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THE USAGE OF THE CRISPR-CAS9 GENETIC EDITING SYSTEM IN NEURODEGENERATIVE DISEASES

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ABSTRACT The CRISPR-Cas9 system has emerged as a revolutionary tool in genetic engineering, offering unprecedented precision in genome editing. This review examines the current state of CRISPR-Cas9 research in the treatment of neurodegenerative diseases, with a focus on Huntington's Disease (HD), Alzheimer's Disease (AD), and Parkinson's Disease (PD). It synthesizes findings from recent studies that have applied CRISPR-Cas9 technology to model these diseases, investigate their mechanisms, and develop potential therapeutic strategies. In HD, CRISPR-Cas9 has shown promise in selectively inactivating mutant HTT alleles. For AD, the technology has been used to edit mutations in genes such as PSEN1 and APP, potentially regulating Aβ production. In PD research, CRISPR-Cas9 has facilitated the creation of animal models and the exploration of epigenetic alterations. While these applications demonstrate the potential of CRISPR-Cas9 in addressing neurodegenerative diseases, challenges remain, including delivery to the central nervous system and potential off-target effects. This review also highlights the ethical considerations and future directions for research in this rapidly evolving field. By collating and analyzing the current research, this paper provides a comprehensive overview of the potential of CRISPR-Cas9 in treating neurodegenerative diseases.

KEYWORDS : CRISPR, Genome Editing, Neurodegeneration, Cas9, Central Nervous System

INTRODUCTION

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) has emerged as a revolutionary tool in genetic engineering since its discovery as a bacterial adaptive immune system. This RNA-guided DNA endonuclease system, first described in its natural context in 1987 and adapted for genome editing in 2012, has rapidly evolved to become one of the most powerful and versatile gene-editing technologies available today (Doudna & Charpentier, 2014; Jinek et al., 2012). The applications of CRISPR-Cas9 in disease treatment are vast and promising. It has shown potential in addressing genetic disorders, cancer, and infectious diseases by allowing precise modifications of disease-causing genes (Hsu et al., 2014). The technology's ability to add, remove, or alter specific DNA sequences offers new avenues for developing innovative therapies (Zhang et al., 2018).

CRISPR-Cas9's capacity for targeted gene editing allows researchers to model neurodegenerative diseases more accurately, investigate disease mechanisms, and potentially develop gene-based therapies. This approach is particularly valuable given the often-monogenic nature of many neurodegenerative conditions (Huang et al., 2016).Globally, numerous studies have explored the potential of CRISPR-Cas9 in treating neurodegenerative diseases.

Research by An et al. (2015) and Xu et al. (2019) has focused on correcting mutations in Huntington's Disease. For Alzheimer's Disease, studies by György et al. (2020) have investigated disrupting the APP gene. In Parkinson's Disease research, Zhou et al. (2015) and Wang et al. (2021) have used CRISPR-Cas9 to create animal models with specific gene knockouts.

Despite the abundance of research in this field, there is a lack of comprehensive reviews collating andanalyzing these diverse studies. This gap in the literature presents a challenge for researchers and clinicians seeking to understand the current state of CRISPR-Cas9 applications in neurodegenerative diseases (De Plano et al., 2022).

A systematic collation and analysis of existing research would offer a clearer picture of the progress made in applying CRISPR-Cas9 to neurodegenerative diseases, highlight promising avenues for future research, and identify potential challenges or limitations in current approaches (Zhou et al., 2023).

This review aims to discuss the general applications of the CRISPR-Cas9 genomic reprogramming tool, its real-life applications, the usage of animal models within the system, and its relation to treating and potentially curing neurodegenerative diseases, with emphasis on Alzheimer's Disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD) (Roy et al., 2020). Specifically, it seeks to answer the following research question:

RQ1: What is the current state of CRISPR-Cas9 research in the treatment of neurodegenerative diseases, particularly Huntington's Disease, Alzheimer's Disease, and Parkinson's Disease?

