Review

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Application of CRISPR/Cas9 in the management of Alzheimer's disease and Parkinson's disease: a review

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Abstract

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) nucleases system (CRISPR/Cas9) is a popular gene-editing technology with an expanding scope in the field of medicine. Recent studies have investigated the role of CRISPR/Cas9 system in the treatment of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Since the risk of occurrence of both conditions is strongly associated with genetic mutations and variations, the use of gene-editing technologies to rectify these genetic errors becomes relevant. The CRISPR/Cas9 system has been tested in AD, which has led to a decrease in either amyloid beta deposition or tau phosphorylation in cells. Likewise, genetic mutations in cells affected by PD have been corrected with promising results in initial studies undertaken. Therefore, the use of the CRISPR/Cas9 system should be expanded among different populations to understand its efficacy and safety in depth among neurodegenerative conditions.

Keywords: Alzheimer's disease, CRISPR/Cas9, gene editing, neurodegenerative disorders, Parkinson's disease

Introduction

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are important causes of morbidity, especially in the elderly population. Both of these are characterized by a common process of neurodegeneration, which stands for a gradual and progressive loss of neurons leading to nervous system dysfunction[1,2]. AD is the most common neurodegenerative disorder, causing dementia globally and affecting an estimated 24 million people worldwide[3]. Particularly in developed nations like Australia, AD has a prevalence of 10–30% in people over 65 years, and the incidence doubles every 10 years after 60 years[4]. Although the most common and strongest risk factor for AD is the genetic factor, other acquired factors such as cerebrovascular diseases, diabetes, hypertension, obesity, and dyslipidemia are associated with an increased risk of the occurrence of AD[5]. On the other hand, PD is the second most common neurodegenerative disease, with an annual incidence rate of 160 per 100 000 people aged 65 years and older. Use of dairy products, exposure to pesticides and methamphetamine, the presence of neoplasms like melanoma, and a history of traumatic brain injuries are some of the risk factors associated with the risk of PD[6].

The mainstay of the management of AD is the use of drugs that target cholinergic or glutamatergic transmission. These drugs can relieve the symptoms and improve cognition. However, there is no curative effect whatsoever[7]. Likewise, PD is currently being managed by the use of drugs with different mechanisms, such as supplementation of dopamine precursors, dopamine agonism, inhibition of metabolizing enzymes, and increased anticholinergic effects. Just like AD, a definite curative treatment is not yet available[8].

Newer advancements in the management of neurodegenerative disorders are focused on tackling a disease at its molecular level, usually targeting a faulty gene or protein to repair, replace, or

HIGHLIGHTS

- The risk of occurrence of neurodegenerative conditions like Alzheimer's disease and Parkinson's disease is strongly associated with genetic mutations.
- The CRISPR/Cas9 system is a gene-editing technology with a great potential to rectify the underlying genetic errors.
- Initial studies on disease-affected cells treated by rectifying the identified genetic mutations have shown promising results.

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remove it from the affected cells[9]. Gene editing is the intentional modification of genomic DNA by insertion, deletion, and replacement at a target site of DNA. This novel technique could be employed in the inactivation of target genes, the acquisition of new genetic traits, and the correction of gene mutations. This process of editing genes has been achieved successfully by an inexpensive and precise system known as the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) nucleases system[10–12].

After the discovery of CRISPR/Cas9 technology by Ishino *et al.* in 1987, CRISPR/Cas9 has been identified as a breakthrough method for genome editing with great promise for treating disorders with few or no therapy choices. The CRISPR/Cas9 system is an essential component of a bacterium's defense mechanism, providing defense against the unwanted incorporation of mobile genetic components like viruses and plasmids[13]. Recent comprehensive studies on CRISPR/Cas9 have greatly increased editing efficiency and reduced off-target effects while being widely employed for fundamental and translational research. The CRISPR/Cas9 system consists of two primary constituents, namely the Cas9 enzyme and the single-guide RNA (sgRNA). The sgRNA identifies the target DNA sequence, considering many criteria throughout the design phase to enhance specificity. On the other hand, the Cas9 protein functions as an endonuclease, facilitating the cleavage of DNA double strands through its molecular scissor-like activity[14–16].

