# The Application and Effectiveness of CRISPR-Cas9 in Alzheimer's Disease and Parkinson's Disease

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**Abstract.** Alzheimer's disease (AD) and Parkinson's disease (PD) are both types of neurodegenerative diseases (ND) that lead to severe failure in the nervous system. Usually, AD and PD are caused by genetic or environmental factors, and patients of AD or PD suffer from life inconveniences. AD and PD are both the major causes of irreversible dementia and can be classified into familiar and sporadic. A greater proportion of patients get sporadic AD and PD, and there hasn't been an effective treatment for the diseases. Widely discussed in the 21st century, it is believed that CRISPR-Cas9 gene therapy may be a possible potential treatment for AD and PD because CRISPR-Cas9 is a such strong genetic modification tool. This passage summarizes papers from the past 5 years to demonstrate the mechanisms of AD and PD, and how these mechanisms related to the applications of CRISPR-Cas9 gene therapy. Also, the benefits and drawbacks of the therapy are discussed in detail.

**Keywords:** CRISPR-Cas9 gene therapy; Alzheimer's disease (AD); Parkinson's disease (PD).

## 1. Introduction

Neurodegenerative disease (ND) is one type of disorder leading to irreversible nerve cell loss in the central or peripheral nervous system. Two of the most common NDs are Alzheimer's disease (AD) and Parkinson's disease (PD). AD is the major cause of irreversible dementia in adults: According to some predictions, the number of people suffering from AD will rise twofold to 100 million in the next thirty years, as 70% of dementia comes from AD [1]. Patients, mostly in middle age, will suffer from severe daily life inconveniences including memory loss, insufficient cognition, and longer reflex arcs. The causes of AD are due to several types of gene mutations, implying that AD related to genomes can be sometimes inherited. This becomes the reason why the results of drug development to cure AD are far less than expected. Over the years, only few drugs passed the whole clinical trial, and over 400 other drugs failed before clinical III with no clinical benefit shown [1].

Parkinson's disease (PD) is another neuron disease involving the degeneration of the nervous system. As a more common disease in the aging population, PD is a rapidly progressed disease that does not have effective control treatments [2]. Patients with severe PD will have syndromes such as fluctuation and fatigue. Similarly, genetic factors may contribute to the rising risk of PD, especially when induced by environmental factors like chemicals inhaled into the body. The progression of drug development in PD is mainly on types of immunotherapies, but the effectiveness is hard to evaluate due to the lack of valid biomarkers [2].

CRISPR-Cas9 gene therapy is one therapy further developed from the CRISPR-Cas9 system, an effective and advanced method for genetic modification of cells of organisms. It gets much attention due to its high potential for curing complex diseases by genome editing. Research shows that it has been proven to become an effective tool for various types of disease treatment, including hematologic diseases and immunological disorders [3]. Other researches indicate that CRISPR-Cas9 may be an alternative for AD or PD treatment, with higher versatility as well as high specificity [4]. However, there are few papers demonstrating the effectiveness and feasibility of CRISPR-Cas9 technology with gene therapy deeply, and few reviews have been done on the other types of neuron diseases such as PD. So, this passage aims to discover the mechanisms of AD and PD and to discuss the function of CRISPR Cas-9 therapies in the treatment of these diseases.

## 2. CRISPR-Cas9

Clustered regulatory interspaced short palindromic repeats (CRISPR) is a natural mechanism found in prokaryotes. Found first in 1987, CRISPR-Cas9 technology is so popular that scientists have studied it for nearly 40 years. However, it wasn't until the 21st century that scientists completely understood the mechanism of the CRISPR-Cas9 system and performed in vitro experiments on mammals [5].

Similar to the lymphocytes in the immune system of humans, CRISPR ensures adaptive immunity and prevents the infection of various types of viruses for the prokaryotes. CRISPR systems are classified into different groups (Type I, II, III, and more) based on the protein involved in the mechanism, and the type II ones are in active research, especially for the CRISPR-Cas9 technology [5]. CRISPR-Cas9 system involves three steps: The prokaryotes first memorize the specific type of intruders by integrating the genetic material of pathogens into the CRISPR locus. Next, molecules including the CRISPR-associated nuclease 9 (Cas9) and trans-activating crRNA (tracrRNA) are produced to assemble a complex [5]. Eventually, when the same intruder releases the genetic material again, a double-strand breakage takes place, producing non-functional fragments of the intruder's genome. In this way, bacteria may display resistance to viral attack including the phages.