The Concept and Evolution of CRISPR

Over the past decade, advancements in brain-related technology have made it possible to visualize and identify neural activity in real-time, furthering our understanding of genomics and opening up new prospects in biotechnology. Genetic engineering, a recent and rapidly growing field, has been instrumental in these developments. The prevalent RNA-guided tool, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)- Cas9 (CRISPR Associated Protein), facilitates endless opportunities for further research (Zhou et al., 2014).

In 2020, Jennifer Doudna and Emmanuelle Charpentier were awarded the Nobel Prize in Chemistry for their groundbreaking research on CRISPR. Their published work inspired a revolution in genetic editing. These palindromic genetic repeats have existed in living organisms for centuries but were only discovered in the early 2020s when their potential and invaluable contribution to science were truly recognized (Doudna & Charpentier, 2014).

CRISPR-Cas is a bacterial antiviral immune defense mechanism that works by recognizing target sites using sgRNA (Single Guide Ribonucleic Acid). The sgRNA includes a 20-base nucleotide spacer and a supporting sequence that holds the Cas9 protein (Wang et al., 2013). Cas9 acts as a molecular cutter, excising specific required portions of nucleotides from the target DNA.

The protein has four parts, two of which are crRNA (CRISPR RNA) and tracrRNA (trans-activating CRISPR RNA) (Wang et al., 2013). As a whole, CRISPR-Cas9 works in three steps: recognition, cleavage, and repair. Meanwhile, the Cas9 protein works to identify and degrade the target DNA.

This is accomplished using sgRNA synthesized from multiple living sources specifically for genetic editing purposes (Fu et al., 2014).However, the CRISPR-Cas9 system has some limitations, including interactions with unintended DNA molecules. This can cause genetic mutations that may foster the development of cancerous cells (Fu et al., 2013; Zhang et al., 2022).

To simplify, sgRNA is engineered to guide the Cas endonuclease to the genomic area of interest. This endonuclease introduces a DSB (Double-Strand DNA Break) at the target, which activates DNA repair machinery pathways, as the DSB is perceived as a threat to the gene (Huang et al., 2021). This repair machinery works to mend breaks in cellular pathways, which could potentially disrupt the functioning of CRISPR-Cas9 (Cota-Coronado et al., 2019).

This method can be directly applied to the central nervous system, which is at risk of developing neurological or neurodegenerative diseases. Mammalian brains are complex, intertwined systems of differentiated neurons, each carrying specialized and specific functions. Genetic sequencing and editing tools such as CRISPR-Cas9 help us further research developments in this area (Roy et al., 2020).

CRISPR and Neurodegenerative Diseases

The neurocircuitry of the brain and the central nervous system is regulated primarily by transcriptional processes, working in tandem with other external processes. This is why many humangenetic problems affect the brain and cause neurodegeneration (Huang et al., 2021). However, the rise of CRISPR-Cas9 offers hope for treating and potentially curing these conditions in the future. These neurological defects are primarily caused by genetic irregularities in the features of the brain and spinal cord, as indicated by neuroscientific literature stating that these neurological and neurodegenerative problems are habitually caused by genetic mutations (Roy et al., 2020).

These alterations in a mammalian brain can cause the gain and loss of functions, making tools like CRISPR-Cas9 integral for tackling these ailments (Zhou et al., 2023).For years, the human genome has been researched through post-mortem samples and stem cell cultures, but CRISPR-Cas9 opens a world of possibilities within research (Cota-Coronado et al., 2019). CRISPR is a versatile and agile tool that enables scientists to edit genes and examine them through spatially and temporally controlled in-vivo genetic manipulation, specifically in the central nervous system of humans and animal models (Huang et al., 2021). Knockout and knock-in models, which are typically mammalian animals that genetically mimic humans, are essential for the process of genetic repression or expression (Cota- Coronado et al., 2019). It is utilized in multiple species, such as pigs, rats, mice, bacteria, zebrafish, somatic cells, and human pluripotent stem cells (Zhou et al., 2023).

