/Cas9 is hence directed at the disruption of miR-33 locus. miR-33 is a factor in suppression of ABCA1 lipid transporter. The deletion of the particle by CRISPR/Cas9 has shown an increase in the number of the transporter proteins, improving the lipid export from neurons. The consequence of this change is boosted lipidation of ApoE4 particles, which by their function resemble benign ApoE2 isoform. Therefore, the rate of amyloid- β degradation increases, contributing to rescuing cells from AD factors. This method does not directly introduce the expression of ApoE2 instead of ApoE4, but it provides the modified sequence, which alters the ApoE4 expression, changing the nature of the protein isoform.

4. ApoE4 effect is prevalent in early amyloid development phase

The study of the period at which the impact of ApoE4 on AD-related factors is the most essential allows us to understand the stage of human development at which the introduction of a treatment would be the most vital. Based on the research of mice models with a human ApoE4 inserted in their astrocytes' chromosomes(Liu et. al., 2017) with a control regulation of the gene expression. The experiment has

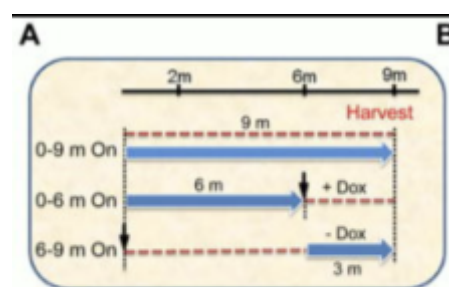


Figure 5: Illustration of apoE induction paradigms at different stages of amyloid pathology (Liu et. al., 2017)

shown that the expression of ApoE4 in the A β -seeding period increased dramatically A β 40 and A β 42, meanwhile expression during the stage of rapid growth has shown little to no impact (Figure 5). This difference in effect is explained by the ApoE4 impairing the A β clearance. The amyloid seeding stage was also shown to be connected with increased A β oligomerisation and plaque creation. This finding suggests that the early periods of mammal development when the A β seeding occurs are the most crucial in ApoE4 effect. This implies that the genetic treatment should be applied through one of two pathways: early detection of the ApoE4 allele and usage of preventive treatment, or usage of treatment rescuing the tissues from AD conditions.

DISCUSSION AND ANALYSIS

Through this study, I research and propose specific models of genetic treatment that can be plausible for performing the switch of allele from $\epsilon 4$ to $\epsilon 2$. These models can combine together, forming specifically engineered decisions, which all have the features that need to be tested. General strategy is based on the development of a carrier which would directly transfer the genetic material across the blood-brain barrier, bind to the specific place in the target cell, which is namely an astrocyte, and induce either new expression of the genes or modification of the original one. The vector would target astrocytes and based on the age of the patient (whether the amyloid seeding is occurring or not) a few adjustments would be made. The model relies on the assumption that the ApoE is one of the most efficient targets for patients with ApoE4 allele, because its isoform causes 40-60% of late-onset Alzheimer's disease. While other targets can be useful in inducing deletion of amyloid- β from the cells, the ApoE gene therapy is special in its ability to impact interconnected phenomena, like amyloid plaques, tau tangles and impaired lipid transport. This makes the particular model have an integrative effect on the organism. When choosing between two kinds of vectors, viral and nanoparticles, it's important to encounter both the advantages and disadvantages of the primer one. Viral vectors are efficient because of the ability to engineer any receptors needed to pass through the BBB and later to enter astrocytes, but because of this reason, they can be a target for the patient's immune system. Generally, AAVs are safe but the addition of some of the receptors can provoke the reaction of the immune system. Nanoparticles, on the other hand, don't have this potential damaging effect, and their properties can be used to maximise transcytosis through the BBB. However, their entrance into the cell requires addition of ligands. The directionality of the vectors also plays a big role in the model, since they require additional receptors or ligands added to the surface for an effective binding to the target astrocytes. Besides the fact they need to pass through the BBB, the vectors also need to have a mechanism to find and interact with the cell. The astrocytes have a great recognition of the neuronal signals, therefore this natural feature can be used for adding specific neuronal receptors and structures able to produce neurotransmitters. Such receptors specific to astrocytes are purinergic, adrenergic, glutamate, GABA.

