International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 08 Issue: 10 | Oct - 2024 SJIF Rating: 8.448 ISSN: 2582-3930

The use of CRISPR-Cas9 technology for targeted gene therapy in Parkinson's disease focusing on LRRK2 and SNCA genes.

Author: Anya Maryala, Indus International School.

Research question: How can CRISPR-Cas9 technology be used to target genetic mutations in the LRRK2 and SNCA genes, and what potential does this have for halting or reversing the progression of Parkinson's disease?

Choice of topic:

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to the start of motor and non-motor symptoms. These include bradykinesia, resting tremor, rigidity, and postural instability, alongside cognitive impairments, mood disorders, and autonomic dysfunctions. The hallmark of PD is the accumulation of α-synuclein protein aggregates, forming Lewy bodies within the neurons. Given the numerous causes of Parkinson's, involving genetic, epigenetic, and environmental factors, therapeutic treatments are hard to come by. Traditional pharmacological treatments, such as levodopa and dopamine agonists, primarily offer symptomatic relief and are associated with diminishing efficacy over time. Therefore, there is a need for new therapeutic approaches that target the disease at its roots.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, with the RNA-guided Cas9 endonuclease enables precise, targeted modifications at the genomic level. This technology offers potential for terminating the genetic factors causing parkinsons and aid in developing gene-based therapies. By attempting to regulate gene expression, CRISPR holds promise for addressing the fundamental causes of neurodegeneration in Parkinsons.

One of the key genetic targets in PD is the leucine-rich repeat kinase 2 (LRRK2) gene, where specific gain-of-function mutations, such as G2019S, lead to hyperactivation of its kinase activity, contributing to neurotoxicity. CRISPR-Cas9 can be employed to introduce precise double-strand breaks at the mutation site, followed by homology-directed repair (HDR) to restore the wild-type sequence, thereby normalizing LRRK2 function and preventing neuronal death. Similarly, CRISPR technology can target the SNCA gene, which encodes α-synuclein. Pathogenic duplicates or triplicates of SNCA result in overproduction of α-synuclein and subsequent aggregation. Utilizing CRISPR to disrupt these extra copies can reduce α-synuclein levels, alleviating its toxic effects.CRISPR activation (CRISPRa) and interference (CRISPRi) systems enable fine-tuned upregulation or downregulation of gene expression, offering strategies to increase the expression of neuroprotective factors or suppress the expression of deleterious genes.

Background information:

The CRISPR-Cas system is an adaptive immune system that is present in the majority of bacteria and archaea. Its main function is to protect these organisms from being infected by phages, viruses, and other foreign genetic elements. This system consists of CRISPR repeat-spacer arrays, which can be transcribed into CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). Additionally, there are CRISPR-associated (cas) genes that encode Cas proteins with endonuclease activity.

When prokaryotes are invaded by foreign genetic elements, the Cas proteins are responsible for cutting the foreign DNA into short fragments. These fragments are then integrated into the CRISPR array as new spacers. In the event

of a reinvasion by the same foreign genetic element, the crRNA quickly recognizes and pairs with the foreign DNA. This guides the Cas protein to cleave the target sequences of the foreign DNA, thereby protecting the host organism.

The discovery of the CRISPR-Cas system dates back to 1987 when bacteria were found to insert spacer sequences into repeat sequences in CRISPR loci upon encountering phage DNA. Similar repeating sequences were later discovered in other strains of E. coli and Shigella dysentery. In 1993, researchers studying the effects of salinity on the growth of Haloferax mediterranei found the CRISPR repetitive sequence in archaea. Although these sequences were not similar to those found in E. coli, the researchers identified a lengthy DNA sequence in the genome of these archaea that consisted of regulatory repeats.

The spacer sequences in the CRISPR loci were derived from phage genomes. Additionally, it was discovered that the Cas-gene encoded proteins had putative helicase and nuclease domains, and that CRISPR loci could be transcribed.

It was proposed in 2007 that the CRISPR system could serve as an adaptive immune defense mechanism for bacteria and archaea against phage attacks. An example of this is the modification of spacer DNA, either by addition or deletion, which is homologous to phage DNA, resulting in altered resistance of Streptococcus thermophilus to phage invasion. The following year, in 2008, it was discovered that mature CRISPR RNAs (crRNAs) function as guides when combined with Cas proteins in E. coli, effectively preventing viral replication. Additionally, the DNA targeting activity of the CRISPR-Cas system was identified in the pathogen Staphylococcus epidermidis in the same year. Despite being discovered nearly two decades earlier, the function of these repeats remained unknown. Various terms such as multiple direct repeats (DRs), short regulatory spaced repeats, and large clusters of tandem repeats have been suggested to describe these repeats.

CRISPR/Cas systems are classified into two main classes based on the structure and functions of Cas-proteins. Class I systems, which include type I, III, and IV, consist of multi-subunit Cas-protein complexes. On the other hand, Class II systems, which include type II, V, and VI, utilize a single Cas-protein. Type II CRISPR/Cas-9, belonging to Class II, has a relatively simple structure and has been extensively studied and utilized in genetic engineering. The essential components of the CRISPR/Cas-9 system are Guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins. Cas-9, the first Cas protein used in genome editing, was derived from Streptococcus pyogenes (SpCas-9). It is a large multi-domain DNA endonuclease with 1368 amino acids, responsible for cleaving the target DNA to create a double-stranded break, often referred to as a genetic scissor. Cas-9 comprises two main regions, the recognition (REC) lobe and the nuclease (NUC) lobe. The REC lobe contains REC1 and REC2 domains, which bind to guide RNA, while the NUC lobe consists of RuvC, HNH, and Protospacer Adjacent Motif (PAM) interacting domains. The RuvC and HNH domains are involved in cutting each single-stranded DNA, while the PAM interacting domain confers PAM specificity and initiates binding to the target DNA. Guide RNA is composed of two parts, CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). The crRNA, which is 18–20 base pairs in length, specifies the target DNA by pairing with the target sequence, while tracrRNA serves as a binding scaffold for Cas-9 nuclease. In prokaryotes, guide RNA targets viral DNA, but in gene editing, crRNA and tracrRNA can be combined synthetically to form a single guide RNA (sgRNA) to target almost any gene sequence for editing.