Genetic factors are the most essential risk factors for irreversible neurodegenerative diseases like AD and PD. Genetic mutations are responsible for around 1% of familial cases of AD. Consequently, the use of genome editing using CRISPR/Cas9 holds potential for addressing familial Alzheimer's disease (FAD) on a broader scale while offering limited or insignificant advantages for sporadic Alzheimer's disease (SAD)[14]. Likewise, PD also has two forms: sporadic Parkinson's disease (SPD) and familial Parkinson's disease (FPD). Both of these forms have a genetic basis for disease, apart from other environmental exposures and lifestyle factors[17]. A wealth of newer studies has reported inspiring results from the CRISPR/Cas9 system in the management of AD and PD. The aim of the review is to comprehensively summarize the application of the CRISPR/Cas9 system in the management of two of the most common neurodegenerative disorders: AD and PD.

Methods

A comprehensive search of electronic databases, including PubMed and Scopus, from inception to January 2023 was conducted. The search terms used included "Alzheimer's disease", "Parkinson's disease", "Parkinsonism", "Dementia", "Neurodegenerative disease", "Gene therapy", "Gene editing", and "CRISPR-Cas9". The Boolean operators "OR" and "AND" were placed between the search terms to create a search strategy and identify the relevant articles in each database. We also searched the reference lists of relevant articles and reviews for additional studies.

The selection process involved an assessment of the abstracts and the major findings of the studies using the following inclusion criteria: any English-language article published in a peer-reviewed journal that reported on the utility of the CRISPR/Cas9 system in the management of either AD or PD. We excluded studies that used gene-editing techniques other than the CRISPR/Cas9 system, other neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), Huntington's disease, etc., and articles with inaccessible full-texts. Duplicate articles were removed using Mendeley Reference Manager. This narrative review study did not require either the ethical approval of the institutional review committee or the informed consent of the participants.

Discussion

Pathophysiology of Alzheimer's disease and Parkinson's disease

The discussion of the utility of gene-editing systems such as CRISPR/Cas9 becomes comprehendible when the underlying pathogenesis of neurodegenerative conditions is elucidated. One of the most commonly recognized hypothetical mechanisms of AD is amyloid beta (Aβ) plaque deposition in the brain. These plaques are recognized as foreign antigens by the host immune system, initiating a cascade of inflammatory responses via the activation of microglia and the release of cytokines. Continuous inflammation in different regions of the brain over a period of time leads to gradual and progressive cellular death and neurodegeneration. The Aβ plaque consists of Aβ peptides produced from amyloid precursor protein (APP) by the enzymatic breakdown of α, β, and γ secretase enzymes[18]. The production of plaque begins with the breakdown of APP by β-secretase enzyme, which releases C-terminal membrane amino-acid fragments. The C-terminal membrane-bound fragment gets further broken down by γ-secretase to produce Aβ1-40 and Aβ1-42 isoforms. Aβ1-42 isoform contributes to the formation of the amyloid plaque because of its propensity to easy deposition. The production of Aβ1-42 is the result of change in the splitting pattern, which is believed to be due to mutations in the APP gene, presenilin-1 (PSEN1), presenilin-2 (PSEN2), and apolipoprotein E (APoE4) gene[7,19,20]. The APP gene mutation results in an upregulation of β-secretase-mediated enzymatic breakdown, consequently resulting in elevated levels of Aβ protein[21]. Likewise, Shea *et al.*[22] reported that PSEN1 mutations are the reason behind increased production of more Aβ42 compared to Aβ40.

Another hypothesis for the development of AD is the hyperphosphorylation of tau proteins present in neurons. Tau proteins are responsible for stabilizing the microtubule assembly that works to maintain the integrity of the cytoskeleton in neurons. Their activity is regulated by phosphorylating enzymes such as cyclin-dependent kinase-5 (CDK5). Overactivity of the CDK5 enzyme leads to hyperphosphorylation of tau proteins, which results in decreased affinity of the tau proteins for microtubules. The hyperphosphorylated tau gets deposited in the cytosol as neurofibrillary tangles (NFTs) negatively affecting synaptic transmission, axonal transport, and signal transduction[7,23]. Ultimately, this results in progressive neuronal degeneration of the affected cells.

