

Gene Therapy for Alzheimer's Disease

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Abstract. According to CDC, Alzheimer's Disease (AD) is the one of the most prevalent type of dementia from which the elderly tends to suffer. It is a progressive cognitive disease that have different stages with different severity. Usually, it begins with mild memory loss and loss function in language and response to the environment. Millions of people aged over 65 around the world are living with Alzheimer's Disease. Until 2022, there are roughly 6.5 million Americans aged 65 or older suffering from AD. By estimation, in 2050, the population of AD is projected to reach around 12.7 million. Based on the fact that AD is a growing progressive illness from which aged groups suffer, it is urgent to look for possible treatments to AD. Currently, most available treatments are drugs that will palliate symptoms of AD, including improving cognitive functions and activities of daily living. However, traditional treatments take a long period to see progress, and patients can easily have drug resistance; they are not efficient enough. Fortunately, with the development in gene therapies, there are more possibilities in treating AD by targeting the pathological region in AD patients. This review systematically introduces treatments to AD, especially gene therapies.

Keywords: Alzheimer's Disease, gene therapy, AAV, CRISPR-Cas9.

1. Alzheimer's Disease

Based on current research on AD, it is a prevalent neurodegenerative disorder and elderly populations are more likely to have AD [1]. Most of patients will suffer from symptoms, such memory loss, from mild to severe, including function loss in language and daily activities. It has been widely recognized that AD is induced by severe stress on endoplasmic reticulum (ER). When there are too many misfolded and unfolded proteins stagnate on ER, the unprocessed protein system (UPR), the responsive mechanism to alleviate ER stress will be aberrantly activated, which will aggravate the ER stress. By measuring and comparing the expression of *Grp78/BiP* and *Chop* mRNA, the ER stress markers, in human cholinergic neuroblastoma cells induced with Amyloid beta ($A\beta$) and normal cells, researchers discovered that the toxic aggregates of $A\beta$ will induce severe ER stress by hyperactivating the UPR [2]. Therefore, $A\beta$ is recognized as one major pathological factor of AD. In addition, tau pathology is also believed to induce AD. Tau is a neuronal protein vital in maintaining the stability of microtubules and it is regulated by phosphorylation. In patients of AD, tau proteins will be aberrantly phosphorylated and they will become paired-helical-filament (PHF) and neurofibrillary tangles (NFTs) [3]. By purifying tau from bovine brain microtubules, researchers found that purified tau will react with antibodies to Alzheimer PHF, indicating that tau protein is part of the Alzheimer PHF and corroborates its pathological role in inducing AD [2]. Current treatments are mainly cholinesterase-inhibitor (CI)-based, N-methyl-D-aspartate-antagonist (NMDA)-based, or combination of CI and NMDA (Table 1). Though these treatments seem promising, they are confined to mostly AD patients in early stage and their functions are mainly alleviating the symptoms rather than eradicating.

Table 1. Current treatments to Alzheimer's Disease

Drug name	Treatment type	Mechanisms	Study reviews
Donepezil/ Rivastigmine/ Galantamine	Cholinesterase inhibitors (CI)	Hamper the decay of acetylcholine between synaptic clefts and restoring neuronal functions, daily activities, and sysyematic functions for mild AD patients	Delay cognitive function degradation evaluated by AD Assessment test in cognitive regions and global clinical rating
Memantine	N-methyl-D-aspartate receptor antagonist (NMDA)	Block the toxicity associated with excess glutamate and regulates glutamate activation	Using memantine for 6 months, AD patients showed improved cognition and ADL; memantine can reduce both psychological and behavioral symptoms of dementia
Combination of memantine and donepezil	CI-NMDA combination	Both blocking the toxic generated by superfluous glutamate and delaying the degradation of CI	Not demonstrated in mild to moderate AD patients

