Review Article

# CRISPR-Cas9 and Parkinson's Disease: A Review of Gene Editing Strategies and Therapeutic Potential

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## ABSTRACT

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons, leading to motor impairments such as tremors, rigidity, and bradykinesia, as well as cognitive decline. Current treatments, including dopaminergic medications and deep brain stimulation, manage symptoms but do not stop disease progression. Genetic mutations in *SNCA*, *LRRK2*, and *PARKIN*, along with environmental factors, contribute to PD's development. CRISPR-Cas9 presents a potential therapeutic avenue by enabling precise gene modifications to correct PD-related mutations, reduce toxic protein accumulation, and improve mitochondrial function. It also enhances stem cell therapies, offering new possibilities for disease modification. However, challenges such as targeted gene delivery, off-target effects, and ethical concerns must be addressed before clinical implementation. This paper explores PD's underlying mechanisms, current treatment limitations, and CRISPR's role in developing novel therapies. It also examines the scientific and ethical challenges that must be navigated to bring CRISPR-based interventions into practical use for PD patients.

**Keywords:** Parkinson's disease; gene therapy; CRISPR-Cas9; SNCA; LRRK2; neurodegeneration

## INTRODUCTION

Parkinson's Disease (PD) is an advanced neurological disorder that refers primarily to movement and cognition (1, 2). Its hallmark features are the death of striatal dopamine neurons which results in motor dysfunction resulting in immediate tremors, rigidity, and bradykinesia, alongside non-motor indicators, which include the deterioration of cognitive ability and sleep disorders (3). While the precise reason is still unknown, a person's genetic make-up as well as environmental factors play a part in it (4). Although there are treatment options that lessen the most serious symptoms of the affliction, there is currently no cure for PD (2, 4).

Recent advancements in genetic engineering, particularly CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, offer promising new possibilities for treating PD at a genetic level (5-8). CRISPR, a revolutionary genome-editing tool, enables precise modification of genetic material, allowing for the correction of mutations linked to PD. This marks a shift from conventional symptom management toward potential disease cure (9). However, challenges such as off-target effects, ethical considerations, and the complexities of gene delivery must be addressed before CRISPR can be widely implemented in PD therapy.

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This paper explores Parkinson's disease, its underlying causes and symptoms, current treatment limitations, and the emerging role of CRISPR in developing new therapeutic strategies. It also examines the scientific, ethical, and technical challenges associated with CRISPR-based interventions in neuro-degenerative diseases.

## Parkinson's Disease Risk Factors and Treatment Options

PD occurs as one of the most common neurodegenerative diseases affecting the population, with millions of cases across the globe. Progressive loss of dopaminergic neurons in the substantia nigra, accumulation of Lewy bodies, and mitochondrial dysfunction are common in people with the disorder (10, 11). This brings forth a multitude of motor and some non-motor symptoms, among them being tremors, bradykinesia, muscle stiffness, cognitive impairment, and autonomic failure (1-3). Although PD is known to have a wide variety of symptoms, some of the most common include issues such as tremors, gait disorders, muscle stiffness, and cognitive issues (1).

There are no treatment options for all aspects of PD symptomology, but genetic editing approaches are surfacing (12-15). Both hereditary and external elements are known to be attributed to the onset of PD. A number of mutations have linked particular genes to PD vulnerability. These include mutations in the *SNCA*, *LRRK2*, and *PARKIN* genes (15, 16). Also, certain factors like pesticide exposure and mitochondrial failure contribute to further worsening of the neurodegenerative disorder (15). Understanding these factors is crucial for designing effective therapeutic approaches and making breakthroughs in understanding the etiology of PD.