CRISPR-Cas9 technology is suitable for gene therapy because of its unique genetic modification characteristics. The CRISPR-Cas9 system involves one single Cas protein so that it is easy to modify to alter the specificity. The protospacer adjacent motif (PAM) is a very short length of nucleotides on the foreign genetic material for recognizing of Cas9 protein. By altering the PAM sequence, CRISPR-Cas9 may perform different targeted cleavage of DNA molecules. CRISPR-Cas9 also has lower cost and high effectiveness compared to other genetic modifying tools like zinc finger endonuclease (ZFN) and transcription activator-like effector nuclease (TALEN) [3], which further expand the potential of gene therapy for treating diseases. Nowadays, as more researches take place, CRISPR-Cas9 technology has made great achievements in curing various types of diseases, including a series of neurodegenerative disorders.

## 3. CRISPR-Cas9 Gene Therapy Effectiveness in AD

### 3.1. AD Mechanism

AD is classified into 2 types: familial (FAD) and sporadic (SAD), each involved in different molecules or genes, and most of the AD cases are SAD. For the FAD, one of the favored hypotheses is related to amyloid, a protein molecule that has severe neurotoxicity to the brain. The precursor protein of amyloid produced during metabolism may be broken down by different secretases. In AD patients, one of the secretases called the β-secretase has greater activity. Compared to other proteases that minimize the toxicity of amyloid monomers, β-secretase results in the formation of Aβ monomers that reassemble to form amyloid plaques [6]. These harmful macromolecules are unable to be cleaved, so they accumulate in the human brain. The hallmark of SAD may be explained by the tubulin-associated unit (Tau). Tau is related to the formation and stabilization of microtubules, and by different DNA splicing methods during transcription, two isoforms of Tau are usually expressed in the brain [6]. In AD, these Tau are abnormal and detached from the microtubules, forming tangles or threads in the cell bodies. In the end, the Tau and Aβ lead to the failure of cholesterol metabolism [6]. Only the oxidized form of cholesterol may go out of the brain, leading to neurodegeneration and other AD symptoms.

### 3.2. How CRISPR-Cas9 Gene Therapy Alleviate AD?

CRISPR-Cas9 gene therapy mainly focuses on the in vivo alternation of specific gene mutations to control the amyloid metabolism to cure AD. For example, CRISPR-Cas9 may correct the mutation of the amyloid precursor protein (APP) gene that makes the β-secretase hyperactive by transferring the wildtype allele. Alternatively, the CRISPR-Cas9 system may target a specific locus on the APP

gene because the C-terminus modification only may silence the gene [6]. There are also studies proving that CRISPR-Cas9 can potentially regulate the gene for Tau and scientists have found the apolipoprotein E4 (APOE4) allele responsible for Tau in SAD [7]. In other situations, an activator or inhibitor of the Cas9 protein may be used: After delivering the CRISPR system into the body, the CRISPR system may not immediately perform a double-strand breakage, to reduce the risk of further mutation of the targeted gene [4].

The CRISPR-Cas9 gene therapy is advantageous for its versatility. To begin with, CRISPR-Cas is helpful for the deep diagnosis of AD by genetic screening to show the alleles under risk to identify the target of the therapy [8]; about 70% of the AD are due to genetic variants. Each with different locus of genetic mutation [6], and CRISPR-Cas9 technology may target any specific allele using the PAM sequence. Altering the PAM sequence and the guide RNA sequence can easily shift the target to another. In addition, this gene therapy proved to be effective in genetic editing. According to experiments in mice, CRISPR-Cas9 can correct permanently up to 75% of the mutated DNA, and alternation of the BACE1 gene (the gene coding the β-secretase) in mice leads to a 70% inhibition of the gene expression [6]. In another experiment where the APP gene is disrupted, CRISPR-Cas9 gene therapy shows no off-target activity in mice [9].

### 3.3. The Drawbacks of CRISPR-Cas9 Technology on AD Treatment

Unfortunately, CRISPR-Cas9 therapy has some limitations for AD treatment. To begin with, the correction of the APP gene is related to early-onset AD or familial AD, which is only a minor part of the whole AD patient group compared to late-onset AD or sporadic AD [6]. Also, the APOE4 allele targeting therapy and treatment of Tau for SAD need further research [7]. Moreover, genetic factors are not the only cause of the AD. About one-third of the sporadic AD causes are irresistible factors, including aging, exposure to chemicals, and hormonal factors [8]. Currently, CRISPR-Cas9 therapy correcting genetic mistakes is not helpful for AD caused by these reasons, and fewer patients may benefit from the CRISPR-Cas9 therapy.

