CRISPR Technology as a Therapeutic for Late Onset Alzheimer's Disease

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Research question:
How can gene therapies alter the accumulation of abnormal buildups of proteins in the brain and act as a potential therapeutic for Alzheimer's disease?

Abstract

Alzheimer's disease (AD), a neurodegenerative disease, is a result of abnormal buildups of protein, such as amyloid and neurofibrillary tangles, in the brain, creating memory loss. While TREM2 normally functions to facilitate the removal of amyloid plaques and neurofibrillary tangles, mutations in TREM2 render it dysfunctional. Specific mutations in genes such as TREM2 have shown to be a big risk factor in AD. Gene therapy, a relatively recent discovery, can help to replace defective or missing genes. Recent advancements in gene therapy, a treatment for diseases, offer a promising avenue for treating AD by addressing defective or missing genes, such as those related to TREM2. One of the notable gene therapy systems is CRISPR. Here, we review CRISPR and our understanding of TREM2 in hopes of finding a potential therapeutic for AD.

Introduction

Around 10.7% of those aged 65 and older in the United States have Alzheimer's Disease (AD), meaning an estimated 6.7 million people are living with a fatal disease ("Alzheimer's Facts and Figures Report"). Not only does this disease harm people with the condition, it also takes a toll on those who live with and take care of them. The estimated total cost of healthcare for those with dementia is around $10,400 to $34,517 per patient (Duttagupta). This disease is not only physically burdening but also economically exhausting. AD affects patients by removing their independence to do everyday things. In addition, AD creates personality changes, which can destroy a person's individuality. Victims lose their memory gradually and many mood and lifestyle choices quickly change.

AD is a neurodegenerative disease

AD is a neurodegenerative disease and is a result of the accumulation of an abnormal buildup of proteins in the brain such as tau and amyloid beta. While these proteins are usually beneficial to the brain at a healthy amount, faulty functions in the brain cause these proteins to start accumulating, eventually destroying the brain. When these buildups are left untreated, the proteins prevent neurons from relaying signals to each other, eventually killing the neuron. These neuronal destructions are typically in parts of the brain relating to memory, specifically in the hippocampus. As a result, patients are unable to recall certain memories and are subject to symptoms such as sudden mood and personality changes. Symptoms of Alzheimer's disease are typically seen in those in their mid-60's or older, which is called late-onset AD, however symptoms are sometimes seen in those under the age of 65, which is considered early-onset (Li et al.).

AD risk factors

Risk factors for AD include genetics, environmental influences, lifestyle, as well as having other diseases. A common misconception is that old age causes AD. However, while age

doesn't cause AD, it is a huge risk factor for AD. Lifestyle choices such as diet and exercise can also affect risk for AD. Environmental influences such as drugs (external influence), hormones (internal influence), and even education level can affect one's susceptibility to developing AD. The accumulation of amyloid beta and tau stem from genetic risk factors such as mutations or missing genes. These genetic risk factors have been shown to be a leading factor in the development of AD. One prevalent and leading genetic risk factor is mutations in the gene Triggering Receptor Expressed on Myeloid cells 2 (*TREM2)* ("What Are the Signs of Alzheimer's Disease? | National Institute on Aging").

TREM2 and Microglia

TREM2 is a protein which triggers an immune response to remove debris from the brain. This protein usually functions by triggering and working with an immune response. TREM2 is a receptor on the surface of microglia, which are cells that help remove cell debris and other material from the brain. In addition, it is responsible for telling the microglia to move to areas with amyloid plaques. Being a cell receptor, the role of TREM2 is to let microglia know when to remove something (Li et al.).

In the brain, TREM2 is responsible for notifying microglia when there is too much amyloid beta or tau. By engulfing the proteins through phagocytosis (the ingestion of bacteria, other cells, and cellular debris), the microglia keep the plaques in check so that they remain at healthy amounts (Li et al.).

