



Review Article

CRISPR Advancements in Correcting Protein Misfolding: Implications for Neurodegenerative Disorders



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Abstract

The genome editing technology based on clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 (CRISPR/Cas9), is revolutionizing research, particularly in the context of human neurodegenerative disorders. This review examines recent advancements in CRISPR/Cas9 and its potential to address the protein misfolding mechanisms underlying these diseases. Proteins, the fundamental units of life, can misfold due to various changes, resulting in aggregation and contributing to devastating illnesses such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, and Huntington's disease. Understanding the pathology of these disorders and the methods used for their detection is vital for developing effective treatments. CRISPR/Cas9 offers a powerful tool for combating neurodegenerative disorders at the molecular level. Its groundbreaking gene-editing capabilities are advancing preclinical and animal studies, paving the way for potential human trials and innovative therapeutic strategies. This review explores the complex challenge of protein misfolding and highlights how CRISPR technology could provide a crucial breakthrough in the fight against neurodegenerative disorders. It offers a synthesis of CRISPR advancements for neurodegenerative disorders. However, it is essential to be aware of the review's limitations, including potential selection bias, the risk of oversimplification, and possible obsolescence in rapidly changing research fields. Despite these considerations, the transformative promise of CRISPR in understanding and potentially treating neurodegenerative diseases warrants continued research and thorough analysis.

Introduction

The technology of clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 (CRISPR/Cas9) has revolutionized genetic editing, offering a precise tool for manipulating genomes across organisms. Derived from bacterial immune systems, it uses the Cas9 protein and guide RNA (gRNA) to target specific DNA sequences.¹ This precision allows for treating genetic disorders, tackling diseases such as cancer, HIV, and sickle cell anemia, and even improving agriculture.^{2,3} By combining CRISPR/Cas9 with tools such as Cre-loxP, researchers are developing even more sophisticated approaches to targeted genome engineering.^{1,4}

Keywords: CRISPR-Cas systems; Gene editing; Neuro-degenerative diseases; Protein folding; Protein aggregates; Proteostasis deficiencies.

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One area where CRISPR/Cas9 holds immense promise is in addressing neurodegenerative diseases. These disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), are marked by the accumulation of misfolded proteins in the brain, leading to neuronal dysfunction.¹ Protein misfolding, where proteins adopt abnormal conformations and aggregate, is a complex phenomenon influenced by factors such as polyglutamine expansions and aging.⁵ This phenomenon plays a central role in various protein misfolding diseases, particularly neurodegenerative disorders.^{5,6}

Understanding protein misfolding is crucial for developing novel diagnostic and treatment strategies. Research has shed light on involved mechanisms, including those related to RNA metabolism, protein synthesis, folding, degradation, and trafficking.⁵ Misfolded protein aggregates are a hallmark of neurodegenerative diseases,^{7,8} but how these aggregates cause neuronal death is still under investigation; they are thought to acquire neurotoxic properties.⁶ The cross-talk between different misfolded proteins indicates a complex interplay within these diseases.⁹

Given the devastating impact of neurodegenerative disorders, there is an urgent need for innovative therapies. Current approach-

es include protein disaggregates, protein-remodeling factors, and chaperones.^{10–13} These strategies can reverse misfolding, prevent it, or disassemble aggregates. Additionally, understanding the roles of protein phosphorylation and flanking amino acid sequences in modulating misfolding will be vital for future interventions.¹⁴

Proper utilization of CRISPR technology has the potential to revolutionize the treatment of neurodegenerative disorders. By targeting and correcting mutations associated with protein misfolding, CRISPR could prevent or reduce toxic aggregate formation. Researchers are actively exploring CRISPR-based therapies for diseases such as AD, PD, and Huntington's disease (HD), aiming to slow or even halt disease progression. However, challenges such as precise delivery into the brain, off-target effects, and ethical considerations must be addressed as research progresses.

This review focuses on the potential of CRISPR/Cas9 for correcting protein misfolding mechanisms implicated in neurodegenerative disorders. We will analyze CRISPR's use in cell and animal models, its success in targeting specific mutations, and the ongoing challenges in clinical translation. Our main objective is to assess the feasibility and potential limitations of CRISPR as a transformative tool for understanding and ultimately treating these devastating diseases.

Protein misfolding and neurodegeneration

Understanding the importance of protein folding in disease

Understanding the factors that initiate protein misfolding is essential for unraveling the mechanisms behind many diseases and revolutionizing our therapeutic approaches. These triggers can range from genetic mutations that directly disrupt protein structure to cellular stressors such as oxidative stress or compromised protein quality control mechanisms.^{13,15} Even the natural decline in cellular quality control associated with aging can increase vulnerability to misfolding.¹⁶ Identifying these initiation factors offers crucial benefits: it allows us to understand disease mechanisms more deeply, guiding researchers toward key therapeutic targets. This knowledge could also enable early diagnosis through biomarkers and risk assessment in susceptible individuals. Furthermore, understanding these triggers paves the way for targeted prevention strategies, potentially involving lifestyle modifications or therapies to reduce disease risk.

Most importantly, this knowledge drives the development of groundbreaking drugs that directly address the root cause of misfolding. These drugs could focus on prevention or enhance the cell's ability to manage misfolded proteins. Targeting the initial stages of misfolding, rather than simply addressing downstream consequences, promises to yield more impactful treatments with the potential to slow or halt the progression of these devastating diseases.

Knowing the role of misfolded proteins in neurodegeneration

The location of protein misfolding, whether inside (intracellular) or outside (extracellular) cells, significantly shapes the mechanisms of neurodegenerative diseases.¹⁷ Intracellular misfolding disrupts vital cellular processes such as protein quality control and organelle function, leading to cellular stress, a key player in neurodegeneration.¹⁷ The formation of intracellular aggregates further exacerbates the situation, impairing cellular transport, compromising mitochondrial health, and activating harmful stress responses.^{17,18}

In contrast, extracellular misfolded proteins, especially those forming aggregates or plaques, induce different detrimental ef-

fects. They can trigger damaging inflammatory responses, activate immune cells, and disrupt the integrity of the blood-brain barrier.¹⁹ Extracellular deposits may also contribute to synaptic dysfunction and neuroinflammation, harming neuronal health.

The relative impact of intracellular versus extracellular misfolded proteins depends heavily on the specific neurodegenerative disease. AD is marked by extracellular beta-amyloid plaques and intracellular tau tangles, contributing significantly to pathology.²⁰ PD, on the other hand, is primarily driven by the intracellular aggregation of *α-synuclein*.²¹

It is crucial to understand the distinct pathogenic mechanisms linked to misfolding location – whether within the cell or in the surrounding environment. Intracellular misfolding directly compromises the cell's function, while extracellular aggregates promote inflammation and damage neighboring cells. The complex interplay between these factors is an active research area, and its understanding is essential for developing targeted and effective therapies.

Understanding the dynamics of protein folding, misfolding, and aggregation

The alpha helix, a right-handed spiral coil, is a common structural feature in the native state of many proteins. Protein folding is the complex process by which a protein transforms from an initially unstructured state into its well-defined, three-dimensional structure.²² This process minimizes the protein's free energy, driven by decreased entropy (reduced possible conformational states).^{23,24} However, local energy minima can sometimes trap the protein in intermediate states, slowing its progression toward the final folded form.²⁵

Misfolding occurs when this precise conformational development goes awry due to various factors. These include genetic mutations in non-reproductive (somatic) cells, errors in gene transcription or protein translation, malfunctions in the cell's protein folding machinery, aberrant post-translational modifications (PTMs) (such as phosphorylation, glycosylation, acetylation, and ubiquitination), disruptions in how proteins are trafficked within the cell, or changes in the protein's environment.²⁶ Misfolded proteins can expose hydrophobic amino acids typically hidden in their core, promoting aggregation driven by hydrophobic interactions.²⁷ This process can lead to disordered aggregates, oligomers, and amyloid fibrils.²⁸ Key factors determining a protein's propensity to aggregate include its hydrophobicity, tendency to form beta-sheet structures, and decreased net electrostatic charge.

The kinetics of protein folding, involving the speed and pathways, are influenced by the amino acid sequence, environment, and the presence of cofactors.²⁹ Folding pathways and energy landscapes help us understand this process. Protein folding kinetics can follow a two-state model (directly from unfolded to folded) or a multi-state model, which includes intermediate states.^{30,31} These intermediate states represent temporary conformations adopted during the folding process and are crucial for understanding folding kinetics.³² Experimental techniques such as Nuclear Magnetic Resonance spectroscopy, protein engineering, and computational models such as molecular dynamics simulations provide valuable insights into these intermediate states and folding pathways.^{33,34}

Various elements can influence the balance between proper folding pathways and those leading to misfolding. Chaperone proteins facilitate accurate protein folding and prevent aggregation.³⁵ Environmental factors such as temperature and pH also affect folding kinetics.³⁶ Furthermore, small molecules or drugs can stabilize

the native state or inhibit aggregation, offering potential therapeutic options for diseases caused by protein misfolding.³⁷

Finding linkage between protein misfolding and neurodegenerative onset

The tendency of certain proteins to form aggregates in neurodegenerative diseases is a complex process driven by several factors. One key element is the protein's structure. Proteins with regions prone to misfolding, often due to exposed hydrophobic areas or unstable structures, are more likely to aggregate.³⁸ Genetic mutations can also be responsible, as changes in the amino acid sequence can make proteins more susceptible to misfolding and clumping. Some proteins linked to neurodegeneration harbor mutations that directly enhance their aggregation potential.³⁹

The cellular environment also plays a crucial role in influencing protein aggregation. Oxidative stress, changes in pH, or reduced availability of molecular chaperones (proteins that help with proper folding) can promote the misfolding and aggregation of certain proteins.⁴⁰ Furthermore, the cell's systems for degrading misfolded or aggregated proteins, such as the ubiquitin-proteasome system and autophagy, can be overwhelmed or malfunction in neurodegenerative diseases. This process leads to a buildup of aggregates as the cell can't effectively remove these harmful structures.⁴¹

Each disease is associated with a specific protein that misfolds, acting as a hallmark of that particular condition.²⁷ Misfolding repeatedly occurs in these disorders as the misfolding protein evades the cell's protein-folding machinery and quality control systems. This allows it to form aggregates that grow into larger fibrous structures.^{42,43} These fibers are called "amyloid fibrils" when formed outside the cell and "intracellular inclusions" when found within cells.^{44,45} Interestingly, despite differences in amino acid sequence, the proteins involved in various neurodegenerative diseases share structural similarities in their aggregated states. This suggests that the accumulation of misfolded protein, rather than the specific protein involved, might be the central driver of these diseases.^{26,46} The aggregated forms often have a prevalence of beta-pleated sheets, a striking contrast to the alpha-helical and globular structures of their native, functional forms.⁴⁷

Unravelling the toxicity of misfolded proteins

In neurodegenerative diseases, misfolded proteins wreak havoc by disrupting essential cellular functions and overwhelming the cell's proteostasis mechanisms, the network responsible for maintaining a healthy protein production, folding, and removal balance. When misfolded proteins accumulate beyond the cell's ability to clear them, they overwhelm quality control systems, triggering stress responses that contribute to neuronal dysfunction and death.^{48,49} This disruption has a cascading effect, leading to impaired synaptic function as misfolded proteins interfere with the release of neurotransmitters, hampering communication between neurons.⁵⁰ These misfolded proteins also trigger a chronic inflammatory response in the brain, as microglia (the brain's immune cells) release inflammatory substances.⁴⁷

Furthermore, misfolded proteins often form toxic intermediates known as soluble oligomers during their aggregation process. These intermediates are even more damaging than the larger, inert plaques and can disrupt cellular membranes, increase oxidative stress, and directly interfere with cellular signaling pathways.⁵¹ Misfolded proteins also harm mitochondria, the cell's energy production centers, leading to energy deficits and the build-up of harmful reactive oxygen species.⁵² Ultimately, the accumulation of misfolded proteins disrupts the delicate proteostasis network,

leading to further buildup of toxic protein aggregates and accelerating cellular dysfunction and neurodegeneration.⁵³ This cascade of damage underlies the cognitive decline and other neurological symptoms characteristic of neurodegenerative diseases.

Importance of PTMs in protein misfolding

PTMs are essential in protein misfolding, a central feature of neurodegenerative diseases.⁵⁴ These modifications, which occur after a protein is created, have far-reaching effects on protein structure, function, stability, and how a protein behaves within a cell. In neurodegenerative diseases, disruptions in PTMs contribute significantly to disease progression.

Phosphorylation, the addition of phosphate groups, is one PTM that dynamically alters a protein's charge and shape, influencing how it interacts with other molecules. Neurodegenerative diseases often showcase aberrant phosphorylation; in AD, the tau protein becomes abnormally phosphorylated.⁵⁵ This pathological change promotes aggregation into neurofibrillary tangles, disrupting tau's normal function and contributing to neuronal damage.

Another critical PTM is ubiquitination, which involves tagging proteins with ubiquitin molecules. This modification often signals the proteasome, the cell's recycling machinery, to degrade the tagged protein. When misfolded proteins escape proper ubiquitination, their clearance mechanisms fail, leading to the dangerous buildup of toxic aggregates within cells.⁵⁶ This breakdown in proteostasis, the cell's careful balance of protein synthesis and removal, is a driving force in neurodegeneration.

Glycosylation, the attachment of sugar molecules, also profoundly affects how proteins fold and behave. Abnormal glycosylation patterns can alter the stability of misfolded proteins and influence their interactions within the cell. In PD, aberrant glycosylation contributes to *α-synuclein* aggregation, an essential protein in the disease process.⁵⁷

Beyond these, other PTMs such as acetylation, methylation, and sumoylation have the power to modulate the structure and function of proteins involved in neurodegenerative diseases.⁵⁸ Changes induced by these modifications can impact where a protein resides in the cell, how it interacts with other molecules, and its tendency to form harmful aggregates.

Overall, PTMs hold significant sway over the fate of proteins involved in misfolding. When these modifications become disrupted, they contribute to the damaging processes seen in neurodegenerative diseases, promoting protein aggregation, compromising cellular function, and ultimately fueling disease progression.

How do neurodegenerative pathologies lead to neuronal death?

The mechanism underlying neuronal demise in neurodegenerative disorders exhibits common characteristics, including the selective loss of neurons, alterations in synaptic connections, and neuroinflammation.^{47,59,60} However, the specific brain regions affected vary across these disorders. In the case of AD, neuronal death predominantly occurs in brain areas crucial for memory and cognitive functions, such as the hippocampus, amygdala, and cortical regions.⁶⁰ PD is characterized by the loss of neurons in the substantia nigra and a decline in dopamine levels within the striatum.⁶¹ HD is distinguished by pronounced neuronal loss in the striatum and cerebral cortex.⁶² ALS manifests as the degeneration of lower motor neurons in the spinal cord and brainstem, accompanied by the loss of upper motor neurons in the motor cortex.⁶³ Transmissible spongiform encephalopathies are characterized by the conspicuous degeneration of brain tissue, with the location and extent of neuronal loss exhibiting variability.⁶⁴

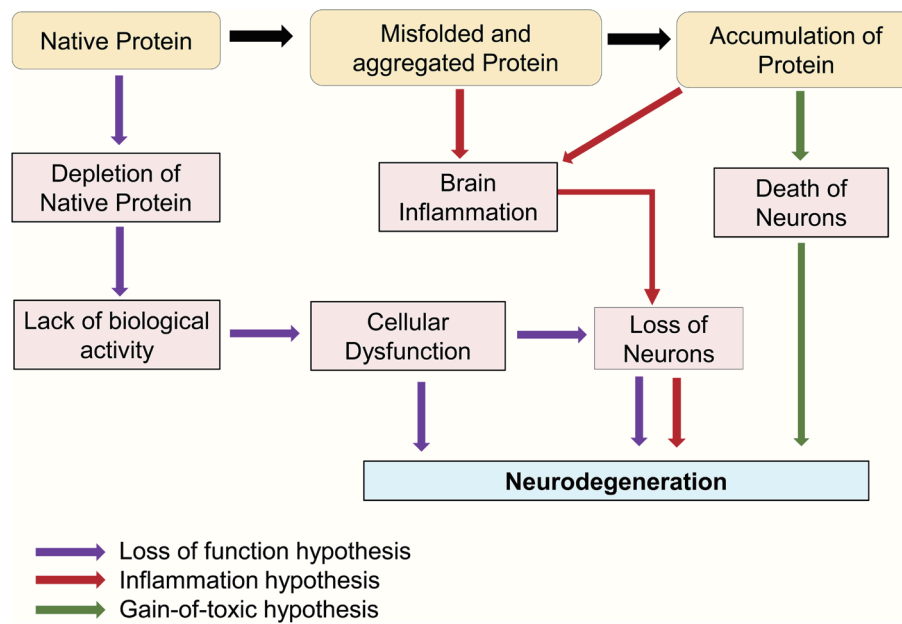


Fig. 1. Illustrating models depicting the mechanism of neurodegeneration linked to the misfolding and aggregation of proteins.

Neuronal loss in these conditions transpires through a programmed cell death process called apoptosis.^{47,65} Three hypotheses have been proposed to elucidate the intricate relationship between protein misfolding and aggregation with neurodegeneration. These hypotheses offer comprehensive explanations for the interplay between these processes, as illustrated in Figure 1. In the loss-of-function model, the crucial factor is the impairment of the native protein's normal function as it gets depleted due to protein misfolding and aggregation.⁶⁶ This model applies to HD, PD, transmissible spongiform encephalopathy, and ALS. Conversely, in the gain-of-toxic activity model, the protein, when misfolded or aggregated, acquires neurotoxic properties.⁶⁷ Within the inflammation model, protein aggregates induce a prolonged inflammatory response within the brain, leading to neurodegeneration.⁶⁸ This phenomenon is evident through several observations, including (a) a widespread increase in astrocyte activity and microglial activation,⁶⁹ (b) the accumulation of inflammatory proteins within cerebral protein aggregates,⁷⁰ (c) elevated levels of inflammatory proteins in the brain,⁷¹ and (d) the administration of NSAIDs (nonsteroidal anti-inflammatory drugs) resulting in a reduction in the occurrence of AD in both animal models and humans.⁷² In numerous diseases, a combination of these mechanisms may operate concurrently.