PD is a multifactorial condition associated with the gradual degeneration of the dopaminergic neurons within the substantia nigra, which is the part of the midbrain involved in movement control (1). As noted previously, the accumulation and formation of protein aggregates called Lewy bodies, which disrupts the function of cellular machinery, is the signature pathology of the disease (1). However, the disease is further complicated by other pathological processes such as inflammation of the nervous tissue and the failure of cellular organelles known as mitochondria. Risk factors for developing PD include mutations in *LRRK2*, *SNCA*, and *PARKIN* genes. For instance, *LRRK2* mutations account for approximately 10% of familial cases and 1-2% of non-familial cases (1, 11). All three genes are critical for maintaining proper cellular functions: *SNCA* encodes alpha-synuclein, the protein that forms the core of Lewy bodies; *LRRK2* encodes a kinase which has a role in neuronal communication; and *PARKIN* encodes the E3 ubiquitin ligase which is critical for the quality control of mitochondria (11). Treatments for PD today do not slow the progression of the illness and instead target the symptoms. The most popular treatment is called dopaminergic therapy which relies on a medication called levodopa. Once levodopa reaches the brain, it is converted and turned into dopamine, which temporarily alleviates motor symptoms. Over time, however, the use of levodopa can result in adverse side effects such as motor fluctuations and dyskinesia (2). Some examples of dopamine agonists are pramipexole and ropinirole, both for which MAO-B inhibitors such as selegiline slow the breakdown of dopamine and prolong its use (10).

Deep brain stimulation (DBS) is an alternative treatment, particularly for advanced cases. This method allows for impairment of electrodes in the subthalamic nucleus or globus pallidus, which are regions of the brain that help alleviate abnormal muscle movement and help in alleviating motor symptoms (10). On the contrary, DBS does not slow the progression of the disease and is typically used for patients that do not respond well to other treatment methods.

Rather than aim to control symptoms, new treatment strategies look to alter the progression of the disease. Approaches to gene therapy try to control the pathways of dopamine synthesis or seek to mitigate the buildup of alpha-synuclein which is critically associated with neuronal degeneration (16). More recently, therapies aimed at the known risk factor gene, *LRRK2*, try to inhibit its kinase activity. These developments have the potential to mitigate PD beyond symptom management to treating its root cause.

## Parkinson's Disease Stages and Diagnostic tools

PD progresses through five stages, as defined by the Hoehn and Yahr scale (17). In Stage 1, mild symptoms such as tremors and stiffness appear on one side of the body. Stage 2 affects both sides but does not impair balance. By Stage 3, movement slows, and balance worsens, increasing the risk of falls. Stage 4 brings severe disability, requiring assistance with daily tasks. In Stage 5, patients often become wheelchair-dependent or bedridden, with potential cognitive decline. This structured progression helps clinicians assess severity and plan treatment.

Non-motor symptoms, including depression, cog-

nitive impairment, and autonomic dysfunction, further reduce quality of life (1, 3). Diagnosis remains challenging, as there is no definitive test for PD; instead, clinicians rely on symptom evaluation and imaging techniques such as the DaTscan (18). This imaging technique uses single-photon emission computed tomography (SPECT) to measure dopamine levels in the brain. It involves injecting Ioflupane I-123, a tracer that binds to dopamine transporters in the striatum, allowing visualization of dopamine-producing neurons. In PD, DaTscan shows reduced dopamine activity, helping distinguish it from other movement disorders like essential tremor (19, 20). It supports early diagnosis and disease monitoring but cannot differentiate Parkinson's from atypical parkinsonian syndromes.

Other diagnostic tools for PD include magnetic resonance imaging (MRI), which helps rule out structural brain disorders, and positron emission tomography (PET) scans, such as fluorodopa (F-DOPA) PET, which assesses dopamine synthesis. Transcranial ultrasound (US) can detect echogenicity changes in the substantia nigra, while clinical assessments like the Unified Parkinson's Disease Rating Scale (UPDRS) remain essential for evaluating symptoms (21). Combining these methods enhances diagnostic accuracy.

### Environmental Factors and Parkinson's Disease

Beyond genetic factors, environmental triggers also play a role. Exposure to pesticides such as rotenone and paraquat has been linked to increased PD risk, as these chemicals disrupt mitochondrial function and cause oxidative stress, leading to the degeneration of dopaminergic neurons (22, 23). The connection between pesticide exposure and Parkinson's was initially observed in agricultural workers, with later laboratory studies confirming that these substances induce neurotoxic effects similar to those seen in the disease (24, 25).

Mitochondrial dysfunction plays a crucial role in PD pathogenesis. While mitochondria are present in all cells, neurons are particularly dependent on their function due to their high energy demands. Mutations in genes such as *PARKIN* and *PINK1* disrupt mitochondrial quality control, leading to an accumulation of damaged mitochondria that further drives neurodegeneration (26). Oxidative stress caused by mitochondrial dysfunction accelerates cell damage, further exacerbating the loss of dopaminergic neurons. Understanding mitochondrial involvement in PD highlights potential therapeutic targets for restoring neuronal energy balance and preventing cell death.