Current research of AD gene therapy also encounters other approaches, including targeting other chemical units than ApoE4. Some of them are based on the restoration of impaired enzymes that catalyze very specific reactions. For instance, neprilysin, which is a major A β -degrading enzyme, when treated with gene therapy reduces A β burden. However, the directionality of action of the enzyme, which is the degradation of amyloid-beta only, and lack of impact on other AD factors such as tau tangles or lipid efflux make the pathway less efficient. The similar issue is with nerve growth factor and brain-derived neurotrophic factor, which are essential for synaptic plasticity only. ApoE impacts most of the factors of AD, which allows gene therapy targeted at it to be the most efficient, if the patient has an $\epsilon 4$ allele.

The feasibility of this model is grounded in the studies evidencing the permeability of the BBB to particular viral vectors and nanoparticles, the effect ApoE switch has on lipid metabolism, the astrocytes producing the ApoE. The limitations to the feasibility are based on the fact that the BBB of model organisms, which were used for studying its permeability, can have different permeabilities compared to

a human barrier. The complexity of the organism and evolutionary history of adaptations can cause this difference, so the studies made on the BBB of model organisms cannot be used as a precise example for the medical treatment. The main obstacle lies in the fact that this is a theoretical model, which relies mostly on the adjacent sources and my conclusions. However, the decent amount of trustworthy information on vectors and genetic material pose a good fundament for the model.

LIMITATIONS

There are a few limitations to the accessible current practical research on ApoE switch impact. These limitations pose the risks to the validity of the developed model. All the studies on this effect were done only in human tissues in vitro or in mice model organisms in vivo, where the treatment was introduced intracerebrally. Therefore, there are no practical investigations of how the injection of the treatment would work when transported through the blood-brain barrier. The numbers and dosages produced in those studies can be not plausible for the case of genetic material being carried in the vectors to the target cells. Hence, experimental trials of this theoretical concept are required to adjust the treatment to clinical purposes. Due to a high efflux rate of the BBB, some of the vectors could be lost to the circulation system, and this number should also be generated practically and then analysed in terms of statistics and causation. ApoE biology can also have different features in those species, which would make it difficult to rely on the model organism data.

Another essential limitation is the impact of ApoE on the peripheral organs. Since this model suggests only BBB-based injections, the vectors with genetic treatment are first introduced to the blood vessels in the great proximity to the internal organs. The issue arises because the $\epsilon 2$ isoform is associated with elevated levels of cholesterol and triglycerides which may be linked to obesity and insulin resistance. This means that if the vector by error pursued the wrong cell before passing through the BBB, the expression of ApoE2 could have started in the periphery, which would lead to deleterious side effects. Because ApoE is not only the astrocytes' protein, the security of vectors should be thoroughly developed through a range of trials.

Relying on literature reviews in some of the sections of the paper also provides some limitations. The main limitation is that the insights and conclusions of those reviews mainly derive from preclinical study of BBB, transporters or other topics. Human organism features are totally excluded from consideration, and that is why those details should be reinvestigated. A biased search of data is another limitation, as reviewers generally rely on data present in their language and may have varying numbers of databases. One of the most contributing limitations is that different reviews are heterogeneous in their methods and data focus, so while overrelying on some specific parts of the topic, they may undermine the other ones, leading to slight inaccuracies.

CONCLUSION

Through the research I summarised the general thoughts in the topic of ApoE-driven AD. The main findings include the insights about specificity of ApoE nature, different transporting systems and genetic material used in the therapy. The nature of BBB is discovered to show the importance of the development of efficient treatment transport. The paper emphasized that astrocytes should be used as a target because of their main role in production of ApoE. It also provided the essential detail about the ApoE4 effect prevailing in amyloid seeding phase, to imply potential preventive treatment for people with $\epsilon 4$ allele. The findings are discussed regarding their feasibility and potential limitations. The model proposed in the research is a treatment delivered through the BBB(or bypassing it) by one of the vectors and using either mRNA complementary to ApoE2 coding sequence or CRISPR/Cas9 to the astrocytes, when the expression is turned on, affecting AD-inducing factors. The main concerns are based on the usage of mice models in the research of both transport systems and ApoE4 effect, and the lack of empirical evidence of the transport through the BBB. Since most of the hand-on studies are performed using intracerebral injections, concerns can be raised about feasibility of the BBB transport. However, explicit research on the vectors passing through the BBB provides a good base for the practical use.