Implementing CRISPR specifically into primate genetic research can help scientists model neurodegenerative diseases like Parkinson's, Alzheimer's, and autism. CRISPR also carries the capability of editing germline cells in relatively larger animals, specifically neuronal cells (Roy et al., 2020). In mammals, the central nervous system operates complex cognitive functions controlled by the prefrontal cortex. Rodents developed in a transgenic manner are chiefly used in laboratories to investigate genetic anomalies, but it is difficult to relate their behavior to that of humans (Huang et al., 2021). Genes in humans do not have a distinguishable one-to-one orthologue with transgenic mice; for example, orthologous genes can have opposing roles if species differ (Cota- Coronado et al., 2019).

Unlike humans, this area is not well-developed in mice, which are popularly used as animal models. This can cause discrepancies in research as a human's prefrontal cortex does not match that of a mouse. Furthermore, the organization of white matter in the brains of both mammals differs, and neurodegenerative diseases like Parkinson's and Alzheimer's have different phenotypes in mice and humans. For this reason, the modelling of neuropsychiatric diseases in mice (to research the human neurological genome) is exceedingly problematic (Cota-Coronado et al., 2019; Huang et al., 2021).

Typically, neurodegenerative diseases are characterized and distinguished by the disappearance of neuronal groups in certain regions of the brain, in relation to age. Animal models can be used to investigate this phenomenon and develop treatments. Using these transgenic models, however, is costly and time-consuming (Zhou et al., 2023). Nevertheless, as stated above, using transgenic rodents enables expression of reformed gene copies, but the risk of mutations through complicated genetic interactions can bring about neurodegenerative diseases to begin with (Huang et al., 2021).

CRISPR CAS 9 in Huntington's Disease

Huntington's disease (HD) is a heritable monogenic autosomal dominant neurological disorder that primarily causes dementia and motor impairments. HD affects approximately 4-8 people out of every 100,000, with a higher prevalence among Europeans (National Institute of Neurological Disorders and Stroke [NINDS], 2023). Symptoms typically begin to manifest between the ages of 35-45, and the disease currently has no known cure (Cleveland Clinic, 2023; Yang et al., 2021; Revilla et al., 2014).HD manifests late in an affected individual's life through pathogenic mechanisms that cause neurodegeneration (Mayo Clinic, 2023).

The expansion of cytosine-adenine-guanine (CAG) repeats in the huntingtin (HTT) gene triggers the disease's gradual yet severe progression, most notably causing motor dysfunction. This gene is located on the short arm of chromosome 4p16.3 (Ross, 2010; Ross & Tabrizi, 2011). The CAG expansion encodes the glutamine amino acid in the first exon of HTT. Protein misfolding and aggregation in the brain lead to psychiatric disruption, cognitive decline, and weak motor coordination (Schulte & Littleton, 2011; Ross & Tabrizi, 2011; McColgan & Tabrizi, 2018).Healthy individuals have fewer than 27 CAG repeats, whilethose with more than 40 repeats typically develop HD (McColgan & Tabrizi, 2018).

The greater the number of repeats, the faster the disease progresses. Furthermore, the disease causes atrophy of the striatum and, in severe cases, atrophy of the cerebral cortex (NINDS, 2023).Due to the disease's singular genetic target, it distinguishes itself from other neurodevelopmental disorders (NDDs), making the discovery of a cure both more likely and feasible in comparison to other NDDs (Cleveland Clinic, 2023). This disease is an exemplary medium for the application of CRISPR- Cas9 genetic editing in the search for a potential cure. Due to CRISPR's ability to target only specific and required segments of DNA using its RNA guide, it can deactivate the mutant HTT allele while leaving normal alleles unaffected (Shin et al., 2016).