2. Gene therapy

2.1. Adeno-associated Virus (AAV)

Adeno-associated Virus (AAV) has already been widely utilized as an efficient and prominent means in *in-vivo* delivery for gene therapy and advanced to target a wide range of cells and tissues in treating genetic diseases since the discovery of adenovirus by Bob Atchison and M. David Hoggan and Wallace Rowe in 1965 [4]. AAV, a kind of icosahedral viruses without membrane-bounded, belongs to the *Dependoparvovirus* genus in the *Parvoviridae* family. They are composed of capsid proteins that enclose a single-stranded DNA genome with an approximately 4.7 kb weight and a 26 Å diameter and contain three reading frames- *rep*, *app*, and *cap*. What makes such small viruses a widely used vector transformed into human bodies is the mild immunogenicity characteristic of AAV. Recombinant AAV (rAAV) is composed of innocuous biomaterials, such as nucleic acids and amino acids, rather than chemical components that have the propensity to trigger unfavored toxicity or severe immunogenicity when introduced into human bodies. In addition, there is no viral gene presented in rAAV, though being a virus itself. Thus, there will be no viral gene expression, which is responsible for some immune reactions, when rAAV presents in the human body [5]. It has been reported that rAAV integrates into the host genome by combining with the glycosylated cell surface of the host and undergoing replication and transcription in the host nucleus (Figure 1). Several clinical trials have already managed to transfer AAVs successfully into human, including the global systems, muscles, central nervous system, heart, and lung [5,6]. Besides, there are also possible applications of AAVs in treating diseases, such as neuronal diseases, ocular diseases, and liver directed diseases.

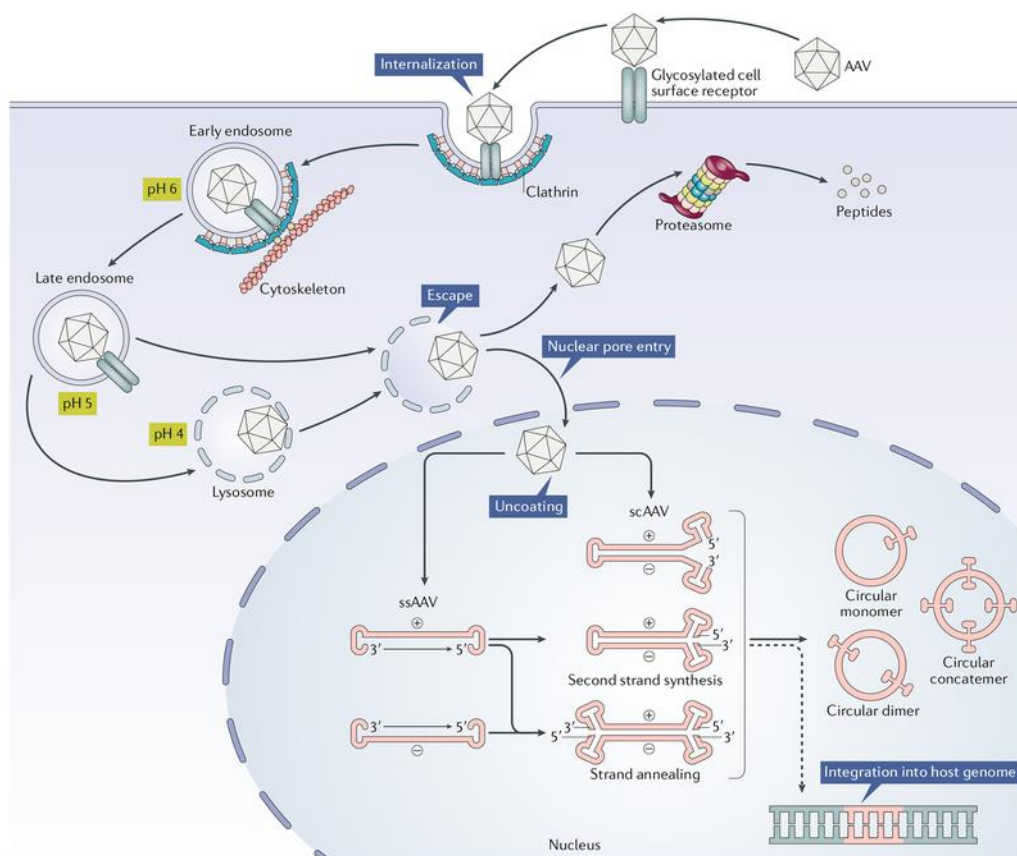


Figure 1. Pathways AAV takes to transduction from outside to the host genome [4]