The interplay of genes and environment in neurodegeneration

Neurodegenerative diseases arise from a complex interplay of loss-of-function, gain-of-toxic activity, and inflammation – all heavily influenced by both genetic and environmental factors. Mutations in genes encoding proteins essential for neuronal function can lead to loss-of-function, disrupting normal cellular processes and contributing to neurodegeneration. Similarly, environmental factors such as exposure to toxins or inadequate nutrition can compromise neuronal function.⁶⁶ Another pathway to degeneration is gain-of-toxic activity, where mutations produce toxic, misfolded proteins or harmful variations of normal proteins. This leads to cellular dysfunction and degeneration, which can also be triggered by

environmental toxins such as pesticides or heavy metals.⁶⁷

Genetic factors influence an individual's susceptibility to chronic inflammation within the central nervous system (CNS), contributing to neurodegenerative processes. This inflammation can be triggered or worsened by environmental factors such as infections, exposure to pollutants, or chronic stress.⁶⁸ Crucially, genetic and environmental factors interact, and a genetic predisposition might dramatically increase vulnerability to specific toxins. Genetics and the environment can also induce epigenetic changes that alter gene expression, influencing the development of neurodegenerative diseases. This complex interplay explains why individuals experience significant variations in these diseases' onset, progression, and severity. Understanding these interconnected mechanisms is essential for developing targeted treatments and personalized medicine strategies, offering improved support for those affected by these devastating disorders.

Involvement of each model in neurodegenerative diseases

Neurodegenerative diseases arise from a complex interplay of mechanisms that disrupt the health and function of neurons. Three major contributors to this damage are loss-of-function, gain-of-toxic activity, and inflammation, each playing distinct roles in different diseases. In disorders such as Alzheimer's and Parkinson's, loss-of-function mechanisms are crucial. Mutations in genes essential for protein processing (such as amyloid precursor protein (*APP*) or *presenilin* (*PSEN*) in Alzheimer's) hinder neurons' ability to function properly, while in Parkinson's, mutations in genes responsible for protein degradation (such as *Parkin* or *PINK1*) lead to an accumulation of waste and eventually the death of dopamine-producing neurons.⁶⁶

Gain-of-toxic activity is another destructive force seen in diseases such as Huntington's and ALS. In Huntington's, an abnormal expansion in the huntingtin (*HTT*) gene creates a toxic form of the HTT protein, and mutations in genes such as superoxide dismutase 1 (*SOD1*) in ALS trigger harmful protein aggregations that directly damage motor neurons.⁶⁷ Inflammation also plays a significant

role in neurodegeneration; in multiple sclerosis, the immune system attacks the protective myelin sheath around neurons, harming their communication ability. Additionally, chronic inflammation, fueled by cells such as microglia and astrocytes, is a significant factor in Alzheimer's, accelerating the disease's progression and causing cognitive decline.⁶⁸

Remembering that neurodegenerative diseases often stem from a complex interaction of genetic and environmental factors is crucial. ALS, for instance, can be caused by specific genetic mutations (e.g., in the *SOD1* gene) and exposure to certain environmental factors. Similarly, genetic variations such as those found in the apolipoprotein E4 (*APOE4*) gene can increase Alzheimer's risk, while factors such as head injuries or vascular problems can further heighten individual susceptibility.

Misfolding disruptions in neurodegeneration

Genetic mutations and environmental factors play a crucial role in developing neurodegenerative diseases. Mutations in genes such as *APP*, *PSEN1*, and *PSEN2* can disrupt the processing of amyloid-beta (β) protein, leading to misfolding and aggregation that forms the characteristic plaques of AD.⁷³ Chronic inflammation and oxidative stress further contribute to this protein misfolding.⁷⁴ Additionally, specific variants of the *APOE* genes, such as *APOE4*, are linked to an increased risk of AD. *APOE* is involved in lipid metabolism and neuronal repair, and its variants can destabilize protein homeostasis, promoting the accumulation of misfolded proteins in the brain.⁷⁵

Genetic mutations play a significant role in the development of PD. Specifically, mutations in the synuclein alpha (*SNCA*) gene, which encodes α -synuclein, are linked to familial forms of the disease.⁷⁶ Misfolding and aggregation of α -synuclein lead to the formation of Lewy bodies, ultimately disrupting cellular function.⁷⁷ Environmental factors, such as exposure to pesticides and mitochondrial dysfunction, can exacerbate this process and contribute to the accumulation of misfolded α -synuclein.⁷⁸ Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene are also associated with an increased risk of both familial and sporadic PD. *LRRK2* plays a crucial role in regulating various cellular processes, and mutations within this gene can disrupt normal protein function. These disruptions lead to aberrant protein folding and aggregation, critical factors in the development of PD.⁷⁹

HD is caused by an expanded cytosine adenine guanine (CAG) repeat in the *HTT* gene, resulting in an elongated polyglutamine tract in the HTT protein.⁸⁰ The mutated HTT misfolds and forms aggregates, leading to neuronal toxicity.⁸¹ Environmental factors may influence the age of onset and progression of symptoms in individuals with HD.⁸²

Mutations in the *SOD1* gene are a known factor in familial ALS. The *SOD1* protein usually plays a protective role, but these mutations cause it to misfold, forming harmful aggregates within motor neurons.⁸³ Environmental factors such as exposure to heavy metals and toxins may further promote *SOD1* misfolding, contributing to the development of ALS.⁸⁴ Another common genetic cause of both familial and sporadic ALS is a hexanucleotide repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) gene. This expansion disrupts normal processes and results in abnormal RNA and protein aggregates, leading to motor neuron degeneration.⁸⁵ Understanding how genetic mutations, environmental factors, and protein misfolding interact is vital for creating effective ALS treatments. CRISPR technology offers significant potential for precision medicine in this field. By directly targeting specific genetic mutations that cause protein misfolding, CRISPR could help ad-

dress the core causes of neurodegenerative diseases, leading to significant breakthroughs in treatment.

Coding genome non-coding genomics

The emergence of robust genome-wide association methodologies promises to explain the genetic etiology of the common sporadic forms of complex diseases. In addition to revealing the genetic susceptibility of neurodegenerative disease, genome-wide association studies (GWAS) should also be an unbiased generator of molecules relevant to disease pathogenesis.⁸⁶ The etiology of neurodegeneration is primarily influenced by genetic variables, which can modify a person's susceptibility to complicated, sporadic illnesses or operate as monogenic causes of heritable disease. The discovery of disease genes and risk loci has produced some of the most significant medical advancements in the past 20 years and priceless insights into pathogenic mechanisms and disease pathways. Our understanding of the genome and the genetic architecture of neurodegenerative illness is quickly developing thanks to large-scale research efforts, creative study designs, and methodological advancements.⁸⁷ Until recently, research to elucidate the genetic basis of neurodegenerative brain disorders (NBDs) concentrated on the coding region of the genome.⁴⁰ However, a significant portion of NBD heritability remains unexplained.⁴⁰ GWAS have identified non-coding variations that are significantly linked to NBDs.^{40,59} The majority of these variations are found in regulatory regions, suggesting they may influence gene expression through enhancers, promoters, and non-coding RNA.^{40,54} GWAS has linked several genetic variations to various neurological disorders.^{13,16,22,27} While these studies have identified susceptibility loci for diseases such as PD and AD, GWAS variations in coding regions only account for a portion of the heritability, 16–36% for PD and 28% for AD.^{22,27,86}

Similarly, in non-coding genomes, non-coding RNAs such as long noncoding RNA Cyranos contribute to neurodegenerative processes by directly interacting with neurological disorders-associated loci or via intricate networks.⁸⁸ Hence, instead of coding the genome noncoding can also be targeted by CRISPR/Cas9.

CRISPR/Cas9 for precision gene editing

CRISPR/Cas9: A genome editing revolution

CRISPR/Cas9-based genome editing has become a powerful tool for inducing mutations in various organisms, including bacteria, zebrafish, rodents, and even large mammals.⁸⁹ Its origins lie in discovering a prokaryotic adaptive immune system against viruses.^{90,91} Initially identified in 1987 as unusual repetitive DNA sequences, CRISPR was later recognized as a system where bacteria store 'memories' of past viral infections, enabling them to defend themselves.^{92,93}

Before CRISPR/Cas9, gene editing relied on methods such as zinc finger nucleases and transcription activator-like effector nucleases.^{94,95} While effective in some cases, these techniques were complex, expensive, and time-consuming.⁹⁶ In contrast, CRISPR/Cas9's ease of use, flexibility, availability of bioinformatic tools, and commercial reagents have made it invaluable for modifying genes *in vitro* and *in vivo*.⁹⁷

The Cas9 endonuclease, especially the SpCas9 (streptococcus pyogenes Cas9) variant, has become the cornerstone of CRISPR-based genome editing, particularly for creating knockouts and precise genetic modifications. Its ability to target virtually any DNA sequence with the appropriate guide RNA makes it a transforma-

tive technology.⁹⁷ CRISPR/Cas9's efficiency, effectiveness, and accuracy have led to its widespread adoption for genome editing across various fields.⁹⁸

While no editing platform is ideal for every situation, CRISPR/Cas9 stands out in many ways. Meganucleases might be sufficient for applications requiring high precision but not necessarily high efficiency. Zinc finger nucleases or transcription activator-like effector nucleases with modified FokI domains for greater specificity can also be useful.⁹⁶ However, their laborious retargeting processes limit their broader use. CRISPR/Cas9, on the other hand, offers exceptional adaptability since retargeting involves changing the single guide RNA (sgRNA).⁹⁹

Additionally, the availability of diverse Cas variants (such as transcriptional activators/repressors and base editors) further expands its capabilities. CRISPR-Cas works with various delivery methods, including ribonucleoproteins (RNPs), viral vectors, and plasmids, increasing its versatility.¹⁰⁰ For safety-critical applications, choosing low off-target Cas variants is crucial, and selection procedures can offset potentially lower modification rates.¹⁰¹ While SpCas9 has been studied extensively, its origin in human pathogens raises concerns about adaptive immunity. Developing highly specific variants such as SpCas9-HF1 and EvoCas9 from non-pathogenic species addresses this issue.¹⁰²

Understanding CRISPR/Cas9 systems

CRISPR/Cas systems are broadly divided into classes based on Cas protein structure and function. Class I systems use multi-subunit Cas-protein complexes, while Class II systems, such as the widely used type II CRISPR/Cas9, employ a single Cas protein.^{103–105}

The heart of the CRISPR/Cas9 system consists of two key components: the gRNA and the Cas9 protein. SpCas9, isolated from *Streptococcus pyogenes*, was the first Cas protein used for genome editing. This large protein acts as a multi-domain DNA endonuclease, creating double-stranded breaks in the target DNA. Structurally, Cas9 has two main lobes: the recognition lobe, which binds the guide RNA, and the nuclease lobe, which contains the RuvC, HNH, and protospacer adjacent motif (PAM) interaction domains responsible for DNA cleavage.^{106–108}

gRNA is composed of trans-activating CRISPR RNA and CRISPR RNA (crRNA). The crRNA, which specifies the target DNA sequence, and trans-activating CRISPR RNA, which acts as a scaffold for Cas9 binding, are often fused into a sgRNA for use in genome editing.¹⁰⁹

Mechanisms of CRISPR/Cas9 precision genome editing

Recognition, cleavage, and repair are the processes that make up the CRISPR/Cas9 genome editing mechanism.^{110,111} The intended sgRNA controls Cas9 and identifies the target sequence in the relevant gene through its complementary base pair in the crRNA. Without sgRNA, the Cas9 protein remains dormant. The Cas9 nuclease produces double-stranded breaks (DSBs) at three base pairs upstream of PAM.⁹⁹ The PAM sequence is a brief (between two and five base pairs long) conserved DNA sequence downstream of the cut location. The PAM sequence at 5-NGG-3 is recognized by the Cas9 protein, the most widely used nuclease in genome editing tools.¹¹² When Cas9 locates a target site with the right PAM, it causes local DNA melting, followed by the synthesis of an RNA-DNA hybrid.

However, the process by which the Cas9 enzyme melts the target DNA sequence is still not fully known. The Cas9 protein is then turned on to start cleaving DNA. Target DNA is broken down into complementary and non-complementary strands by the HNH

and RuvC domains, respectively, resulting primarily in blunt-ended DSBs. The host cellular machinery then fixes the DSB.¹¹³ In the CRISPR/Cas9 mechanism, the Cas9 protein creates DSBs that must be repaired using either the non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways.¹¹⁴ NHEJ accelerates DSB repair by connecting DNA fragments via an enzymatic mechanism without foreign homologous DNA and is active throughout the cell cycle. It is the most dynamic and dominant cellular repair mechanism.

However, an error-prone mechanism can result in small random insertions or deletions (indels) at the cleavage site, resulting in frameshift mutations or premature stop.¹¹⁵ HDR is a very accurate technique that necessitates using a homologous DNA template.¹¹⁶ The cell cycle is mainly active in the late S and G2 stages. HDR needs many donor (exogenous) DNA templates containing the desired sequence when editing CRISPR. By placing a donor DNA template with sequence homology at the anticipated DSB location, HDR performs the exact gene insertion or replacement.¹¹⁷ Porteus *et al.*¹¹⁸ state that single-base-pair changes account for many human genetic disorders. These substitutions profoundly affect protein function, requiring more complex and precise editing to recreate in model systems. The efficiency of precise genome editing (PGE) approaches, however, is often less than a tenth of that of methods that create less-specific indels, and much work has been done to increase PGE efficiency. These improvements include developing the best guide RNA and mutation-bearing donor DNA templates, modifying the DNA repair pathways that govern how edits are produced due to Cas-induced cuts, and creating Cas9 fusion proteins that introduce edits through alternate methods.¹¹⁹

CRISPR/Cas9 mediated remediation of neurodegenerative disease

A few years after its discovery, the CRISPR/Cas9 genome editing tool has significantly influenced many fields, including health and agriculture. Researchers are confident that this technology will evolve to heal and cure illnesses, create more nutrient-dense crops,⁹⁴ and eradicate infectious diseases.¹²⁰ Here, the new CRISPR/Cas9 applications and ongoing clinical studies that are being looked into are briefly highlighted for the treatment of neurodegenerative illnesses, which usually occur due to mutations that result in protein misfolding. The causes of protein misfolding are multifaceted: (i) somatic mutations in gene sequences leading to the production of proteins incapable of proper folding; (ii) transcriptional or translational errors causing aberrant proteins that fail to fold correctly; (iii) breakdowns in the protein folding and chaperone systems; (iv) mistakes in PTMs or protein trafficking; (v) structural alterations triggered by environmental shifts; or (vi) the induction of protein misfolding through seeding and cross-seeding mechanisms.¹²¹ CRISPR/Cas9 gene editing can provide new mechanistic insights into disease etiology and facilitate precise gene therapy.^{121,122} Gene mutations cause the majority of these disorders. For example, AD is caused by autosomal dominant mutations in one of three genes: *APP*, *PSEN1* and *PSEN2*.¹²³ With its advanced versions, the CRISPR/Cas9 system is of great promise for treating human neurodegenerative disorders by correcting their mutational effect, which causes protein misfolding.¹²⁴

Because of its dependable and durable nature, CRISPR/Cas9 can be used as a programmable tool to produce DNA double-strand breaks *in vivo* and *in vitro*.^{125,126} To treat human diseases, CRISPR/Cas9 has developed into a simple and versatile RNA-directed system for genome editing that can be applied to a wide range of organisms and cell types, including rats, mice, zebrafish, pigs, human

somatic cells, and human pluripotent stem cells.^{127,128} Stem cells are an ideal platform for genome editing technologies because of their ability to self-renew and release endogenous proteins such as coagulation factor IX, VEGF, FGF-b, Ang1, and sRAGE.¹²⁹ However, high passage numbers have been shown to have a deleterious effect on stem cell self-renewal activity.¹³⁰ As a result, it is critical to generate therapeutic genome-edited stem cell lines that meet safety requirements while also meeting criteria such as high efficacy, quality, and reproducibility. Therefore, several groups are still investigating methods of producing effective combinations of gene editing tools and target cells for treating specific diseases. Some have found positive combinations such as neural stem cells with CRISPR/Cas9 for brain tumors, human embryonic stem cells for myocardial infarction, etc. Therapeutic improvements for neurological disorders are difficult due to the limited accessibility of the human CNS and an insufficient understanding of disease causes. Many neurological illnesses lack precise treatments, resulting in a high disease burden and poor outcomes for affected patients. The induced pluripotent stem cell (iPSC) method generates human neural cells, allowing for disease modeling and therapy development. Genome editing, specifically CRISPR-Cas9 technology, has increased the potential of iPSCs, resulting in new models for various illnesses, including AD and PD.¹³¹ CRISPR-iPSC-based techniques have accelerated neurological disease research and bring us closer to a treatment for diseases including AD, PD, HD, ALS, and others.¹³²

Delivery system of CRISPR-Cas9

CRISPR/Cas9 can be transported into different types of stem cells by carrier-independent (e.g., physical and mechanical administration) and carrier-dependent (e.g., nanoparticles, extracellular vesicles, viral-like particles, and viruses) methods. Viral delivery is the most prevalent approach for CRISPR/Cas9. Several virus delivery systems, including adeno-associated viruses (AAV), adenoviral vectors, and lentiviral vectors, have been used for this purpose.¹³³ AAVs are often employed in CRISPR genome editing for the following reasons: First, AAVs can infiltrate the host cell and remain independently of the host cell genome, allowing the provirus to express continuously and stably for up to one to two years, which is useful for disease treatment. Second, AAVs' varied capsids allow them to infect a wide range of tissues.¹³⁴ Third, AAVs can resist changes in pH and temperature while retaining constant activity.¹³⁵ One major obstacle in gene therapy remains the successful delivery of specific cells, tissues, and organs for PGE using CRISPR/Cas components.¹³⁶ Achieving a high delivery efficiency is necessary to produce successful gene editing outcomes. When selecting a delivery vector, caution should be exercised to understand the detrimental effects of vectors fully. One of the primary challenges is effectively delivering CRISPR/Cas9 components to the target cells or tissues. To facilitate the efficient release and internalization of the CRISPR/Cas9 components into the target cells, new approaches such as advanced versions of nanoparticles, biomaterials, etc. must be engineered to encapsulate and safeguard them during delivery.¹³⁷⁻¹³⁹

Decoding the enigma of protein misfolding with CRISPR/Cas9

Investigating protein misfolding genes via CRISPR studies

The CRISPR/Cas9 system was investigated for several uses in genome editing and gene therapy, focusing on genes linked to

protein misfolding disorders. Due to the presence and accumulation of typical proteins or peptides, such as β -amyloid peptides and phosphorylated tau proteins in AD, *α -synuclein* in PD, and mutant HTT in HD, older people are at greater risk of acquiring neurodegenerative disorders.¹⁴⁰ The ability of patients to detoxify or activate self-healing processes is generally diminished in these disorders, mainly attributed to aging. Table 1 summarizes some of the major findings, recommendations, and potential applications of CRISPR/Cas9 in resolving neuro-diseases in humans.^{100,141-159} Several studies showcasing the application of CRISPR to target genes linked to protein misfolding have been comprehensively discussed in the following section:

AD: A CRISPR perspective

It has been proven that amyloid β -peptide and tubulin-associated protein accumulation are related to AD. These peptides are heavily accumulated in brain interstitial fluid and neurons.^{160,161} The *PSEN1M146L* allele that causes AD can be selectively disrupted using the CRISPR/Cas9 method, and the abnormal A42/40 ratio that causes the disease in people who carry this mutation can be partially fixed. Researchers engineered a mouse model of the humanized *APOE4* gene, a known risk factor for AD, in 2020, using CRISPR technology. This model was used to investigate the role of *APOE4* in protein misfolding and neurodegeneration.^{162,163} Several groups have developed Cas9 activator nano complexes to speed up the distribution of Cas9 activators; these non-viral delivery systems have excellent potential therapeutic uses, especially for CNS illnesses.^{164,165} The CRISPR-Cas9 system is employed to reduce *BACE-1* expression in AD by utilizing guide RNAs to target and bind to specific sequences within the *BACE-1* gene, facilitating the Cas9 enzyme to induce precise DNA cleavage, subsequently triggering cellular repair mechanisms that result in reduced *BACE-1* expression, thereby mitigating the production of A β plaques associated with Alzheimer's pathology.