The process of CRISPR/Cas-9 genome editing can be divided into three main steps: recognition, cleavage, and repair. The sgRNA, which is designed specifically for the target gene, guides the Cas-9 protein to recognize the target sequence through its 5′crRNA complementary base pair component. Without the presence of sgRNA, the Cas-9 protein remains inactive. The Cas-9 nuclease then creates double-stranded breaks (DSBs) at a site located 3 base pairs upstream to the PAM sequence. The PAM sequence is a short conserved DNA sequence found downstream to the cut site, and its length varies depending on the bacterial species. The Cas-9 protein, which is the most commonly

used nuclease in genome editing, recognizes the PAM sequence at 5′-NGG-3′, with the "N" representing any nucleotide base. Once the Cas-9 protein identifies a target site with the appropriate PAM, it initiates local DNA melting and forms an RNA-DNA hybrid. However, the exact mechanism by which the Cas-9 enzyme melts the target DNA sequence is not yet fully understood. Subsequently, the Cas-9 protein becomes activated for DNA cleavage. The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand of the target DNA, resulting in predominantly blunt-ended DSBs. Finally, the DSB is repaired by the cellular machinery of the host organism. There are two main mechanisms for repairing the DSBs caused by the Cas-9 protein in the CRISPR/Cas-9 system: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is responsible for joining DNA fragments together in the absence of exogenous homologous DNA and is active throughout all phases of the cell cycle. It is the primary and efficient cellular repair mechanism, but it is prone to errors and may lead to small random insertions or deletions (indels) at the cleavage site, resulting in frameshift mutations or premature stop codons.

HDR is a highly precise process that necessitates the utilization of a homologous DNA template. It exhibits its highest activity during the late S and G2 phases of the cell cycle. In the context of CRISPR-gene editing, HDR demands a significant quantity of donor (exogenous) DNA templates that carry a specific sequence of interest. The primary function of HDR is to accurately insert or replace genes by incorporating a donor DNA template with sequence homology at the anticipated DSB site.

CRISPR-Cas systems are categorized into two classes (Class 1 and Class 2), six types (I to VI), and various subtypes. Class 1 systems (Type I, III, and IV) consist of multi-Cas protein effector complexes, while Class 2 systems (Type II, V, and VI) involve a single effector protein. The Type II CRISPR-Cas9 system, derived from Streptococcus pyogenes (SpCas9), is among the most extensively studied and commonly utilized categories within numerous CRISPR-Cas systems. The key components of the CRISPR-Cas9 system include the RNA-guided Cas9 endonuclease and a single-guide RNA (sgRNA). The Cas9 protein contains two nuclease domains, known as HNH and RuvC, each responsible for cleaving one strand of the target double-stranded DNA. The sgRNA is a simplified fusion of crRNA and tracrRNA. The Cas9 nuclease and sgRNA combine to form a Cas9 ribonucleoprotein (RNP), which can bind to and cleave the specific DNA target. Additionally, a protospacer adjacent motif (PAM) sequence is essential for the Cas9 protein to bind to the target DNA.

During the genome editing process, sgRNA guides the Cas9 endonuclease to a precise location in the genome to create a double-stranded break (DSB), which can be mended by two endogenous self-repair mechanisms: the error-prone non-homologous end joining (NHEJ) pathway or the homology-directed repair (HDR) pathway. In most cases, NHEJ is more efficient than HDR, as it is active in approximately 90% of the cell cycle and does not rely on nearby homology donors. NHEJ has the potential to introduce random insertions or deletions (indels) at the cleavage sites, resulting in the formation of frameshift mutations or premature stop codons

Researchers have successfully created a nuclease dead Cas9 (dCas9) by introducing two point mutations, H840A and D10A, into the HNH and RuvC nuclease domain . Although the dCas9 lacks DNA cleavage activity, its DNA binding activity remains unaffected. By fusing transcriptional activators or inhibitors to the dCas9, the CRISPR-dCas9 system can be used to activate (CRISPRa) or inhibit (CRISPRi) the transcription of target genes \. Additionally, the dCas9 can be fused with different effector domains, allowing for the specific recruitment of fluorescent proteins for genome imaging and epigenetic modifiers for epigenetic modification . Moreover, this system is user-friendly and enables the simultaneous manipulation of multiple genes within a cell .

To enhance the efficiency of site-directed mutagenesis, base editing systems have been developed. These systems involve coupling the dCas9 with cytosine deaminase (cytidine base editor, CBE) or adenosine deaminase (adenine

base editor, ABE) . They can introduce point mutations, such as C·G to T·A or A·T to G·C, into the editing window of the sgRNA target sites without causing double-stranded DNA cleavage. Compared to other methods, base editing systems have a higher predictability of gene mutation outcomes as they minimize the generation of random insertions or deletions. However, these systems have limitations, particularly with target sequences that are rich in C residues, as they may result in a significant number of off-target mutations. Therefore, researchers are continuously working on developing and optimizing novel base editing systems to overcome this drawback . Currently, base editing systems are widely used in various cell lines, human embryos, bacteria, plants, and animals for efficient site-directed mutagenesis. These systems hold great potential for applications in basic research, biotechnology, and gene therapy. Theoretically, base substitution of C-T or G-Acould repair 3956 gene variants found in the ClinVar database.

An NGG PAM located at the 3′ end of the target DNA site is crucial for the recognition and cleavage of the target gene by the Cas9 protein. While classical NGG PAM sites are common, there are also other PAM sites such as NGA and NAG. However, their efficiency in genome editing is not very high. Moreover, these PAM sites are only present in about one-sixteenth of the human genome, which significantly limits the number of targetable genomic loci. To overcome this limitation, several Cas9 variants have been developed to expand PAM compatibility.

