How can CRISPR be used to treat Alzheimer's disease?

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Abstract

Alzheimer's disease (AD) affects about 1 in 9 people above the age of 65 in the US, with projected healthcare costs reaching $1.1 trillion by 2050. Despite the severity of AD, there is no current cure, making it critical to improve current research and care. AD can be caused by a combination of genetic and environmental factors, complicating treatment efforts. However, CRISPR technology provides potential to treat the disease by manipulating certain genes involved in its pathogenesis. CRISPR treatments are currently being explored in mouse models of AD and human cells, and have the potential for improving therapeutics in human patients. The goal of this paper is to discuss how incorporating CRISPR into AD therapeutics can translate to improved care in humans.

Intro to Alzheimer's disease:

Alzheimer's disease (AD) is a neurodegenerative disease that affects around 24 million people worldwide (Mayeux & Stern, 2012). About 1 in 9 people above the age of 65 in the US are affected by late-onset AD (Alzheimer's Association, 2024). Additionally, some patients develop early-onset AD, which can occur around age 30, although rare (National Institute on Aging, 2022). Health care costs for AD are estimated to reach $1.1 trillion by 2050 (Stefanacci, 2011). The prevalence of this disease makes research for it crucial, as there is no current cure.

AD is the most common form of dementia. It affects connections between neurons in the brain and causes brain cells to degenerate. First, it damages connections among neurons in the entorhinal cortex and hippocampus, which is the part of the brain involved in memory. It later affects the cerebral cortex, which is the part of the brain involved with language, reasoning, and behavior (National Institute on Aging, 2024). As a result, the symptoms of AD include memory loss, impaired thinking and behavior, deficits in problem-solving, difficulty in completing tasks, changes in sleeping patterns, and increased anxiety or aggression. Symptoms of AD will progress and worsen over time. Unfortunately, these symptoms are common amongst numerous other neurological diseases and disorders (Johns Hopkins Medicine, 2024). This, coupled with the lack of simple and reliable tests to diagnose AD (NHS, 2024), make this disease difficult to diagnose.

Alzheimer's disease does not have a single cause, but can be caused by a variety of genetic, lifestyle, and environmental factors (National Institutes on Aging, 2023). Two major causes of AD include a buildup of amyloid beta plaques and neurofibrillary tangles. Amyloid beta plaques are accumulations of misfolded proteins that form in the spaces between neurons (Robertson, 2023). Neurofibrillary tangles, or tau tangles, are abnormal collections of tau proteins inside the neurons (National Institute on Aging, 2024). Plaques form when the beta secretase and gamma secretase enzymes interact improperly with the amyloid precursor protein (APP), a protein in the cell membrane that helps neurons grow and repair, leading to the accumulation of insoluble amyloid beta fragments (Osmosis, 2016). These fragments aggregate outside neurons, forming

amyloid beta plaques that disrupt neuron-to-neuron signaling and often trigger an immune response, causing inflammation that can damage surrounding neurons. Tangles develop inside neurons due to the buildup of beta-amyloid plaques which leads to the activation of kinase, an enzyme that transfers phosphate to the tau protein. The tau protein is a protein in the neurons to stabilize microtubules. The kinase alters the shape of the tau protein, causing it to stop supporting the microtubule and clump up with other tau proteins into a tangle. Neurons with tangles and non-functioning microtubules can't signal as well, and may undergo apoptosis. This causes brain atrophy and the gyri to get narrower, contributing to memory loss and loss of functioning.

Both plaques and tangles can be induced by a variety of different genetic mutations. The total number of AD risk genes is around 30 (Bertram, 2019), but the genes that are most closely associated with Alzheimer's disease are APP, APOE, PSEN1, and PSEN2 (National Institute on Aging, 2023). Apolipoprotein E (*APOE*) is the most common risk gene for late-onset AD (Mayo Foundation for Medical Education and Research, 2023), whereas APP, Presenilin 1 (*PSEN1*), and Presenilin 2 (*PSEN2*) are the most prevalent risk factors for early-onset AD (Lanoiselée et al., 2017). APOE has multiple forms. APOE e2 is thought to be protective and reduce the risk of AD, but it is least commonly found in patients. On the contrary, APOE e4 increases the risk and severity of AD. Mutations in any of these genes result in a buildup of amyloid beta plaques and tangles inside neurons and greatly increases one's risk for early-onset AD. These toxic plaques and tangles lead to the death of neurons and nerve cells and ultimately lead to the symptoms of AD.

Treating Alzheimer's disease is difficult, which is why we need to explore new approaches. One possible therapeutic is Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). CRISPR technology offers the potential to precisely edit genes associated with AD, which would allow for more personalized and effective AD treatments.

Intro to CRISPR:

CRISPR technology is used to edit the genome of organisms with high precision. It's a natural adaptive immune system found in bacteria and archaea (Synthego). It utilizes a Cas9 nuclease, which is a protein that is capable of cutting DNA, along with a guide RNA (gRNA) that directs the Cas9 to a specific gene of interest. The gRNA is a 20 base pair sequence designed to bind to a particular region of the DNA. The Cas9 protein can recognize and bind to the gRNA, allowing scientists to design specific gRNAs to target and edit particular genes.