Treatment using CRISPR

Attempts to investigate or treat HD using CRISPR- Cas9 primarily focus on editing the HTT gene. The approach involves first correcting mutations in HD patients, followed by introducing mutations into healthy cells. A study conducted by Shin et al. (2016), despite correcting the HTT mutation, failed to remove a gene from the cell lines that could have impacted the regulation of the HTT gene expression (Heman-Ackah et al., 2016). Similarly, Xu et al. (2023) repeated this procedure using both CRISPR- Cas9 technology and piggyBac, which uses a genetically engineered transposase enzyme for the insertion of a gene in the genome of an organism. Cell lines resultant from iPSCs helped maintain karyotypes and allowed their further differentiation into effective and synaptically active neurons. The phenotype for these edited cells was normal, whereas the iPSCs derived from an HD patient displayed various symptoms such as extremely high susceptibility to growth factors and discrepancies in mitochondrial respiratory processes (Xu et al., 2023).

CRISPR-Cas9 was first used in 2014 to generate isogenic cell models of HD. The exon 1 region in the HTT gene was cut, and researchers introduced human induced iPSCs in the area, having 21, 72, or 97 CAG repeats (Zhou et al., 2023). Multiple studies have been replicated in a similar manner to generate isogenic HD human iPSCs, concluding with observations such as phenotypic abnormalities, impaired neuronal differentiation, and defects in gene expression (Zhou et al., 2023).

Thus, these isogenic cellular models for HD are also capable and useful resources for further discoveries in this area. However, knockout mice models remain the preferred and primary means through which HD is researched. Through CRISPR-Cas9's versatile specific deactivation ability, knock-in mice models can help to test hypotheses related to HD (Zhou et al., 2023).Presently, research relating CRISPR-Cas9 to HD is focused on targeting the transcription of DNA to lessen the amount of mRNA of the mutated HTT gene. Non-coding RNA is also used to decrease the translation of RNA (Huang et al., 2021).

CRISPR IN Alzheimer's Disease

Alzheimer's Disease (AD) is a genetic, sporadic, age-associated neurodegenerative condition characterized primarily by progressive memory loss. This occurs through the formation and progressively abnormal accumulation of a large transmembrane protein, amyloid-beta 42 (Aβ42), paired with the phosphorylation of tubulin- associated unit proteins (Tau) and the elevated activation of glial cells, which support and protect nerve cells (Alzheimer's Association, 2015). Together, these factors can cause a myriad of symptoms, including amnesia, cognitive deficit, neuronal loss, and hippocampal pyramidal cell granule vacuolar degeneration (Reitz et al., 2011).AD is linked to mutations in genes such as presenilin 1 (PSEN1), presenilin 2 (PSEN2), Beta- secretase 1 (BACE), amyloid precursor protein (APP), and apolipoprotein-E (ApoE) (Alzheimer's Association, 2015). Specifically, PSEN1 is a component of γ-secretase, an enzyme complex involved in the generation of the Aβ peptide, which deposits as plaque in the AD brain. The PS1 gene contains an M146L mutation that eventually leads to the formation and development of AD (Wong et al., 2018).

This PSEN1M146L mutation leads to the generation of a novel protospacer-adjacent motif (PAM) site—PAM is typically used to

mark protein targeting sites as it can bind to the Cas protein—which led researchers to design a guide RNA (gRNA) to selectively disrupt the mutation without disturbing other vital proteins (Kim et al., 2019).The first reported case ofAD affected a 51-year-old woman suffering from symptoms like rapid memory loss, aggression, and sleeping disorders. In 1906, psychiatrist Alois Alzheimer studied this case and found cell clusters in the cortex and diffuse atrophy in the brain (Alzheimer's Association, 2015).