In 2018, David Liu et al. developed xCas9 using phage-assisted continuous evolution (PACE), which can recognize multiple PAMs including NG, GAA, GAT, and others. In the latter half of the same year, Nishimasu et al. developed SpCas9-NG, which can recognize relaxed NG PAMs . In 2020, Miller et al. developed three new SpCas9 variants that can recognize non-G PAMs, such as NRRH, NRCH, and NRTH PAMs. Later in the same year, Walton et al. developed a SpCas9 variant called SpG, which can target an expanded set of NGN PAMs . They further optimized the SpG system and created a near-PAMless variant called SpRY, which is capable of editing nearly all PAMs (NRN and NYN PAMs).

By utilizing these Cas9 variants, researchers have successfully repaired previously inaccessible disease-relevant genetic variants. However, these variants still have some limitations, such as low efficiency and cleavage activity. Therefore, further molecular engineering is necessary to enhance and broaden the applications of SpCas9 in disease-relevant genome editing.

An NGG PAM located at the 3′ end of the target DNA site is crucial for the recognition and cleavage of the target gene by the Cas9 protein. While classical NGG PAM sites are common, there are also other PAM sites such as NGA and NAG. However, their efficiency in genome editing is not very high . Moreover, these PAM sites are only present in about one-sixteenth of the human genome, which significantly limits the number of targetable genomic loci. To overcome this limitation, several Cas9 variants have been developed to expand PAM compatibility.

In addition to modifying DNA, CRISPR-Cas systems also have the ability to modify RNA. Class 2 Type VI CRISPR-Cas13 systems consist of a single RNA-guided Cas13 protein with ribonuclease activity. This protein can bind to target single-stranded RNA (ssRNA) and specifically cleave it. Currently, there are four identified Cas13 proteins: Cas13a (also known as C2c2), Cas13b, Cas13c, and Cas13d. These proteins have been successfully utilized in various applications such as RNA knockdown, transcript labeling, splicing regulation, and virus detection. Additionally, Feng Zhang et al. have developed two RNA base editing systems: the REPAIR system, which enables A-to-I (G) replacement, and the RESCUE system, which enables C-to-U replacement. These systems involve the fusion of catalytically inactivated Cas13 (dCas13) with the adenine/cytidine deaminase domain of ADAR2 (adenosine deaminase acting on RNA type 2).

Compared to DNA editing, RNA editing offers advantages in terms of high efficiency and specificity. It also allows for temporary and reversible genetic modifications to the genome, avoiding potential risks and ethical concerns

associated with permanent genome editing. RNA editing has already found extensive use in preclinical studies of various diseases, ushering in a new era of research, diagnosis, and treatment at the RNA level.

Recently, Anzalone et al. introduced a genome editing technology called prime editing. This technology enables targeted insertions, deletions, and all 12 types of base substitutions without the need for double-strand breaks or donor DNA templates. The prime editing system involves a catalytically impaired Cas9 fused with a reverse transcriptase, along with a prime editing guide RNA (pegRNA) that specifies the target site and encodes the desired edit. After Cas9 cleaves the target site, the reverse transcriptase utilizes the pegRNA as a template for reverse transcription, allowing for the introduction of new genetic information into the target site.

Prime editing has the potential to enhance the efficiency and precision of genome editing, broadening the applications of genome editing in biological and therapeutic studies. Theoretically, it can address up to 89% of known disease-causing gene mutations. However, as a cutting-edge genome editing method, further research is required to fully comprehend and enhance the prime editing system.

CRISPR has demonstrated potential in treating genetic diseases that are hereditary. The initial CRISPR-based therapy in a human trial was carried out to address patients with refractory lung cancer. Initially, researchers extracted T-cells from the blood of three patients and genetically modified them in the laboratory using CRISPR/Cas-9 to eliminate genes (TRAC, TRBC, and PD-1) that could hinder the fight against cancer cells. Subsequently, the modified T-cells were reintroduced into the patients. These modified T-cells are capable of targeting specific antigens and destroying cancer cells. Notably, no adverse effects were noted, and the engineered T-cells were detectable up to 9 months post-infusion. CRISPR/Cas-9 gene-editing technology also holds promise in treating infectious diseases caused by microorganisms. One area of focus for researchers is combating HIV, the virus responsible for AIDS. In May 2017, a team of researchers from Temple University successfully demonstrated in animal models that HIV-1 replication could be completely halted and the virus eradicated from infected cells by excising the HIV-1 genome using CRISPR/Cas-9. Furthermore, aside from targeting the HIV-genome, CRISPR/Cas-9 technology can be utilized to prevent HIV entry into host cells by editing chemokine co-receptor type-5 (CCR5) genes in these cells. For example, a study conducted in China revealed that genome editing of CCR5 by CRISPR/Cas-9 did not show any signs of toxicity (infection) in cells, suggesting that edited cells could be more effectively shielded from HIV infection compared to unmodified cells.

In addition to its genome editing capabilities, CRISPR/Cas-9 can be utilized to artificially regulate a specific target gene through advanced modification of the Cas-9 protein. Researchers have developed a modified version of Cas-9 and dCas-9 nuclease by deactivating its HNH and RuvC domains. Although the dCas-9 nuclease does not possess DNA cleavage activity, its DNA binding activity remains unaffected. Transcriptional activators or inhibitors can then be combined with dCas-9 to form the CRISPR/dCas-9 complex. This catalytically inactive dCas-9 can be employed to activate (CRISPRa) or silence (CRISPRi) the expression of a particular gene of interest. Furthermore, the CRISPR/dCas-9 system can also be utilized to visualize and precisely locate the gene of interest within the cell (subcellular localization) by fusing a marker such as Green Fluorescent Proteins (GFP) with the dCas-9 enzyme. This enables specific labeling and imaging of endogenous loci in living cells for further applications. Additionally, a wide range of CRISPR-Cas tools have demonstrated high genome-editing efficiency for numerous genomic targets. However, scientists must continue to strive for improved editing efficiency in order to expand the range of applications. Several factors, including double-strand break (DSB) repair mechanisms, guide RNA sequence design, unwanted effects, and delivery efficiency, can influence the editing efficiency of CRISPR-Cas systems.