Mutations in TREM2 removes the brains watchguard

Microglia relies on TREM2 to help with the maintenance of neuronal systems, injury repair, homeostasis, and brain growth. However, because of mutations in *TREM2*, the microglia are unable to know when there is an overwhelming amount of tau and amyloid beta. This results in a reduction of microglial phagocytosis, resulting in an abnormal protein buildup and eventually in neurodegeneration . Mutations in the *TREM2* gene from birth are highly implicated in late-onset AD (Li et al.).

TREM2 mutations have been shown to cause neurodegeneration

Studies in mice have shown how the *Trem2* R47H variant results in lowered gene expression and microglial removal of amyloid plaques (Li et al.). Because the mutated R47H variant doesn't create an immune response in the brain, there is no implication of microglial cell removal, meaning there is no limit to how big protein plaques can grow (Fig. 1). Subsequently, there is a buildup in the amyloid beta and tau proteins, which is known to be a cause of neurodegeneration (Li et al.).

Mutations in TREM2 are common

Not only are TREM2 mutations a huge risk factor in AD, it is also very common (Li et al.). Studies have shown that 4 of 5 patients in a family affected by AD were shown to have a variant in the *TREM2* gene, meaning 80% of those with late-onset AD in this family have AD due to a mutated *TREM2* gene (Li et al.). While only 6.7% of all AD patients have *TREM2* mutations, this still amounts to tens of thousands of people in the US alone.

However, as mutations in *TREM2* are a huge genetic risk factor in the development of AD, gene therapies are a possible solution in restoring healthy TREM2 function in the brain.