Regarding PD, the underlying ultimate pathology is the degeneration of dopaminergic neurons in the substantia nigra of the basal ganglia. A number of genetic factors that influence the risk of PD have been identified by numerous studies. Mutations in the alpha-synuclein gene (SNCA), UCH-L1, MAPT/STH, PARKIN, and PINK1 genes are widely studied in the risk of developing PD[24–27]. A missense mutation in SNCA gene known as Ala53Thr (A53T) was reported to have a strong association

with PD, according to Spira *et al*.[28]. Both PARKIN and PINK1 genes are involved in a process called 'mitophagy', by which lysosomes remove dysfunctional mitochondria from the cells. Loss-of-function mutation of these genes lead to compromised mitophagy, resulting in the accumulation of dysfunctional mitochondria in neuronal cells. A continuous state of accumulation of dysfunctional mitochondria is implicated in the preferential degeneration of dopaminergic neurons[17]. Dominations in the DJ-1 gene, which exerts antioxidant effects through the DJ-1 protein, are also associated with an increased risk of PD[29]. Likewise, mutations in the LRRK2 gene are associated with autosomal dominant PD[30]. Another common mutation leading to an increased risk of PD is a mutation in the GBA gene. The mutated GBA gene, which has been associated with Gaucher's disease, has an approximately four-fold increased risk of occurrence of PD[31,32].

Utility of CRISPR/Cas9 in neurodegenerative diseases

Neurodegenerative disorders such as AD, PD, ALS, and Huntington's disease are widely recognized as significant health concerns affecting a substantial number of individuals on a global scale. These diseases like AD and PD have garnered considerable attention due to their high prevalence in elderly and the considerable impact they have on affected individuals and their families[1].

Even though we have identified numerous genetic associations and underlying pathophysiology of AD and PD in familial cases, the molecular mechanisms underlying the pathophysiology of SPD and SAD remain elusive. The majority of cases involving AD and PD manifest in a sporadic manner, as indicated by previous research[6,14,22,33]. The confirmation of these neurodegenerative disorders can pose challenges due to the reliance on brain autopsy, which is widely regarded as the most established and definitive diagnostic approach. Therefore, it is imperative to acquire a comprehensive understanding of the unique characteristics and presentations of the disease in question to effectively differentiate authentic AD or PD from other associated disorders. This technology is applied to manipulate genes associated with the condition through knockouts, knock-ins, or alterations. Additionally, it allows for the selective activation or repression of crucial genes, as well as the introduction of epigenetic changes. Overall, the emergence of CRISPR/Cas9 technology has presented novel opportunities for understanding and treating neurodegenerative disorders[34,35]. The capacity to accurately manipulate genetic material holds promise for forthcoming therapeutic interventions that have the potential to greatly enhance patient outcomes (Fig. 1).

Application of CRISPR/Cas9 in Alzheimer's disease

The utilization of CRISPR/Cas9 technology has become increasingly prevalent within the realm of AD research. The efficacy of the CRISPR/Cas9 system in rectifying mutations associated with cancer has been well documented. With the same principle in mind, the scope of utility of the CRISPR/Cas9 system has been expanded from the diagnosis to the treatment of AD. Currently, there is widespread utilization of this technology in the creation of disease models, the identification of culprit genes through screening, and the implementation of targeted gene therapy[36].

The CRISPR/Cas9 system has been tested to rectify the mutations in the PSEN1 gene, APP gene, and the APoE4 gene. Autosomal dominant mutations in genes such as PSEN1 can be corrected with the use of this technology, according to recent studies. In 2016, the mutations in the PSEN1 gene were rectified by using induced pluripotent stem cells derived from AD patients by two studies[37,38]. Furthermore, another study provided evidence that the application of the CRISPR/Cas9 system for the purpose of disrupting PSEN1 genes in N2a cells resulted in the elimination of the inherent γ-secretase background[39]. A recent study utilized the CRISPR/Cas9 technique to specifically interfere with the PSEN1M146L allele. This intervention may also have the ability to partially restore the imbalanced Aβ42/40 ratio[40]. A similar restoration in the ratio of Aβ42/40 and normal electrophysiology of affected neurons has been described when the PSEN2N141I mutation was corrected with CRISPR/Cas9 by Ortiz-Virumbrales *et al*.[41].

APP mutations have also been selected as targets for elimination using the CRISPR/Cas9 system in a handful of studies. A study demonstrated a decrease in the expression of Aβ protein in fibroblasts derived from AD patients after eliminating Swedish APP (APPswe) mutations[21]. Furthermore, another study introduced a novel mutation (E674K) via a CRISPR/Cas9-mediated system to alter the APP gene. The alanine codon was modified to threonine in HEK293T cells and SH-SY5Y cells, which harbor the APP gene with deaminated cytosine 1 and cytosine 2 positions. They also demonstrated a decrease in the accumulation of Aβ peptide as a result of the successful introduction of the A673T mutation in 53% of HEK293T cells[42].