In vivo experiments using these methods have been successful in reducing *BACE-1* expression or elevating *ADAM10* expression in animal models of AD. In both instances, the outcome was a decrease in amyloid β production and a concurrent improvement in AD-like pathology.

CRISPR insights into PD

PD is a neurodegenerative disorder in which the substantia nigra and basal ganglia of the brain lose their dopaminergic (DA) neurons, and toxic deposits known as Lewy bodies accumulate in these areas of the brain. According to clinical tests, individuals with this illness exhibit involuntary movements, including shaking, stiffness, and trouble balancing and coordination.¹⁶⁶ Researchers used a clever CRISPR-dCas9-mediated approach to modify the methylation state of the *SNCA* gene, which is increased in PD patients.¹⁶⁷ Specifically, they recruited a specific histone lysine demethylase, JARID1A, to the *SNCA* promoter of PD-iPSCs and reported that their system was sufficient to decrease the expression of *α -synuclein*.¹⁶⁸ This study paved the way for a cutting-edge PD treatment strategy. Similar to AD, PD has experienced the implementation of CRISPR/Cas9 technology to reverse known PD-causing mutations directly or to eliminate the expression of mutant genes.

The overproduction of *α -synuclein*, reactive microgliosis, neurodegeneration, and Parkinson's-related motor symptoms were all significantly reduced after the *A53T-SNCA* gene was deleted using the CRISPR/Cas9 system.¹⁶⁹ In 2021, Li *et al.* created an effective rhesus monkey model of PD using gene editing.¹⁷⁰ For

Table 1. Findings and potential applications of CRISPR/Cas9 in neurodegenerative disorders

Pathology	Target gene	Model	Result	Reference
Alzheimer's disease	<i>Bace1</i> gene	5XFAD mouse, APP knock-in (KI) mouse	Decreased <i>APP</i> and reduced levels of Ab	143,144
	<i>APP</i> gene	Mouse	Decrease in amyloid β peptide levels. Improved cognitive function in mouse models	100,145
	<i>APOE4</i> gene	Mouse iPSCs	Transition from the <i>APOE4</i> allele to the <i>APOE3</i> allele. Reduction in Tau Hyper-phosphorylation	146,147 148
Parkinson's disease	<i>Vps35 D620N</i>	Mouse	Mouse model of PD	149,150
	<i>α-synuclein</i> gene	Pig	Pig model of PD. Suppression of <i>α-synuclein</i> expression; Attenuation of motor symptoms in animal models	151–153
	<i>DNAJC6</i>	ESCs	Stem cell model of PD	154
Huntington's disease	<i>HTT</i> gene	Pig	Pig model of HD	155,156
	<i>HTT</i> gene	HD140Q-Ki mice	Decreased early neuropathological alterations in the striatum. Correction of CAG repeat expansion; Alleviation of motor deficits in cell and mouse models	149,157
Amyotrophic lateral sclerosis	<i>C9orf72</i>	Mouse	Reduction in synaptic dysfunction	149,158
	<i>SOD1</i> gene	<i>SOD1G93A</i> mutant neonatal mice	Diminished motor neuron quantity. Weakened muscle strength.	141,142,159

ALS, amyotrophic lateral sclerosis; APOE4, apolipoprotein E4; APP knock-in (KI) mouse, amyloid precursor protein knock-in mouse; *Bace1*, beta-secretase 1 gene; *C9orf72*, chromosome 9 open reading frame 72; CAG, cytosine adenine guanine; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9; *DNAJC6*, DnaJ heat shock protein family (Hsp40) member C6; ESCs, embryonic stem cells; HD140Q-Ki mice, transgenic mouse model expressing human huntingtin with 140 CAG repeats; *HTT*, huntingtin; iPSCs, induced pluripotent stem cells; PD, Parkinson's disease; *SOD1*, superoxide dismutase 1; *SOD1G93A* mutant neonatal mice, transgenic mice expressing a mutant form of *SOD1* with a glycine to alanine substitution at position 93; *Vps35 D620N*, vacuolar protein sorting 35 D620N mutation; 5XFAD mouse, transgenic mouse model for Alzheimer.

this study, a viral vector CRISPR/Cas9 system was used to target the *PINK1* locations in the substantia nigra under MRI supervision. This group of adult monkeys demonstrated a rapid (six to ten months) PD progression process with all the typical symptoms of the disease, such as bradykinesia, tremor, and postural instability, as well as critical pathological indicators of PD, such as severe nigral DA neuron loss and obvious *α -synuclein* pathology. In addition, an intriguing study on nigral neurons used the CRISPR/Cas9 system to remove the genes for *PAR-KIN (PRKN)*, *DJ-1 (PARK7)*, and *ATP13A2 (PARK9)*. Examining transcriptome and proteome data, it was discovered that oxidative stress is a common dysregulation mechanism across all isogenic cell types.¹⁷¹

Exploring ALS through CRISPR

Recent research showed that the CRISPR/Cas9 system may successfully expel hexanucleotide repeat expansion from the *C9ORF72* gene, the most common genetic cause of ALS and frontotemporal dementia.¹⁴¹ Transgenes (*hSOD1-G93A*) responsible for ALS have been edited using CRISPR/Cas9 technology in two transgenic mice.¹⁷² In two separate hSOD1-G93A transgenic mouse models of ALS (*G1H* and *G1L*), they demonstrated that the *in vivo* gene-editing method efficiently targets *hSOD1*, leading to a disease-free state.¹⁷² A study described a CRISPR/Cas9 approach that is quick, easy, and effective for correcting specific point mutations associated with ALS in human iPSCs without using antibiotic selection.¹⁷³ They either corrected mutations such as *I114T* in *SOD1* in patient iPSCs or generated mutations such as *G94A* in *SOD1* and *H517Q* in fused in sarcoma in control iPSCs.¹⁷³ In one study, CRISPR-Cas9 therapy

was used to fix the *SOD1* mutant gene, which led to an increase in the number of motor neurons, a delay in the onset of the disease, and a longer lifespan in ALS animals.¹⁴² Several research studies have provided evidence that mutations in the *C9orf72* gene impact subsequent protein synthesis and can lead to the early death of neurons by either causing a harmful gain or loss-of-function.^{141,174} Consequently, reducing the expression of *C9orf72* is a potentially promising approach to slowing down the progression of ALS disease.

CRISPR-driven approaches to HD

Located on chromosome 4, the *HTT* gene is responsible for the neurodegenerative condition known as HD (HD). Poly-glutamine (CAG) repeats are overexpressed in the altered gene, resulting in aberrant, harmful protein synthesizing.¹⁷⁵ Shin and associates used a modified CRISPR/Cas9 approach based on SNPs that changed PAMs.¹⁷⁶ With a thorough understanding of the *HTT* gene haplotype structure, this approach targeted CRISPR/Cas9 regions unique to each patient. Selectively, inactivating the mutant *HTT* allele for a specific diplotype.¹⁷⁶ Additionally, this study resulted in the same mice living longer and showing improvement in specific motor deficits, highlighting the potential of CRISPR/Cas9 technology as a treatment option for HD and reiterating its potential for treating other autosomal dominant neurodegenerative disorders.¹⁷⁷

CRISPR/Cas9 for precision editing in misfolding disorders

The CRISPR/Cas9 technique can potentially reverse or change the genetic abnormalities that cause disorders linked to protein misfolding. Accumulation of misfolded proteins and resultant tissue

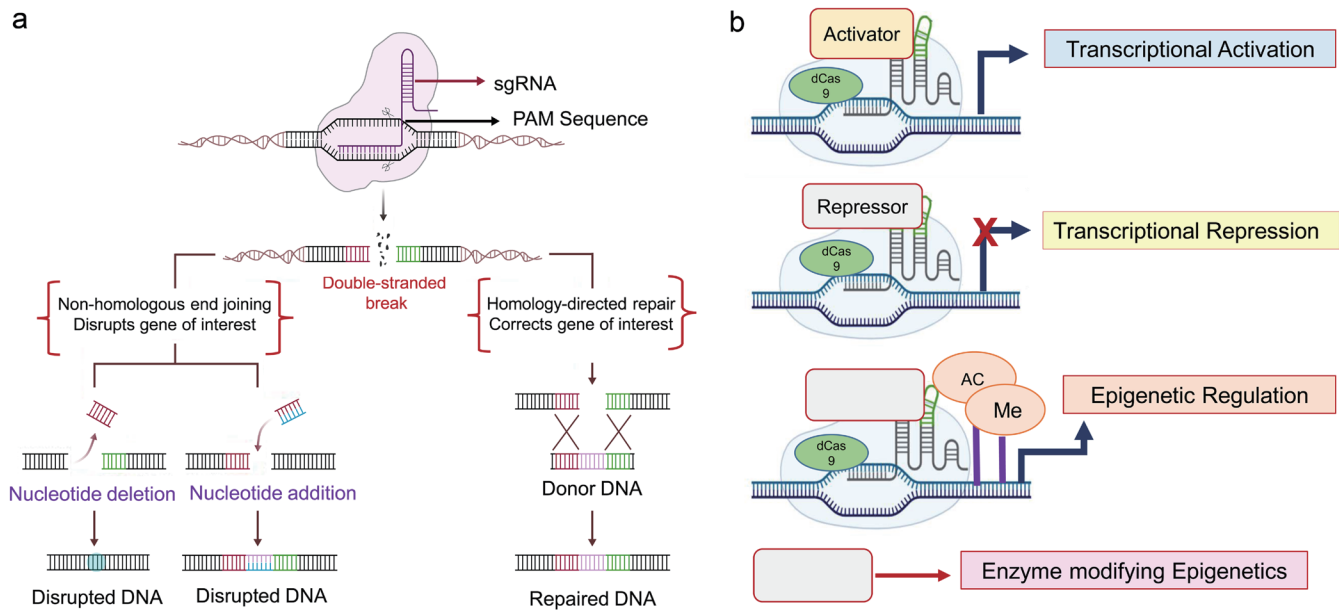


Fig. 2. CRISPR/Cas9 mediated gene editing in PD. (a) CRISPR/Cas9-based gene editing mechanism in PD; (b) Regulation of transcription using CRISPR and Cas9. AC, activating Cas; dCas9, dead Cas9; Me, methylation; PAM, protospacer adjacent motif; PD, Parkinson's disease; sgRNA, single guide RNA; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9.

damage are key characteristics of diseases associated with protein misfolding. These disorders can be mitigated or potentially cured through, correcting or modifying the underlying genetic mutations responsible for improperly folding proteins.

Therapeutic approach for PD using CRISPR/Cas9

Structural investigations and *in vitro* tests were used to demonstrate the mechanism of CRISPR/Cas9 in PD. In brief, CRISPR/Cas9 works by sequential processes. The Cas9 endonuclease is first expressed, and then an sgRNA sequence with a 20-nt complementary sequence to the target DNA is created. Cas9 will finally clear the PAM site, close to the 3' end of the target area.¹⁷⁸ Cas9 has an auto-inhibitory configuration when not linked to sgRNA because the RuvC domain blocks the HNH domains' active sites.

When sgRNA is attached, it changes into a DNA-recognition-competent conformation that opens a central channel among the two lobes for DNA binding.¹⁷⁹ The sgRNA-Cas9 complex adheres to the PAM through the PI domain after first searching for it through three-dimensional diffusion in the desired DNA. DNA strand separation starts at the PAM-proximal region and allows for synthesizing an sgRNA-DNA duplex.¹⁸⁰ When the target DNA and gRNA have structural similarities, strands unwind and split.¹⁸¹ Creating a complete R loop in the PAM-proximal area triggers another conformational change in the HNH domain, which causes DNA breaks by triggering the nuclease activity of both the RuvC and HNH domains. An intense connection between sgRNA and DNA further accelerates this process.^{182,183} Before being released for reuse by other cellular components, Cas9 is tightly attached to the DNA cleaved as its target.¹⁰⁹ The DSBs that Cas9 generates are connected *via* either NHEJ or HDR (Fig. 2a). Because NHEJ links the broken ends of the targeted DNA to create sporadic DNA indels, it is very error-prone.

In contrast, HDR uses an intricate repair strategy to fix the damage by incorporating a similar donor DNA template.¹⁸⁴ These two methods allow for direct homologous recombination or random

insertion/deletion mutation in the presence of a donor template.¹⁸⁵ Cas9 (dCas9) nuclease deactivation also creates a potent tool for modifying gene transcription. The combination of activator domains such as V16 and VP64 activates the target gene, while the fusion of dCas9 with a repressor domain such as Krüppel-associated box makes the repression of desired genes possible. Connecting DNA methyltransferase 3A, DNA demethylase ten-eleven-translocation, or p300 core with dCas9 results in epigenetic modification.¹⁸⁵ The dCas9-Regulatory Domain Complex engages the appropriate gene activators/repressors to perform the regulatory function after binding to the target DNA region (Fig. 2b).

Thus, CRISPR advancements in correcting protein misfolding extend to epigenetic modifications by enabling precise editing of the underlying DNA sequence, influencing gene expression, and potentially mitigating neurodegenerative effects associated with aberrant protein folding.

CRISPR/Cas9 therapeutics for protein misfolding in AD

Point mutations or deletions in the genes encoding the *APP*, *PSEN1*, and *PSEN2* usually cause an early-onset dominant inherited type of AD.¹⁷² Subunits of γ -secretase, *PSEN1*, and *PSEN2*, contribute to the increased synthesis of the β -amyloid peptide,¹⁸⁶ thereby changing the cleavage point in APP to cause mutation. Such mutations can be corrected via CRISPR/Cas9.¹⁸⁷ iPSC-derived nerve cells originated from basal forebrain cholinergic iPSC neurons of a patient with the *PSEN2*N141I mutation have been altered using CRISPR/Cas9 to fix a *PSEN2* predominant alteration (Fig. 3).¹⁸⁸ By using the CRISPR/Cas9 technology, amyloid- β -induced protein cysteine oxidative change can be successfully mitigated in HT22 cells by downregulating the quantity of the Thioredoxin-interacting protein (Txnip), which proves to be an effective target in the cure of AD.¹⁸⁹

Applying CRISPR/Cas9 in HD treatment

Certain guide RNAs direct the Cas9 protein to target particular sections of DNA. A specific recognition area known as the PAM

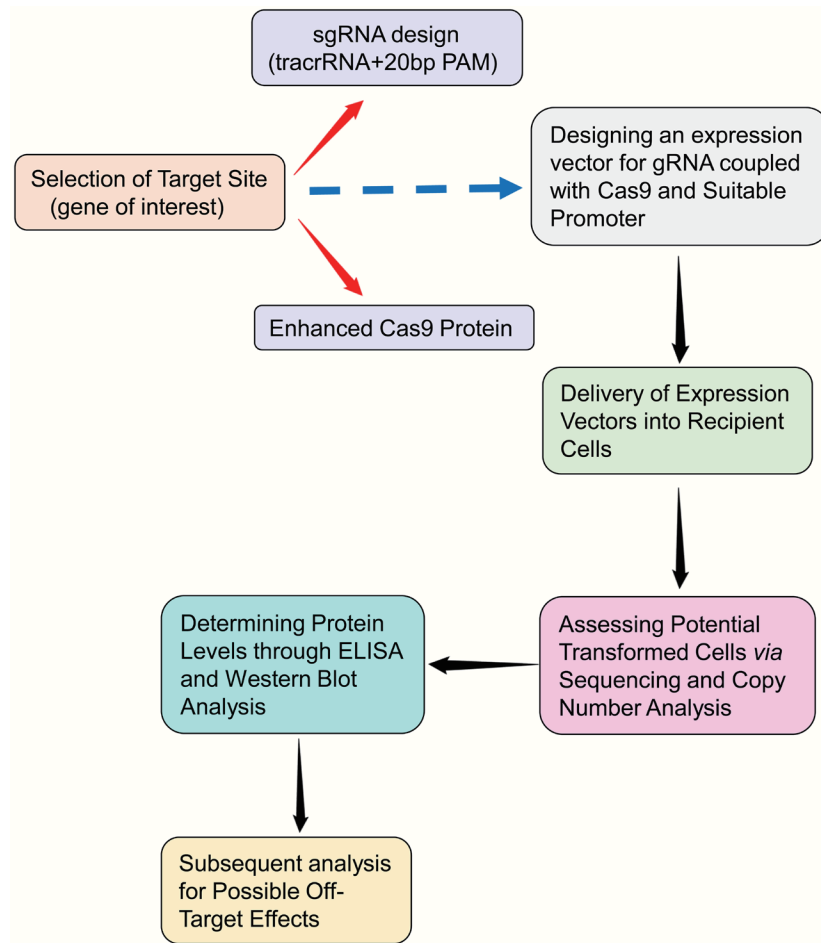


Fig. 3. Depiction of CRISPR/Cas9 mediated genome alteration in AD. AD, Alzheimer's disease; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9; ELISA: enzyme-linked immunosorbent assay; gRNA, guide RNA; PAM, protospacer adjacent motif; sgRNA, single guide RNA; tracrRNA, trans-activating CRISPR RNA.

sequence appears next in gene editing procedures. The initial protein used in gene editing, known as PAM, typically has 2–5 nucleotides and comprises PAM, nucleotide guanine guanine, or nucleotide adenine guanine nucleotides.¹⁹⁰ Synthetic guide RNAs are coupled with Cas9 nuclease forms to create complexes that can be directed to specific DNA locations.¹⁹¹ HD can be effectively treated using the CRISPR/Cas9 method, which modifies the *HTT* gene's encoding without permanently altering the genome (Fig. 4).^{192–194} Additionally, the *mHTT* genes responsible for the genetic changes linked to the CAG-expanded gene in patient-derived fibroblasts are inactivated using the CRISPR/Cas9 system. Thus, a total drop occurs in RNA and *mHTT* protein.¹⁹⁵ Therefore, applying CRISPR/Cas9 involves the precise targeting and editing of the mutant *HTT* gene, focusing on selectively modifying the expanded CAG repeat region. This intricate genetic intervention is designed to curtail the production of the deleterious mutant HTT protein, ultimately alleviating the neurodegenerative consequences linked to HD.