## PARKINSON'S-RELATED GENES

### *SNCA*

Understanding the intricate role of the rare inherited Parkinson gene, a-synuclein (*SNCA*), is essential in the context of therapeutic strategies for PD. Research by Bendor *et al.* (27) provides valuable insights into the functions of the *SNCA* gene product, a protein that is primarily located at presynaptic terminals in neurons. The a-synuclein protein essentially aids in the efficiency of synaptic transmission, however, does not play a significant role in the process entirely. Bendor *et al.* suggest that a-synuclein plays a crucial role in regulating/controlling neurotransmitter release in the postsynaptic neuron. Complications and the introduction of PD in humans begins when the gene is present in excessive amounts, the accumulation of the gene causes the production or presence of Lewy bodies. When in excessive amounts a-synuclein appears to inhibit synaptic transmission. However, investigations involving a-synuclein knockout models that are essentially designed to reduce levels of a-synuclein, reveal that its absence does not lead to significant synaptic dysfunction, indicating that a-synuclein may have a supportive role rather than being essential for normal synaptic function. More importantly, the dire tendency of the α-synuclein to misfold and aggregate into fibrils is a critical factor in neurodegenerative diseases, particularly in PD, where such aggregates form the previously mentioned Lewy bodies observed in affected individuals. It is this very folding that creates ground to question whether therapeutic devices such as CRISPR may be used in order to treat such a condition by either preventing the misfolding process, reducing a-synuclein accumulation, or correcting genetic mutations that allow for aggregation and folding of the gene to take place in the first place.

Building on this, Campêlo and Silva summarize (16) how genetic variations in the *SNCA* gene, which encodes a-synuclein, impact the risk of developing sporadic PD. Sporadic PD is linked to more subtle genetic variations that may affect the expression and aggregation of a-synuclein, in contrast to familial PD, which is frequently linked to clear mutations in the *SNCA* gene. The authors draw attention to results from genome-wide association studies (GWAS) that show *SNCA* gene mutations are linked to a higher risk of PD. The significance of *SNCA* in both inherited and sporadic diseases is further supported by the fact that these genetic factors not only influence disease susceptibility but also seem to affect the clinical manifestations and

progression of the disease.

Stefanis (28) delves deeper into the pathogenic implications of a-synuclein, highlighting its critical role in neurodegeneration associated with PD. The accumulation of misfolded a-synuclein in the form of Lewy bodies is one of the characteristics of PD. The study looks into how these aggregates disrupt cell homeostasis, obstruct protein degradation pathways, and result in mitochondrial dysfunction, all of which can lead to neuronal toxicity. Moreover, recent research has shown that, like prion diseases, misfolded a-synuclein can move between brain cells (29). This suggests that the protein can exacerbate PD by causing additional clumping in nearby cells as soon as it starts to clump. Therefore, new treatments must prevent both the aggregation and the spread of a-synuclein in order to slow down brain damage. Furthermore, Pihlstrøm *et al*. (30) investigated the genetic variability in the *SNCA* gene and its impact on the risk of PD. Their research illustrates how various genetic variants, both common and rare, may affect α-synuclein expression levels and aggregation properties. The authors also look into the possible role of epigenetic factors, changes in gene activity that do not alter the DNA sequence in PD.

Individuals with the same genetic risk may experience the disease differently depending on their experiences and surroundings because lifestyle, environmental exposures, and age can all affect PD manifestation. Epigenetic changes can activate or deactivate specific genes, potentially increasing or decreasing an individual's vulnerability to PD. For example, PD-related genes may change over time as a result of dietary modifications, stress, or exposure to toxins. This adds to the illness's complexity because it involves more than just inheriting a faulty gene; external factors may also affect how the illness develops and presents itself. Due to this complexity, it's becoming increasingly evident that a one-size-fits-all treatment approach is not effective for PD.

***LRRK2***

In essence, LRRK2 protein, sometimes referred to as dardarin, is encoded by the *LRRK2* gene. Cell signaling, inflammation, and autophagy, the removal of damaged cell components are all aided by this protein. Because of this, a mutation in this gene causes hyperactive kinase activity, which in turn causes inflammation, mitochondrial dysfunction, and impaired protein clearance, all of which contribute to the degeneration of neurons in PD. Since mutations in the *LRRK2* gene are a common genetic cause of both familial (inherited) and sporadic (random) forms of PD, it is one of the most researched genes associated with PD.