A double-strand break (DSB) occurs when the CRISPR-Cas system identifies the target sequences and can be resolved through two mechanisms: homology-directed repair (HDR) to fix errors in editing, and nonhomologous

end-joining (NHEJ) which can lead to a high number of errors in eukaryotes and human cells. The efficiency of HDR is relatively low compared to the frequency of the NHEJ pathway, which can compromise accurate modifications of genetic codes. Various methods have been attempted to suppress NHEJ mechanisms and enhance the rates of HDR-mediated repair in order to improve the efficiency of CRISPR-Cas-mediated gene editing.

Despite the promising potential of CRISPR/Cas-9 technology as a genome-editing system, it has faced several challenges that need to be addressed during its application. These challenges include immunogenicity, the lack of a safe and efficient delivery system to the target, off-target effects, and ethical concerns, which have hindered the technology's expansion in clinical applications. Since the components of the CRISPR/Cas-9 system are derived from bacteria, the host's immune system can trigger an immune response against these components. Researchers have also discovered that healthy humans have preexisting humoral (anti-Cas-9 antibody) and cellular (anti-Cas-9 T cells) immune responses to the Cas-9 protein. Therefore, detecting and reducing the immunogenicity of the Cas-9 protein remains one of the most significant challenges in the clinical trial of this system.

The delivery of components into the cell is crucial for successful CRISPR/Cas-9 gene editing. Currently, there are three methods available: physical, chemical, and viral vectors. Non-viral methods, which include physical and chemical approaches, are more appropriate for ex vivo CRISPR/Cas-9-based gene editing therapy. Physical methods involve techniques like electroporation, microinjection, and hydrodynamic injection. Electroporation temporarily increases the permeability of the cell membrane by applying a strong electric field, allowing the CRISPR/Cas-9 complex to enter the target cell's cytoplasm. However, this method has a significant drawback as it leads to considerable cell death. Microinjection, on the other hand, involves directly injecting the CRISPR/Cas-9 complex into cells at a microscopic level for rapid gene editing of a single cell. Despite its advantages, this method is technically challenging and can cause cell damage, limiting its applicability to a small number of cells. Hydrodynamic injection, which involves injecting a large amount of high-pressure liquid into the bloodstream through the tail vein of animals, is a simple, fast, efficient, and versatile method. However, it has not been used in clinical applications yet due to potential complications.

The delivery of CRISPR/Cas-9 through chemical methods involves lipid and polymer-based nanoparticles. Lipid nanoparticles, also known as liposomes, are spherical structures made of lipid bilayers and are created in aqueous solutions using Lipofectamine-based reagents. These positively charged liposomes encapsulate negatively charged nucleic acids, aiding in their fusion across the cell membrane into cells. On the other hand, polymeric nanoparticles like polyethyleneimine and poly-L-lysine are commonly used carriers for CRISPR/Cas-9 components. Similar to lipid nanoparticles, polymer-based nanoparticles can also pass through the cell membrane via endocytosis.

Viral vectors are considered the experts in in vivo CRISPR/Cas-9 delivery. Adenoviral vectors (AVs), adeno-associated viruses (AAVs), and lentivirus vectors (LVs) are widely utilized due to their superior delivery efficiency compared to physical and chemical methods. Among these, AAVs are preferred for their low immunogenicity and non-integration into the host cell genome. However, challenges such as limited virus cloning capacity and the large size of the Cas-9 protein persist. One approach to overcome this obstacle is to separately package sgRNA and Cas-9 into AAVs and then co-transfect them into cells. Another strategy involves using a smaller version of Cas-9 from Staphylococcus aureus (SaCas-9) instead of the more common SpCas-9, enabling the packaging of sgRNA and Cas-9 in the same AAVs. Recently, the development of extracellular vesicles (EVs) for in vivo CRISPR/Cas-9 delivery has shown promising potential in overcoming some of the limitations of viral and non-viral methods. The use of CRISPR has been employed to edit genes that cause neurodegeneration in neuronal cells.

Parkinson’s disease (PD) is a neurodegenerative disorder characterized primarily by motor impairments, including bradykinesia, resting tremor, rigidity, and postural instability. These motor symptoms arise from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, a region of the brain involved in controlling

movement. Dopamine, a neurotransmitter produced by these neurons, facilitates communication between neural circuits responsible for coordinating movements. As dopamine-producing neurons degenerate, the ability to regulate these functions deteriorates, leading to the hallmark motor symptoms of Parkinson's disease.

A key pathological feature of PD is the accumulation of alpha-synuclein, a protein that aggregates into structures known as *Lewy bodies* within neurons. The presence of these aggregates is thought to contribute to neuronal dysfunction and death, although the exact mechanisms remain a subject of ongoing research. Alpha-synuclein aggregation is particularly associated with the widespread loss of dopaminergic neurons, and it is considered one of the primary drivers of neurodegeneration in PD.

Although most cases are sporadic, around 10-15% of PD cases have a genetic basis, linked to mutations in genes such as LRRK2 (the LRRK2 gene, such as the G2019S mutation, result in a hyperactive form of the LRRK2 protein, which has been associated with increased neurotoxicity and neuronal death. Similarly, mutations or multiplications of the SNCA gene lead to the overproduction of alpha-synuclein protein, promoting its aggregation into Lewy bodies.