2.2. CRISPR-Cas 9

CRISPR-Cas 9 genome editing technology also plays a leading role in gene therapies. CRISPR is a group of DNA sequences discovered in prokaryotic organism and it acts as an immune defensive response. They are derived from the DNA fragments of the previous infected bacteriophages in the prokaryotes and are utilized to monitor and destroy invaded DNA pieces from the same infection sources [7]. Cas-9 is an immunological protein that defends against certain bacterial infection, and it can cut DNA thus altering the cell's genome. When they are delivered into human, the gRNA will aid the Cas enzyme to target the specific DNA sequence with PAM, which is complementary with the gRNA. Then, Cas 9 will cut the target sequence and either knock out the section with the help of the nonhomologous DNA end joining (NHEJ) or knock in another sequence by homology-directed recombination repair (HR) as shown in Figure 2 [7]. Due to its simplicity, high efficiency and accuracy, CRISPR/Cas 9 has been widely applied as a possible treatment to virus infections, genetic disorders, cancer, and detection of pathogens. It has been tested to be effective in treating HIV-1/AIDS by targeting the HIV-1 genome to reduce infections and eliminate the latent viral reservoirs [8]. Meanwhile, CRISPR/Cas system plays a pivotal role in immune and cancer therapies by editing T cells in patients [9].

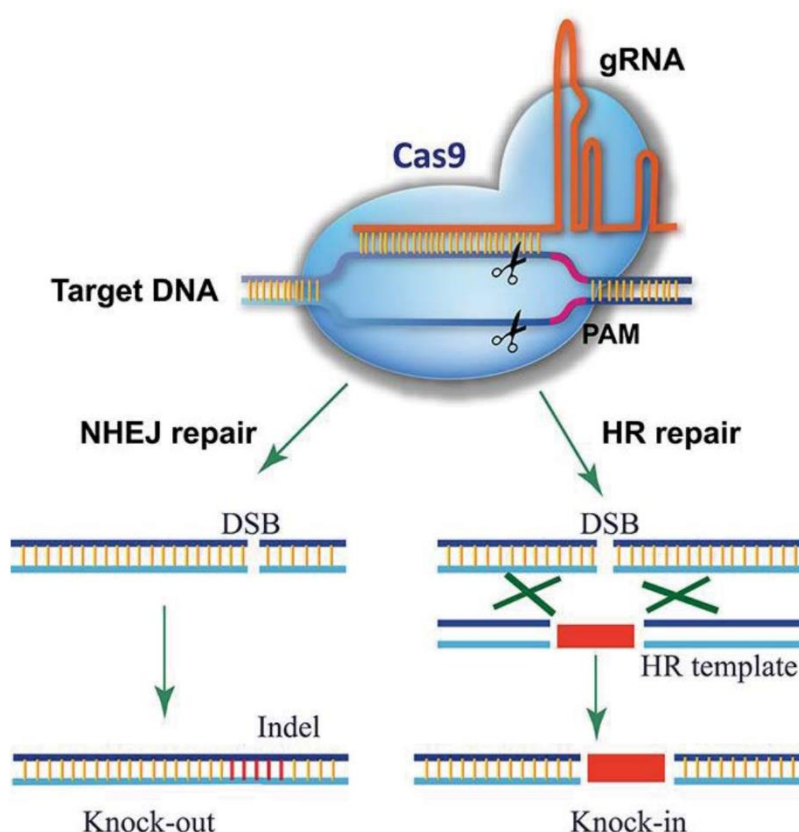


Figure 2. Mechanisms of CRISPR/Cas 9 when deliver into human [10]

3. Gene therapies in AD

On account of the limitations of traditional treatments, researchers have turned to gene therapy to look for possible treatments in for Alzheimer's Disease.