One of the strongest predictors of sporadic AD development, APoE4 gene, was investigated at first using CRISPR/Cas9 technology by Lin *et al*. in 2018[43]. The study revealed that the impact of APoE4 on Aβ metabolism varied across different cell types. Moreover, another study explored potential therapeutic targets associated with APoE4. The researchers employed the CRISPR/Cas9 technique to rectify the E4 allele to the E3/E3 genotype in induced pluripotent stem cells of AD patients. Following the research, it was observed that E3 neurons exhibited greater resistance to ionomycin-induced cytotoxicity and displayed a decrease in tau phosphorylation in comparison to E4 neurons[44]. The application of CRISPR/Cas9 in rectifying potential targets of genetic mutations of AD is illustrated in Figure 2. The details of the interventional studies that employed targeted gene therapy to achieve favorable outcomes are provided in the Table 1.

Application of CRISPR/Cas9 in Parkinson's disease

The evidence of use of CRISPR/Cas9 gene-editing technology in the potential management of PD is limited in the literature. The details of the interventional studies that employed targeted gene therapy to achieve favorable outcomes are provided in Table 2. The studies published so far in this area have focused more on uncovering the different associated mutations and their hypothesized pathways. For instance, the CRISPR/Cas system was used to eliminate PARKIN (PRKN), DJ-1, and ATP13A2 (PARK9) genes from dopaminergic neurons in a study[49]. Likewise, recent studies have identified loss-of-function mutations in the DNAJC6 gene, which encodes the HSP40 auxilin protein. A study uncovered that disruptions in DNAJC6-mediated endocytosis can impede the WNT-LMX1A signal pathway in the process of midbrain dopamine