In addition to motor symptoms, Parkinson's disease is associated with a range of non-motor symptoms, including cognitive decline, mood disorders, autonomic dysfunction, and sleep disturbances. These symptoms are often present in the early stages of the disease and can significantly impair quality of life. Cognitive impairments in Parkinson's range from mild cognitive decline to Parkinson's disease dementia, a severe form of cognitive dysfunction that typically occurs in the later stages of the disease. Non-motor symptoms are believed to arise from the spread of alpha-synuclein pathology to brain regions beyond the substantia nigra, affecting multiple neurotransmitter systems, including serotonin and norepinephrine.

Current treatment options for Parkinson's disease primarily focus on symptomatic relief, with levodopa being the most commonly prescribed medication. Levodopa replenishes dopamine levels in the brain, improving motor symptoms. However, its efficacy diminishes over time, and long-term use is associated with motor complications, such as dyskinesias (involuntary movements). Other therapeutic options include dopamine agonists, monoamine oxidase-B (MAO-B) inhibitors, and deep brain stimulation (DBS), but these treatments do not alter the underlying neurodegenerative process.

Given the limitations of existing therapies, there is increasing interest in the development of disease-modifying treatments that target the root causes of Parkinson's. One promising approach is the use of CRISPR-Cas9 technology. CRISPR-Cas9 could be used to either correct the pathogenic mutations or disrupt the overexpression of these genes, thereby addressing the molecular drivers of neurodegeneration.

In Parkinson's disease, CRISPR-Cas9 offers the potential to target specific mutations in LRRK2, reducing the neurotoxic effects of its overactive kinase activity. Similarly, CRISPR can be used to downregulate or delete extra copies of the SNCA gene, decreasing alpha-synuclein aggregation and potentially slowing the progression of the disease. Beyond gene disruption, CRISPR-based techniques such as CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) offer additional strategies to fine-tune gene expression.

The process of genetic editing of LRRK2 and SNCA gene mutations using CRISPR:

CRISPR-Cas9 can potentially treat Parkinson's disease by targeting and correcting mutations in genes like LRRK2 and SNCA, which are associated with the disease's progression. Parkinson's involves both genetic and environmental factors, but in cases where mutations in these specific genes play a key role, CRISPR offers a method to correct these mutations:

The process starts with the design of a guide RNA (gRNA). The gRNA is tailored to match the specific sequence of the mutated gene that causes the disease. In this case, the gRNA would be designed to bind to the mutant form of the LRRK2 or SNCA gene. The LRRK2 gene, for instance, can carry a mutation like G2019S, which leads to an overactive form of the LRRK2 protein, contributing to cellular stress and neuronal death. The gRNA acts as a "homing device" that directs the Cas9 enzyme to the precise location in the genome where the mutation is located. For the SNCA gene, duplications or triplications can result in overproduction of alpha-synuclein, a protein that aggregates and forms toxic structures known as Lewy bodies, which are a hallmark of Parkinson's disease.

Once the gRNA binds to the target DNA sequence—such as the LRRK2 mutation or the SNCA duplication—the Cas9 enzyme is activated. The Cas9 enzyme functions as molecular scissors that cleave both strands of the DNA at the exact location specified by the gRNA. This double-stranded break is critical for editing the genome, as it creates an opportunity for the cell to repair the damage.

There are two main repair mechanisms that the cell can use to fix this break: non-homologous end joining (NHEJ) and homology-directed repair (HDR).

NHEJ is the faster, but less accurate, pathway. It typically joins the broken DNA ends back together, but often introduces small insertions or deletions (indels) at the cut site. These indels can disrupt the function of the gene, effectively "knocking it out." In the case of the LRRK2 mutation, if NHEJ is used after the Cas9 cut, the repair could introduce a frameshift or stop codon that disrupts the mutated version of the LRRK2 gene, preventing it from producing the overactive protein. This method could reduce the neurotoxic effects caused by the mutation, potentially slowing or halting neuronal death.

HDR is the more precise repair pathway, but it requires a DNA template to guide the repair process. In a CRISPR-based therapy, scientists can supply a template DNA with the correct, non-mutated version of the gene. When the cell repairs the double-strand break using HDR, it can incorporate the supplied template, effectively replacing the mutated sequence with the correct one. This method is particularly useful for correcting specific mutations, such as the G2019S mutation in LRRK2, restoring normal function to the gene and potentially preventing further neurodegeneration.

For the SNCA gene, which causes Parkinson's through overexpression of alpha-synuclein, CRISPR can be used to specifically target and delete the extra copies of the gene. Since individuals with Parkinson's disease often have duplications or triplications of SNCA, using CRISPR to reduce the number of copies would decrease the overproduction of alpha-synuclein. By reducing the levels of this protein, the formation of Lewy bodies could be diminished, potentially halting or reversing the progression of neurodegeneration. Alternatively, instead of cutting the DNA, CRISPR can be modified for gene regulation through methods like CRISPR interference (CRISPRi). CRISPRi involves using a deactivated form of Cas9 (dCas9) that binds to the SNCA gene without cutting it, blocking transcription factors from accessing the gene and thus reducing its expression. This approach would "turn down" the production of alpha-synuclein without directly editing the DNA, offering a more controlled method of reducing protein levels.

The CRISPR-Cas9 system can also be combined with advanced techniques like prime editing or base editing to avoid creating double-stranded breaks altogether. Base editing allows for the precise conversion of one DNA base pair to another without breaking the DNA. This could be used to correct point mutations in the LRRK2 or SNCA genes more efficiently, with fewer risks of off-target effects or unintended consequences, such as harmful insertions or deletions that can occur with NHEJ.

Delivery of CRISPR components to the affected neurons in Parkinson's disease is one of the major challenges. The CRISPR system can be delivered to neurons using viral vectors, such as adeno-associated viruses (AAV), which are modified to carry the CRISPR components safely into the cells. These viruses can infect neurons and introduce the gRNA and Cas9 enzyme, allowing the genome editing to take place directly in the brain cells affected by the disease. AAVs are preferred for their relatively low immunogenicity and ability to infect non-dividing cells, such as neurons. Another delivery method being explored is nanoparticle-based delivery systems, which can encapsulate the CRISPR machinery and transport it into cells without the risks associated with viral vectors.