In addition, the different methods of CRISPR-Cas9 therapy transferring into the human body have their disadvantages. Depending on the type of CRISPR-Cas9 tool used to cure AD, the selection of the non-viral vectors varies [6]. Furthermore, some of the non-viral physical methods require advanced technology, which reduces the availability of the patients. For example, plasmid DNA is optional for Cas9 transportation, but only standard laboratories may carry out the delivery [10]. Also, vectors need to be more efficient to transfer the CRISPR-Cas9 system to ensure greater effectiveness, and even though non-viral vectors are low-cost and more versatile, they are not as efficient as viral vectors for transport. However, the use of viruses occasionally leads to severe side effects due to random mutation [6]. The most common vector for transportation is the AAV, with a lower risk of side effects including the integration of the genome into the human chromosome. However, the capacity of AAV is only up to 4.7kb, making it impossible to take multiple gene editing [8]. The lentivirus may deal with the issue. Nevertheless, the risk of provoking an immune response as well as the spreading failure throughout the brain increases significantly [8].

Lastly, the developed studies are limited to animals in vivo or human body cells in vitro [10]. Due to the regulation of gene therapy, most of the experiments are based on mice having the same allele compared to humans, and few of the researchers take a further step into the clinical trials. Other researches focus on the pluripotent stem cell-derived neurons in vitro. Nevertheless, there are not sufficient researches to further explore the safety and effectiveness of CRISPR-Cas9 therapy in vivo, thus making it harder to convince the public to treat by using gene therapy.

## 4. CRISPR-Cas9 Gene Therapy Effectiveness in PD

### 4.1. PD Mechanism

Similar to AD, PD are divided into familial PD and sporadic PD, but both of the cases show the loss of dopaminergic neurons [11]. In the cerebral hemisphere of the brain, there is a group of nuclei

called basal ganglia. They are responsible for the motion, cognition, and emotion. In the basal ganglia, a specific part called substantia nigra is vital for the production of dopamine. In PD, the number of neurons decreases significantly so the concentration of dopamine decreases significantly. As a kind of neurotransmitter, dopamine helps to transmit signals across the synapses of different neuron cells. Therefore, the neurons in the brain of PD patients fail to communicate efficiently with each other, leading to uncontrollable or slow movements and failure in coordination or balance. There is another popular hypothesis claiming that the involvement of non-dopaminergic neuron pathways throughout other parts of the brain also contributes to PD, providing another way of treatment [2]. However, further observations are needed to confirm this idea.

The causes of PD are still in active research. According to clinical studies, scientists make a list of many possible PD-leading factors. Nevertheless, some of the observations hardly reappear, and the meta-analysis on these potential environmental risk factors suggests a lack of relationship between the factors and PD except the chemical exposure [2]. No matter what kind of factors, it is well-accepted that environmental and other factors lead to the change in genes, which has a direct relationship with PD. Similar to AD, many loci of genes being identified are involved in different molecular mechanisms. In PD, mutation of these genes mainly leads to the failure of vesicles and lysosomes as well as the mitochondria [2]. Lysosomes will perform autolysis, and mitochondria in the neuron burst, leading to neuronal death. Looking into the details, among a series of PD-related genes (PARK), the PARK-SNCA gene implicitly interferes with the lysosome function by changing the structure of the α-synuclein protein that plays a crucial role in the vesicle fusing process [2]. The mutation of the gene may lead to the toxic misfolding and abnormal tertiary structure of the α-synuclein. Besides, the lysosome inside the neuron may not correctly fuse with vesicles, releasing the digestive enzymes into the cell body.

### 4.2. How CRISPR-Cas9 Gene Therapy Alleviate PD?

CRISPR-Cas9 gene therapy aims to alleviate the symptoms of PD by restoration of the dopaminergic neurons. CRISPR-Cas9 may directly modify the dopaminergic neurons losing substantia nigra by inserting genes for dopamine production. Tyrosine hydroxylase (TH) produces levodopa, the precursor of dopamine, and L-amino acid decarboxylase (AADC) converts the precursor into dopamine [12]. CRISPR-Cas9 can also perform genetic knockouts of mutated genes, including a series of PD-related genes (PARK). For instance, the PARK-GBA, PARK-PARAKIN gene, and the PARK-LRRK2 gene are the most common PD-causing mutations. [2].