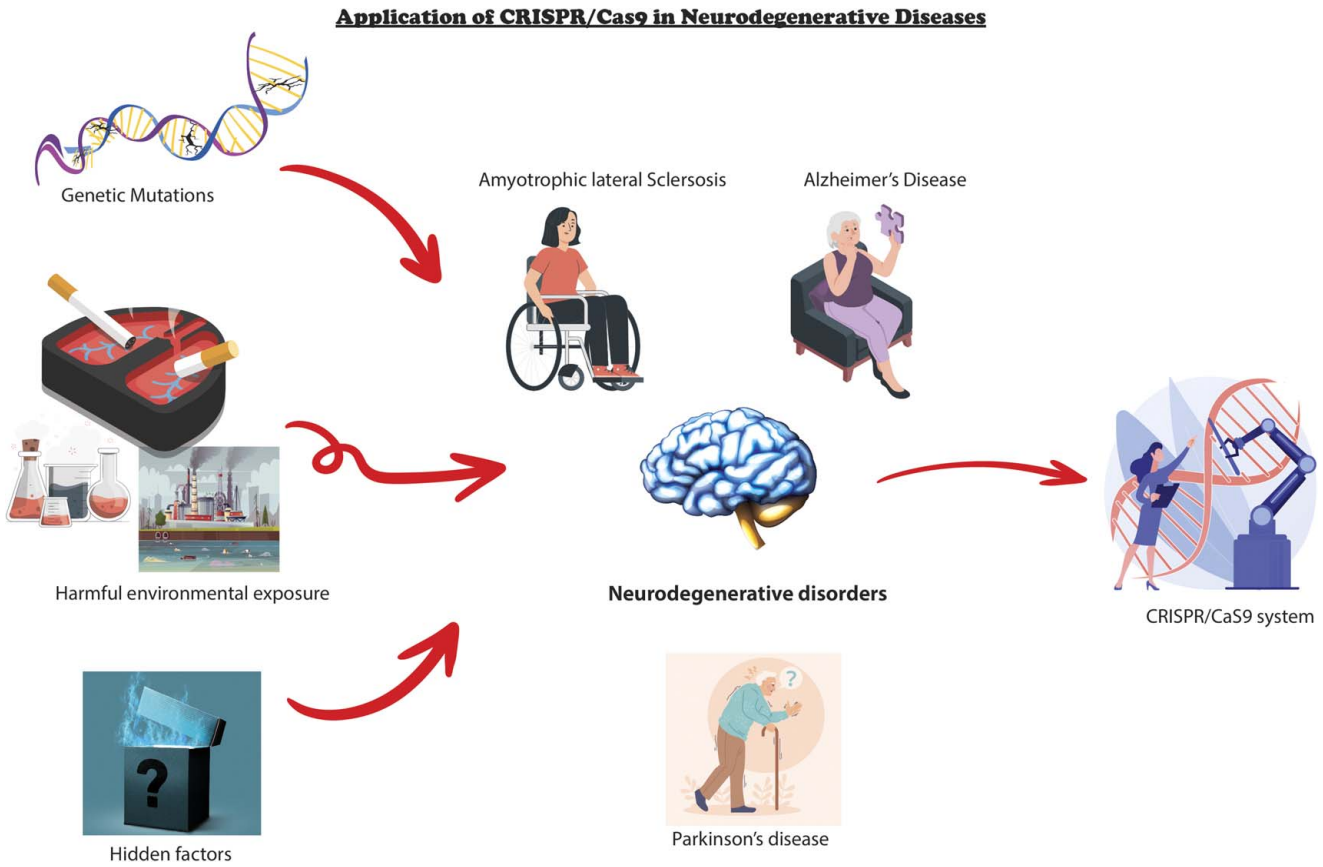

Figure 1. Application of CRISPR/Cas9 in neurodegenerative diseases.

(mDA) neuron development. Consequently, production of mDA neurons that are susceptible to vulnerability is created due to diminished expression of LMX1A during the developmental process[50].

As early as 2015, a study conducted an examination of the PARK2 and PINK1 genes using CRISPR/Cas9 and somatic cell nuclear transfer methodologies within a model of the domestic pig. The same study reported a success rate of ~38% in obtaining

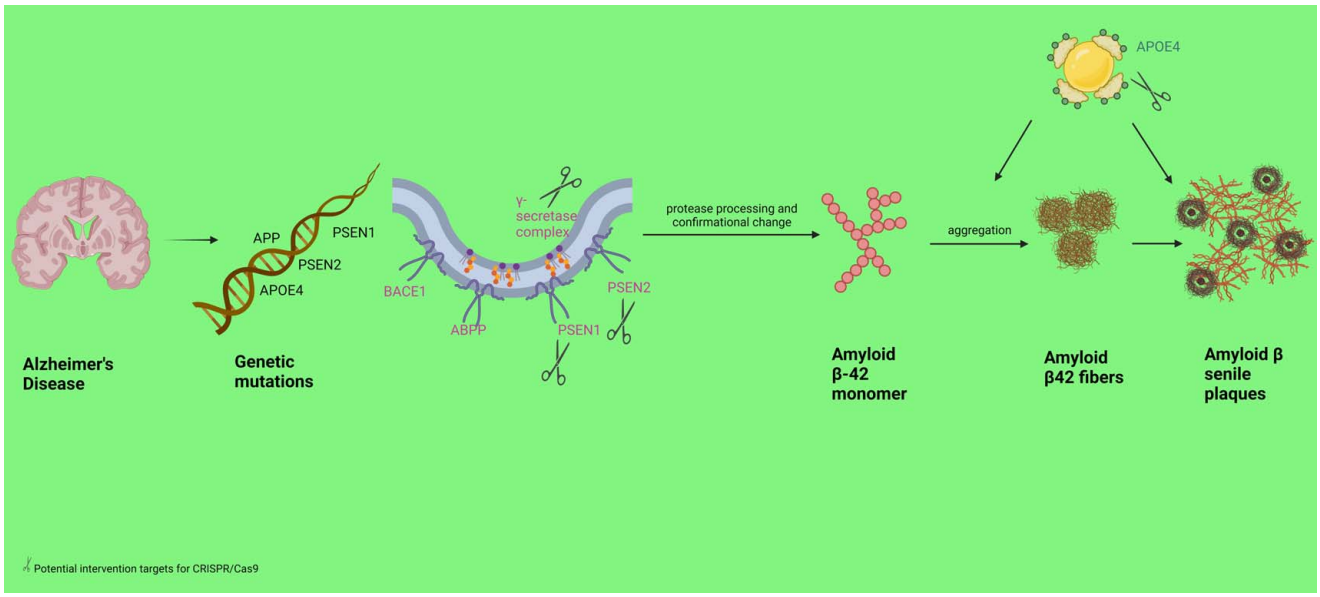

Figure 2. Application of CRISPR/Cas9 in Alzheimer's disease.