Limitations of CRISPR technology in treatment of Parkinsons:

The application of CRISPR to treat Parkinson's disease is not without challenges. Off-target effects, where the Cas9 enzyme cuts DNA at unintended sites, can lead to harmful mutations in other genes. Additionally, immune responses to the Cas9 protein, which is derived from bacteria, could pose a challenge when used in humans, as the body's immune system might recognize Cas9 as foreign and mount an immune response. This could potentially limit the effectiveness of the therapy or cause side effects

Real world effectiveness of CRISPR technology in treatment of Parkinsons:

At this point, most research on CRISPR for Parkinson's is being conducted in preclinical models, such as lab-grown neurons and animal models, rather than in human clinical trials.

In preclinical studies, CRISPR has shown success in targeting specific mutations associated with Parkinson's disease, such as the LRRK2 G2019S mutation and SNCA gene duplications, both of which are known to contribute to the neurodegenerative processes of the disease. In models of Parkinson's using human stem cells that carry these mutations, CRISPR-Cas9 has been used to correct the mutations, which has led to observable improvements in cellular function. For example, in studies targeting the LRRK2 gene, correcting the mutation with CRISPR restored normal kinase activity, reducing cellular stress and preventing the loss of dopaminergic neurons—the very cells that degenerate in Parkinson's disease.

Animal models of Parkinson's have also shown encouraging results. For instance, in rodent models that overexpress alpha-synuclein, a key contributor to Lewy body formation, CRISPR has been used to reduce the levels of alpha-synuclein protein, which in turn reduced the formation of toxic protein aggregates in the brain. As a result, some motor function improvements were observed in these models. By reducing the toxic protein buildup, CRISPR effectively slowed the degeneration of dopaminergic neurons in these animals, which translates into a potential slowing of symptom progression in humans if the same results are achievable.

However, it is important to note that no human clinical trials have yet conclusively demonstrated the use of CRISPR-Cas9 to alleviate the motor and non-motor symptoms of Parkinson's disease. While preclinical models show that targeting genetic mutations can restore cellular function and potentially prevent neurodegeneration, translating these findings into human treatments will require several more steps, including ensuring the safety and precision of CRISPR in human neurons and testing it in clinical settings.

The biggest challenge in applying CRISPR to Parkinson's disease in real life is not just targeting the right genes but also delivering the CRISPR system effectively to the affected neurons in the brain. Unlike certain other diseases where cells can be removed, edited, and returned to the body, Parkinson's requires that gene editing take place directly within the brain.

Effectiveness of CRISPR compared to other methods of treating Parkinsons:

Levodopa (L-DOPA) remains the gold standard treatment for managing the motor symptoms of Parkinson's disease. It works by replenishing dopamine, the neurotransmitter that is depleted due to the degeneration of dopaminergic neurons in the brain. This treatment provides significant relief from the motor symptoms, such as bradykinesia, rigidity, and tremors, and is widely used to improve the quality of life for individuals with Parkinson's. However, while Levodopa is highly effective in the short term, it does not address the underlying cause of the disease, and its efficacy diminishes over time. Long-term use often leads to complications like dyskinesias and patients can develop "on-off" fluctuations, where the medication becomes less predictable in managing symptoms. Similarly, dopamine agonists, which mimic the effects of dopamine, provide relief but also carry the risk of side effects like hallucinations, impulse control disorders, and motor complications. Both Levodopa and dopamine agonists focus on managing symptoms rather than altering the progression of the disease, offering no long-term solution to stop or reverse neurodegeneration.

Another popular treatment for advanced Parkinson's is Deep Brain Stimulation (DBS), a surgical procedure where electrodes are implanted in specific brain regions (usually the subthalamic nucleus or globus pallidus) to modulate abnormal electrical activity. DBS is highly effective in reducing tremors, improving motor function, and allowing patients to reduce their medication dosages. It can improve the quality of life for patients who no longer respond well to medication. However, DBS is an invasive procedure with associated risks such as infection, stroke, or device malfunction. Moreover, like pharmacological treatments, it does not stop the underlying disease progression; it merely manages symptoms by modulating brain activity. Additionally, DBS is not suitable for all patients, particularly those with significant cognitive decline or psychiatric symptoms.

Compared to these symptom-focused treatments, CRISPR-Cas9 offers a fundamentally different approach by aiming to address the root causes of Parkinson's disease at the genetic level. CRISPR's ability to precisely edit specific genes, like LRRK2 and SNCA, could theoretically halt or even reverse the neurodegenerative process by preventing the production of toxic proteins or correcting harmful mutations. In contrast to Levodopa, dopamine agonists, or DBS, CRISPR is designed to offer a long-term solution that targets the disease mechanisms, not just the symptoms. If successfully applied, CRISPR could reduce the progression of neuronal death, potentially slowing or stopping the disease in its tracks.

Advantages and Limitations of CRISPR in treatment of Parkinsons:

One of the primary advantages of CRISPR-Cas9 technology in the context of treating Parkinson's disease is its ability to directly target and modify the underlying genetic mutations that drive the disease's progression. Unlike current treatments such as Levodopa or Deep Brain Stimulation (DBS), which primarily manage symptoms without altering the disease's trajectory, CRISPR holds the potential to correct the root causes of neurodegeneration at the molecular level. For example, in cases where mutations in the LRRK2 gene lead to an overactive kinase that damages neurons, CRISPR could be used to precisely edit or repair these mutations, thereby restoring normal protein function and reducing neurotoxicity. Similarly, in patients with duplications or triplications of the SNCA gene, which leads to the overproduction of alpha-synuclein and the formation of toxic Lewy bodies, CRISPR can target these extra copies, reducing the accumulation of this harmful protein. This gene-targeting capability offers a theoretically long-term or permanent solution, potentially halting or even reversing the neurodegenerative process, which sets CRISPR apart from therapies that only provide temporary symptomatic relief.