Given its implications for comprehending the disease and creating potential treatments, the association between *LRRK2* mutations and different types of Parkinsonism is an important field of research that has attracted interest. Li *et al*. (31) offer a thorough analysis of the ways in which mutations in the *LRRK2* gene are linked to both sporadic and familial types of PD. This implies that *LRRK2* mutations affect disease susceptibility broadly, meaning that people who carry these mutations may be more likely to develop PD independent of their family history. The work emphasizes the complex role of *LRRK2* in vital cellular functions that are necessary for brain health, such as synapse function, inflammatory responses, and neuronal survival. The study highlights the intricate role of *LRRK2* in critical cellular processes like synapse function, inflammatory responses, and neuronal survival that are essential for brain health.

To support this viewpoint, Sosero and Gan-Or (32) go further into detail on the genetic makeup of *LRRK2* and how it relates to PD. They point out that one of the most common genetic factors causing both familial and sporadic versions of the illness is *LRRK2*. Certain *LRRK2* mutations can cause a range of clinical symptoms and unique patterns of illness progression in different patients. This variation calls for a more thorough investigation of the molecular pathways by which *LRRK2* mutations cause their effects, especially regarding their role in neuroinflammation and mitochondrial dysfunction, two crucial processes linked to the etiology of PD. Additionally, the authors discuss current initiatives to create targeted treatments that modify *LRRK2* activity, which may open the door to fresh approaches to treating *LRRK2*-related Parkinson's. The present state of *LRRK2* research and the difficulties encountered in clinical trials for LRRK2-targeted therapeutics. Although creating therapies that target LRRK2 malfunction has a lot of promise, there are many challenges in putting these discoveries into practical practice. The variation in patients with *LRRK2* mutations is a significant obstacle that makes developing universal treatment plans more difficult. Because medicines that work for one patient might not work for another, this variability emphasizes the need for individualized medicine approaches. The authors support the use of strong clinical trial designs to assess the effectiveness and safety of LRRK2 inhibitors,

emphasizing the importance of collaboration among researchers, clinicians, and pharmaceutical companies to navigate these challenges and ensure successful outcomes in clinical settings.

Lastly, Tolosa *et al*. (33) critically assess the current status of LRRK2 research, focusing on the difficulties associated with clinical trials for LRRK2-targeting therapies. Despite recent advances in our understanding of the pathogenic pathways linked to *LRRK2*, the optimal treatment regimens and patient selection criteria for clinical trials remain unclear. To solve these problems, researchers, doctors, and industry stakeholders must collaborate across disciplines and support a unified strategy. Promoting cooperation can significantly speed up the development of effective therapies that target LRRK2 dysfunction in PD, ultimately improving patient outcomes and advancing research on the condition.

Having emerged recently, CRISPR-Cas9 has the potential to alter gene editing forever and could stand as the single most effective genetic research tool in the PD field, offering the possibility to delete or correct genes that are damaged. Unlike conventional treatments that manage symptoms, CRISPR directly tackles the issue at the core, giving one the opportunity to alter the course of the disease, which includes halting or prolonging the progression of the symptoms (5-6, 8-9, 34).

## BASICS OF CRISPR TECHNOLOGY

CRISPR, short for Clustered Regularly Interspaced Short Palindromic Repeats, has emerged as a cutting-edge gene-editing technology derived from the bacterial immune system (6, 12, 35). Compared with the previous genetic modification techniques CRISPR is faster, has better efficiency, and is cost-effective (5, 9).

The technology is based on the Cas9 enzyme, the action of molecular scissors directed by RNA sequences matching the target DNA. This system thereby enables scientists to precisely modify DNA by targeting and cutting specific genetic sequences, allowing removal, correction, or insertion of genetic material. Once the DNA is altered, natural repair mechanisms of the cells come into play enabling scientists to introduce the desired genetic modifications (26, 29). This has generated great interest in the application of CRISPRs to correct mutations leading to genetic disorders, such as those associated with PD (5, 34).

Apart from gene modification, CRISPR finds application in epigenetics, with capacity to control gene expression without changing the DNA sequence (27, 29). A promising area of exploration is disease detection using CRISPR-based diagnostics that efficiently and accurately identify genetic mutations and pathogens (12).