CRISPR-Cas9 has its strength in different perspectives. Firstly, the combination of CRISPR-Cas9 and the iPSCs cultivation produces many disease models. For instance, to complete a model for PD, an introduction with two different mutations in healthy iPSCs may use CRISPR-Cas9 technology [13]. These samples enable the researchers to fully understand the mechanism and the function of each mutated gene. Therefore, the application of CRISPR-Cas9 gene therapy may proceed quickly to be more advanced for curing diseases, including those targeting PD-causing genes. For instance, the targeting of a decarboxylase by CRISPR-Cas9 using viruses as the vector has entered a phase I clinical trial, where 15 patients with advanced PD was tested [12]. According to the 15-patient-involved phase I clinical trial, the delivery of the gene therapy into the brain leads to over 40% restoration of putamen [13]. There is also a phase II double-blinded clinical trial on another enzyme carried out by multiple centers [2].

Additionally, CRISPR-Cas9 may be artificially modified to improve its efficacy so that it will not perform frequent unwanted off-target effects, including the cleavage of the wrong locus, leading to the loss of genes or parts of chromosomes. One of the clinical trials with 12 PD patients using CRISPR-Cas9 and pluripotent stem cells explicit no significant side effects [2]. In this way, the CRISPR-Cas9 system displays several advantages, both used as a monotherapy and combined with other stem cell transplantation. These trials evaluate the potential and feasibility of the CRISPR-Cas9 gene therapy, which makes it more accessible in the future.

Furthermore, CRISPR-Cas9 therapy may be combined with the use of stem cells. Induced pluripotent stem cells (iPSCs) is a case that may both used on the development of disease models and the treatment of PD. By inserting the mutation genes inside the iPSCs, scientists can create different neuron samples to further explore the consequences of the mutated genomes on dopaminergic neurons. For the treatment of PD, scientists extract healthy iPSCs with no mutations to obtain new differentiated cells. The differentiated macrophages, for example, can trigger the compensation mechanism of α-synuclein by degrading it [13]. This is helpful for the maintenance of normal dopaminergic neurons. Besides, iPSCs from healthy people may differentiate into new functional neurons. By replenishing the dopaminergic neurons and replacing non-functional neurons, the symptoms of PD may be alleviated. Other kinds of stem cell therapies including embryonic stem cells (ESC) and neural stem cells (NSC) are also suitable for a combination of CRISPR-Cas9, with a similar method compared to the iPSCs [13].

### 4.3. The Disadvantages of CRISPR-Cas9 in PD

The biggest disadvantage of CRISPR-Cas9 as well as the combined stem cell therapies is the ethical concern. A case in point is a PD patient using pluripotent stem cells. Even though that the stem cell is extracted from his own body, the case is widely discussed and controversial because of ethical problems. Although CRISPR-Cas9 technology combined with pluripotent stem cells is considered to have low side effects, the risk of genetic modification for humans is bound to be discussed. Also, the usage of embryonic stem cells in PD may trigger an immune response so that the neurons replanted will be killed by the immune system [2]. There is also a chance for the cells to grow into tumors. Similar to the situation in AD, gene therapy research on humans is difficult to conduct. According to the data of clinical trial registration on neurodegenerative disease (including AD and PD), gene therapy only occupies less than 5% of the 9000 trials [14]. Therefore, further research should be done to understand fully the mechanism of PD, to deal with the side effects of the CRISPR-Cas9 stem cell combined therapy, and to discuss more on the human clinical trials.

## 5. Conclusion

CRISPR-Cas9 gene therapy is widely discussed and in active research because of its effectiveness and strong genetic modification ability to correct genetic disorders. It is a technology that shows great potency in curing a variety of diseases, including hematopoietic and immunological diseases as well as the NDs. Previous research papers indicate that CRISPR-Cas9 therapy may become the possible treatment of AD and PD, and recent researches have further achievements on that.

The mechanisms of AD depend on the specific type: The abnormal activity of beta-secretase or the Tau is supposed to lead to FAD and SAD respectively. CRISPR-Cas9 targets the genes that code for these proteins. After entering into the human body, CRISPR-Cas9 may target the specific locus for silence; In contrast, the mechanisms of PD tend to be more unknown, especially for environmental causes. CRISPR-Cas9 gene therapy is helpful for PD because whatever type of PD has a strong relationship with genetic factors in the dopaminergic neurons. In this case, the CRISPR system targets the PARK genes to restore other fundamental cell structures to ensure the survival of the neurons. CRISPR-Cas9 may also insert genes for dopaminergic production.