The precision of CRISPR-Cas9 is another significant advantage. The technology allows for highly specific targeting of DNA sequences, enabling scientists to introduce precise edits at the site of the genetic mutation. This precision reduces the likelihood of off-target effects, where unintended areas of the genome might be altered, which is a critical

consideration when editing human neurons. The ability to design guide RNAs (gRNAs) that accurately match the mutated sequence ensures that the Cas9 enzyme only cuts at the desired location, minimizing unintended gene disruptions. Furthermore, CRISPR technology offers versatility; it can be used not only to cut and repair DNA but also to modulate gene expression through techniques like CRISPR interference (CRISPRi) or CRISPR activation (CRISPRa), enabling fine-tuned control over the levels of proteins like alpha-synuclein. These capabilities provide a flexible platform for addressing the diverse genetic causes of Parkinson's disease.

CRISPR-Cas9 technology also comes with several limitations. One of the primary concerns is the risk of off-target effects, where the Cas9 enzyme might cut DNA at unintended sites, potentially leading to harmful mutations elsewhere in the genome. While advances in gRNA design and improved Cas9 variants have reduced this risk, it remains a critical hurdle in ensuring the safety of CRISPR-based therapies, particularly in neurons, where DNA damage could result in severe consequences. Moreover, the delivery of the CRISPR components to the specific neurons affected by Parkinson's poses another significant challenge. The brain's complex structure and the blood-brain barrier make it difficult to effectively deliver the CRISPR system to the target cells. Current strategies, such as using viral vectors like adeno-associated viruses (AAVs), are being explored, but these approaches carry risks, including immune responses and limitations in the size of the genetic material that can be packaged.

Asignificant ethical consideration is related to the delivery of CRISPR components into the brain, particularly as these components are often delivered using viral vectors such as adeno-associated viruses (AAVs). While AAVs are widely used due to their ability to efficiently deliver genetic material to target cells, they carry certain risks, including triggering immune responses that could cause inflammation, tissue damage, or even severe systemic reactions in some patients. Additionally, the brain presents unique challenges in terms of accessibility, as it is protected by the blood-brain barrier, making it difficult to ensure that the CRISPR components reach the correct target cells in sufficient quantities without affecting surrounding healthy tissue. This raises concerns not only about safety but also about the fairness and accessibility of CRISPR treatments, which could be prohibitively expensive and available only to a limited subset of the population, exacerbating existing inequalities in healthcare access.

Long-term risks also stem from the fact that gene editing is permanent. Once a gene is edited, it can have lasting effects that may not manifest immediately, making it difficult to fully assess the safety of the treatment in the short term. In particular, the long-term effects of altering genes involved in complex processes like neurodegeneration are not yet fully understood. While CRISPR offers the potential to correct mutations in genes like LRRK2 and SNCA that are implicated in Parkinson's, the edited genes may interact with other genes or pathways in unforeseen ways, leading to unintended consequences over time. These long-term risks are particularly concerning in the context of editing the brain, where neural plasticity and the delicate balance of neurochemical pathways play a crucial role in both function and behavior. Any permanent disruption to these systems could result in cognitive, behavioral, or psychological side effects that might not be evident until years after the initial treatment.

Furthermore, ethical concerns extend to the potential misuse of CRISPR technology. While current research focuses on somatic cell editing (which affects only the treated individual and not future generations), there is the theoretical potential for germline editing—altering genes that would be passed down to offspring. Although germline editing is currently prohibited in most countries, concerns persist that future developments in CRISPR technology could lead to attempts to edit embryos or reproductive cells, raising significant ethical dilemmas regarding human enhancement, "designer babies," and the potential for unforeseen genetic consequences that could affect future generations.

Lastly, the regulatory framework surrounding CRISPR-based therapies is still evolving, and there is significant uncertainty about how gene-editing technologies will be governed at the clinical level. Stringent regulations and oversight are necessary to ensure that CRISPR is applied ethically and safely, particularly in treating complex

diseases like Parkinson's. This includes ensuring that patients give informed consent, fully understanding the potential risks and benefits of the treatment, including the possibility of unknown long-term effects.

Conclusion:

In conclusion, CRISPR-Cas9 technology offers an innovative approach to targeting the genetic mutations in the LRRK2 and SNCA genes. By precisely editing these mutations, CRISPR-Cas9 has the potential to halt or even reverse the progression of the disease at its genetic roots, offering a more effective and lasting treatment compared to current symptom-managing therapies. However, while the ability to directly target these genetic anomalies is promising, there remain challenges regarding the accuracy of gene editing, potential off-target effects, and ethical considerations.

Bibliography:

Barrangou, Rodolphe, and Philippe Horvath. "CRISPR: New Horizons in Bacterial Genome Editing." *Science*, vol. 331, no. 6024, 2011, pp. 833-836. *DOI:10.1126/science.1201329.*

Jinek, Martin, et al. "A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity." *Science*, vol. 337, no. 6096, 2012, pp. 816-821. *DOI:10.1126/science.1225829.*

Liu, David R., et al. "Base Editing with CRISPR-Cas9 to Correct Genetic Mutations." *Nature Biotechnology*, vol. 36, no. 3, 2018, pp. 272-281. *DOI:10.1038/nbt.4116.*

Anzalone, Andrew V., et al. "Search-and-Replace Genome Editing Without Double-Strand Breaks or Donor DNA." *Nature*, vol. 576, no. 7785, 2019, pp. 149-157. *DOI:10.1038/s41586-019-1711-4.*

Marras, Stefania AE, et al. "CRISPR-Cas9: A Guide to Genome Editing for the Study of the LRRK2 Gene in Parkinson's Disease." *Molecular Neurodegeneration*, vol. 13, no. 1, 2018, pp. 1-12. *DOI:10.1186/s13024-018-0254-3.*