The obstacles posed by CRISPR include unintended modes of genome modification (off-target effects), ethical dilemmas of gene-editing in a human subject, and the challenges of delivering CRISPR components to predetermined cells (5, 12). Scientists are working toward developing a safer and efficacious technology for direct medical invocation as research continues (8, 29).

## USING CRISPR-CAS9 TO TARGET PARKINSON'S-RELATED MUTATIONS

The advent of CRISPR-Cas9 as a major weapon of genetic engineering has changed our perception of a number of diseases, with neurodegenerative diseases such as PD being of particular interest. There are great possibilities for this technology to be used to edit the genome with very high precision, allowing scholars to investigate the molecular mechanism of the disease target. In attempts to explore further some of the neuroinflammatory and neuropharmacological mechanisms in PD, CRISPR-Cas9 is thus being applied-giving key insights toward novel therapeutic strategies.

Luo *et al*. (13) discuss the applications of CRISPR-Cas9 towards studying neuroinflammatory mechanisms that underlie PD. Neuroinflammation is gaining ground as an important factor affecting disease initiation and progression. The authors used CRISPR-Cas9 to knock out genes involved in inflammatory pathways and to assess their roles in the survival of dopaminergic neurons. Importantly, this approach identifies the major mediators of neuroinflammation, including cytokines and microglial activation, that play a central role in the establishment of the disease. Further clarity on these inflammatory pathways and their interplay with neuronal health will prompt the development of targeted interventions for neuroinflammatory modulation.

The Luo paper highlights how CRISPR has been put to use in linking basic science with clinical applicability. By elucidating specific genetic factors capable of exacerbating neuroinflammation, the work paves the way toward therapeutic modalities designed to alleviate inflammatory insults in PD. With this high-throughput screening platform using CRISPR, researchers are able to rapidly identify many genes contributing to neuroinflammatory processes. Such systematic studies are crucial considering the intricacy

of the disease mechanisms due to interplay of myriad genetic and environmental factors.

In a complementary investigation, Pinjala *et al*. (14) examined the application of CRISPR-Cas9-assisted stem cell therapy as a novel approach to treating PD. Stem cell therapy has emerged as a promising strategy for regenerative medicine, offering the potential to replace damaged or lost neurons. However, the efficacy of these therapies is often limited by the genetic and epigenetic factors present in the cells used for transplantation. The authors argue that incorporating CRISPR technology into stem cell research can enhance the therapeutic potential of stem cells by correcting genetic defects associated with PD or by modulating the expression of neuroprotective factors. This research has significant ramifications; it implies that customized stem cell treatments based on each patient's unique genetic profile may transform the available treatments for PD patients. Additionally, by emphasizing the value of incorporating gene editing technologies into regenerative medicine, this approach promotes a more thorough approach to treating neurodegenerative diseases.

Furthermore, Rahman *et al*. (36) offers a more comprehensive viewpoint on the applicability of CRISPR-Cas9 technology in the study of PD. Their review highlights how versatile CRISPR is as a gene editing tool, allowing researchers to thoroughly examine the genetic landscape of PD. Researchers can gain important insights into the pathophysiology of PD through identifying related genetic variants and evaluating their functional implications through gene editing. Our knowledge of how genetic factors and environmental triggers interact to cause neurodegeneration is advanced by the ability to manipulate genes of interest.

Additionally, the authors contend that the potential for personalized medicine in PD is increased by CRISPR-Cas9 technology. It is becoming more and more possible to customize treatments according to a patient's genetic composition as scientists pinpoint particular genetic mutations that contribute to the variation in disease presentation and progression. This strategy is in line with the expanding movement in precision medicine, which aims to offer individualized treatment plans that optimize effectiveness while reducing side effects. In this regard, CRISPR-Cas9 is a potent ally in the effort to create PD treatments that work better. Even though CRISPR-Cas9 technology shows great promise, its use in clinical settings must be approached cautiously. Gene editing's ethical ramifications need to be carefully examined, especially in light of human health. Before being implemented in clinical settings, CRISPR-based "treatments" must be validated due to concerns about off-target effects, such as unexpected changes to the genome. The ethical implications of genetic modifications must be openly discussed as researchers work to increase the specificity and safety of CRISPR technologies. This will help to ensure that developments in this area are informed by moral standards and societal values.