CRISPR-Cas9 gene therapy displays its great potential with high efficacy and specificity to alleviate or cure diseases, ease of modification and control, and high suitability to combine with other molecules or therapies. For instance, in AD, CRISPR-Cas9 may perform timing cleavage with the help of activators. In PD, gene therapy helps to carry out treatments with different kinds of stem cells which further improve the effectiveness of both treatments. CRISPR-Cas9 gene therapy also displays benifits other than treatments, including the diagnosis and experimental sample modeling. On the other hand, this therapy still has its disadvantages. There is a not perfect viral vector to transport CRISPR-gene in vitro safely and effectively. The ethical issues on genetic modification prevent some of the further research on humans, and many experiments use mice instead. However, experiments

on mice are less convincing to assess the efficacy of CRISPR-Cas9 gene therapy. In addition, few of the research trials pass the clinical trials, and fewer patients may benefit from it. Therefore, it is suggested that more research should be done to obtain details on the characteristics of NDs such as AD and PD. For the transition of CRISPR-Cas9 into human beings, safer viral vectors or any alternatives should be in more active research. Researchers should also carry out quantitative analysis on other kinds of mammals and clinical trials to assess the potential of CRISPR-Cas9 gene therapy with less bias and higher evidence-based quality.

## References

[1] S. A. Tatulian, "Challenges and hopes for Alzheimer's disease," Drug Discovery Today, vol. 27, no. 4, pp. 1027–1043, Apr. 2022, doi: 10.1016/j.drudis.2022.01.016.

[2] J. Jankovic and E. K. Tan, "Parkinson's disease: etiopathogenesis and treatment," J Neurol Neurosurg Psychiatry, vol. 91, no. 8, pp. 795–808, Aug. 2020, doi: 10.1136/jnnp-2019-322338.

[3] H. Mollanoori and S. Teimourian, "Therapeutic applications of CRISPR/Cas9 system in gene therapy," Biotechnol Lett, vol. 40, no. 6, pp. 907–914, Jun. 2018, doi: 10.1007/s10529-018-2555-y.

[4] L. Lu, X. Yu, Y. Cai, M. Sun, and H. Yang, "Application of CRISPR/Cas9 in Alzheimer's Disease," Front. Neurosci. vol. 15, p. 803894, Dec. 2021, doi: 10.3389/fnins.2021.803894.

[5] S.-W. Wang et al., "Current applications and future perspective of CRISPR/Cas9 gene editing in cancer," Mol Cancer, vol. 21, no. 1, p. 57, Feb. 2022, doi: 10.1186/s12943-022-01518-8.

[6] A. S. Hanafy, S. Schoch, and A. Lamprecht, "CRISPR/Cas9 Delivery Potentials in Alzheimer's disease Management: A Mini Review," Pharmaceutics, vol. 12, no. 9, p. 801, Aug. 2020, doi: 10.3390/pharmaceutics12090801.

[7] E. Konstantinidis et al., "CRISPR-Cas9 treatment partially restores amyloid-β 42/40 in human fibroblasts with the Alzheimer's disease PSEN1 M146L mutation," Molecular Therapy - Nucleic Acids, vol. 28, pp. 450–461, Jun. 2022, doi: 10.1016/j.omtn.2022.03.022.

[8] N. C. Barman et al., "CRISPR-Cas9: A Promising Genome Editing Therapeutic Tool for Alzheimer's disease—A Narrative Review," Neurol Ther, vol. 9, no. 2, pp. 419–434, Dec. 2020, doi: 10.1007/s40120-020-00218-z.

[9] Y. Duan et al., "Brain-wide Cas9-mediated cleavage of a gene causing familial Alzheimer's disease alleviates amyloid-related pathologies in mice," Nat Biomed Eng, vol. 6, no. 2, pp. 168–180, Jul. 2021, doi: 10.1038/s41551-021-00759-0.

[10] B. Yip, "Recent Advances in CRISPR/Cas9 Delivery Strategies," Biomolecules, vol. 10, no. 6, p. 839, May 2020, doi: 10.3390/biom10060839.

[11] L. M. De Plano, G. Calabrese, S. Conoci, S. P. P. Guglielmino, S. Oddo, and A. Caccamo, "Applications of CRISPR-Cas9 in Alzheimer's Disease and Related Disorders," IJMS, vol. 23, no. 15, p. 8714, Aug. 2022, doi: 10.3390/ijms23158714.

[12] T. B. Stoker and R. A. Barker, "Recent developments in the treatment of Parkinson's Disease," F1000Res, vol. 9, p. 862, Jul. 2020, doi: 10.12688/f1000research.25634.1.

[13] P. Pinjala et al., "CRISPR/Cas9 assisted stem cell therapy in Parkinson's disease," Biomater Res, vol. 27, no. 1, p. 46, May 2023, doi: 10.1186/s40824-023-00381-y.

[14] A. Klinkovskij, M. Shepelev, Y. Isaakyan, D. Aniskin, and I. Ulasov, "Advances of Genome Editing with CRISPR/Cas9 in Neurodegeneration: The Right Path towards Therapy," 2023.