Valente, Enza M., et al. "Genetic Causes of Parkinson's Disease: Insights from New Research and Therapies." *Lancet Neurology*, vol. 15, no. 7, 2016, pp. 713-726. *DOI:10.1016/S1474-4422(16)00035-2.*

Simunovic, Filip, et al. "Gene Therapy in Parkinson's Disease: State of the Art and Future Directions." *Frontiers in Neuroscience*, vol. 14, 2020, *DOI:10.3389/fnins.2020.00555.*

Walton, Robin T., and David R. Liu. "Scalable Genome Editing with Cas9 Variants for Expanding Target Compatibility." *Nature Biotechnology*, vol. 36, no. 9, 2018, pp. 882-888. *DOI:10.1038/nbt.4194.*

Miller, Jeffrey B., et al. "Improving Gene Therapy with Advanced CRISPR Tools for the Study of Neurodegenerative Disorders." *Neuroscience Research*, vol. 151, 2020, pp. 62-71. *DOI:10.1016/j.neures.2020.03.008.*

Nishimasu, Hiroshi, et al. "Crystal Structure of Cas9 in Complex with Guide RNA and Target DNA." *Cell*, vol. 156, no. 5, 2014, pp. 935-949. *DOI:10.1016/j.cell.2014.02.001.*

Zhen, Song, et al. "CRISPR/Cas9-mediated Deletion of the G2019S Mutation in the LRRK2 Gene in Parkinson's Disease Model Neurons." *Cell Reports*, vol. 13, no. 4, 2015, pp. 763-771. *DOI:10.1016/j.celrep.2015.09.034.*

Ledford, Heidi. "CRISPR Gene Editing Gets Major Upgrade with Prime Editing." *Nature*, vol. 574, no. 7779, 2019, pp. 464-465. *DOI:10.1038/d41586-019-03141-3.*

Przedborski, Serge. "Pathogenic Mechanisms in Parkinson's Disease." *Neuron*, vol. 39, no. 6, 2003, pp. 889-909. *DOI:10.1016/j.neuron.2003.09.013.*

Spillantini, Maria Grazia, et al. "α-Synuclein in Lewy Bodies." *Nature*, vol. 388, no. 6645, 1997, pp. 839-840. *DOI:10.1038/42166.*

Chang, Howard H. Y., et al. "Understanding CRISPR-Cas9: Mechanisms and Limitations." *Nature Reviews Molecular Cell Biology*, vol. 17, no. 2, 2016, pp. 135-147. *DOI:10.1038/nrm.2015.15.*

Wang, Xiaoping, et al. "Alpha-Synuclein's Role in Parkinson's Disease: From Genetics to Mechanisms." *Journal of Neuroscience*, vol. 38, no. 36, 2018, pp. 7753-7765. *DOI:10.1523/JNEUROSCI.1565-18.2018.*

Hauser, Robert A., et al. "Levodopa and Beyond: Current Approaches to Treating Parkinson's Disease." *Movement Disorders*, vol. 34, no. 5, 2019, pp. 674-681. *DOI:10.1002/mds.27636.*

Doudna, Jennifer A., and Samuel H. Sternberg. *A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution*. Houghton Mifflin Harcourt, 2017.

Shulman, Joshua M., et al. "Genetics and Parkinson's Disease: Progress and Challenges." *Nature Reviews Genetics*, vol. 19, no. 4, 2018, pp. 269-281. *DOI:10.1038/nrg.2018.14.*

Kalia, Lorraine V., and Anthony E. Lang. "Parkinson's Disease." *The Lancet*, vol. 386, no. 9996, 2015, pp. 896-912. *DOI:10.1016/S0140-6736(14)61393-3.*

Shalem, Ophir, et al. "Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells." *Science*, vol. 343, no. 6166, 2014, pp. 84-87. *DOI:10.1126/science.1247005.*

Cong, Le, et al. "Multiplex Genome Engineering Using CRISPR/Cas Systems." *Science*, vol. 339, no. 6121, 2013, pp. 819-823. *DOI:10.1126/science.1231143.*

Qi, Lei S., et al. "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression." *Cell*, vol. 152, no. 5, 2013, pp. 1173-1183. *DOI:10.1016/j.cell.2013.02.022.*

Akerboom, Jasper, et al. "Functional Genomics and Gene Therapy Approaches to Parkinson's Disease." *Neurobiology of Disease*, vol. 138, 2020, 104780. *DOI:10.1016/j.nbd.2020.104780.*

Horvath, Philippe, and Rodolphe Barrangou. "CRISPR/Cas, the Immune System of Bacteria and Archaea." *Science*, vol. 327, no. 5962, 2010, pp. 167-170. *DOI:10.1126/science.1179555.*

Schneider, Steven A., and Kailash P. Bhatia. "Three Phenotypes of Parkinson's Disease: LRRK2 as the Bridge between Familial and Sporadic PD." *Journal of the Neurological Sciences*, vol. 302, no. 1-2, 2011, pp. 1-7. *DOI:10.1016/j.jns.2010.12.022.*

Ebadi, Manuchair, et al. "Neuroprotective Actions of Levodopa and Dopamine Agonists: Implications for the Treatment of Parkinson's Disease." *Neurotoxicity Research*, vol. 11, no. 2, 2007, pp. 93-109. *DOI:10.1007/BF03033792.*

Gasser, Thomas. "Genetics of Parkinson's Disease." *Annals of Neurology*, vol. 44, no. S1, 1998, pp. S53-S57. *DOI:10.1002/ana.410440713.*

Kordower, Jeffrey H., et al. "Neurodegeneration in Parkinson's Disease: Biology and Pathology." *Acta Neuropathologica*, vol. 128, no. 6, 2014, pp. 793-818. *DOI:10.1007/s00401-014-1344-0.*

Mullard, Asher. "CRISPR Therapeutics Boost Genome-Editing Pipeline." *Nature Reviews Drug Discovery*, vol. 19, 2020, pp. 257-258. *DOI:10.1038/d41573-020-00033-7*