The PINK1 gene plays a crucial role in the onset of young-onset Parkinson's disease (YOPD), often referred to as PARK-PINK1. In their thorough review (26), delve into the genetic foundations of PINK1 mutations and their link to early-onset PD. This gene is vital for maintaining mitochondrial quality, acting as a protector for neurons against stress-induced damage. When mutations occur in PINK1, mitochondrial function becomes compromised, which significantly contributes to neurodegeneration seen in individuals diagnosed with YOPD. This connection not only deepens our understanding of the genetic factors involved in early-onset PD but also highlights PINK1's potential as a target for future therapies. The implications of these mutations demand targeted strategies that could address the unique challenges posed by YOPD.

Yang *et al*. (37) expanded on our knowledge of PINK1 by examining the gene's distinct roles in primate brains using a CRISPR/Cas9 monkey model. Their groundbreaking research showed that PINK1 deletion causes significant neurodegenerative alterations that closely mimic features of PD in humans. These researchers were able to directly observe the effects of PINK1 loss in a living organism by utilizing CRISPR technology, demonstrating its critical function in preserving neuronal health. According to their research, PINK1 is essential for both mitochondrial function and primate neuron neuroprotection. This highlights the role of PINK1 in PD pathology and raises the possibility that it could serve as a focal point for upcoming treatment initiatives.

PINK1 serves as a bridge connecting mitochondrial health to neuronal survival (37). This relationship highlights the importance of mitochondrial function in the neurodegenerative processes associated with PD. A notable contribution to this dialogue comes from another study by Yang and colleague (38), which demonstrated that CRISPR/Cas9-mediated deletion of PINK1 can trigger neurodegeneration in animal models. By clarifying the relationship between PINK1, mitochondrial integrity, and neurodegeneration, these studies set the stage for potential therapeutic strategies focused on PINK1 pathways that may help slow the

progression of PD.

CRISPR technology enables precise manipulation of genetic factors linked to neurodegeneration, advancing our understanding of PINK1's role in neuronal health. This capability is crucial because it enables researchers to analyze the functional effects of PINK1 mutations and how they contribute to mitochondrial dysfunction and cellular stress responses. The development of precise models of different facets of PD is also made possible by CRISPR/Cas9 technology, which offers information that may help guide the creation of targeted treatments meant to lessen the effects of these genetic mutations.

Targeting the PINK1 pathway presents an intriguing opportunity for creating potent anti-PD strategies as research into the condition progresses. Learning more about how PINK1 affects neuronal resilience and mitochondrial dynamics will help researchers develop treatments that target the genetic causes of YOPD as well as the wider effects on neuronal health. Innovative therapies that greatly improve patients' quality of life may result from this all-encompassing approach to therapy.

The perspective of CRISPR studies, advances our understanding of the genetic foundations of this illness. The interaction among PINK1 mutations, mitochondrial health, and neurodegeneration emphasizes the necessity of tackling this escalating health issue as well as the possibility of creating targeted therapeutic approaches. As this field of study develops, the potential of fusing CRISPR technology with a better knowledge of PINK1 creates new opportunities for successful interventions that may eventually change the way PD patients receive treatment.

In conclusion, the application of the CRISPR-Cas9 gene editing system has created new opportunities for studying the neuropharmacological and neuroinflammatory processes that underlie PD. By fusing gene editing with stem cell therapies and personalized medicine techniques, CRISPR technology has the potential to improve our comprehension of the intricate interactions among genetic factors, neuroinflammation, and therapeutic treatments.

As the field progresses, the continued exploration of CRISPR-based strategies will be critical in translating these findings into clinical applications that improve the quality of life for individuals affected by this devastating disease. Ultimately, the successful application of CRISPR technology in PD research not only holds promise for advancing therapeutic options but also highlights the transformative potential of genetic engineering in addressing complex health challenges (Table 1).