Further research showed that the likelihood of developing AD increases with age, and it is projected that around 152 million people will be affected by 2050, emphasizing the urgent need for effective treatments and potential cures for AD in the human population (Alzheimer's Association, 2015).Current treatments for AD provide only symptomatic relief to affected patients without slowing or stopping disease progression. Usually, cholinesterase inhibitors and N-methyl-D- aspartate receptor antagonists are used for this relief (Chopade et al., 2013). Two etiologies have been attributed to AD: positive and negative lesions, while factors such as cholinergic system damage, nervous system inflammation, and oxidative stress contribute to its development.

Risk factors associated with AD include old age, with two-thirds of all cases occurring in women due to decreased estrogen secretion during menopause (Alzheimer's Association, 2015).In familial cases of AD (FAD), genetic mutations account for only about 1% of total cases. However, gene editing in sporadic AD (SAD) may be negligible or ineffective since it is caused by environmental factors rather than genetic mutations present in FAD. Regardless, both FAD and SAD involve dysregulated metabolism of Aβ; thus limiting Aβ production may help slow disease progression regardless of type (Reitz et al., 2011).

Treatment using CRISPR Cas 9

CRISPR-Cas9 is capable of creating AD models to represent a realistic AD phenotype to investigate pathogenesis, screen harmful genes, and attempt to find a cure (De Plano et al., 2022). AD has garnered significant attention in this field due to its high prevalence and the subsequent lack of cure and effective therapies. The CRISPR-Cas9 system has shown its ability to normalize electrophysiology. Through the editing of mutations in the PSEN1 and PSEN2 genes derived from induced pluripotent stem cells (iPSCs) of AD patients, the regulation of Aβ secretion has been made possible (Sun et al., 2019). The Swedish mutation (double mutation K670M, N671L) of amyloid precursor protein (APP) can also be disrupted by CRISPR-Cas9, as demonstrated by György et al. (2018).

To treat AD effectively, multiple approaches must be tested to reach affected cells in the central nervous system (CNS), including the penetration of the blood-brain barrier (BBB), which usually breaks down or malfunctions during the onset of AD, before dementia, neurodegeneration, and brain atrophy occur (Bhardwaj et al., 2022). A study utilized a combination of Cas9 and engineered reverse transcriptase (prime editing) to introduce the Icelandic APP mutation into human cell lines for behavioral studies. Having been applied successfully, it was also discovered to be capable of targeting pathogenic mutations and even replacing them. Using this technique, the London mutation in familial AD could potentially be targeted and replaced as well (Tan et al., 2018).

In a particular study, a CRISPR-Cas9 system obtained from Streptococcus pyogenes was used to disrupt the PSEN1^M146L mutated allele in human fibroblasts. This resulted in the observable disruption of more than 50% of mutant alleles from the treated sample, which was evident in reduced extracellular ratios of Aβ42/40. Treatment with CRISPR-Cas9 also affected PS1 conformation and showed a decrease in PS1 levels altogether. This demonstrates that CRISPR-Cas9 is effective in targeting the PSEN1^M146L mutation, thereby also countering the phenotype associated with AD (Heman-Ackah et al., 2016).AD's associated genetic mutations allow for immense scope in research using CRISPR-Cas9 gene editing. Through the application of theoretical approaches to real-life therapy, AD can potentially begin to be treated more effectively (Reitz et al., 2011).

CRISPR in Parkinson's Disease

Parkinson's disease (PD) is primarily characterized by the degeneration of dopaminergic neurons in the subthalamic nucleus, caused by Lewy bodies with misfolded α-synuclein (SN) (Klein & Westenberger, 2012). It is associated with mutations in several genes, including Protein deglycase DJ-1, PTEN-induced putative kinase 1 (PINK1), SNCA (synuclein alpha), and leucine-rich repeat kinase-2 (LRRK2) (Klein & Westenberger, 2012). The eventual death of these neurons in the central nervous system (CNS) occurs frequently in the human population, making PD the second-most common neurodegenerativedisease. It affects 60-140 people per 100,000, with the incidence increasing, especially in the geriatric population (Tran et al., 2020).