CRISPR-Cas9 gene editing works by making double stranded breaks in DNA, and then allowing the DNA to repair itself. The primary repair pathways used for gene editing are non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ repair is the most common and typically leads to an insertion or deletion of nucleotides, while with HDR, the addition of a DNA repair template leads to a gene knock-in (Synthego). NHEJ is a quicker repair method, but often leads to more errors and thus gene knockouts. In contrast, HDR is more precise and allows for gene knock-ins or the fixing of mutations, but overall, it has lower efficiency. Having information on these repair mechanisms allows scientists to determine the best way to alter a gene.

dCas9 is a deactivated version of the Cas9 protein, meaning it cannot create a double stranded break in DNA. However, the gRNA is still able to guide it to the gene of interest. Scientists have tethered activator or repressor proteins to dCas9 in order to have precise control over gene expression. This approach, known as CRISPR activator (CRISPRa) or CRISPR interference (CRISPRi) allows for upregulation or downregulation of the gene of interest, respectively, without modifying the genome permanently (Karlson et al., 2021). CRISPR/dCas9 technology has allowed scientists to study gene function and potentially develop therapeutic strategies for various genetic diseases.

In the lab, CRISPR has various applications, including creating genetically modified organisms, investigating gene function, and gene knockout studies (Xu & Li, 2020). Treating genetic disorders and diseases is one of the primary focuses for the development of CRISPR-based therapeutics. In addition to Alzheimer's disease, CRISPR is being researched as a potential cure for other genetic diseases such as sickle cell disease, diabetes, immune disorders, and various cancers (Roberts, 2024). Although CRISPR is not yet used in clinical settings for patient treatment, it is a promising option that, with more research and development, can treat many genetic diseases that currently lack effective treatments.

CRISPR & AD:

To date, CRISPR has been used in various animal models in a laboratory setting, specifically on treating Alzheimer's disease. For example, in 2023, Brent D. Aulston, a postdoctoral researcher at the University of California San Diego, presented findings on CRISPR-based gene therapy for Alzheimer's in mouse models. They edited the APP gene in the brains of mice that were genetically modified to exhibit signs of Alzheimer's disease. The results showed that the CRISPR therapeutic reduced the accumulation of amyloid beta plaques by up to 50-60%, decreased neuroinflammation, and restored memory capabilities in the mice (Aulston, 2023). A full safety evaluation revealed no negative side effects. While the gene sequence targeted in mice is slightly different from that in humans, efforts are ongoing to determine the best CRISPR enzyme for targeting the human sequence to achieve the highest efficiency and lowest off-target risks (Alzheimer's Association, 2023). The targeted sequence is relevant for both familial and sporadic Alzheimer's disease, making the approach broadly applicable to any patient with the disease, rather than being mutation-specific.

Another potential way that CRISPR can be used to treat late-onset Alzheimer's disease is by altering the expression of APOE e4 without changing the genetic code using dCas9. In 2023, researchers attempted this in human induced pluripotent stem cells (hiPSC)-derived neurons. These cells were originally obtained from Alzheimer's disease patients who had the APOE e4 variant and were reprogrammed into neurons and human brain organoids ("mini brains") in order to mimic the characteristics of the human AD brain. Scientists used the CRISPRi technique to decrease the expression of the e4 allele of the APOE gene, which increases the risk for AD, in these cultured neurons and organoids without altering the expression of the neutral APOE e3 allele (Practical Neurology, 2023). They lowered APOE e4 levels by 50-70%, which resulted in

an overall reduction in the development of AD, highlighting an effective technique to specifically target AD risk genes without altering non-pathogenic genes.

Additionally, researchers have used zinc finger nucleases, which is another form of gene editing, to convert the APOE e4 variant to either APOE e2 or APOE e3 (Wang et al., 2018). APOE e4 promotes amyloid beta plaque production, while APOE e2 is protective against AD and APOE e3 is neutral. By making this conversion, they were able to reserve and prevent AD pathology. This approach could potentially be achieved using CRISPR as well to increase precision and efficiency.

Scientists have also targeted PSEN1 and PSEN2 which are risk genes for early-onset AD. CRISPR was used to disrupt a PSEN1 mutation (Konstantinidis et al., 2022) or correct a PSEN2 mutation (Ortiz-Virumbrales et al., 2017) in cells derived from AD patients, which lead to the reversal of AD phenotypes. More specifically, by altering mutations that lead to AD pathophysiology, scientists can decrease the overall levels of amyloid beta plaques, thus reducing AD.

Conclusion:

CRISPR technology holds significant promise for treating AD by targeting and modifying genes associated with its pathogenesis. Studies in AD mouse models and patient-derived cells have demonstrated that CRISPR therapeutics can reduce amyloid beta plaques and neurofibrillary tangles, decrease neuroinflammation, and restore cognitive functions. This can be achieved by editing AD risk genes like APP, APOE, PSEN1, and PSEN2.

CRISPR Limitations:

The studies conducted demonstrate possible avenues for using CRISPR-based therapies in the treatment of Alzheimer's disease. However, translating these findings to human patients will require extended research and consideration. CRISPR enzymes must be optimized for human-specific targets to ensure effectiveness and safety, given the genetic differences between animal models and human patients. Additionally, the potential risk of off-target effects, which are alterations at a site other than the intended target, must be minimized before clinical application of CRISPR (Thorne, 2024). Researchers have made a lot of progress in improving the accuracy of CRISPR, but the introduction of a double stranded break is still a safety concern. In vivo stability, which is the stability of CRISPR within a living organism, is a concern as well. It is crucial for the CRISPR components to stay active long enough to make the necessary edits, without being broken down, in order for the treatment to be effective. Host immunogenic responses are also a challenge, since the body's immune system may recognize the CRISPR components as foreign and start an immune response against them. This would limit the effectiveness of the gene editing and could cause other complications as well.

Ethics of CRISPR:

There are many ethical concerns surrounding CRISPR technology that prevent it from being used in a clinical setting. These include the risk of off-target effects, since they may cause health issues or genetic mutations. Another problem with incorporating gene editing into treatment options is equitability, since getting gene therapy would be very expensive and therefore only available to those who can afford it. Lastly, a major moral issue surrounding CRISPR is the possibility of needing to edit the genome of human embryos, since the use of human embryos in science research remains controversial within the United States.

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