Table 1

CRISPR/Cas9 interventional studies on Alzheimer's disease

Study	Targeted gene	Outcomes
Pires *et al.*[37]	PSEN1	Production of a gene-corrected induced pluripotent stem cell line by substituting the point mutation with the wild-type sequence
Poon *et al.*[38]	PSEN1	Production of a gene-corrected induced pluripotent stem cell line by correcting for the single base pair mutation
Sun *et al.*[39]	PSEN1	Elimination of inherent γ-secretase background by disrupting PSEN1 genes in N2a cells
Konstantinidis *et al.*[40]	PSEN1	Reduction of extracellular Aβ42/40 ratios by disrupting the *PSEN1* allele in human fibroblasts
Ortiz-Virumbrales *et al.*[41]	PSEN1	Correction of the electrophysiological deficit by rectifying the PSEN2 point mutation in basal forebrain cholinergic neurons generated from induced pluripotent stem cells
György *et al.*[21]	APP	Reduction of Aβ levels in fibroblasts after eliminating Swedish APP mutations
Guyon *et al.*[42]	APP	Decrease in the accumulation of Aβ peptide by introduction of the A673T mutation in HEK293T cells
Wadhwani *et al.*[44]	APOE4	Production of stem-cell-derived E3 neurons that are less susceptible to ionomycin-induced cytotoxicity by correcting E4 allele to the E3/E3 genotype

Table 2

CRISPR/Cas9 interventional studies on Parkinson's disease

Study	Targeted gene or protein	Outcome
Zhou *et al.*[45]	PINK1, PARK2	Generation of single or double gene-targeted porcine fetal fibroblasts
Kantor *et al.*[46]	SNCA	Reduction in *SNCA* levels of human-induced pluripotent stem cell (hiPSC)-derived dopaminergic neurons by targeted DNA methylation editing
Yoon *et al.*[47]	A53T-SNCA	Reduction of the overexpression of α-synuclein, reactive microgliosis, dopaminergic neurodegeneration, and parkinsonian motor symptoms by gene deletion of A53T-SNCA significantly
Inoue *et al.*[48]	p13 protein	Prevention of toxin-induced motor deficits and the loss of dopaminergic neurons in the substantia nigra of heterozygous p13 knockout mices