Researchers have investigated the potential of CRISPR/Cas9 to target the mutant *HTT* gene in HD using both *in vitro* and *in vivo* approaches. *In vitro* studies have used cell culture models such as human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) derived from HD patients. These cells were modified with CRISPR/Cas9 to disrupt the expanded CAG repeat region of

the mutant *HTT* gene. Researchers then analyzed the edited cells for reduced mutant *HTT* expression and changes in associated cellular characteristics.^{196–198}

Similarly, *in vivo* studies employ animal models such as mice harboring the human mutant *HTT* gene. Researchers directly introduced CRISPR components (gRNAs and Cas9) into specific tissues, often the brain, using delivery methods such as viral vectors. The goal was to edit the mutant *HTT* gene within the living organism. Subsequently, they assessed the edited mice for reduced mutant *HTT* expression and improvements in behavioral and neurodegenerative symptoms associated with HD.^{197,198}

In specific studies targeting the mutant *HTT* gene using CRISPR/Cas9, both *in vitro* and *in vivo* methodologies have been employed to assess the efficacy of mutant *HTT* editing. For instance, a study might use a viral vector to deliver CRISPR components selectively to neurons in the mouse brain, aiming to edit the mutant *HTT* gene and observe subsequent effects on disease progression.¹⁹⁷

CRISPR/Cas9 strategies for ALS

SOD1, *C9orf72*, *TAR DNA-binding protein 4*, and *RNA-binding protein fused in sarcoma* are the most widely investigated ALS genes. They are thought to be responsible for about 75% of famil-

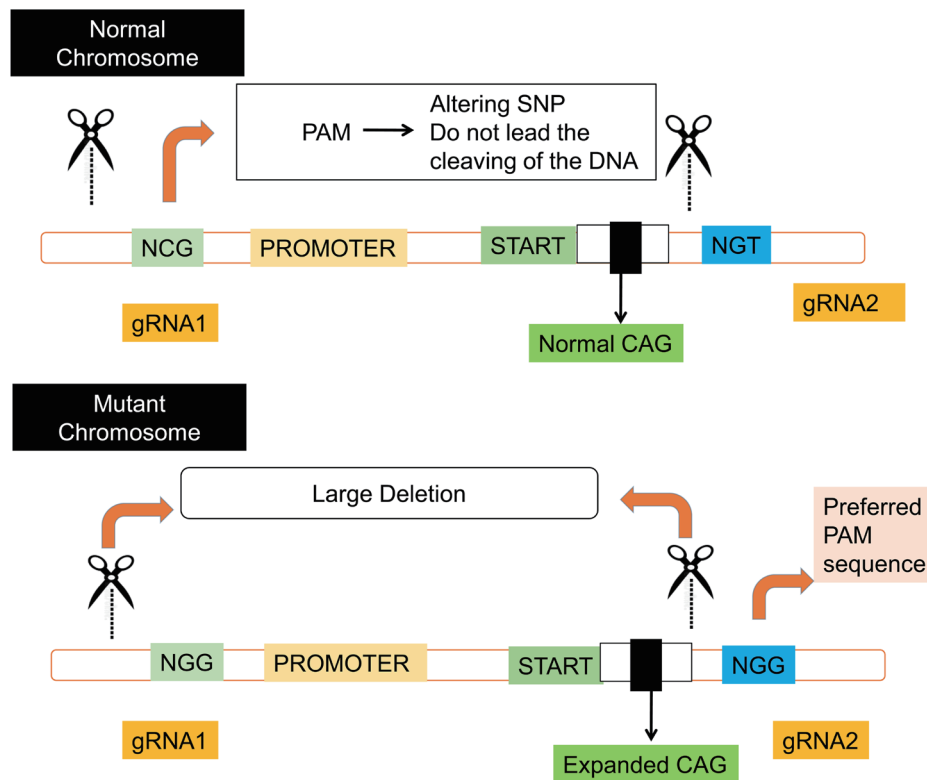


Fig. 4. Method of CRISPR/Cas9 mediated gene editing in HD.¹⁹³ CAG, a codon in DNA that codes for the amino acid glutamine or a trinucleotide repeat sequence found in certain genes; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9; gRNA, guide RNA; HD, Huntington's disease; NCG, nucleotide cytosine guanine; NGG, nucleotide guanine guanine; NGT, PAM, protospacer adjacent motif; SNP, single nucleotide polymorphism.

ial ALS cases.¹⁵⁹

To use Cas nuclease to target specific DNA sequences, sgRNA must be placed near PAM sequences. Common methods for introducing the Cas protein-coding gene and sgRNA plasmids into cells include electroporation and lipid-based transfection. Other techniques for delivering CRISPR/Cas9 include the formation of RNP complexes and their incorporation within lentiviruses or AAV, as discussed in references.¹⁹⁹⁻²⁰¹ DSBs in Genomic DNA are caused by Cas nuclease binding once they enter the nucleus and are repaired by the cell's repair machinery.²⁰² When a donor template is present, these DSBs are frequently fixed *via* non-homologous end joining NHEJ or HDR. NHEJ-generated insertions and deletions knock out a protein-encoding gene at a cleavage site, whereas HDR causes insertions or substitutes segments of DNA with a donor template.^{203,204} This approach raises potential safety issues, which could lead to harmful significant deletions, unfavorable chromosomal rearrangements, or unfavorable mutagenesis effects.²⁰⁵ To address this constraint and enhance the safety and versatility of the CRISPR/Cas9 system, a groundbreaking category of genome editing tools known as CRISPR single-base editors has emerged recently. By integrating a Cas9 nickase with nucleobase deaminases, CRISPR single-base editors can accurately and directly introduce point mutations into chromosomal DNA without creating double-strand breaks.²⁰⁶ So far, there are two primary categories of base editors that have been created: adenine base editors (ABEs), which facilitate A/T to G/C conversions, and cytosine base editors (CBEs), which enable the transformation of G/C base pairs into T/A base pairs.²⁰⁷

CRISPR/Cas9-based disease modeling stands out as a superior, more precise, efficient, and versatile method when contrasted with conventional disease modeling techniques (Fig. 5). This groundbreaking approach has brought about a profound transformation in genetic and disease research by empowering the development of exceptionally targeted and precise ALS disease models. Consequently, it has significantly advanced our comprehension of ALS disease mechanisms and the potential for therapeutic advancements.

Successful neurodegenerative disorder tests and their implications for treatment

Successful neurodegenerative disorder tests represent a cornerstone of modern healthcare, offering invaluable insights into disease pathology, prognosis, and treatment response. Their continued refinement and integration into clinical practice hold immense promise for improving outcomes and quality of life for individuals affected by these debilitating conditions. Table 2 provides instances of effective experiments in the treatment of neurodegenerative disorders and their significance.^{142,147,149,196,208-210}

CRISPR-mediated phenotypic and functional improvement

CRISPR-based therapies offer exciting potential for addressing a wide range of diseases where the underlying problem is protein aggregation, which is the clumping of misfolded proteins that cause cellular damage. This hallmark feature is characteristic of various neurodegenerative disorders, including AD and PD. In Alzheimer's, CRISPR might directly edit genes such as *APP* or *PSEN1*,

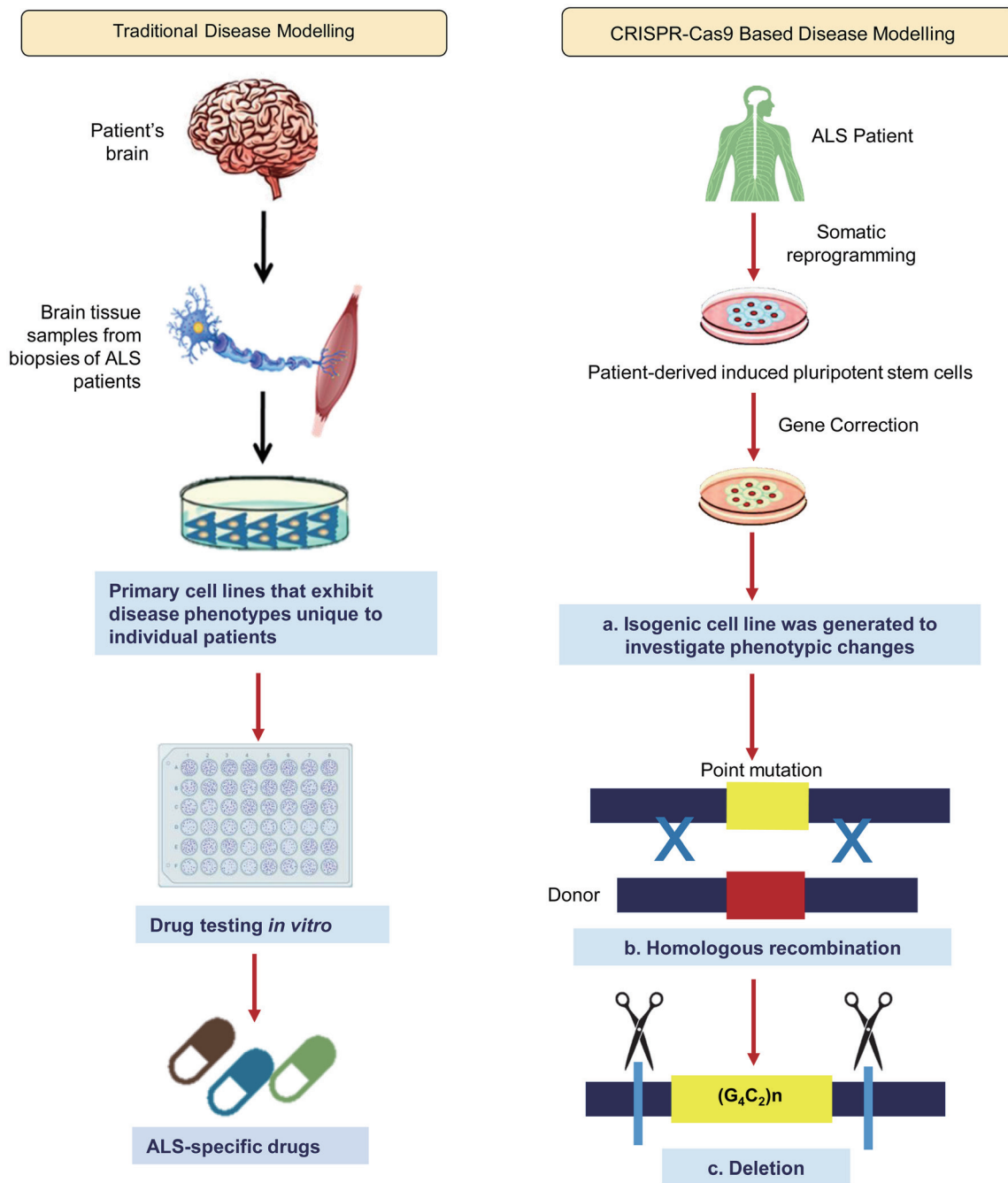


Fig. 5. Disease modeling using CRISPR/Cas9 versus conventional methods. ALS, amyotrophic lateral sclerosis; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9.

which are involved in forming A β plaques.²¹¹ CRISPR could potentially lessen plaque buildup by reducing the A β production or altering its aggregation properties. A similar approach could be applied to HD, where CRISPR could disrupt cellular pathways that promote the aggregation of the mutant HTT protein, potentially delaying the onset or reducing the severity of Huntington's symptoms.²¹²

The potential of CRISPR extends beyond neurodegenerative diseases. In transthyretin amyloidosis, CRISPR could boost genes

responsible for clearing misfolded transthyretin protein, enhancing cellular "housekeeping" and mitigating disease.²¹³ Even in prion diseases such as Creutzfeldt-Jakob disease, where normal prion proteins convert into a misfolded form, researchers are exploring using CRISPR to disrupt the genes involved in this conversion.²¹⁴ Additionally, while not strictly neurodegenerative, cystic fibrosis involves protein misfolding. Here, CRISPR-based therapies might correct mutations in the *CFTR* gene, restoring functional CFTR protein production, potentially leading to improved lung function

Table 2. Instances of effective experiments in the treatment of neurodegenerative disorders and outlining their significance

Neurodegenerative Disorder	Experimental Treatment	Key Findings/Implications	References
Alzheimer's disease	In animal models, targeting the <i>APOE4</i> gene variant, a risk factor for Alzheimer's, with CRISPR/Cas9 showed potential for reducing the risk or severity of the disease.	Insights into the role of genetics in Alzheimer's and the possibility of gene-editing to modify risk factors.	147,149
Parkinson's disease	Research on gene editing in animal models has shown the possibility of targeting genes such as <i>LRKK2</i> to reduce neurodegeneration in Parkinson's.	Exploration of gene-editing as a potential treatment strategy for PD.	208
Huntington's disease	Correction of the <i>HTT</i> gene mutation in animal models resulted in reduced disease symptoms and improved motor function.	Potential for gene-editing therapies to mitigate the effects of HD by targeting the causative mutation.	209,196
Amyotrophic lateral sclerosis	Preclinical studies demonstrated the potential to correct <i>SOD1</i> mutations in motor neurons, reducing disease progression in animal models.	Hope for gene-editing interventions in ALS therapy by addressing genetic mutations associated with the disease.	142,210

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; *APOE4*, apolipoprotein E4; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; HD, Huntington's disease; *HTT*, huntingtin; *LRKK2*, leucine-rich repeat kinase 2; PD, Parkinson's disease; *SOD1*, superoxide dismutase 1.

and addressing the underlying cause of the disease.

While extensive research is still needed, early studies highlight the potential of CRISPR-based therapies in reducing the adverse effects of protein aggregation. Mitigating this aggregation could improve cellular function, slow disease progression, and ultimately lead to better management of these complex diseases. Long-term studies are crucial to fully understand the impact of CRISPR on protein aggregation and its potential to transform treatment approaches.

Impacts of CRISPR-based therapy on disease phenotypes and protein aggregation

CRISPR-based therapies offer exciting potential for addressing diseases caused by protein aggregation, where misfolded proteins clump together and damage cells. This hallmark feature is observed in various neurodegenerative disorders, including AD and PD. CRISPR interventions could target this problem in several ways. For instance, in AD, CRISPR might directly edit genes such as *APP* or *PSEN1*, which are involved in forming A β plaques.²¹¹ Altering these genes could potentially reduce plaque buildup. Similarly, in HD, CRISPR could disrupt cellular pathways that promote the aggregation of the mutant HTT protein, slowing or preventing the formation of harmful protein clusters.²¹²

Beyond neurodegenerative diseases, CRISPR holds promise for other conditions involving protein misfolding. In transthyretin amyloidosis, CRISPR could boost genes responsible for clearing misfolded transthyretin protein, enhancing cellular "housekeeping" and mitigating disease.²¹³ Researchers are even exploring using CRISPR to disrupt the expression of genes that convert normal prion proteins into their misfolded form, potentially opening up new avenues for combating prion diseases such as Creutzfeldt-Jakob.²¹⁴ Additionally, while not primarily a neurodegenerative disease, cystic fibrosis involves protein misfolding; CRISPR-based therapies might correct mutations in the *CFTR* gene, restoring functional protein production.

While extensive research is still needed, early studies highlight the potential of CRISPR-based therapies in reducing the adverse effects of protein aggregation. Mitigating this aggregation could improve cellular function, slow disease progression, and ultimately lead to better management of these complex diseases. Long-term studies are crucial to fully understand the impact of CRISPR

on protein aggregation and its potential to transform treatment approaches.

Progression and breakthroughs in CRISPR technology

Although precision genetic engineering for gene repair or gene replacement therapy has trailed behind, CRISPR/Cas9 has found considerable application in the prior decade in loss-of-function mutations. Only a few research studies have successfully replaced genetic mutations with HDR using CRISPR/Cas9 *in vivo*.²¹⁵ *Ex vivo* applications in monogenic illnesses are increasingly frequent, although substantial on-target indels may appear, necessitating time-consuming and expensive quality control and comprehensive screening of edited cells.²¹⁵ Many studies investigate fusing different proteins and small molecules to Cas9 to address these constraints and favor HDR DNA repair over the more widespread NHEJ.²¹⁶ Thus, accurate genome editing using CRISPR/Cas9 necessitates high-efficiency HDR.²¹⁷ As a result, significant advances have been made to improve the efficiency of HDR, including cell cycle control, localization of regulatory proteins in HDR, epigenetic alteration, and local donor saturation, which can favor HDR in gene knock-in experiments employing Cas9 direct fusion variants.