3.1. AAV

Delivering Nerve Growth Factor (NGF) with the use of AAV shows alleviation of AD symptoms [11]. NGF specifically plays a role in forestalling the neuronal demise and triggers the function of basal forebrain cholinergic neurons that are increasingly declined in AD patients. In a clinical trial first initiated in 2001, researchers tested on how early-stage AD patients react to AAV2-NGF treatment. They studied a large series of brain samples from 10 AD patients having surviving time ranging from 1 to 10 years after the NGF treatment, and found that by injecting NGF into AD patients, the related symptoms were alleviated and biological responses, including neuronal sprouting, hypertrophy, and expression of neuronal functional marker were induced [12] (Figure 3). Labeled cholinergic neurons expressed hypotrophy near the regions of NGF delivery when compared to remote regions and control group (Figure 3A), and the activation of neuronal functions were demonstrated by the activation of CREB signaling as the yellow arrows pointed in figure 3B. Meanwhile, AAV2 was selected as the transfer vector due to its specific expression of human NGF and safety: nerve growth factors tested in AD patients require chronic delivery that achieve accurate central delivery and restricted distribution to the pathological cholinergic neurons in the basal forebrain [12]. These biological responses validate the effectiveness of AAV2-NGF treatment in curing Alzheimer's Disease.