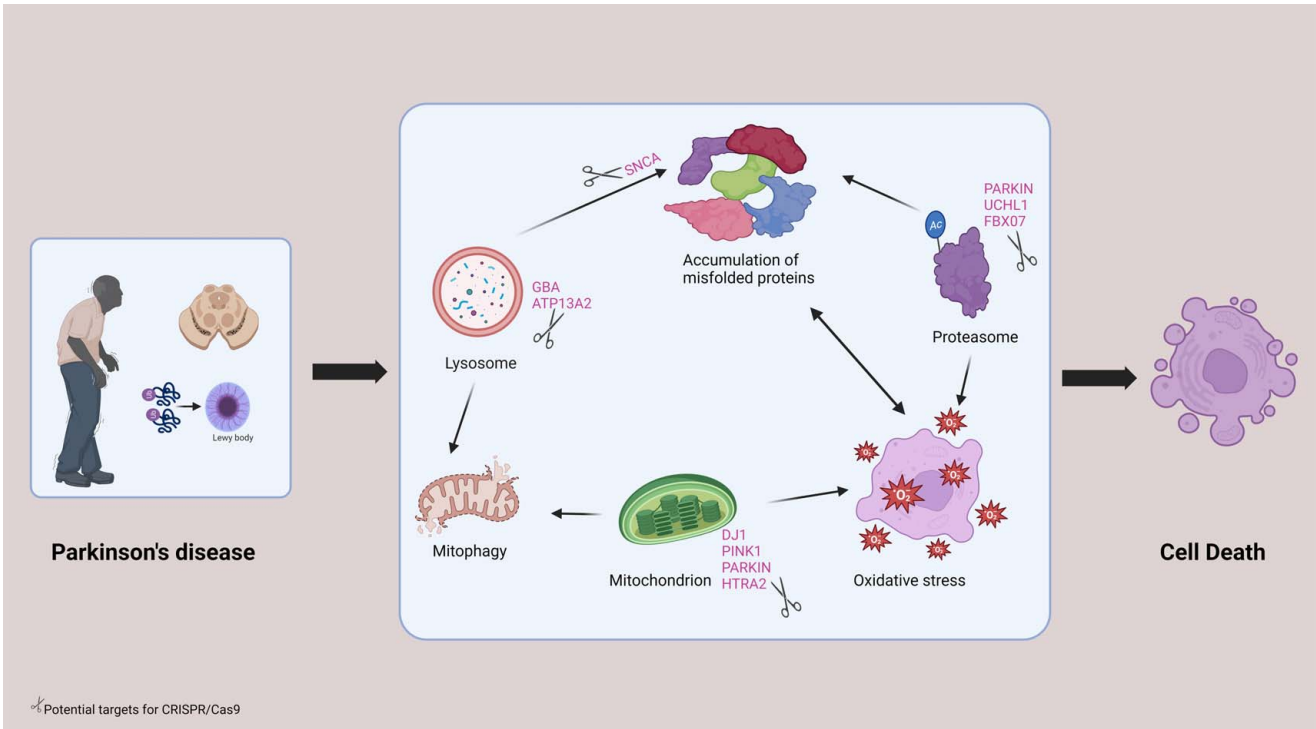

Figure 3. Application of CRISPR/Cas9 in Parkinson's disease.

homozygous cell colonies with a double-knockout for the PARK2 and PINK1 genes[45]. An intriguing development of an innovative lentiviral vector that integrates CRISPR/Cas9 technology to selectively suppress the expression of SNCA mRNA and protein has been described in the literature. This targeted intervention resulted in the restoration of phenotypic abnormalities associated with PD[46]. A recent study demonstrated that the utilization of the CRISPR/Cas9 system for the purpose of eliminating the A53T-SNCA gene mutation resulted in notable improvements in various aspects associated with PD. These improvements included the reduction of α-synuclein overproduction, prevention of dopaminergic neurodegeneration, mitigation of reactive microgliosis, and alleviation of motor symptoms[47]. Furthermore, Chen *et al*. used human-induced pluripotent stem cells obtained from individuals with PD to study A53T and SNCA-triplication autosomal dominant mutations. The research findings presented in the study indicate that the lack of SNCA is associated with a reduced susceptibility to Lewy pathology[51, 52].

Inoue and colleagues provided promising evidence that the expression of a novel 13-kDa protein (p13), which is involved in inducing mitochondrial dysfunction and apoptosis in dopaminergic neurons, could be a potential target gene-editing system. Employing the CRISPR/Cas9 system, they also produced p13-deficient mice with no motor dysfunction or dopaminergic neuron destruction following treatment with neurotoxin that can lead to mitochondrial dysfunction[48]. This suggests that the CRISPR/Cas9 system could be explored as a potential therapeutic approach for PD. The application of CRISPR/Cas9 in rectifying potential targets of genetic mutations of PD is illustrated in Figure 3.

Conclusion

The utilization of CRISPR/Cas9 gene-editing technology in the diagnosis and treatment of neurodegenerative disorders with a genetic pathophysiological basis is a relatively new concept. Recent studies have shown promising results in effectively targeting the known mutations of certain genes associated with AD and PD. The ability to precisely edit genes associated with these diseases offers a promising approach to understanding disease mechanisms and developing potential therapies. Future research should focus on optimizing the delivery and specificity of CRISPR/Cas9, minimizing off-target effects, and conducting rigorous preclinical and clinical trials. With these advancements, CRISPR/Cas9 gene editing could potentially revolutionize the therapeutic landscape of neurodegenerative diseases.

Ethical approval

No ethical approval was obtained for this review.

Consent

Informed consent was not obtained for this review.

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Author contribution

Conceptualization: N.T.; methodology: N.T., M.A.F.E., N.R., and T.K.; validation: N.T. and H.S.B.; formal analysis and investigation: N.T., M.A.F.E., N.R., and T.K.; resources: N.T. and D.S.; data curation: N.T., M.A.F.E., N.R., and T.K.; writing – original draft preparation: N.T., M.A.F.E., N.R., T.K., and D.S.; writing – review and editing: N.T. and D.S.; visualization: N.T. and T.K.; supervision: T.K.; project administration: N.T. and D.S. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest disclosure

The authors have no conflicts of interest to declare.

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