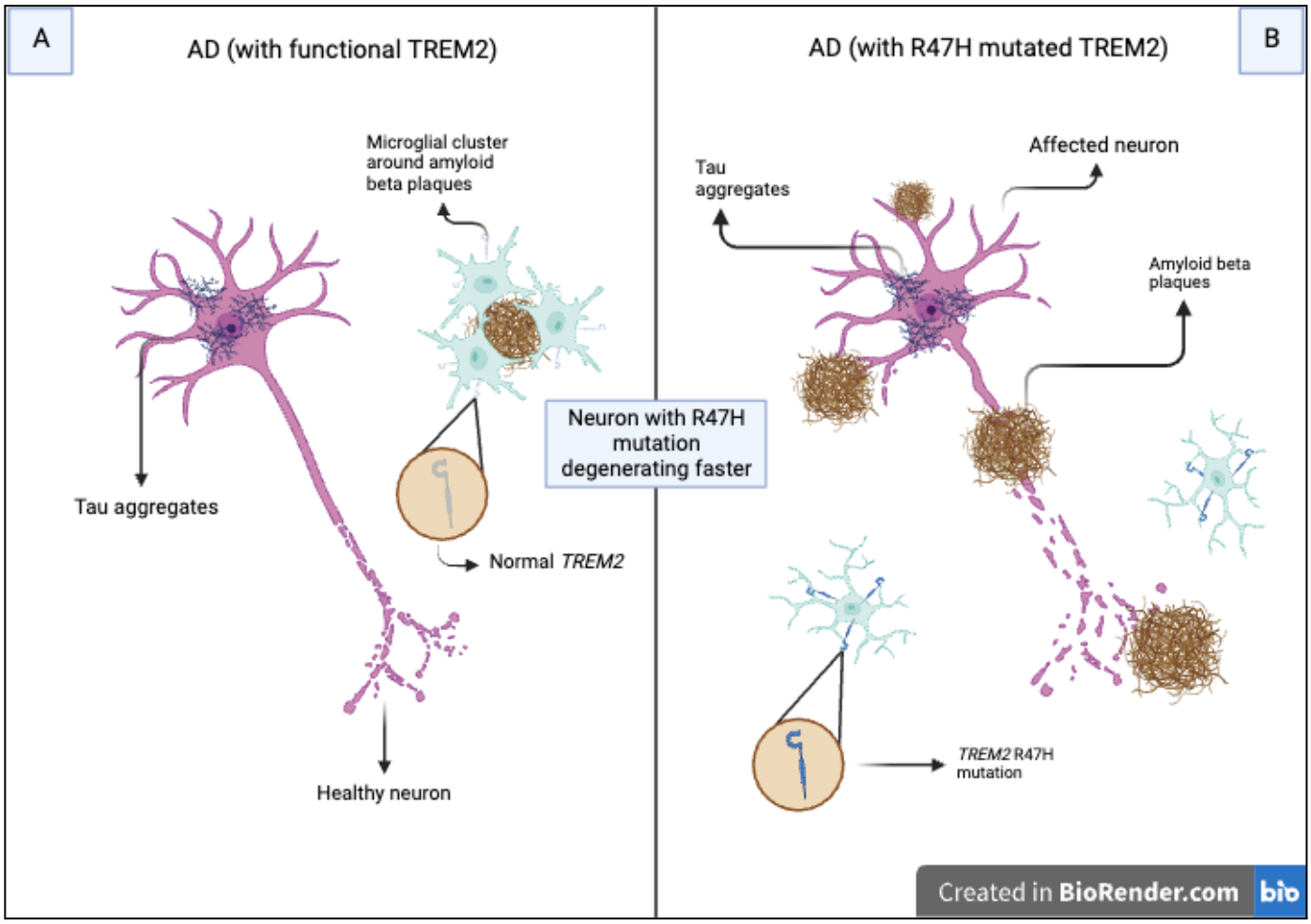

Figure 1: Diagram of Functional and Mutated TREM2 & How They Affect Neurons. Depicted here are two scenarios of AD pathology. Panel A shows the classic microglial response to AB plaques, through the engagement of TREM2. Panel B illustrates pathology when TREM2 is mutated and there is little to no microglial response.

Gene Therapy

How do they work?

Gene therapy is a technique in which genes are modified to treat or cure a disease (Sheikh). It can be used to fix practically anything gene related, which means it can possibly be used to treat AD. In gene therapy, scientists need to first identify the sequences which need to be replaced in the DNA. After finding the correct sequence (template strand) in the DNA,

scientists are then able to use a system to correct the sequence inside of the body. Different systems have different ways of correcting a sequence. Among these systems are CRISPR, RNAi, and TALEN (Douch). CRISPR technology is the focus of this paper.

How does CRISPR work?

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) requires two parts: Cas9 and a guide RNA. Cas9 is a protein that unwinds and opens up DNA sequences while guide RNA's tell it the sequence of the mutation (Fig. 2). In a system called homology directed repair, Cas9 cuts out the mutation and gives the DNA a template DNA to rebuild itself. This template DNA often has the corrected sequence and can be used to fix mutations (Douch).

Clinical application of CRISPR

Mice models back up technique

Many mouse models have been used to test intranasal CRISPR treatments. For example, Mazieres and colleagues used intranasal delivery of CRISPR to help treat chronic anxiety in mice. Their study targeted a receptor cell known to play a role in anxiety and depression. Anxious mice tend to spend more time in the dark while mice without anxiety stay in the light more often. The mice who received this treatment were seen to have a decrease in the receptor expression. Those mice who had received treatment resulted in spending more time in areas with light compared to those who did not (Mazieres).