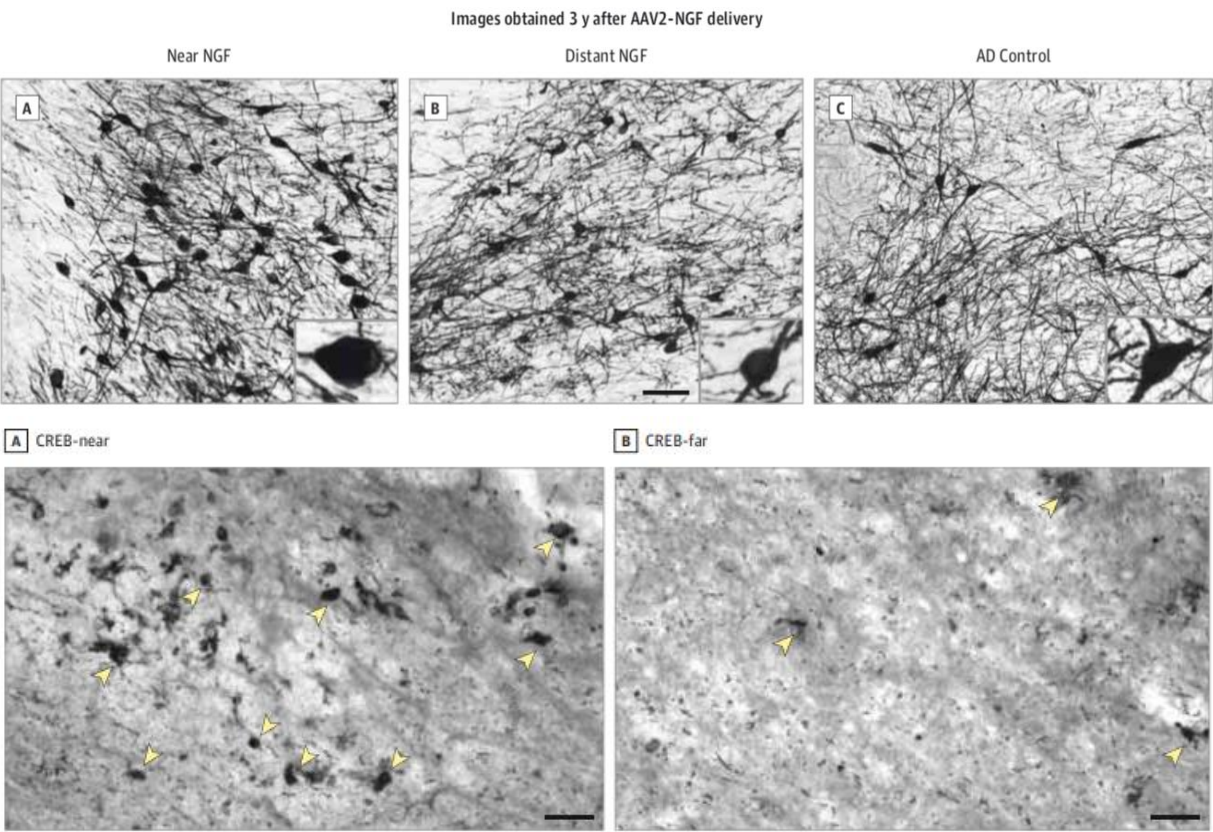


Figure 3. A. Labeled cholinergic neurons located near the NGF delivery regions

B. CREB signaling activation [12]

In addition to AAV2, AAV9-Nurr1 and Foxa2 treatment is also capable of alleviating AD symptoms. Nuclear receptor-related factor 1 (Nurr1) plays an essential role in the midbrain of human by regulating adult dopamine neurons, and forkhead box protein A2 (Foxa2) is a cofactor to Nurr1-mediated glial. By using AAV9 as vectors, researchers injected AAV9-Nurr1 and Foxa2 intracardinally, and the expression Nurr1 and Foxa2 triggered the production of neuronal inciting factors, such as the trophic factors in neuron. In condition of AD with toxic A β plaques, these factors showed prominent decline and increased expression of inflammation, and the injection of Nurr1 and Foxa2 along with AAV9 as the vector indicated to reduce these symptoms in mice models (Figure 4) Besides reducing A β pathologies, combination treatment of Nurr1 and Foxa2 was also capable of restoring neuronal functions by reducing pathologies in tau proteins, alleviating aging, and preventing synaptic loss in the mice models [13].

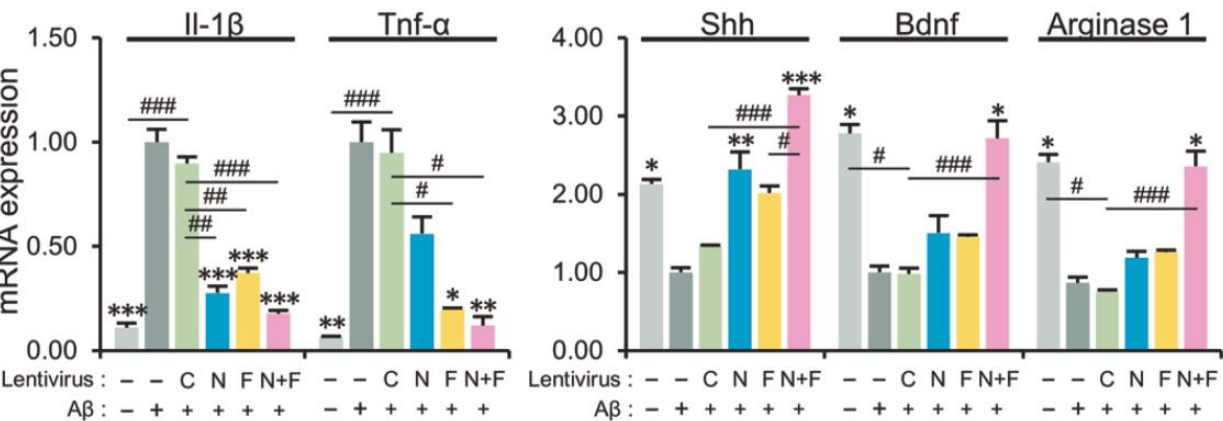


Figure 4. Nurr1 and Foxa2 combination incited the production of neuronal trophic factors and suppressed inflaming in mice models [13]

3.2. CRISPR-Cas 9

Furthermore, CRISPR-Cas 9 gene editing has been proven to alleviate AD symptoms in AD mice. CRISPR-Cas9 nanocomplexes are combination of amphiphilic R7L10 peptide and Cas9-single guided-RNA and they are able to precisely target Bace1 and Th gene, which are AD related genes. By introducing CRISPR-Cas9 nanocomplexes into AD mice, researchers discovered that these nanocomplexes will target Bace1 to suppress A β accumulation and cognitive defects. In experiments, Cas9 nanocomplexes reduced expression of Bace1 and Th, thus suppressing the inflammation and reducing A β accumulation and A β 42 production in AD mice models. The accumulation of A β 42 is responsible for cognition decline by inducing neuronal cell loss in AD patients [14].