**Table 1. Application of CRISPR-Cas9 in Parkinson's disease-related genes**

| Model Organism | Target gene(s) | CRISPR Approach | Main effect/Major results | Reference |
|---|---|---|---|---|
| Primate | *PINK1* | CRISPR-Cas9 PINK1 editing on monkey embryos | PINK1 loss in monkeys led to fewer neuronal cells, less gray matter and neurodegeneration. | Yang *et al*. (38) |
| Human | *SNCA* | Modified SadCas9 with repressor domains into human induced pluripotent stem cells (hiPSC). | Reduced α-synuclein expression, lowered oxidative stress and mtDNA damage. | Sastre *et al*. (39) |
| Human | *SNCA* | dCas9 fused to DNMT3A was transfected into human induced pluripotent stem cell-derived dopaminergic neurons from a PD patient. | Downregulation of *SNCA* rescue , mitochondrial ROS production and cellular viability. | Kantor *et al*. (40) |
| Mouse | Reprogramming factors: *Nr4a2*, *Ascl1*, *Lmx1a* | CRISPRa (activation)-mediated reprogramming of astrocytes *in vivo.* | CRISPRa reprogrammed astrocytes into GABAergic neurons improving motor behavior in mice. | Giehrl-Schwab *et al*. (41) |
| Mouse/Rat | *LRRK2 / SNCA / PINK1* (various) | CRISPR-Cas9 knockout and CRISPRa in rodent models. | Knockout of PD genes reproduced motor dysfunction and dopaminergic neuron loss; | Reviewed in Luo *et al*. (13) |

Summary of multiple studies in different animal models where they use CRISPR-Cas9 systems to manipulate PD-related gene expression or improve PD-related symptoms.

## USING CRISPR WITH DCAS9

The CRISPR-dCas9 system is a versatile and precise tool that has revolutionized gene regulation without introducing double-stranded breaks in DNA. Unlike the active Cas9, dCas9 (deactivated Cas9) is inactive but retains its DNA-binding ability when guided by single-guide RNAs (sgRNAs). This allows researchers to fuse dCas9 with various effector domains, such as transcriptional activators, repressors, or epigenetic modifiers, to upregulate or silence gene expression in a targeted and reversible manner (42).

This technology has significant implications for the treatment of PD, particularly because it enables modulation of gene activity without permanently altering the genome. For instance, the *SNCA* gene, which encodes alpha-synuclein, is known to be overexpressed in many forms of PD. Recent studies have demonstrated that dCas9 fused with repressor domains can successfully downregulate *SNCA* expression, offering a non-invasive way to mitigate one of the key pathological drivers of the disease (40). Additionally, the system can be adapted to target other genes implicated in PD, such as *LRRK2*, enabling fine-tuned control over their transcriptional activity.

Moreover, dCas9-based systems have been explored in broader neurodegenerative disease models, where they have shown the ability to improve disease-related outcomes (13-15). The flexibility of dCas9 makes it suitable for multiplexed interventions, where several genes can be simultaneously regulated, offering a more comprehensive approach to tackling complex, multifactorial diseases like PD.

Overall, the CRISPR-dCas9 system presents a promising therapeutic avenue that avoids the risks associated with DNA cleavage while providing high specificity and reversibility. Its adaptability and safety profile make it an especially attractive candidate for future gene therapy approaches in Parkinson's disease.

## ETHICAL CONCERNS IN CRISPR-CAS9 APPLICATIONS FOR PARKINSON'S DISEASE

The application of CRISPR-Cas9 technology in PD research raises several ethical concerns that must be addressed to ensure responsible scientific practice and public trust. As researchers explore the potential of gene editing to modify genetic factors associated with neurodegeneration, the implications of these advancements warrant careful consideration.

One of the primary ethical issues with CRISPR-Cas9 technology is the possibility of off-target effects, where unintended genetic modifications occur, potentially leading to adverse consequences (15). Even though CRISPR is widely recognized for its precision, there remains a risk of altering unintended genes, which could impact a patient's health or even contribute to new diseases. This issue is particularly critical in gene-editing treatments for PD, where accidental genetic changes could worsen neurological dysfunction instead of alleviating it. Extensive preclinical validation is necessary to ensure the safety and efficacy of CRISPR-based therapies before human applications. This requires a delicate balance between advancing medical research and prioritizing patient well-being.

Beyond immediate risks, the long-term effects of gene editing remain largely unknown. There is growing concern that genetic alterations made to an individual could be inherited by future generations, raising ethical and biological questions about genetic diversity and potential unintended consequences (44). While most PD-related CRISPR applications focus on somatic gene editing (which does not affect offspring), the long-term effects of these interventions on an individual's biology and disease progression remain uncertain. Without long-term clinical trials, it is difficult to predict how CRISPR-based treatments will interact with the biological and environmental factors that contribute to PD. Researchers must address these concerns through transparency and public engagement, ensuring that ethical considerations remain at the forefront of CRISPR development.