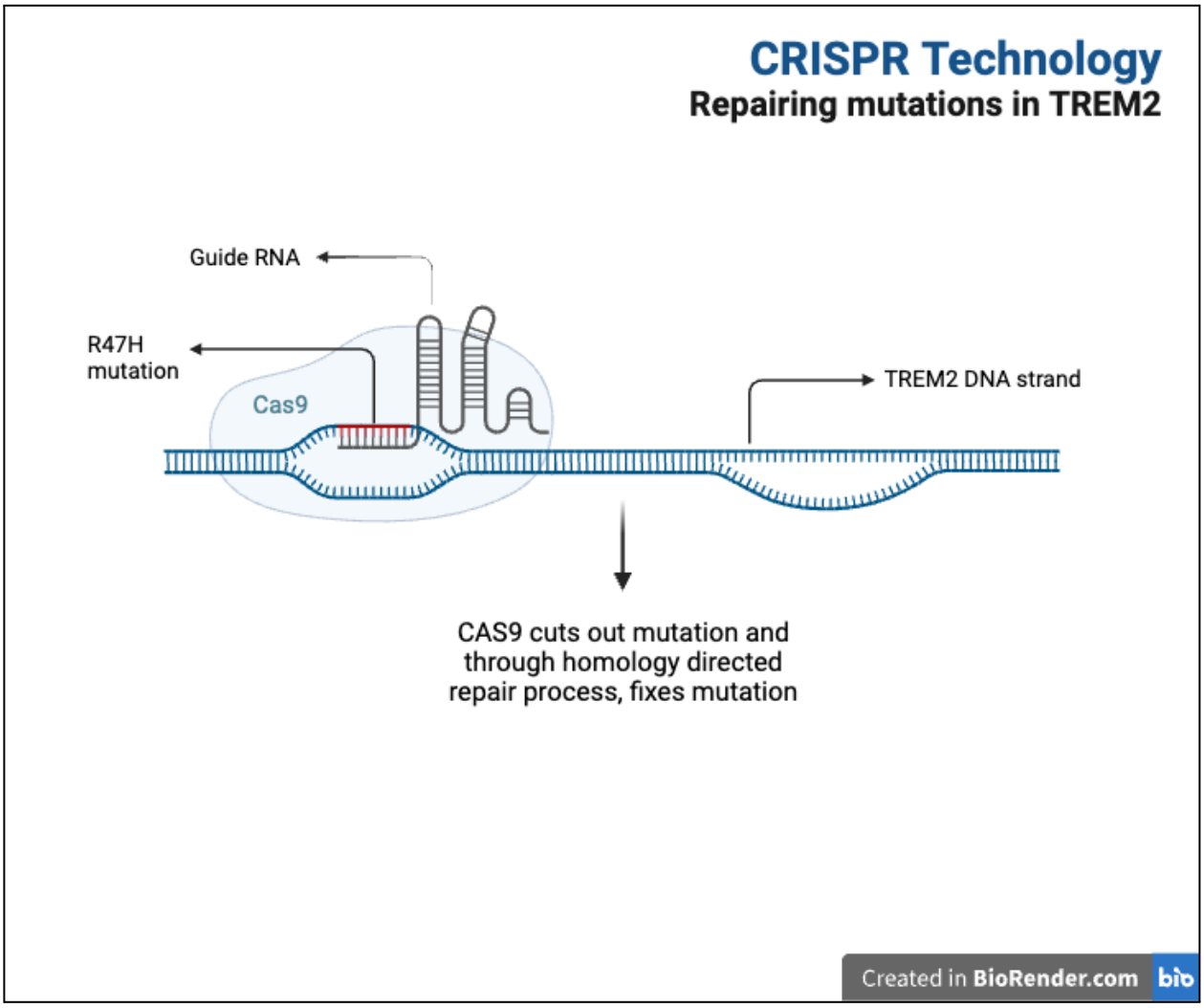

Figure 2: Diagram of TREM2 Mutation & CRISPR Cas9 Repair. TREM2 DNA strand (unwound) with CRISPR Cas9 enzyme and guide RNA repairing R47H mutation.

CRISPR delivery into the brain

There are delivery techniques for CRISPR such as injection or inhaling. While direct injection into the brain (intracranial) is a more direct delivery technique, there is the risk of hemorrhage (internal bleeding in the brain) and tissue damage, which has happened in a different disease treatment (Bao et al.). A safer technique, called intranasal, utilizes a nasal spray in which the components needed for CRISPR are transported in a carrier package. Often, these carrier packages are in the form of viral vectors or liposomes (Fig 3). Viral vectors are viruses that have been genetically modified to remove the viral genes that adversely impact organisms. Instead of carrying viral genes, these viruses now deliver only therapeutic genes. A prime example of a viral vector is the Adeno-Associated Virus (AAV). Liposomes are spherical sacs of phospholipid molecules that encase a water droplet. These can be formed to artificially

carry drugs. These viral vector or liposome packages encase CRISPR components so that they can safely reach the target destination without facing an immune response from the body (Li et al.). Without these carriers, the components would be destroyed by the body before they even reach the target destination.