In its familial form, PD can be both autosomal recessive and autosomal dominant (Klein & Westenberger, 2012). While the development of the disease is caused by mutations in the LRRK2, PARK2, and SNCA genes, its dominant nature arises from mutations in SNCA, LRRK2, and UCHL1, while its recessive variant is associated with mutations in PINK1, DJ-1, and PARK2 genes. Genetic editing through advanced tools such as the CRISPR-Cas9 system can potentially correct these mutations through intensive studies on their functions and products.As the illness progresses, the pathology extends beyond the basal ganglia, leading to additional non-motor symptoms such as dyskinesias, distressing sensory experiences, autonomic dysfunction, and neuropsychiatric manifestations.

PD is classified as either familial or sporadic depending on the affected individuals' family background. Patients with familial PD exhibit symptoms that run in the family and make up a small portion (5-15%) of documented cases, while those with sporadic PD acquire the disease without any known genetic predisposition (Klein & Westenberger, 2012; Tran et al., 2020). The genetic basis of PD pathology is complex and involves multiple factors. Over 90 different gene variations, including SNCA, GBA, LRRK2, and MAPT, have been linked to the development of Parkinson's disease. Currently, there is no medication or surgery that can cure PD.

Existing treatments aim to enhance the patient's quality of life and slow down the development of motor symptoms associated with the condition (Kalia & Lang, 2015; Poewe et al., 2017; Paff et al., 2020; Sonninen et al., 2020). Commonly used treatments for PD are dopamine replacement therapy through levodopa and deep brain stimulation (DBS). As Parkinson's disease progresses, the effectiveness of levodopa in improving motor function decreases and its side effects become more noticeable (Poewe et al., 2020).

Hence, it is crucial to develop new treatment approaches to advance towards finding a cure for PD.

Treatment using CRISPR Cas9

Recent research has shown promise for innovative treatment approaches targeting epigenetic alterations in human cell genomes, especially in PD. It is understood that increased levels of SNCA linked to gene duplication play a role in the development of PD. Simultaneously, neurons require a typical physiological amount of SNCA to preserve their function.

A method utilizing a combination of dCas9 and DNMT3A was employed to control the expression of the SNCA gene by targeting DNA methylation in its first intron, thereby regulating its transcription (Zhou et al., 2023). By utilizing this method, targeted DNA methylation was carried out in the initial section of the SNCA gene within neurons that had three copies of SNCA derived from iPSCs of a patient with PD.

This led to a reduction in the production of α- synuclein mRNA and proteins in neurons, normalizing the abnormal characteristics of the dopaminergic neurons' phenotype. These findings suggest that the CRISPR- dCas9- DNMT3A technology has potential as a novel therapeutic option for Parkinson's disease by utilizing epigenetics. Different research has investigated the possibility of using gene therapy to treat PD by focusing on various molecules involved in its development. One method includes reinstating the function of malfunctioning genes through delivering the appropriate genes.

The typical motor symptoms seen in PD arise due to a lack of dopamine in the striatum. The main reason for this insufficiency is the deterioration of nigrostriatal neurons. The enzyme tyrosine hydroxylase (TH) transforms the amino acid tyrosine into L-DOPA, playing a crucial role in dopamine synthesis. Subsequently, L-DOPA is transformed into dopamine by aromatic L-amino acid decarboxylase

(AADC) (Palfi et al., 2002).

Animal models provide valuable means to study a variety of neurological disorders like PD. Because Parkinson's Disease has multiple causes, researchers have employed different methods to induce the condition in animal models. These methods involvethe use of drugs and toxins that cause dysfunctions linked to midbrain dopamine signaling. It also involves genetic engineering techniques to simulate the genetic variations of PD. 6-OHDA and MPTP have been widely utilized to create pharmacological models of PD (Dawson et al., 2018). While these models were suitable for treating the motor symptoms of PD, they were not effective in developing therapeutic strategies (Athauda & Foltynie, 2016).