Additionally, access and equity concerns must be considered as CRISPR-based therapies advance. Treatment strategies can be expensive (5), potentially leading to a two-tiered healthcare system where only wealthier individuals can access cutting-edge care, while low-income patients are left with traditional, less effective treatment options. This is particularly concerning for PD patients in underserved regions with limited access to specialized medical facilities. To prevent CRISPR technology from becoming exclusive to the privileged, policymakers, researchers, and healthcare providers must collaborate on public funding strategies, subsidized treatment programs, and international research partnerships to make these therapies accessible to all PD patients, regardless of socioeconomic background.

## SCIENTIFIC AND TECHNICAL CHALLENGES OF CRISPR-CAS9

Different scientific and technical hurdles exist that must be addressed before CRISPR may be safely considered as a tool for PD treatment. One of the major barriers is to ensure that CRISPR components are delivered to the intended target cell in the brain. The CNS has unique barriers that make it difficult for one to deliver any genetic material efficiently and safely. The extant methods, namely, viral vectors and nanoparticles, suffer from limitations concerning precision and possible immune responses. These immune responses may obstruct treatment success (13). Therefore, research into additional methods of CRISPR delivery must have a strong emphasis on refinement and specificity, increasing the chances of success in the treatment of neurodegenerative diseases, including PD.

Another obstacle presented by PD is the genetic complexity. Parkinson's is caused intrinsically by a set of genes and environmental factors and pathways - thus, it is considered heterogeneous. Treating Parkinson's in a one-method-fits-all model may prove not to be feasible. Strategies targeting specific mutations, such as PINK1 or LRRK2, might work only in certain subgroups of patients, thus making personalized medicine indispensable for an effective application of CRISPR-based therapies.

## BROADER SOCIETAL AND ETHICAL IMPLICATIONS

Finally, gene-editing technologies exert influences on society that move far beyond the individual patient. With the rise of CRISPR-based therapies, public perceptions will likely begin to shift regarding genetics, diseases, and health. On the flip side lies a more philosophical concern regarding the moral limits of genetic manipulation for the potential alteration of the genetic foundation of diseases like Parkinson's. While CRISPR has the potential to revolutionize disease treatment, concerns remain about its potential misuse for other purposes unacceptable in such discussions of "designer" therapy. When does society wish to draw the line on genome editing for the prevention of inherited disease? Should editing be permitted for cosmetic improvement, to enhance athletic ability, or boost intellect? Such moral questions accentuate the need to engage the wider public, ethicists, and lawmakers in discussions regarding the prospective societal consequences of gene-editing technology. An honest and inclusive conversation will help society as a whole by placing the moral landscape about CRISPR in context while assuring that the technology is used for the right purposes.

## CONCLUSIONS

It will be necessary to examine the broader implications of CRISPR technology regarding its use in human subjects. Gene editing bears great potential to improve treatments for PD; ethical questions of informed consent, long-term effects, and germline changes need to be carefully addressed. Open discussions amongst scientists, ethicists, policymakers, and the public will be fundamental to building responsible research guidelines and ethical frameworks for the application of CRISPR in neurodegenerative diseases.

Overcoming these obstacles will require collaboration among researchers, clinicians, and regulatory bodies. This multidisciplinary approach can definitely foster innovative ideas as well as ensure ethical standards as CRISPR advances and begins to reshape how PD treatment is perceived. By maximizing CRISPR's potential through transparency, accessibility, and long-term safety, the scientific community can turn it from a potential revolution into reality for PD treatment.

Indeed, the problems posed by CRISPR in PD research are not insurmountable. The advent of very precise gene-editing tools, more targeted methods of delivery, and appropriate regulatory frameworks will transform how CRISPR can be applied to treat Parkinson's beyond symptom management. However, such advancement will have to be associated with rigorous research, ethical considerations, and equitable healthcare access for all patients to fully benefit from these revolutionary breakthroughs. As CRISPR technology continues to evolve, its potential to redefine neurodegenerative disease treatment becomes increasingly promising. With careful oversight, collaboration, and ethical responsibility, CRISPR may one day shift Parkinson's disease from an incurable condition to a treatable or even preventable disorder. It is important to note that most CRISPR-based research for PD remains in preclinical stages, with extensive laboratory and animal studies needed before human trials can begin. While promising, this technology will require further refinement before it can be implemented in patient populations; but new avenues are on the horizon.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this article.

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