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REFERENCES

1. Iqbal, K., del C. Alonso, A., Chen, S., Chohan, M.O., El-Akkad, E., Gong, C.-X., Khatoon, S., Li, B., Liu, F., Rahman, A., Tanimukai, H. and Grundke-Iqbal, I. (2005). Tau pathology in Alzheimer disease and other tauopathies. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1739(2-3), pp.198–210.
2. João Pedro Ferrari-Souza, Lussier, F.Z., Douglas Teixeira Leffa, Therriault, J., Tissot, C., Bellaver, B., Pamela C.L. Ferreira, Malpetti, M., Wang, Y., Guilherme Povala, Andréa Lessa Benedet, Ashton, N.J., Chamoun, M., Servaes, S., Gleb Bezgin, Min Su Kang, Stevenson, J., Nesrine Rahmouni, Pallen, V. and Nina Margherita Poltronetti (2023). APOE $\epsilon 4$ Associates with Microglial Activation Independently of A β Plaques and Tau Tangles. *Science Advances*, 9(14).
3. dos Santos Rodrigues, B., Kanekiyo, T. and Singh, J. (2019). ApoE-2 Brain-Targeted Gene Therapy Through Transferrin and Penetratin Tagged Liposomal Nanoparticles. *Pharmaceutical Research*, [online] 36(11).
4. Ingusci, S., Verlengia, G., Soukupova, M., Zucchini, S. and Simonato, M. (2019). Gene Therapy Tools for Brain Diseases. *Frontiers in Pharmacology*, 10.

5. Hasty, A.H., Linton, M.F., Brandt, S.J., Babaev, V.R., Gleaves, L.A. and Fazio, S. (1999). Retroviral Gene Therapy in ApoE-Deficient Mice. *Circulation*, 99(19), pp.2571–2576.
6. Ho, D.Y., Fink, S.L., Lawrence, M.S., Meier, T.J., Saydam, T.C., Dash, R. and Sapolsky, R.M. (1995). Herpes simplex virus vector system: analysis of its in vivo and in vitro cytopathic effects. *Journal of Neuroscience Methods*, 57(2), pp.205–215.
7. Nilsson, P., Iwata, N., Muramatsu, S.-i., Tjernberg, L.O., Winblad, B. and Saido, T.C. (2010), Gene therapy in Alzheimer's disease – potential for disease modification. *Journal of Cellular and Molecular Medicine*, 14: 741-757.
8. Carrell, E.M., Yong Hong Chen, Ranum, P.T., Coffin, S.L., Singh, L.N., Tecedor, L., Keiser, M.S., Hudry, E., Hyman, B.T. and Davidson, B.L. (2023). VWA3A-derived ependyma promoter drives increased therapeutic protein secretion into the CSF. *Molecular Therapy — Nucleic Acids*, 33, pp.296–304.
9. Lehár, J., Zimmermann, G.R., Krueger, A.S., Molnar, R.A., Ledell, J.T., Heilbut, A.M., Short, G.F., Giusti, L.C., Nolan, G.P., Magid, O.A., Lee, M.S., Borisy, A.A., Stockwell, B.R. and Keith, C.T. (2007). Chemical combination effects predict connectivity in biological systems. *Molecular Systems Biology*, 3(1), p.80.
10. Liu, C.-C., Zhao, N., Fu, Y., Wang, N., Linares, C., Tsai, C.-W. and Bu, G. (2017). ApoE4 Accelerates Early Seeding of Amyloid Pathology. *Neuron*, 96(5), pp.1024-1032.e3.
11. Xu, Q., Bernardo, A., Walker, D., Kanegawa, T., W. Mahley, R. and Huang, Y. (2006). Profile and Regulation of Apolipoprotein E (ApoE) Expression in the CNS in Mice with Targeting of Green Fluorescent Protein Gene to the ApoE Locus. *Journal of Neuroscience*, 26(19), pp.4985–4994.
12. Boyles, J.K., Pitas, R.E., Wilson, E., Mahley, R.W. and Taylor, J.M. (1985). Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *Journal of Clinical Investigation*, 76(4), pp.1501–1513.
13. Haley, T.J. and McCormick, W.D. (1957). Pharmacological Effects Produced by Intracerebral Injection of Drugs in the Conscious Mouse. *British Journal of Pharmacology*, 12(1), pp.12–15.
14. Jiang, F. and Doudna, J.A. (2017). CRISPR–Cas9 structures and mechanisms. *Annual Review of Biophysics*, [online] 46(1), pp.505–529.
15. Hu, J., Liu, C.-C., Chen, X.-F., Zhang, Y., Xu, H. and Bu, G. (2015). Opposing Effects of Viral Mediated Brain Expression of Apolipoprotein E2 (apoE2) and apoE4 on apoE Lipidation and A β Metabolism in apoE4-targeted Replacement Mice. *Molecular Neurodegeneration*, 10(1).
16. Zhang, W., Mehta, A., Tong, Z., Esser, L. and Voelcker, N.H. (2021). Development of Polymeric Nanoparticles for Blood–Brain Barrier Transfer—Strategies and Challenges. *Advanced Science*, [online] 8(10), p.2003937.