Moreover, by using CRISPR-Cas9 treatment to correct the PSEN1 M146L mutation has been shown to restores amyloid-beta 42/40 to alleviate the AD symptoms. Mutations on the M146L position in the PSEN1 (PS1) gene will trigger early-onset of AD by creating tighter forms of PS1 gene, bringing C- and N- terminus closer, which will influence the APP recognition and increase the production of A β 42 and A β 40. By noticing PSEN1 M146L as the pathological factors of AD, researchers then applied the CRISPR-Cas9 treatment from *Streptococcus pyogenes* to specifically disrupt the mutations at PSEN1 M146L in human fibroblasts to normalize the A β 42/40 ration and the conformation of PS1 genes. They discovered that CRISPR-Cas9 strategy successfully restored the ratio of A β 42/40. (Figure 5) In addition, by inducing the CRISPR-Cas9 system that had edited the PSEN1 M146L mutation to a distant dependent physical energy-transferring process, known as the fluorescence resonance energy transfer and calculating the efficiency of the energy transfer (E_{FERT}), researchers found that there was a significant decrease in E_{FERT} when treated with CRISPR-Cas9 editing [15]. This demonstrated that CRISPR-Cas9 treatment altered the pathological conformation of PS1 genes.

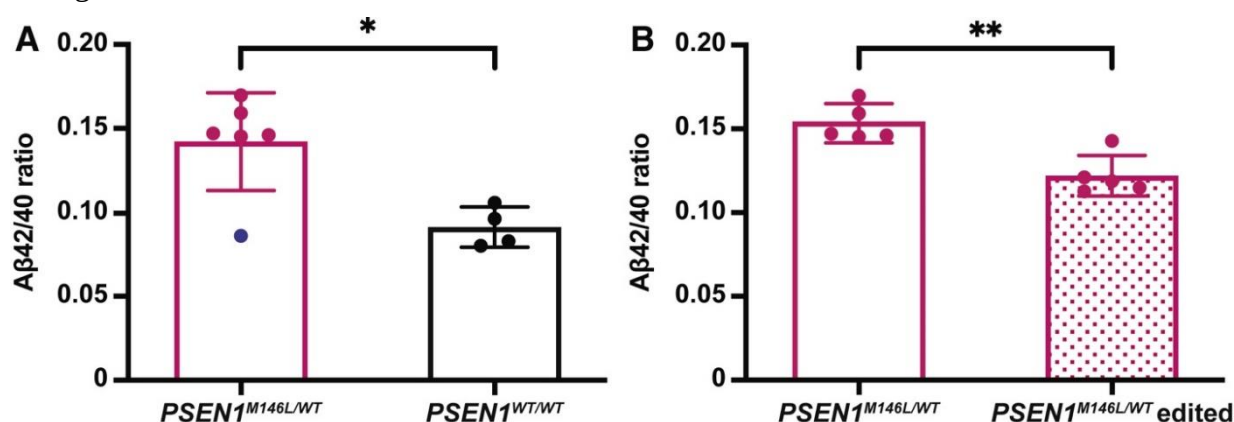


Figure 5. CRISPR-Cas9 treatment decrease the A β 42/40 ratio

A. Mutations on PSEN1 M146L showed higher ration of A β 42/40 comparing to the PSEN1 WT

B. Using CRISPR-Cas9 to edit the mutations on PSEN1 M146L, the ratio of A β 42/40 was restored when comparing to the PSEN1 M146L mutation group [15]

4. Conclusions

In a nutshell, current effective treatments to AD are mainly AD symptoms alleviating drugs, including Donepezil, Rivastigmine, Galantamine, which inhibit CI, Memantine that are known as NMDA or CI and NMDA combination therapy. With the development in gene therapies, AAV treatment and CRISPR-Cas9 editing treatment, there are more possible treatments to AD, such as AAV2-NGF treatment, AAV9-Nurr1 and Foxa2 treatment, CRISPR-Cas9 nanocomplexes treatment, and CRISPR-Cas9 PSEN1 M146L treatment. By utilizing gene therapies, AD pathological factors can be targeted more precisely and efficiently, and symptoms of AD can be drastically reduced as shown in clinical trials. However, current gene therapies treatments on AD are limited, and only a few are tested in clinical trials. More importantly, gene correcting, or delivery strategies cannot

guarantee one hundred percent correctness in locating the pathological parts and ensuring the effectiveness of the treatments in AD patients. It is highly possible that not all AD patients will react actively and positively with the gene altering treatments. In experiment testing CRISPR-Cas9 on PSEN1 M146L mutation, only three of five treated fibroblast samples showed prominent decrease and one significant increase in E_{FRET} [11]. Therefore, a lot of further researches are still required to search for more possible treatments to AD and bring those to clinical trials, and to stabilize the gene therapy strategies.

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