Hence, the focus has shifted to creating genetic models of PD. It is not surprising that CRISPR has become a widely used method in genetic animal modeling for PD tissues.Zhou et al. (2015) used CRISPR-Cas9 for accurate genome editing to create double-knockout pigs for Parkin and PINK1 through SCNT methods. Piglets from this study showed no immunofluorescence staining of anti-Parkin and anti-PINK1 antibodies if they had double knockouts. Every piglet born had the same genetic makeup, and no unintended mutations were found. Following that, a research study by Wang et al. (2016) produced Bama miniature pigs with triple knockouts of Parkin, DJ-1, and PINK1. During the experiment, researchers injected CRISPR-Cas9 gRNAs targeting Parkin, DJ-1, and PINK1 into pronuclear embryos along with Cas9 mRNA, and then transplanted them. Immunostaining fibroblasts from the gene-edited pigs showed no staining for DJ-1 protein, with decreased expression of Parkin and PINK1 genes but not complete absence (Zhu et al., 2018).

These studies show how the CRISPR-Cas9 system saves time and money, but the models showed no differences in neuroinflammation or behavioral deficits compared to wild-types. This is likely due to the piglets being in their early stages of life. However, it is still uncertain whether double- or triple-knockout pigs will effectively mimic human PD symptoms (Zhou et al., 2023).

CONCLUSION

The CRISPR-Cas9 system has emerged as a powerful tool in the field of genetic engineering, offering unprecedented precision and efficiency in genome editing. This review has explored its potential applications in the treatment of neurodegenerative diseases, focusing on Huntington's Disease (HD), Alzheimer's Disease (AD), and Parkinson's Disease (PD). Findings indicate that CRISPR-Cas9 holds significant promise for addressing the genetic underpinnings of these complex disorders.

In HD, CRISPR-Cas9 has shown potential in selectively inactivating the mutant HTT allele while leaving normal alleles unaffected. Studies using HD-iPSCs have demonstrated the feasibility of genetic correction, offering hope for future therapies (Yan et al., 2022).

For AD, CRISPR-Cas9 has been used to edit mutations in genes such as PSEN1 and PSEN2, regulating Aβ secretion and potentially slowing disease progression (Zhou et al., 2023). In PD research, CRISPR-Cas9 has been employed to create animal models and explore epigenetic alterations, particularly in regulating the expression of the SNCA gene (Kalia & Lang, 2015).

This review has some limitations. First, this study is limited by the availability and quality of existing research on CRISPR-Cas9 applications in neurodegenerative diseases. Second, the scope of this study was limited to three major neurodegenerative diseases (HD, AD, and PD), potentially overlooking applications of CRISPR-Cas9 in other neurological disorders. Third, this review primarily focused on preclinical studies and theoretical applications, with limited data from human clinical trials, which are crucial for assessing the true therapeutic potential of CRISPR-Cas9 in neurodegenerative diseases. Lastly, the study did not extensively cover the ethical implications and regulatory challenges associated with gene editing technologies, which are important considerations in the clinical application of CRISPR-Cas9.

The implications of this research are far-reaching. CRISPR-Cas9 technology could potentially revolutionize the treatment of neurodegenerative diseases, moving beyond symptomatic relief to address the underlying genetic causes. This could lead to more effective therapies, improved quality of life for patients, and potentially even cures for these currently incurable conditions.

Further research in this field can focus on improving the specificity and efficiency of CRISPR-Cas9 editing to minimize off-target effects, developing more effective delivery methods for CRISPR-Cas9 components to the central nervous system, conducting long-term studies to assess the safetyand efficacy of CRISPR-Cas9-based therapies in animal models and eventually in clinical trials.In conclusion, while CRISPR-Cas9 technology offers exciting possibilities for treating neurodegenerative diseases, it is crucial to approach this field with cautious optimism. Continued research, rigorous testing, and ethical considerations will be essential as we navigate the path towards translating these promising laboratory findings into safe and effective clinical treatments.

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