17. Zhao, Y., Gan, L., Ren, L., Lin, Y., Ma, C. and Lin, X. (2022). Factors Influencing the Blood-Brain Barrier Permeability. *Brain Research*, 1788, p.147937.
18. Jackson, R.J., Keiser, M.S., Meltzer, J.C., Fykstra, D.P., Dierksmeier, S.E., Soroush Hajizadeh, Kreuzer, J., Morris, R., Melloni, A., Nakajima, T., Tecedor, L., Ranum, P.T., Carrell, E., Chen, Y., Nishtar, M.A., Holtzman, D.M., Haas, W., Davidson, B.L. and Hyman, B.T. (2024). APOE2 gene therapy reduces amyloid deposition, and improves markers of neuroinflammation and neurodegeneration in a mouse model of Alzheimer disease. *APOE2 Gene Therapy Reduces Amyloid Deposition and Improves Markers of Neuroinflammation and Neurodegeneration in a Mouse Model of Alzheimer Disease*, 32(5).
19. Bors, L. and Erdő, F. (2019). Overcoming the Blood–Brain Barrier. Challenges and Tricks for CNS Drug Delivery. *Scientia Pharmaceutica*, 87(1), p.6.
20. Boyoung Kim, Minju Yuk, Moonsu Park, Hongji Ryu, Junhang Park, Hyerin Yu, Sungsoo Park, Jin Tae Hong, Key-Hwan Lim, Sang-Bae Han, Nan Song, Hanseul Park, CRISPR editing of miR-33 restores ApoE lipidation and amyloid- β metabolism in ApoE4 sporadic Alzheimer's disease, *Brain*, Volume 148, Issue 12, December 2025, Pages 4400–4415
21. Pardridge, W.M. (2020). Blood-Brain Barrier and Delivery of Protein and Gene Therapeutics to Brain. *Frontiers in Aging Neuroscience*, [online] 11. doi:<https://doi.org/10.3389/fnagi.2019.00373>.
22. Kepp, K.P., Robakis, N.K., Høilund-Carlsen, P.F., Sensi, S.L. and Vissel, B. (2023). The Amyloid Cascade hypothesis: an Updated Critical Review. *Brain: A Journal of Neurology*, 146(10), p.awad159..
23. Fiala, J.C. (2007). Mechanisms of amyloid plaque pathogenesis. *Acta Neuropathologica*, 114(6), pp.551–571.
24. Suri, S., Heise, V., Trachtenberg, A.J. and Mackay, C.E. (2013). The forgotten APOE allele: A review of the evidence and suggested mechanisms for the protective effect of APOE ϵ 2. *Neuroscience & Biobehavioral Reviews*, 37(10), pp.2878–2886.
25. Ralhan, I., Do, A.D., Bae, J.-Y., Feringa, F.M., Cai, W., Chang, J., Chik, K., Lee, N.Y.J., Gerry, C.J., van der Kant, R., Jackson, J., Ricq, E.L. and Ioannou, M.S. (2026). Protective ApoE Variants Support Neuronal Function by Effluxing Oxidized Phospholipids. *Neuron*, [online] 114(4), pp.661-678.e10.
26. Arjomandnejad, M., Dasgupta, I., Flotte, T.R. *et al.* Immunogenicity of Recombinant Adeno-Associated Virus (AAV) Vectors for Gene Transfer. *BioDrugs* 37, 311–329 (2023).

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