HDR uses a donor DNA template to repair the DSB precisely.²¹⁸ Supplying an external repair template may introduce a broad range of precise genomic alterations, such as fixing pathogenic SNPs and specifically targeting deletions and inserting target gene sequences.^{219,220} HDR frequencies are frequently modest in mammalian cells compared to indel formation.²²¹ This process restricts its use and makes further enrichment procedures in mammalian cells impractical for therapeutic purposes. Although HDR may yield specific genomic modifications in the presence of donor templates, its poor efficiency compared to NHEJ and MMEJ repair remains an obstacle to genome editing applications.²²² Cas9-based approaches, such as base editing and prime editing, allow for targeted replacements and small insertions without needing a DSB (Fig. 6).²²³ These techniques, however, are confined to single-nucleotide alterations or insertions of less than 50 bases and have been linked to off-target RNA editing.²²⁴ As a result, HDR remains the most adaptable approach for targeted replacements and insertions. As a result, numerous approaches for increasing HDR frequency in PGE in mammalian cells have been developed. Many strategies

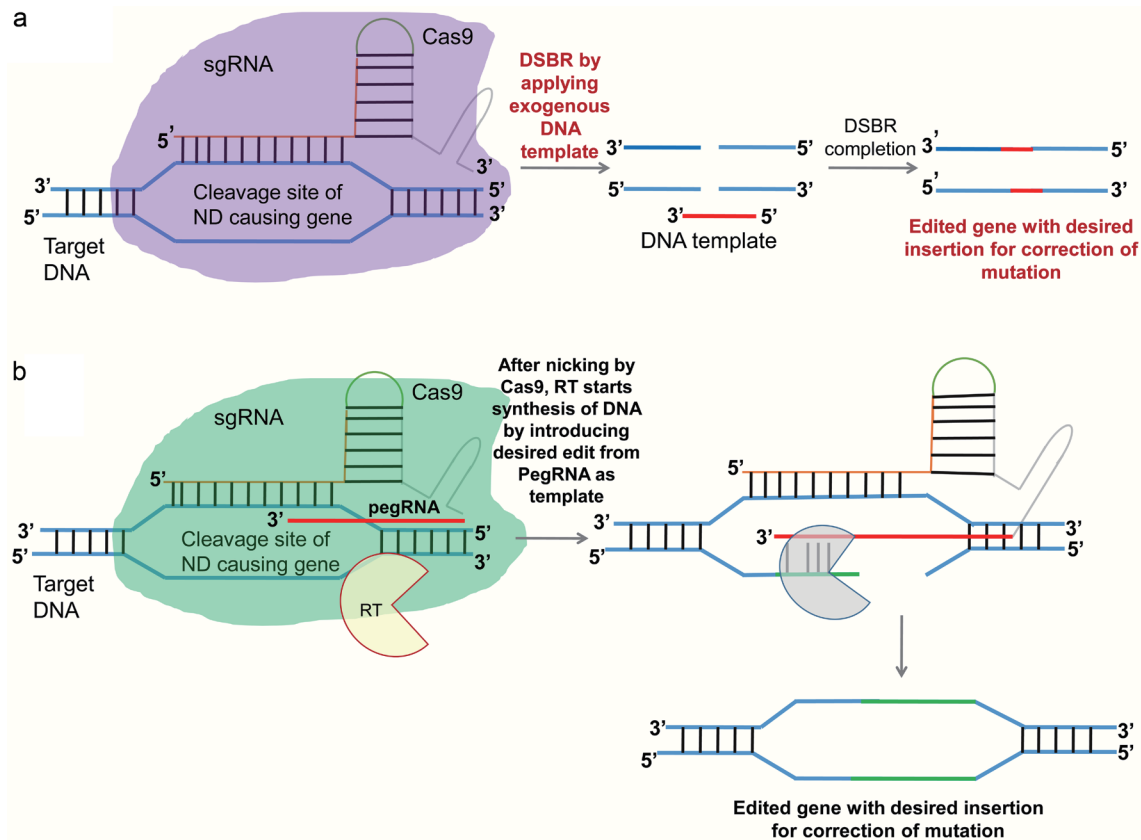


Fig. 6. General overview of the advanced CRISPR/Cas9 gene-editing tools. (a) Exogenous desired DNA template to introduce the desired nucleotide sequence in the correction of the neurodegenerative disease-causing gene. (b) The prime editor consists of Cas9 nickase, reverse transcriptase, and pegRNA (Prime editing guide RNA). Cas9, CRISPR-associated protein 9; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9; DSBR, double-strand break repair; ND, nucleotide deletion; PegRNA, prime editing guide RN; RT, reverse transcriptase or retrotranscriptase; sgRNA, single guide RNA.

have been employed to increase HDR frequencies, which utilize cellular manipulations to control cell cycle progression,²²⁵ inhibit NHEJ pathway,²²⁶ or upregulate HDR-related.²²⁷ Other methods use Cas9-protein fusions to covalently attach HDR ssDNA templates to Cas9, recruit HDR factors, change the epigenetic state, or temporally control Cas9 expression.²²⁸ These techniques improve HDR results but may harm genomic integrity and cell fitness. They frequently also demand complicated conjugation or exact timing.²²⁹

However, widespread uses of HDR are also restricted by its dependence on sister chromatids or exogenously supplied homologous template DNA segments.²²¹ A broad spectrum of organisms' genomes can now be edited using DSB-free CRISPR/Cas9-based editing methods such as Base editing and Prime editing.²³⁰

Base editors allow the conversion of a targeted base to another without requiring donor repair templates or DSB. Base editors let you convert a targeted base to another without using a donor repair template or DSB. The base editors are composed of an enzymatically defective Cas protein, usually called nCas9 (Cas9 nickase) or dCas9 (dead Cas9), and a cytidine or adenosine deaminase, which results in ABEs or CBEs, respectively. Whereas ABEs mediate the conversion of A to G by A-to-I deamination, CBEs produce C-to-T substitutions by C-to-U deamination and subsequent DNA replication.²³¹ Prime editing is the first genome-editing method that supports all 12 base-to-base conversions, insertions, and dele-

tions without DSBs or donor DNA. Its diverse editing capabilities can fix up to 89% of human genetic illnesses. PEs are made up of nCas9 (H840A) coupled with an engineered reverse transcriptase and a prime-editing guide RNA that specify the target site and encode the desired modification.²³² Although these are highly remarkable, due to some limitations such as low editing efficiency in both donor-dependent and donor-independent methods in therapy, there is still a need to develop more efficient solutions that are user-friendly, secure, and effective solutions.

Confronting the complexities of misfolded proteins in neurodegenerative disease management

The formation and progression of the neurodegenerative illnesses previously discussed are linked by the buildup of misfolded proteins and the resulting disruption of cellular functions. The specific proteins involved in each disease acquire aberrant conformations and do not fold properly, but many nuances remain to be figured out. These misfolded proteins frequently aggregate and build up in the brain, disrupting organelles' function (such as the mitochondria or the endoplasmic reticulum), impairing protein degradation pathways, or changing the distribution of cellular components, among other effects.²³³

Genetic mutations, environmental stress, post-translational alterations, chaperone failure, abnormalities in proteostasis, or structural changes are a few causes of protein misfolding.¹⁹ This pro-

Table 3. Drug-target pairs for neurodegenerative diseases associated with misfolded proteins

Compound name	Company	Disease indication	Mechanism of action	Status
TRx0237	TauRx Therapeutics	Alzheimer's disease	Tau aggregation inhibitor	Phase II clinical trials completed
AADvac1	Axon Neuroscience SE	Alzheimer's disease	Active tau-based immunotherapy	Phase I clinical trials completed
ACI-35	AC Immune AG	Alzheimer's disease	Phospho-tau vaccine	Phase I trial active
Arimoclomol	OrphazymeApS	Amyotrophic lateral sclerosis	HSP activation	Phase II/III active
Nuedexta	Avanir Pharmaceuticals	Amyotrophic lateral sclerosis and Parkinson's disease	Unknown for PD treatment; NMDA receptor antagonist	FDA approved
Deferiprone	Generic	Parkinson's disease	Iron chelator	Phase II recruiting
Istradefylline	Kyowa Hakko Kirin	Parkinson's disease	Adenosine A2A receptor antagonist	Approved in Japan; no FDA approval

AC Immune AG, analyst consultant immune aktiengesellschaft; ACI-35, AC Immune AG's vaccine for Alzheimer's disease; FDA, Food and Drug Administration; NMDA, N-Methyl-D-Aspartate.

cess may result in the earlier cascade of molecular events affecting health. Sometimes, changes to the proteins' coding genes can directly impact how the proteins fold. These modifications or mutations could affect the protein's structure or amino acid sequence, increasing the likelihood of misfolding.¹⁹ Examples are *HTT* or *presenilin* gene mutations, which cause HD and AD, respectively.

PD and HD are both neurodegenerative conditions that have in common an aging-dependent buildup of misfolded proteins and selective neurodegeneration. For instance, the clinical characteristic of PD in patient's brains is the presence of cytoplasmic misfolded proteins, known as Lewy bodies, which comprise ubiquitinated α -synuclein, *Parkin*, *syphilin*, and neurofilaments.²³⁴ On the other hand, aggregates or inclusions created by mutant-HTT within an enlarged polyQ tract can be found in the brains of HD patients.²³⁵ Using animal models has tremendously aided in researching neurological diseases and discovering therapeutic targets.²³⁶ The ability of CRISPR/Cas9 to specifically target any gene in one or two alleles of the embryonic genome opens up a new possibility for utilizing this cutting-edge technology to develop animal models of neurodegenerative diseases, as many neurodegenerative diseases can result from genetic DNA abnormalities.²³⁷ Developing large animal models of human diseases using conventional gene-targeting technology was difficult because few embryonic stem cell lines were available.²³⁸ Disease models in large animals are essential for analyzing the etiology and management of both neuropsychiatric illnesses.

For instance, CRISPR/Cas9-mediated mutations can resemble *Parkin* and *Pink1* gene knockouts because the loss of function of these genes can lead to PD.²³⁹ CRISPR/Cas9 generates the same muscular atrophy phenotype seen in patients by functionally disrupting the dystrophin gene in founder monkeys.²⁴⁰ *Parkin* or *Pink1* will completely disappear when both alleles are altered by CRISPR/Cas9, simulating the genetic changes seen in PD patients. Additionally, deleting the *Parkin* and *Pink1* genes will enable investigations of the synergistic impact of the loss of these crucial genes because CRISPR/Cas9 may target several genes in the same cells. In fact, by focusing on the *Parkin* and *Pink1* genes, CRISPR/Cas9 has been utilized to create pig models of PD. Tables 3 and 4 show several drug-target pairs for neurodegenerative diseases associated with misfolded proteins and ongoing gene therapy clinical trials for neurodegenerative disorders, respectively.

Challenges in CRISPR-mediated neurodegenerative disease research

CRISPR-Cas9 therapy for neurodegenerative diseases faces the hurdle of delivering the gene-editing tools to the brain safely and effectively. Researchers must ensure these components reach the specific brain regions the disease affects without damaging other areas. Additionally, minimizing the risk of off-target effects, where unintended changes occur elsewhere in the genome, is crucial for patient safety. Some of the challenges are discussed below.

Off-target effects

A major challenge in CRISPR/Cas9-based therapy is the comparatively limited effectiveness of HDR in contrast to NHEJ.²⁴¹ NHEJ excels in generating minor insertions or deletions (indels) for disrupting mutations.²⁴² Substantial endeavors have been directed toward augmenting the efficacy of HDR. Recent innovations involve an altered CRISPR/Cas9 system utilizing a mutated Cas9 domain to yield a CRISPR nickase.²⁰⁸ This adapted system has exhibited heightened accuracy and efficiency, resulting in a notable reduction in unintended off-target effects.²⁰⁴ One of the most intricate challenges revolves around reducing unintended effects when utilizing the CRISPR/Cas protein system. Numerous researchers have endeavored to create modified systems exhibiting heightened precision and more effective gene targeting, a pursuit notably evident in the extensive research being conducted. The phenomenon becomes even more imperative in clinical trials where precise drug targeting is paramount, yet the absence of fully perfected methods for precise drug delivery remains a notable limitation.²⁴³ As a result, extensive studies were conducted to elevate the efficiency and precision of the CRISPR/Cas protein system, focusing on prioritizing HDR over NHEJ.

Complex multigene interactions

Neurodegenerative diseases present a complex challenge due to their multifactorial nature. Often, these diseases involve mutations or variations in multiple genes,^{86,87} intricate genetic interactions,⁸⁸ and the influence of epigenetic factors and environmental triggers.^{19,54,55,74} To tackle this complexity, researchers are turning to sophisticated CRISPR technologies. Recent developments, such as multiplexed CRISPR systems, allow for the simultaneous targeting of several genes,⁹⁸ offering the potential to correct multiple

Table 4. Ongoing gene therapy clinical trials for neurodegenerative disorders (Clinicaltrials.gov.org)

Disorders	Trial code	Delivery route	Gene therapy	Phase
Alzheimer's disease	NCT00876863	Direct basal forebrain injection	AAV2-NGF	Phase II
Huntington's disease	NCT02519036	Intrathecal injection	ASOs to <i>HTT</i> messenger RNA	Phase III
	NCT03225833, NCT03225846	Intrathecal injection	ASOs to <i>HTT</i> mutant pre-messenger RNA	Phase I
Parkinson's disease	NCT03065192, NCT01793543	Intrapataminal injection	AAV2-AADC	Phase I
	NCT01621581	Intrapataminal injection	AAV2-GDNF	Phase I
	NCT02418598	Intrapataminal injection	AAV2-AADC	Phase II
	NCT00400634, NCT00985517	Intrapataminal injection	AAV2-neurturin	Phase II
	NCT00627588	Intrapataminal injection	Lentivirus-AADC	Phase I
	NCT00643890	Injection into the sub thalamic nucleus	AAV2-GAD	Phase II
Amyotrophic lateral sclerosis	NCT01041222	Intrathecal injection	ASOs to SOD1	Phase I

AADC, aromatic L-amino acid decarboxylase; AAV2, adeno-associated virus serotype 2; ASOs, antisense oligonucleotides; GDNF, glial cell line-derived neurotrophic factor; *HTT*, huntingtin; NCT, national clinical trial; NGF, nerve growth factor; SOD1, superoxide dismutase 1.

disease-causing mutations simultaneously. Additionally, CRISPR tools are being adapted to address epigenetic modifications alongside DNA changes,^{98,108} which could be critical in diseases where epigenetic dysregulation plays a role.^{54,55}

CRISPR may also be a powerful tool when used synergistically with other therapeutic strategies. For example, combining CRISPR-based gene correction with drug delivery could address genetic and environmental factors contributing to neurodegenerative diseases.¹⁰² Furthermore, animal and cellular disease models generated using CRISPR technology provide researchers with vital insights into the complex genetic interplay underlying these diseases.^{111,112}

Ethical and regulatory frameworks

Integrating CRISPR/Cas9 technology into preclinical or clinical trials is riddled with many ethical considerations. Chief among these ethical concerns are apprehensions regarding CRISPR technology's potential and technical constraints. The plausibility of unintended off-target effects, incomplete editing, and limited efficacy is a restraining factor for using CRISPR in clinical applications.²⁴⁴ There is also an element of uncertainty regarding the enduring impact on modified organisms and whether the corrections will be hereditary. Moreover, the application of CRISPR is hampered by the incomplete comprehension of genetic compositions and biological traits.²⁴⁵

Ethical guidelines and regulatory oversight

The potential of CRISPR technology to make heritable changes to the human genome raises profound ethical concerns, prompting ongoing efforts to establish clear guidelines and regulatory frameworks.^{5,9} Key concerns include ensuring equity and access to potential CRISPR-based therapies,⁵ establishing robust informed consent mechanisms,^{5,9} and fostering open dialogue between scientists, ethicists, and the public.^{4,5} These measures are crucial to address societal implications, build trust, and ensure the responsible use of this powerful technology.

Implementing conscientious and transparent practices is particularly important when considering germline editing, where the

potential for unintended long-term consequences and potential misuse must be carefully weighed.^{4,5,9} As CRISPR technology advances, it's imperative to address these ethical challenges alongside scientific progress.⁹ This process is essential for unlocking the potential of CRISPR in neurodegenerative disease treatment while ensuring its responsible and equitable application.

Safety and long-term monitoring

Ensuring safety and assessing long-term consequences present a challenge that necessitates extensive preclinical examination and sustained monitoring of treated individuals. Safety concerns may arise due to unintended off-target effects,^{114,115} immune responses,^{18,52} or unforeseen consequences of gene editing.¹⁰¹ Hence, it is critical to do comprehensive preclinical testing to detect and alleviate any hazards.^{118,120} Researchers must conduct comprehensive safety assessments of the medication in animal models before initiating human trials.^{111,120}

Long-term monitoring is crucial to assess the sustained efficacy of CRISPR-based therapies and to monitor for any delayed or unexpected side effects.^{101,102} Neuro-degenerative diseases are often chronic and progressive, and their treatments need to demonstrate lasting benefits.¹²² By continuously monitoring treated individuals over an extended period, we can gain valuable insights into the long-term effects and efficacy of CRISPR interventions.

Future perspectives in CRISPR-mediated neurodegenerative disease research

The CRISPR system encounters challenges when it comes to gene editing.²⁴¹ CRISPR/Cas9 holds significant promise, yet its recent identification and use in human applications impede its clinical study utility. The application of CRISPR/Cas in the context of neurodegenerative diseases confronts several formidable challenges, each requiring innovative strategies for resolution: Understanding CRISPR/Cas9 delivery mechanisms.

The foremost challenge in working with the CRISPR system is effectively delivering it to target cells.^{246,247} This is crucial for minimizing off-target effects and ensuring precise gene editing.

Various physical and viral delivery systems have been explored, including electroporation, nanoparticles, and lipid-mediated transfection. Physical methods are generally safer and less expensive but aren't always valuable for *in vivo* situations, where viral vectors are more common. While physical delivery is effective for creating knockout cell lines and animal models, its efficacy in *in vivo* applications can be relatively low. Viral delivery systems, particularly AAV and lentivirus, are recognized as highly efficient for introducing plasmid-based CRISPR/Cas9 into mammalian cells, both *in vitro* and *in vivo*.²⁴⁸⁻²⁵⁰ AAVs are known for their non-pathogenic and low immunogenic properties and their ability to infect various cell types. However, AAVs have size limitations.^{247,251} Dual AAVs have been explored to deliver Cas-9 encoding DNA and sgRNA separately to address the issue.^{247,252,253} Lentivirus-mediated CRISPR/Cas9 offers high infection efficiency even in non-dividing cells,^{254,255} making it ideal for gene modification in tissues such as the liver and brain.