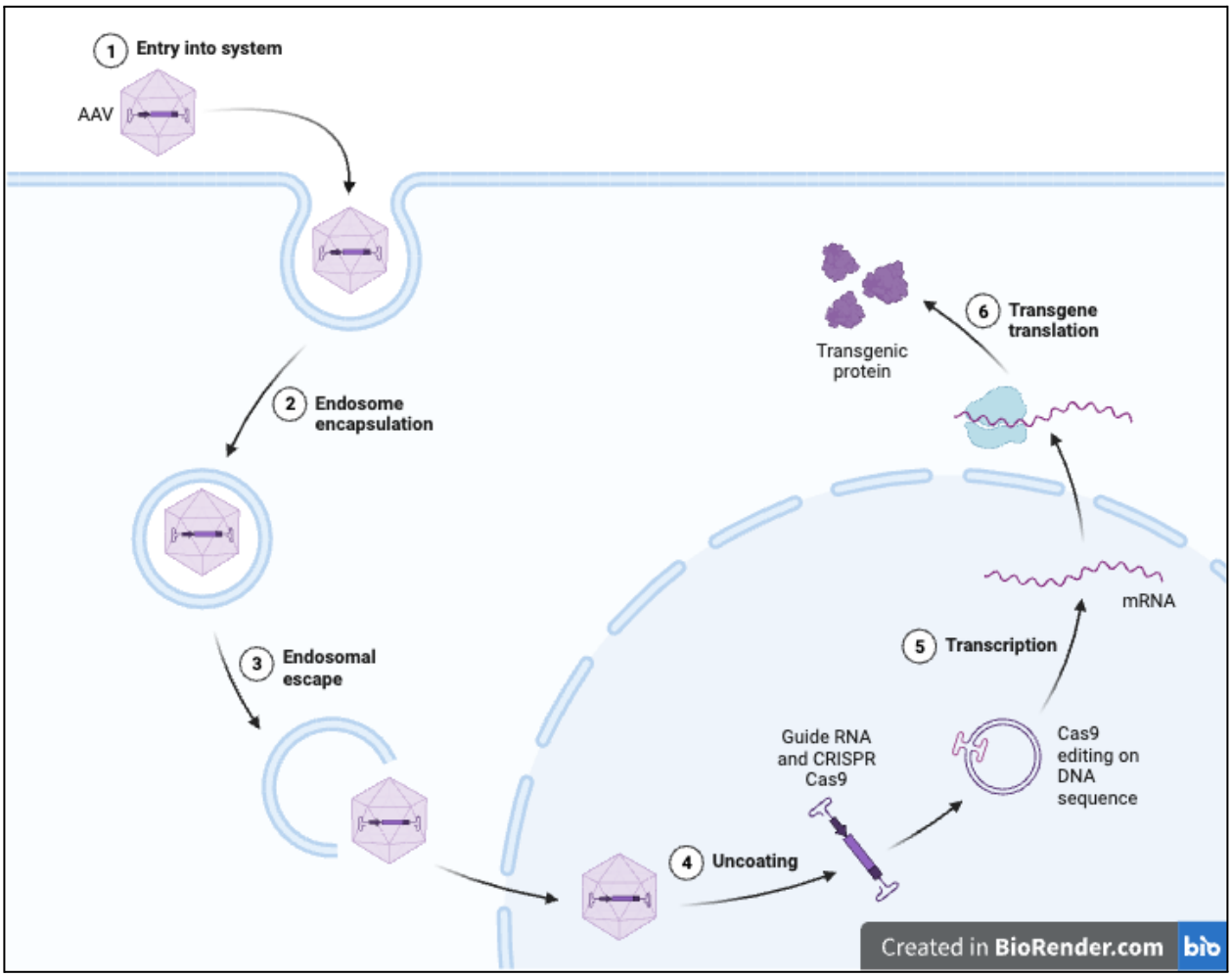

Figure 3: Diagram of AAV Delivery Technique. AAVs enter the cell through endocytosis (1) but are able to escape the endosome through mechanism X (3). CRISPR exits the endosome (3) and enters the cell, uncoating itself from the protective shell (4). CRISPR Cas9 edits DNA and creates an mRNA (5), eventually turning it into a protein (6).

Safety of drug delivery methods

These two methods (intracranial and intranasal) have previously been practiced in clinical trials. While the intracranial technique was used in a non-AD related clinical trial, the results were fatal. Not only can brain hemorrhages occur as a result of intracranial injection, but these injections can also result in paralysis, loss of vision, stroke, and death (Bao et al.). In contrast, intranasal treatments are safer compared to intracranial injections. However, one downside of

intranasal treatments is decreased efficiency, as treatment took several days to reach proper dosage in a trial on mice (Palmgren). Although intranasal treatments may take longer to fulfill, it is a much safer alternative to intracranial injections as there have been no casualties seen in the pre-clinical trial done on mice (Palmgren).

As gene therapies have helped with many genetic diseases, gene therapy is a viable solution for AD.

Design for theoretical clinical trial

For a theoretical clinical trial, CRISPR would be administered as a gene therapy technique. As the R47H mutation is the most common mutation linked to AD, it poses an excellent target for therapeutic intervention. Using CRISPR technology and homology directed repair, the R47H mutation could be corrected to its functional sequence and thus support normal microglial phagocytosis (Fig. 4). As the R47H mutation only occurs in late-onset AD, clinical trials should target those 65 and older.