Introducing CRISPR components into the brain for neurodegenerative disease treatment carries the risk of triggering immune responses, potentially leading to inflammation and unintended consequences that could impact the safety and efficacy of the therapy.²⁵⁶ Understanding and managing these immune responses is crucial for successfully developing CRISPR-based interventions. Researchers are actively investigating the immune system's innate (non-specific) and adaptive (specific) arms to address these challenges. The innate immune system might recognize elements such as viral vectors or the bacterial Cas9 protein as foreign,^{18,91} while the adaptive immune system could develop antibodies specifically targeting Cas9, limiting the effectiveness of repeated treatments.²

To mitigate these risks, researchers are exploring several strategies. These include modifying delivery vehicles to be less visible to the immune system (e.g., using nanoparticles or exosomes),¹⁰⁴ ensuring only transient expression of Cas9 to minimize prolonged exposure,¹²⁰ and potentially co-administering targeted immunosuppressants to dampen the immune response temporarily.⁹¹ Extensive preclinical testing in animal models is essential to evaluate how different CRISPR systems interact with the immune system, allowing for the refinement of these mitigation strategies.¹¹¹ As these approaches move into clinical trials, careful immune monitoring of patients will be paramount to ensure the continued optimization and safe application of CRISPR-based therapies for neurodegenerative diseases.¹⁰¹

Improving patient-specific approaches

Neurodegenerative disorders often manifest genetic diversity among patients, and the genetic anomalies contributing to these conditions can vary from individual to individual.^{126,130} Consequently, a uniform treatment approach may not be as effective in addressing the heterogeneous genetic factors that underlie these diseases.¹²⁹ Patient-specific strategies entail thoroughly examining the patient's unique genetic mutations and developing a CRISPR-based therapy that precisely targets and corrects these specific mutations.^{127,131} This personalized approach has the potential to yield more tailored and potentially more efficient treatments for each patient.^{128,132} Furthermore, it can reduce the risk of unintended genetic alterations and enhance the overall safety and effectiveness of the CRISPR-based treatment.¹²⁷

Hence, it is critical to conduct comprehensive preclinical testing to detect and alleviate any hazards. Researchers must conduct comprehensive safety assessments of the medication in animal models before initiating human trials.^{111,120} By customizing the treatment in this manner, researchers aspire to directly confront the

fundamental genetic factors behind neurodegenerative diseases, offering individualized care that could lead to improved treatment outcomes.^{128,132}

However, it's crucial to acknowledge that implementing patient-specific approaches in clinical settings demands comprehensive genetic analysis and the development of tailored treatment plans, which can be intricate and resource-intensive.^{129,131}

A primary focus in CRISPR research lies in continuously enhancing its precision and minimizing the potential for off-target DNA modifications. To achieve these goals, several innovative strategies are being explored. Researchers are engineering Cas9 variants with heightened fidelity, incorporating mutations that refine their DNA-binding and cutting mechanisms for reduced off-target effects.²⁵⁷ Additionally, the remarkable diversity of naturally occurring CRISPR systems offers potential alternative Cas enzymes, some of which demonstrate superior specificity compared to the commonly used Cas9.²⁵⁸ Furthermore, the mode of CRISPR/Cas9 delivery can influence precision. Direct delivery of pre-assembled Cas9 protein and guide RNA complexes, known as RNP delivery, has shown the potential to reduce off-target edits compared to traditional DNA-based delivery methods.²⁵⁹ These cutting-edge advancements offer the promise of CRISPR systems with exceptional accuracy, enabling safer and more effective therapeutic applications in the future.

Enhancing delivery modalities

One of the most significant hurdles in developing CRISPR-based therapies for neurodegenerative diseases lies in the safe and effective delivery of gene-editing components to the affected brain regions. The blood-brain barrier (BBB), a highly selective barrier designed to protect the brain from toxins and pathogens, poses a particular challenge for drug delivery. Researchers are actively developing specialized delivery modalities to overcome this obstacle, including nanoparticles, viral vectors, and alternative systems. Nanoparticles, such as lipid-based or gold nanoparticles, can encapsulate CRISPR components and be modified with molecules to help them cross the BBB.^{104,133} Researchers are also exploring how to use receptors naturally found on the BBB to shuttle in CRISPR-carrying nanoparticles or viral vectors and even investigating focused ultrasound to disrupt the BBB and enhance the delivery of therapeutics temporarily.¹⁹

Viral vectors, particularly AAVs, are often used for gene therapies, and researchers are optimizing them for CRISPR delivery. The focus is on identifying AAV serotypes best suited for crossing the BBB and targeting specific cell types within the brain.^{134,135} Beyond these traditional approaches, innovative strategies are constantly emerging. These include using exosomes (naturally occurring extracellular vesicles) and cell-penetrating peptides, which may offer unique advantages for delivering CRISPR components to the brain.¹⁰⁴ Continued research into these diverse delivery modalities will be crucial to refining the precision, safety, and efficacy of CRISPR-based therapies for neurodegenerative diseases.¹⁰⁴

Advances in base editing

Traditional CRISPR-Cas9 systems, while powerful, introduce DSBs in DNA that can lead to unintended mutations as the cell attempts to repair the damage.^{114,115} To address this limitation, advancements such as base editing with CBEs and ABEs offer a more precise alternative, allowing for the direct conversion of one specific DNA base to another without creating DSBs.^{98,102} This process significantly reduces the risk of off-target effects.⁹⁸

Building upon this precision, prime editing takes gene editing

even further by using a modified Cas9 enzyme fused to reverse transcriptase and a specially designed guide RNA.^{98,119} This system can directly “write” new genetic information into a specified DNA location, enabling small insertions, deletions, and all possible base-to-base conversions with unprecedented control.^{98,119}

Base editing and prime editing hold immense promise for neurodegenerative diseases, where even single-point mutations can have devastating consequences.¹²² Their ability to precisely correct disease-causing mutations without the risks associated with traditional CRISPR/Cas9 could revolutionize treatment approaches.^{122,124} While still in their early stages, these technologies are rapidly evolving, with researchers focusing on improving their efficiency, reducing off-target effects even further, and developing effective delivery methods for the brain.^{102,125} These advancements have the potential to usher in a new era of safer and more targeted therapies for neurodegenerative diseases.

Implications and insights

Protein misfolding is a key feature in numerous diseases, including neurodegenerative disorders such as Alzheimer’s, Parkinson’s, and Huntington’s. CRISPR/Cas9 gene editing presents a revolutionary approach to potentially address this issue by directly targeting underlying genetic mutations or modulating the pathways involved in protein folding and cellular responses to misfolded proteins. Despite challenges in efficient delivery to affected tissues and minimizing off-target effects, advancements in CRISPR technology are rapidly progressing.²⁶⁰ Understanding the complex biological networks involved in misfolding diseases is essential for optimal target selection. While ethical considerations are necessary, ongoing research demonstrates the immense potential of CRISPR/Cas9 to address protein misfolding, with promising results in cell culture and animal models highlighting its potential to transform the treatment of these diseases.

Major findings

Protein misfolding is central to numerous neurodegenerative diseases, making it a prime target for therapeutic intervention. The CRISPR/Cas9 gene editing system offers a revolutionary approach to modifying genes responsible for these misfolded proteins directly. This system uses a gRNA to target a specific DNA sequence. The Cas9 enzyme creates a double-stranded break, enabling precise gene modification.²⁶⁰ Researchers have successfully applied CRISPR/Cas9 to target genes implicated in neurodegenerative diseases in cell cultures. For instance, a study demonstrated a reduction in *α-synuclein* aggregates, a hallmark of PD, after correcting a mutation in the SNCA gene. While these successes are promising, challenges such as potential off-target effects and efficient delivery of CRISPR/Cas9 components to affected cells remain active research areas.^{114,115,121}

Importantly, studies demonstrate that CRISPR/Cas9 gene editing can reduce misfolded protein levels, as assessed by techniques such as immunostaining and western blotting.²⁶¹ Furthermore, correcting misfolded proteins using CRISPR/Cas9 has been linked to improvements in various cellular health markers. These improvements include increased cell viability, decreased indicators of cellular stress, and restored mitochondrial function, which are frequently disrupted in neurodegenerative diseases.²⁶²

However, recent findings suggest that cells might sometimes respond to CRISPR/Cas9 editing by activating compensatory mechanisms (Fig. 7). A recent study reported that editing a gene implicated in AD triggered the upregulation of a related protein,

partially offsetting the intended therapeutic benefit.²⁶³ These compensatory mechanisms illuminate the interconnected nature of biological networks involved in neurodegenerative diseases. This emphasizes that a multi-target CRISPR/Cas9 editing approach could provide a more comprehensive and lasting therapeutic solution, focusing on multiple points in the protein misfolding pathway.

Connections to recent work

The transformative reach of CRISPR/Cas9 gene editing extends far beyond neurodegenerative diseases. Significant advances in correcting disease-related gene mutations have been reported across numerous conditions. These successes include promising breakthroughs in treating sickle cell disease and beta-thalassemia, where CRISPR/Cas9 has been used to modify genes involved in haemoglobin production.^{264,265} Furthermore, researchers have successfully employed CRISPR/Cas9 to address cystic fibrosis by targeting the mutated *CFTR* gene and restoring its function in cell models.²⁶⁶

While the direct use of CRISPR-Cas9 to target protein misfolding is still a developing field, recent studies demonstrate its growing potential. For example, a study successfully reduced the aggregation-prone mutant huntingtin protein, a key target in HD, using CRISPR/Cas9.^{77,212} This work aligns with these pioneering efforts by focusing on the fundamental problem of protein misfolding. Importantly, these findings on compensatory mechanisms raise crucial considerations for refining future CRISPR-based therapies.

Interestingly, evidence of compensatory mechanisms in response to CRISPR-mediated gene editing is also emerging in other disease areas. In Duchenne muscular dystrophy, studies have reported that the CRISPR-based correction of the dystrophin gene could trigger the upregulation of a related protein, utrophin.²⁶⁷ This potentially influences therapeutic outcomes. While the in-depth study of such mechanisms in the context of CRISPR editing is ongoing, these studies highlight the vital significance of neurodegenerative diseases. This process emphasizes the need for further research to fully understand the broader biological consequences of CRISPR editing, ultimately enabling the design of therapies that anticipate and effectively manage these cellular adaptations.

Limitations

While the successful use of CRISPR-Cas9 in correcting disease-related gene mutations is demonstrated in cell cultures across conditions such as sickle cell disease, beta-thalassemia, and cystic fibrosis,^{264–266} translating these findings into safe and effective human treatments presents several challenges. It’s important to acknowledge that cell cultures represent a simplified environment and cannot fully replicate the complexity of a living organism. Recent studies, including work on HD,^{77,212} suggest the potential of CRISPR-Cas9 to target protein misfolding directly. However, further research is needed to address crucial concerns such as the potential for off-target edits, where unintended DNA sequences might be modified. Rigorous safety validation and ongoing refinements of the CRISPR/Cas9 system are essential to mitigate the risk of these off-target effects. Recent studies contribute to these efforts by highlighting compensatory mechanisms following CRISPR-based intervention, as observed in other diseases such as Duchenne muscular dystrophy.²⁶⁷ Understanding such cellular responses is vital for developing therapies that anticipate and effectively manage potential adaptations, ultimately advancing the promise of CRISPR/Cas9 toward clinical applications.

Future directions

Recent findings on compensatory mechanisms highlight the need



Fig. 7. Exploring the role of CRISPR advancements in correcting protein misfolding in neurodegenerative diseases. APOE, apolipoprotein E; APP, amyloid precursor protein; BACE 1, beta-secretase 1; C9orf72, chromosome 9 open reading frame 72; CAG, cytosine-adenine-guanine repeat; CRISPR, clustered regularly interspaced short palindromic repeats; DNAJC6, DnaJ heat shock protein family (Hsp40) member C6; HTT, huntingtin; SOD1, superoxide dismutase 1; Vps35 D620N, vacuolar protein sorting 35 D620N mutation.

for multi-target CRISPR/Cas9 strategies in neurodegenerative diseases.^{60,65,86} Future studies should explore the benefits of simultaneously editing multiple genes involved in the misfolding pathway to achieve more effective and long-lasting therapeutic effects.^{98,129} This approach can potentially counteract compensatory mechanisms and provide more comprehensive treatment.¹⁰²

A significant hurdle in realizing the potential of CRISPR/Cas9 for these diseases lies in developing safe and efficient methods to deliver the editing components to the brain.^{104,133} Advances in delivery strategies, such as viral vectors or nanoparticle formulations,^{104,134,135} are crucial for overcoming the blood-brain barrier and achieving targeted delivery to affected neurons.

Despite these challenges, CRISPR/Cas9 gene editing holds revolutionary potential for treating neurodegenerative diseases.^{122,123}

By meticulously investigating compensatory mechanisms, continuously refining the technology, and pushing the boundaries of targeted delivery, CRISPR-based therapies offer the exciting prospect of addressing the root cause of these disorders.¹²⁴

Conclusions

The intersection of CRISPR/Cas9 technology and the immense challenges posed by protein misfolding in neurodegenerative disorders represents a pivotal turning point in biomedical research. This comprehensive review has delved into the intricate web connecting protein misfolding, neurodegeneration, and the potential of CRISPR-based interventions. We've explored the mechanisms of protein folding and the devastating consequences of misfolding,

establishing a foundation for understanding how CRISPR/Cas9 might offer new therapeutic avenues.

CRISPR/Cas9 has evolved from its origins in bacterial immunity to become a remarkably precise tool for genome editing. This review meticulously outlines its mechanisms, emphasizing its ability to target the genetic roots of protein misfolding. Our exploration of neurodegenerative diseases, including AD, PD, ALS, and HD, underscores CRISPR's potential to address various disease-causing mutations.

Crucially, this review highlights the translational potential of CRISPR/Cas9. Successful case studies demonstrate its promise in correcting protein misfolding, offering a path toward personalized medicine tailored to individual patients' genetic profiles. This process opens a new era of precise, targeted therapies.

While CRISPR represents remarkable progress, challenges and ethical considerations remain. This review emphasizes ongoing research on enhancing CRISPR safety, refining targeting, mitigating off-target effects, and addressing immune responses. The imperative for ethical frameworks to guide the responsible use of this powerful technology is also stressed.

This review spotlights the transformative role of CRISPR in addressing protein misfolding, a central driver of neurodegenerative disease. Synthesizing current knowledge and outlining future directions provides a roadmap for tackling these devastating illnesses. By expanding our understanding of delivery mechanisms, base editing, and patient-specific approaches, along with continued ethical vigilance, we can harness the full potential of CRISPR/Cas9. This research ultimately offers a beacon of hope, moving us closer to treatments capable of changing the lives of those affected by these life-altering conditions.

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Conflict of interest

The authors have no conflict of interest related to this publication.

Author contributions

Study concept and design (KY, AY, AA, RA), acquisition of data (AA, AY, DK), analysis and interpretation of data (AA, AY, KY, DK), drafting of the manuscript (AA, DK, AY), critical revision of the manuscript for important intellectual content (AY, KY, RA), administrative, technical, or material support (AY, KY), and study supervision (AY, KY, AA, RA). All authors have made a significant contribution to this study and have approved the final manuscript.

References

[1] Yang F, Liu C, Chen D, Tu M, Xie H, Sun H, *et al*. CRISPR/Cas9-loxP-Mediated Gene Editing as a Novel Site-Specific Genetic Manipula-

tion Tool. *Mol Ther Nucleic Acids* 2017;7:378–386. doi:10.1016/j.omtn.2017.04.018, PMID:28624213.

- [2] Simhadri VL, McGill J, McMahon S, Wang J, Jiang H, Sauna ZE. Prevalence of Pre-existing Antibodies to CRISPR-Associated Nuclease Cas9 in the USA Population. *Mol Ther Methods Clin Dev* 2018;10:105–112. doi:10.1016/j.omtm.2018.06.006, PMID:30073181.
- [3] Wada N, Ueta R, Osakabe Y, Osakabe K. Precision genome editing in plants: state-of-the-art in CRISPR/Cas9-based genome engineering. *BMC Plant Biol* 2020;20(1):234. doi:10.1186/s12870-020-02385-5, PMID:32450802.
- [4] Akinci E, Hamilton MC, Khowpinitchai B, Sherwood RI. Using CRISPR to understand and manipulate gene regulation. *Development* 2021;148(9):dev182667. doi:10.1242/dev.182667, PMID:33913466.
- [5] Nollen EA, Garcia SM, van Haaften G, Kim S, Chavez A, Morimoto RI, *et al*. Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. *Proc Natl Acad Sci U S A* 2004;101(17):6403–6408. doi:10.1073/pnas.0307697101, PMID:15084750.
- [6] Soto C, Estrada LD. Protein misfolding and neurodegeneration. *Arch Neurol* 2008;65(2):184–189. doi:10.1001/archneurol.2007.56, PMID:18268186.
- [7] Morales R, Estrada LD, Diaz-Espinoza R, Morales-Scheihing D, Jara MC, Castilla J, *et al*. Molecular cross talk between misfolded proteins in animal models of Alzheimer's and prion diseases. *J Neurosci* 2010;30(13):4528–4535. doi:10.1523/JNEUROSCI.5924-09.2010, PMID:20357103.
- [8] Moreno-Gonzalez I, Soto C. Misfolded protein aggregates: mechanisms, structures and potential for disease transmission. *Semin Cell Dev Biol* 2011;22(5):482–487. doi:10.1016/j.semcdb.2011.04.002, PMID:21571086.
- [9] Morales R, Moreno-Gonzalez I, Soto C. Cross-seeding of misfolded proteins: implications for etiology and pathogenesis of protein misfolding diseases. *PLoS Pathog* 2013;9(9):e1003537. doi:10.1371/journal.ppat.1003537, PMID:24068917.
- [10] Shorter J. Designer protein disaggregases to counter neurodegenerative disease. *Curr Opin Genet Dev* 2017;44:1–8. doi:10.1016/j.gde.2017.01.008, PMID:28208059.
- [11] Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci* 2014;7:42. doi:10.3389/fnmol.2014.00042, PMID:24860424.
- [12] Jackrel ME, Shorter J. Protein-Remodeling Factors As Potential Therapeutics for Neurodegenerative Disease. *Front Neurosci* 2017;11:99. doi:10.3389/fnins.2017.00099, PMID:28293166.
- [13] Chaudhuri TK, Paul S. Protein-misfolding diseases and chaperone-based therapeutic approaches. *FEBS J* 2006;273(7):1331–1349. doi:10.1111/j.1742-4658.2006.05181.x, PMID:16689923.
- [14] Duennwald ML, Jagadish S, Muchowski PJ, Lindquist S. Flanking sequences profoundly alter polyglutamine toxicity in yeast. *Proc Natl Acad Sci U S A* 2006;103(29):11045–11050. doi:10.1073/pnas.0604547103, PMID:16832050.
- [15] Kampinga HH, Mayer MP, Mogk A. Protein quality control: from mechanism to disease : EMBO Workshop, Costa de la Calma (Mallorca), Spain, April 28 - May 03, 2019. *Cell Stress Chaperones* 2019;24(6):1013–1026. doi:10.1007/s12192-019-01040-9, PMID:31713048.
- [16] Balchin D, Hayer-Hartl M, Hartl FU. In vivo aspects of protein folding and quality control. *Science* 2016;353(6294):aac4354. doi:10.1126/science.aac4354, PMID:27365453.
- [17] Ajmal MR. Protein Misfolding and Aggregation in Proteinopathies: Causes, Mechanism and Cellular Response. *Diseases* 2023;11(1):30. doi:10.3390/diseases11010030, PMID:36810544.
- [18] Matus S, Glimcher LH, Hetz C. Protein folding stress in neurodegenerative diseases: a glimpse into the ER. *Curr Opin Cell Biol* 2011;23(2):239–252. doi:10.1016/j.ceb.2011.01.003, PMID:21288706.
- [19] Majerova P, Michalicova A, Cente M, Hanes J, Vegh J, Kittel A, *et al*. Trafficking of immune cells across the blood-brain barrier is modulated by neurofibrillary pathology in tauopathies. *PLoS One* 2019;14(5):e0217216. doi:10.1371/journal.pone.0217216, PMID:31120951.
- [20] Sharma K, Pradhan S, Duffy LK, Yeasmin S, Bhattarai N, Schulte MK. Role of Receptors in Relation to Plaques and Tangles in Alzheimer's Disease Pathology. *Int J Mol Sci* 2021;22(23):12987. doi:10.3390/

- ijms222312987, PMID:34884789.
- [21] Vidović M, Rikalovic MG. Alpha-Synuclein Aggregation Pathway in Parkinson's Disease: Current Status and Novel Therapeutic Approaches. *Cells* 2022;11(11):1732. doi:10.3390/cells11111732, PMID:35681426.
 - [22] Gomes CM, Faisca PFN. Protein Folding: An Introduction. Cham: Springer 2019;doi:10.1007/978-3-319-00882-0.
 - [23] Khan P, Parkash A, Islam A, Ahmad F, Hassan MI. Molecular basis of the structural stability of hemochromatosis factor E: A combined molecular dynamic simulation and GdmCl-induced denaturation study. *Biopolymers* 2016;105(3):133–142. doi:10.1002/bip.22760, PMID:26537310.
 - [24] Naz H, Shahbaaz M, Bisetty K, Islam A, Ahmad F, Hassan MI. Effect of pH on the structure, function, and stability of human calcium/calmodulin-dependent protein kinase IV: combined spectroscopic and MD simulation studies. *Biochem Cell Biol* 2016;94(3):221–228. doi:10.1139/bcb-2015-0132, PMID:27032767.
 - [25] Shakhnovich E. Protein folding thermodynamics and dynamics: where physics, chemistry, and biology meet. *Chem Rev* 2006;106(5):1559–1588. doi:10.1021/cr040425u, PMID:16683745.
 - [26] Yadav K, Yadav A, Vashistha P, Pandey VP, Dwivedi UN. Protein Misfolding Diseases and Therapeutic Approaches. *Curr Protein Pept Sci* 2019;20(12):1226–1245. doi:10.2174/1389203720666190610092840, PMID:31187709.
 - [27] Ahmad A, Uversky VN, Khan RH. Aberrant liquid-liquid phase separation and amyloid aggregation of proteins related to neurodegenerative diseases. *Int J Biol Macromol* 2022;220:703–720. doi:10.1016/j.ijbiomac.2022.08.132, PMID:35998851.
 - [28] Owen MC, Gnutt D, Gao M, Wärmländer SKTS, Jarvet J, Gräslund A, *et al*. Effects of in vivo conditions on amyloid aggregation. *Chem Soc Rev* 2019;48(14):3946–3996. doi:10.1039/c8cs00034d, PMID:31192324.
 - [29] Jaenicke R. Protein folding: local structures, domains, subunits, and assemblies. *Biochemistry* 1991;30(13):3147–3161. doi:10.1021/bi00227a001, PMID:2009257.
 - [30] Lane TJ, Schwantes CR, Beauchamp KA, Pande VS. Probing the origins of two-state folding. *J Chem Phys* 2013;139(14):145104. doi:10.1063/1.4823502, PMID:24116650.
 - [31] Malhotra P, Udgaonkar JB. How cooperative are protein folding and unfolding transitions? *Protein Sci* 2016;25(11):1924–1941. doi:10.1002/pro.3015, PMID:27522064.
 - [32] Matthews CR. Pathways of protein folding. *Annu Rev Biochem* 1993;62:653–683. doi:10.1146/annurev.bi.62.070193.003253, PMID:8352599.
 - [33] Englander SW, Mayne L. The nature of protein folding pathways. *Proc Natl Acad Sci U S A* 2014;111(45):15873–15880. doi:10.1073/pnas.1411798111, PMID:25326421.
 - [34] Childers MC, Daggett V. Insights from molecular dynamics simulations for computational protein design. *Mol Syst Des Eng* 2017;2(1):9–33. doi:10.1039/C6ME00083E, PMID:28239489.
 - [35] Saibil H. Chaperone machines for protein folding, unfolding and disaggregation. *Nat Rev Mol Cell Biol* 2013;14(10):630–642. doi:10.1038/nrm3658, PMID:24026055.
 - [36] Veitshans T, Klimov D, Thirumalai D. Protein folding kinetics: time-scales, pathways and energy landscapes in terms of sequence-dependent properties. *Fold Des* 1997;2(1):1–22. doi:10.1016/S1359-0278(97)00002-3, PMID:9080195.
 - [37] Sweeney P, Park H, Baumann M, Dunlop J, Frydman J, Kopito R, *et al*. Protein misfolding in neurodegenerative diseases: implications and strategies. *TranslNeurodegener* 2017;6:6. doi:10.1186/s40035-017-0077-5, PMID:28293421.
 - [38] Fink AL. Protein aggregation: folding aggregates, inclusion bodies and amyloid. *Fold Des* 1998;3(1):R9–23. doi:10.1016/S1359-0278(98)00002-9, PMID:9502314.
 - [39] Bolshette NB, Thakur NK, Bidkar AP, Trandafir C, Kumar P, Gogoi R. Protein folding and misfolding in the neurodegenerative disorders: a review. *Rev Neurol (Paris)* 2014;170(3):151–161. doi:10.1016/j.neurol.2013.11.002, PMID:24613386.
 - [40] Dahl JU, Gray MJ, Jakob U. Protein quality control under oxidative stress conditions. *J Mol Biol* 2015;427(7):1549–1563. doi:10.1016/j.jmb.2015.02.014, PMID:25698115.
 - [41] Wang Y, Le WD. Autophagy and Ubiquitin-Proteasome System. *Adv Exp Med Biol* 2019;1206:527–550. doi:10.1007/978-981-15-0602-4_25, PMID:31777002.
 - [42] Naidu G, Nagar N, Poluri KM. Mechanistic Insights into Cellular and Molecular Basis of Protein-Nanoplastic Interactions. *Small* 2024;20(5):e2305094. doi:10.1002/sml.202305094, PMID:37786309.
 - [43] Rochet JC, Lansbury PT Jr. Amyloid fibrillogenesis: themes and variations. *Curr Opin Struct Biol* 2000;10(1):60–68. doi:10.1016/s0959-440x(99)00049-4, PMID:10679462.
 - [44] Almeida ZL, Brito RMM. Structure and Aggregation Mechanisms in Amyloids. *Molecules* 2020;25(5):1195. doi:10.3390/molecules25051195, PMID:32155822.
 - [45] Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 2006;75:333–366. doi:10.1146/annurev.biochem.75.101304.123901, PMID:16756495.
 - [46] Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, *et al*. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 2003;300(5618):486–489. doi:10.1126/science.1079469, PMID:12702875.
 - [47] Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci* 2003;4(1):49–60. doi:10.1038/nrn1007, PMID:12511861.
 - [48] Khan AN, Khan RH. Protein misfolding and related human diseases: A comprehensive review of toxicity, proteins involved, and current therapeutic strategies. *Int J Biol Macromol* 2022;223(Pt A):143–160. doi:10.1016/j.ijbiomac.2022.11.031, PMID:36356861.
 - [49] Cuanalo-Contreras K, Mukherjee A, Soto C. Role of protein misfolding and proteostasis deficiency in protein misfolding diseases and aging. *Int J Cell Biol* 2013;2013:638083. doi:10.1155/2013/638083, PMID:24348562.
 - [50] Ghiglieri V, Calabrese V, Calabresi P. Alpha-Synuclein: From Early Synaptic Dysfunction to Neurodegeneration. *Front Neurol* 2018;9:295. doi:10.3389/fneur.2018.00295, PMID:29780350.
 - [51] Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J Mol Med (Berl)* 2003;81(11):678–699. doi:10.1007/s00109-003-0464-5, PMID:12942175.
 - [52] Briston T, Hicks AR. Mitochondrial dysfunction and neurodegenerative proteinopathies: mechanisms and prospects for therapeutic intervention. *Biochem Soc Trans* 2018;46(4):829–842. doi:10.1042/BST20180025, PMID:29986938.
 - [53] Di Domenico F, Lanzillotta C. The disturbance of protein synthesis/degradation homeostasis is a common trait of age-related neurodegenerative disorders. *Adv Protein Chem Struct Biol* 2022;132:49–87. doi:10.1016/bs.apcsb.2022.05.008, PMID:36088079.
 - [54] Del Monte F, Agnetti G. Protein post-translational modifications and misfolding: new concepts in heart failure. *Proteomics Clin Appl* 2014;8(7-8):534–542. doi:10.1002/prca.201400037, PMID:24946239.
 - [55] Guha S, Johnson GVW, Nehrke K. The Crosstalk Between Pathological Tau Phosphorylation and Mitochondrial Dysfunction as a Key to Understanding and Treating Alzheimer's Disease. *Mol Neurobiol* 2020;57(12):5103–5120. doi:10.1007/s12035-020-02084-0, PMID:32851560.
 - [56] Le Guerroué F, Youle RJ. Ubiquitin signaling in neurodegenerative diseases: an autophagy and proteasome perspective. *Cell Death Differ* 2021;28(2):439–454. doi:10.1038/s41418-020-00667-x, PMID:33208890.
 - [57] Yoo H, Lee J, Kim B, Moon H, Jeong H, Lee K, *et al*. Role of post-translational modifications on the alpha-synuclein aggregation-related pathogenesis of Parkinson's disease. *BMB Rep* 2022;55(7):323–335. doi:10.5483/BMBRep.2022.55.7.073, PMID:35733294.
 - [58] Schaffert LN, Carter WG. Do Post-Translational Modifications Influence Protein Aggregation in Neurodegenerative Diseases: A Systematic Review. *Brain Sci* 2020;10(4):232. doi:10.3390/brainsci10040232, PMID:32290481.
 - [59] Martin JB. Molecular basis of the neurodegenerative disorders. *N Engl J Med* 1999;340(25):1970–1980. doi:10.1056/NEJM199906243402507, PMID:10379022.
 - [60] Hyman BT, Van Hoesen GW. Neuron numbers in Alzheimer's disease: cell-specific pathology. *Neurobiol Aging* 1987;8(6):555–556. doi:10.1016/0197-4580(87)90133-3, PMID:3431631.

- [61] Forno LS. The Neuropathology Of Parkinson'S Disease. In: Hefti F, Weiner WJ (eds). Progress in Parkinson Research. Boston (MA): Springer; 1988:11–21. doi:10.1007/978-1-4613-0759-4_2.
- [62] Myers RH, Vonsattel JP, Paskevich PA, Kiely DK, Stevens TJ, Cupples LA, *et al.* Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. *J Neuropathol Exp Neurol* 1991;50(6):729–742. doi:10.1097/00005072-199111000-00005, PMID:1836225.
- [63] Hughes JT. Pathology of amyotrophic lateral sclerosis. *Adv Neurol* 1982;36:61–74. PMID:7180695.
- [64] Gray F, Chrétien F, Adle-Biassette H, Dorandeu A, Ereau T, Delisle MB, *et al.* Neuronal apoptosis in Creutzfeldt-Jakob disease. *J Neuropathol Exp Neurol* 1999;58(4):321–328. doi:10.1097/00005072-199904000-00002, PMID:10218627.
- [65] Mattson MP. Apoptosis in neurodegenerative disorders. *Nat Rev Mol Cell Biol* 2000;1(2):120–129. doi:10.1038/35040009, PMID:11253364.
- [66] Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, *et al.* Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 1994;264(5166):1772–1775. doi:10.1126/science.8209258, PMID:8209258.
- [67] El-Agnaf OM, Jakes R, Curran MD, Middleton D, Ingenito R, Bianchi E, *et al.* Aggregates from mutant and wild-type alpha-synuclein proteins and NAC peptide induce apoptotic cell death in human neuroblastoma cells by formation of beta-sheet and amyloid-like filaments. *FEBS Lett* 1998;440(1-2):71–75. doi:10.1016/s0014-5793(98)01418-5, PMID:9862428.
- [68] Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease—a double-edged sword. *Neuron* 2002;35(3):419–432. doi:10.1016/s0896-6273(02)00794-8, PMID:12165466.
- [69] Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, *et al.* Early and progressive accumulation of reactive microglia in the Huntington disease brain. *J Neuropathol Exp Neurol* 2001;60(2):161–172. doi:10.1093/jnen/60.2.161, PMID:11273004.
- [70] McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res Brain Res Rev* 1995;21(2):195–218. doi:10.1016/0165-0173(95)00011-9, PMID:8866675.
- [71] Xia MQ, Hyman BT. Chemokines/chemokine receptors in the central nervous system and Alzheimer's disease. *J Neurovirol* 1999;5(1):32–41. doi:10.3109/13550289909029743, PMID:10190688.
- [72] Yates SL, Burgess LH, Kocsis-Angle J, Antal JM, Dority MD, Embury PB, *et al.* Amyloid beta and amylin fibrils induce increases in proinflammatory cytokine and chemokine production by THP-1 cells and murine microglia. *J Neurochem* 2000;74(3):1017–1025. doi:10.1046/j.1471-4159.2000.0741017.x, PMID:10693932.
- [73] Hernandez-Sapiens MA, Reza-Zaldívar EE, Márquez-Aguirre AL, Gómez-Pinedo U, Matias-Guiu J, Cevallos RR, *et al.* Presenilin mutations and their impact on neuronal differentiation in Alzheimer's disease. *Neural Regen Res* 2022;17(1):31–37. doi:10.4103/1673-5374.313016, PMID:34100423.
- [74] Migliore L, Coppèdè F. Environmental-induced oxidative stress in neurodegenerative disorders and aging. *Mutat Res* 2009;674(1-2):73–84. doi:10.1016/j.mrgentox.2008.09.013, PMID:18952194.
- [75] Parhizkar S, Holtzman DM. APOE mediated neuroinflammation and neurodegeneration in Alzheimer's disease. *Semin Immunol* 2022;59:101594. doi:10.1016/j.smim.2022.101594, PMID:35232622.
- [76] Petrucci S, Ginevrino M, Valente EM. Phenotypic spectrum of alpha-synuclein mutations: New insights from patients and cellular models. *Parkinsonism RelatDisord* 2016;22(Suppl 1):S16–S20. doi:10.1016/j.parkreldis.2015.08.015, PMID:26341711.
- [77] Srinivasan E, Chandrasekar G, Chandrasekar P, Anbarasu K, Vickram AS, Karunakaran R, *et al.* Alpha-Synuclein Aggregation in Parkinson's Disease. *Front Med (Lausanne)* 2021;8:736978. doi:10.3389/fmed.2021.736978, PMID:34733860.
- [78] Huang M, Barges-Carot A, Riaz Z, Wickham H, Zenitsky G, Jin H, *et al.* Impact of Environmental Risk Factors on Mitochondrial Dysfunction, Neuroinflammation, Protein Misfolding, and Oxidative Stress in the Etiopathogenesis of Parkinson's Disease. *Int J Mol Sci* 2022;23(18):10808. doi:10.3390/ijms231810808, PMID:36142718.
- [79] Jeong GR, Lee BD. Pathological Functions of LRRK2 in Parkinson's Disease. *Cells* 2020;9(12):2565. doi:10.3390/cells9122565, PMID:33266247.
- [80] Kaye J, Reisine T, Finkbeiner S. Huntington's disease mouse models: unraveling the pathology caused by CAG repeat expansion. *Fac Rev* 2021;10:77. doi:10.12703/r/10-77, PMID:34746930.
- [81] Folger A, Wang Y. The Cytotoxicity and Clearance of Mutant Huntingtin and Other Misfolded Proteins. *Cells* 2021;10(11):2835. doi:10.3390/cells10112835, PMID:34831058.
- [82] Chao TK, Hu J, Pringsheim T. Risk factors for the onset and progression of Huntington disease. *Neurotoxicology* 2017;61:79–99. doi:10.1016/j.neuro.2017.01.005, PMID:28111121.
- [83] Kaur SJ, McKeown SR, Rashid S. Mutant SOD1 mediated pathogenesis of Amyotrophic Lateral Sclerosis. *Gene* 2016;577(2):109–118. doi:10.1016/j.gene.2015.11.049, PMID:26657039.
- [84] Nowicka N, Juranek J, Juranek JK, Wojtkiewicz J. Risk Factors and Emerging Therapies in Amyotrophic Lateral Sclerosis. *Int J Mol Sci* 2019;20(11):2616. doi:10.3390/ijms20112616, PMID:31141951.
- [85] Babić Leko M, Župunski V, Kirincich J, Smilović D, Hortobágyi T, Hof PR, *et al.* Molecular Mechanisms of Neurodegeneration Related to C9orf72 Hexanucleotide Repeat Expansion. *Behav Neurol* 2019;2019:2909168. doi:10.1155/2019/2909168, PMID:30774737.
- [86] Gandhi S, Wood NW. Genome-wide association studies: the key to unlocking neurodegeneration? *Nat Neurosci* 2010;13(7):789–794. doi:10.1038/nn.2584, PMID:20581814.
- [87] Pihlström L, Wiethoff S, Houlden H. Genetics of neurodegenerative diseases: an overview. *Handb Clin Neurol* 2017;145:309–323. doi:10.1016/B978-0-12-802395-2.00022-5, PMID:28987179.
- [88] Frydas A, Wauters E, van der Zee J, Van Broeckhoven C. Uncovering the impact of noncoding variants in neurodegenerative brain diseases. *Trends Genet* 2022;38(3):258–272. doi:10.1016/j.tig.2021.08.010, PMID:34535299.
- [89] Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. *Comput Struct Biotechnol J* 2020;18:2401–2415. doi:10.1016/j.csbj.2020.08.031, PMID:33005303.
- [90] Hille F, Charpentier E. CRISPR-Cas: biology, mechanisms and relevance. *Philos Trans R Soc Lond B Biol Sci* 2016;371(1707):20150496. doi:10.1098/rstb.2015.0496, PMID:27672148.
- [91] Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. *J Bacteriol* 2018;200(7):e00580–17. doi:10.1128/JB.00580-17, PMID:29358495.
- [92] Wang JY, Pausch P, Doudna JA. Structural biology of CRISPR-Cas immunity and genome editing enzymes. *Nat Rev Microbiol* 2022;20(11):641–656. doi:10.1038/s41579-022-00739-4, PMID:35562427.
- [93] Rath D, Amlinger L, Rath A, Lundgren M. The CRISPR-Cas immune system: biology, mechanisms and applications. *Biochimie* 2015;117:119–128. doi:10.1016/j.biochi.2015.03.025, PMID:25868999.
- [94] Kumar D, Yadav A, Ahmad R, Dwivedi UN, Yadav K. CRISPR-Based Genome Editing for Nutrient Enrichment in Crops: A Promising Approach Toward Global Food Security. *Front Genet* 2022;13:932859. doi:10.3389/fgene.2022.932859, PMID:35910203.
- [95] Bottino P, Pastrone L, Curtioni A, Bondi A, Sidoti F, Zanotto E, *et al.* Antiviral Approach to Cytomegalovirus Infection: An Overview of Conventional and Novel Strategies. *Microorganisms* 2023;11(10):2372. doi:10.3390/microorganisms11102372, PMID:37894030.
- [96] González Castro N, Bjelic J, Malhotra G, Huang C, Alsaifir SH. Comparison of the Feasibility, Efficiency, and Safety of Genome Editing Technologies. *Int J Mol Sci* 2021;22(19):10355. doi:10.3390/ijms221910355, PMID:34638696.
- [97] El-Ashry HA. The CRISPR/Cas system: Gene Editing by bacterial defense. *Nov Res Microbiol J* 2023;7(5):2101–2115. doi:10.21608/nrmj.2023.317036.
- [98] Adli M. The CRISPR tool kit for genome editing and beyond. *Nat Commun* 2018;9(1):1911. doi:10.1038/s41467-018-04252-2, PMID:29765029.
- [99] Ceasar SA, Rajan V, Prykhodzij SV, Berman JN, Ignacimuthu S. Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9. *BiochimBiophys Acta* 2016;1863(9):2333–2344. doi:10.1016/j.bbamcr.2016.06.009, PMID:27350235.
- [100] Zhang Y, Chen H, Li R, Sterling K, Song W. Amyloid β -based therapy