Name of the treatment CRISPR treatment: **CRISPR47H**

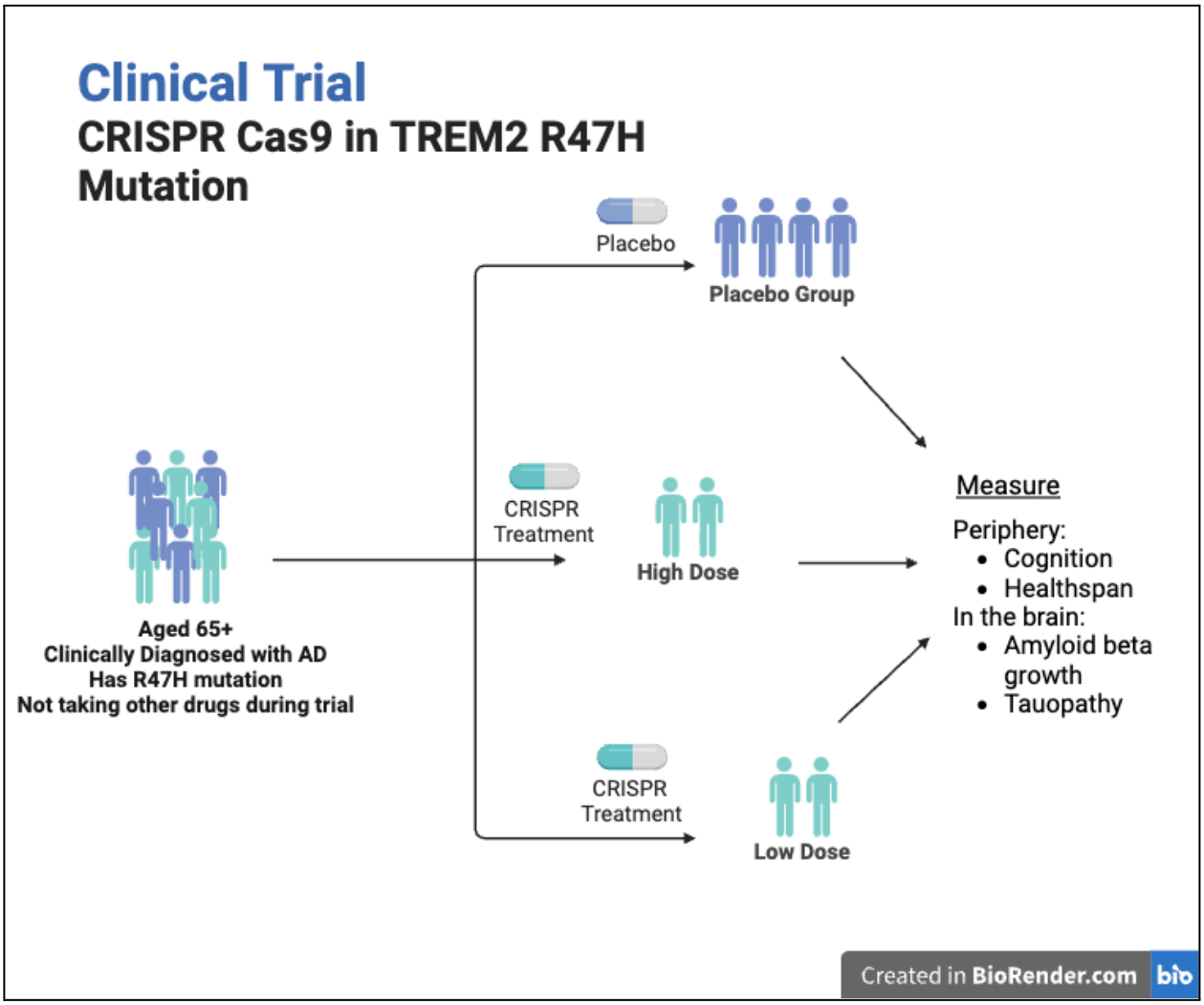

Figure 4: Diagram of Theoretical Clinical Trial. Two test groups, one split into two subgroups: high and low dosage. At the end of trial, periphery and in-brain activity are measured.

Dosages and timing of treatments

As intranasal treatments take several rotations to reach proper dosage, patients would receive treatment once a week on one specific day. However, as there have been no clinical trials on humans yet, the dosage given to a patient is still undecided. The dosage given will be decided in a lab. If the Food and Drug Administration (FDA) approves of safety and accessibility, treatments could possibly be given at home.

Outcomes and measurements

Two different categories of outcomes will be measured in the clinic: peripherally, such as one's cognition and healthspan, and in-brain, such as amyloid beta growth and tauopathy. These will not be measured right away, but over the years, following patients for 7-10 years potentially. Measuring cognition could be measured with drawing a clock, for example,

observing if patients remember where to place each number, while measuring healthspan will be recorded throughout the years. In-brain activity such as amyloid beta growth and tauopathy will be measured in the clinic and compared to patients who haven't received treatment, also a long term process, with data collected throughout the years. While it may be close to impossible to reverse the effects of degeneration, a slowing of cognitive decline would be a successful outcome.

Conclusion and Discussion

AD is a neurodegenerative condition and is highly linked to many genetic risk factors. AD is a genetic disease and mutations or missing genes in cells related to AD can make one more prone to developingAD. Mutations in TREM2, a cell receptor, can render microglial phagocytosis unable to remove harmful protein plaques such as amyloid beta and tau (Li et al.). While AD is a fatal disease, gene therapies are a promising future treatment option for AD (LaFee). By fixing DNA sequences in mutated genes, CRISPR can possibly correct the R47H mutation in *TREM2*, which is the most common mutation in *TREM2*. In this research paper, CRISPR technology has been reviewed to see its possible application in correcting the R47H mutation, possibly slowing or stopping the progression of AD, such as by stopping plaque formation, tauopathy, and cognition decline.

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