- for Alzheimer's disease: challenges, successes and future. *Signal Transduct Target Ther* 2023;8(1):248. doi:10.1038/s41392-023-01484-7, PMID:37386015.
- [101] Tao J, Bauer DE, Chiarle R. Assessing and advancing the safety of CRISPR-Cas tools: from DNA to RNA editing. *Nat Commun* 2023;14(1):212. doi:10.1038/s41467-023-35886-6, PMID:36639728.
- [102] Li T, Yang Y, Qi H, Cui W, Zhang L, Fu X, *et al*. CRISPR/Cas9 therapeutics: progress and prospects. *Signal Transduct Target Ther* 2023;8(1):36. doi:10.1038/s41392-023-01309-7, PMID:36646687.
- [103] Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. *Curr Opin Microbiol* 2017;37:67–78. doi:10.1016/j.mib.2017.05.008, PMID:28605718.
- [104] Liu Z, Dong H, Cui Y, Cong L, Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. *Microb Cell Fact* 2020;19(1):172. doi:10.1186/s12934-020-01431-z, PMID:32883277.
- [105] Khan Z, Ali Z, Khan AA, Sattar T, Zeshan A, Saboor T, *et al*. History and Classification of CRISPR/Cas System. In: Ahmad A, Khan SH, Khan Z (eds). *The CRISPR/Cas Tool Kit for Genome Editing*. Singapore: Springer; 2022:29–52. doi:10.1007/978-981-16-6305-5.
- [106] Mei Y, Wang Y, Chen H, Sun ZS, Ju XD. Recent Progress in CRISPR/Cas9 Technology. *J Genet Genomics* 2016;43(2):63–75. doi:10.1016/j.jgg.2016.01.001, PMID:26924689.
- [107] Huang CH, Lee KC, Doudna JA. Applications of CRISPR-Cas Enzymes in Cancer Therapeutics and Detection. *Trends Cancer* 2018;4(7):499–512. doi:10.1016/j.trecan.2018.05.006, PMID:29937048.
- [108] Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, *et al*. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell* 2014;156(5):935–949. doi:10.1016/j.cell.2014.02.001, PMID:24529477.
- [109] Jiang F, Doudna JA. CRISPR-Cas9 Structures and Mechanisms. *Annu Rev Biophys* 2017;46:505–529. doi:10.1146/annurev-biophys-062215-010822, PMID:28375731.
- [110] Shao M, Xu TR, Chen CS. The big bang of genome editing technology: development and application of the CRISPR/Cas9 system in disease animal models. *DongwuxueYanjiu* 2016;37(4):191–204. doi:10.13918/j.issn.2095-8137.2016.4.191, PMID:27469250.
- [111] Zarei A, Razban V, Hosseini SE, Tabei SMB. Creating cell and animal models of human disease by genome editing using CRISPR/Cas9. *J Gene Med* 2019;21(4):e3082. doi:10.1002/jgm.3082, PMID:30786106.
- [112] Gleditsch D, Pausch P, Müller-Esparza H, Özcan A, Guo X, Bange G, *et al*. PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. *RNA Biol* 2019;16(4):504–517. doi:10.1080/15476286.2018.1504546, PMID:30109815.
- [113] Collias D, Beisel CL. CRISPR technologies and the search for the PAM-free nuclease. *Nat Commun* 2021;12(1):555. doi:10.1038/s41467-020-20633-y, PMID:33483498.
- [114] Xue C, Greene EC. DNA Repair Pathway Choices in CRISPR-Cas9-Mediated Genome Editing. *Trends Genet* 2021;37(7):639–656. doi:10.1016/j.tig.2021.02.008, PMID:33896583.
- [115] Allen F, Crepaldi L, Alsinet C, Strong AJ, Kleshchevnikov V, De Angeli P, *et al*. Predicting the mutations generated by repair of Cas9-induced double-strand breaks. *Nat Biotechnol* 2018;doi:10.1038/nbt.4317, PMID:30480667.
- [116] Smirnikhina SA, Zaynitdinova MI, Sergeeva VA, Lavrov AV. Improving Homology-Directed Repair in Genome Editing Experiments by Influencing the Cell Cycle. *Int J Mol Sci* 2022;23(11):5992. doi:10.3390/ijms23115992, PMID:35682671.
- [117] Möller L, Aird EJ, Schröder MS, Kobel L, Kissling L, van de Venn L, *et al*. Recursive Editing improves homology-directed repair through retargeting of undesired outcomes. *Nat Commun* 2022;13(1):4550. doi:10.1038/s41467-022-31944-7, PMID:35931681.
- [118] Porteus M. Genome Editing: A New Approach to Human Therapeutics. *Annu Rev Pharmacol Toxicol* 2016;56:163–190. doi:10.1146/annurev-pharmtox-010814-124454, PMID:26566154.
- [119] Richardson C, Kelsh RN, J Richardson R. New advances in CRISPR/Cas-mediated precise gene-editing techniques. *Dis Model Mech* 2023;16(2):dmm049874. doi:10.1242/dmm.049874, PMID:36847161.
- [120] Wang JY, Doudna JA. CRISPR technology: A decade of genome editing is only the beginning. *Science* 2023;379(6629):eadd8643. doi:10.1126/science.add8643, PMID:36656942.
- [121] Sahoo S, Shivani K, Padhy AA, Kumari V, Mishra P. Principles, Methods, and Applications of Protein Folding Inside Cells. In: Saudagar P, Tripathi T (eds). *Protein Folding Dynamics and Stability*. Singapore: Springer; 2023:251–284. doi:10.1007/978-981-99-2079-2_13.
- [122] De Plano LM, Calabrese G, Conoci S, Guglielmino SPP, Oddo S, Caccamo A. Applications of CRISPR-Cas9 in Alzheimer's Disease and Related Disorders. *Int J Mol Sci* 2022;23(15):8714. doi:10.3390/ijms23158714, PMID:35955847.
- [123] Querfurth HW, LaFerla FM. Alzheimer's disease. *N Engl J Med* 2010;362(4):329–344. doi:10.1056/NEJMra0909142, PMID:20107219.
- [124] Raikwar SP, Kikkeri NS, Sakuru R, Saeed D, Zahoor H, Premkumar K, *et al*. Next Generation Precision Medicine: CRISPR-mediated Genome Editing for the Treatment of Neurodegenerative Disorders. *J Neuroimmune Pharmacol* 2019;14(4):608–641. doi:10.1007/s11481-019-09849-y, PMID:31011884.
- [125] Fellmann C, Gowen BG, Lin PC, Doudna JA, Corn JE. Cornerstones of CRISPR-Cas in drug discovery and therapy. *Nat Rev Drug Discov* 2017;16(2):89–100. doi:10.1038/nrd.2016.238, PMID:28008168.
- [126] Oakes BL, Fellmann C, Rishi H, Taylor KL, Ren SM, Nadler DC, *et al*. CRISPR-Cas9 Circular Permutants as Programmable Scaffolds for Genome Modification. *Cell* 2019;176(1-2):254–267.e16. doi:10.1016/j.cell.2018.11.052, PMID:30633905.
- [127] Henao-Mejia J, Williams A, Rongvaux A, Stein J, Hughes C, Flavell RA. Generation of Genetically Modified Mice Using the CRISPR-Cas9 Genome-Editing System. *Cold Spring Harb Protoc* 2016;2016(2):pdb.prot090704. doi:10.1101/pdb.prot090704, PMID:26832688.
- [128] Vejnar CE, Moreno-Mateos MA, Cifuentes D, Bazzini AA, Giraldez AJ. Optimized CRISPR-Cas9 System for Genome Editing in Zebrafish. *Cold Spring Harb Protoc* 2016;2016(10). doi:10.1101/pdb.prot086850, PMID:27698232.
- [129] Lee J, Bayarsaikhan D, Arivazhagan R, Park H, Lim B, Gwak P, *et al*. CRISPR/Cas9 Edited sRAGE-MSCs Protect Neuronal Death in Parkinson's Disease Model. *Int J Stem Cells* 2019;12(1):114–124. doi:10.15283/ijsc18110, PMID:30836725.
- [130] Crisostomo PR, Wang M, Wairiuko GM, Morrell ED, Terrell AM, Seshadri P, *et al*. High passage number of stem cells adversely affects stem cell activation and myocardial protection. *Shock* 2006;26(6):575–580. doi:10.1097/01.shk.0000235087.45798.93, PMID:17117132.
- [131] McTague A, Rossignoli G, Ferrini A, Barral S, Kurian MA. Genome Editing in iPSC-Based Neural Systems: From Disease Models to Future Therapeutic Strategies. *Front Genome Ed* 2021;3:630600. doi:10.3389/fgeed.2021.630600, PMID:34713254.
- [132] Sen T, Thummer RP. CRISPR and iPSCs: Recent Developments and Future Perspectives in Neurodegenerative Disease Modelling, Research, and Therapeutics. *Neurotox Res* 2022;40(5):1597–1623. doi:10.1007/s12640-022-00564-w, PMID:36044181.
- [133] Xu CL, Ruan MZC, Mahajan VB, Tsang SH. Viral Delivery Systems for CRISPR. *Viruses* 2019;11(1):28. doi:10.3390/v11010028, PMID:30621179.
- [134] Weinmann J, Weis S, Sippel J, Tulalamba W, Remes A, El Andari J, *et al*. Identification of a myotropic AAV by massively parallel in vivo evaluation of barcoded capsid variants. *Nat Commun* 2020;11(1):5432. doi:10.1038/s41467-020-19230-w, PMID:33116134.
- [135] Rayaprolu V, Kruse S, Kant R, Venkatakrishnan B, Movahed N, Brooke D, *et al*. Comparative analysis of adeno-associated virus capsid stability and dynamics. *J Virol* 2013;87(24):13150–13160. doi:10.1128/JVI.01415-13, PMID:24067976.
- [136] Zou Y, Sun X, Yang Q, Zheng M, Shimoni O, Ruan W, *et al*. Blood-brain barrier-penetrating single CRISPR-Cas9 nanocapsules for effective and safe glioblastoma gene therapy. *Sci Adv* 2022;8(16):eabm8011. doi:10.1126/sciadv.abm8011, PMID:35442747.
- [137] Ramamoorth M, Narvekar A. Non viral vectors in gene therapy-an overview. *J Clin Diagn Res* 2015;9(1):GE01–GE06. doi:10.7860/JCDR/2015/10443.5394, PMID:25738007.
- [138] Maeder ML, Gersbach CA. Genome-editing Technologies for Gene and Cell Therapy. *Mol Ther* 2016;24(3):430–446. doi:10.1038/mt.2016.10, PMID:26755333.
- [139] Asmamaw Mengstie M. Viral Vectors for the in Vivo Delivery of CRISPR Components: Advances and Challenges. *Front Bioeng Biotechnol* 2022;10:895713. doi:10.3389/fbioe.2022.895713, PMID:35646852.
- [140] Lamptey RNL, Chaulagain B, Trivedi R, Gothwal A, Layek B, Singh J. A Review of the Common Neurodegenerative Disorders: Current

- Therapeutic Approaches and the Potential Role of Nanotherapeutics. *Int J Mol Sci* 2022;23(3):1851. doi:10.3390/ijms23031851, PMID:35163773.
- [141] Meijboom KE, Abdallah A, Fordham NP, Nagase H, Rodriguez T, Kraus C, *et al.* CRISPR/Cas9-mediated excision of ALS/FTD-causing hexanucleotide repeat expansion in C9ORF72 rescues major disease mechanisms in vivo and in vitro. *Nat Commun* 2022;13(1):6286. doi:10.1038/s41467-022-33332-7, PMID:36271076.
- [142] Gaj T, Ojala DS, Ekman FK, Byrne LC, Limsirichai P, Schaffer DV. In vivo genome editing improves motor function and extends survival in a mouse model of ALS. *Sci Adv* 2017;3(12):eaar3952. doi:10.1126/sciadv.aar3952, PMID:29279867.
- [143] Guan L, Han Y, Yang C, Lu S, Du J, Li H, *et al.* CRISPR-Cas9-Mediated Gene Therapy in Neurological Disorders. *Mol Neurobiol* 2022;59(2):968–982. doi:10.1007/s12035-021-02638-w, PMID:34813019.
- [144] Wilson CA, Doms RW, Lee VM. Intracellular APP processing and A beta production in Alzheimer disease. *J Neuropathol Exp Neurol* 1999; 58(8):787–794. doi:10.1097/00005072-199908000-00001, PMID:104 46803.
- [145] György B, Lööv C, Zaborowski MP, Takeda S, Kleinstiver BP, Commins C, *et al.* CRISPR/Cas9 Mediated Disruption of the Swedish APP Allele as a Therapeutic Approach for Early-Onset Alzheimer's Disease. *Mol Ther Nucleic Acids* 2018;11:429–440. doi:10.1016/j.omtn.2018.03.007, PMID:29858078.
- [146] Jackson RJ, Keiser MS, Meltzer JC, Fykstra DP, Dierksmeier SE, Melloni A, *et al.* APOE2 gene therapy reduces amyloid deposition, and improves markers of neuroinflammation and neurodegeneration in a mouse model of Alzheimer disease. *bioRxiv* 2023:2023. doi:10.1101/2023.08.14.552850, PMID:37645718.
- [147] Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 2016;533(7603):420–424. doi:10.1038/nature17946, PMID:27096365.
- [148] Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, *et al.* APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. *Neuron* 2018;98(6):1141–1154.e7. doi:10.1016/j.neuron.2018.05.008, PMID:29861287.
- [149] Jiang H, Tang M, Xu Z, Wang Y, Li M, Zheng S, *et al.* CRISPR/Cas9 system and its applications in nervous system diseases. *Genes Dis* 2024;11(2):675–686. doi:10.1016/j.gendis.2023.03.017, PMID:37692518.
- [150] Ishizu N, Yui D, Hebisawa A, Aizawa H, Cui W, Fujita Y, *et al.* Impaired striatal dopamine release in homozygous Vps35 D620N knock-in mice. *Hum Mol Genet* 2016;25(20):4507–4517. doi:10.1093/hmg/ddw279, PMID:28173004.
- [151] Pinjala P, Tryphena KP, Prasad R, Khatri DK, Sun W, Singh SB, *et al.* CRISPR/Cas9 assisted stem cell therapy in Parkinson's disease. *Biomater Res* 2023;27(1):46. doi:10.1186/s40824-023-00381-y, PMID:371 94005.
- [152] Yin P, Li S, Li XJ, Yang W. New pathogenic insights from large animal models of neurodegenerative diseases. *Protein Cell* 2022;13(10):707–720. doi:10.1007/s13238-022-00912-8, PMID:35334073.
- [153] Zhu XX, Zhong YZ, Ge YW, Lu KH, Lu SS. CRISPR/Cas9-Mediated Generation of Guangxi Bama Minipigs Harboring Three Mutations in α -Synuclein Causing Parkinson's Disease. *Sci Rep* 2018;8(1):12420. doi:10.1038/s41598-018-30436-3, PMID:30127453.
- [154] Wulansari N, Darsono WHW, Woo HJ, Chang MY, Kim J, Bae EJ, *et al.* Neurodevelopmental defects and neurodegenerative phenotypes in human brain organoids carrying Parkinson's disease-linked DNAJC6 mutations. *Sci Adv* 2021;7(8):eabb1540. doi:10.1126/sciadv.abb1540, PMID:33597231.
- [155] Qin Y, Li S, Li XJ, Yang S. CRISPR-Based Genome-Editing Tools for Huntington's Disease Research and Therapy. *Neurosci Bull* 2022;38(11):1397–1408. doi:10.1007/s12264-022-00880-3, PMID:35608753.
- [156] Yan S, Tu Z, Liu Z, Fan N, Yang H, Yang S, *et al.* A Huntingtin Knockin Pig Model Recapitulates Features of Selective Neurodegeneration in Huntington's Disease. *Cell* 2018;173(4):989–1002.e13. doi:10.1016/j.cell.2018.03.005, PMID:29606351.
- [157] Yang S, Chang R, Yang H, Zhao T, Hong Y, Kong HE, *et al.* CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. *J Clin Invest* 2017;127(7):2719–2724. doi:10.1172/JCI92087, PMID:28628038.
- [158] Perkins EM, Burr K, Banerjee P, Mehta AR, Dando O, Selvaraj BT, *et al.* Altered network properties in C9ORF72 repeat expansion cortical neurons are due to synaptic dysfunction. *Mol Neurodegener* 2021;16(1):13. doi:10.1186/s13024-021-00433-8, PMID:33663561.
- [159] Fang T, Je G, Pacut P, Keyhanian K, Gao J, Ghasemi M. Gene Therapy in Amyotrophic Lateral Sclerosis. *Cells* 2022;11(13):2066. doi:10.3390/cells11132066, PMID:35805149.
- [160] Tutar Y, Özgür A, Tutar L. Role of Protein Aggregation in Neurodegenerative Diseases. In: Kishore U (ed). *Neurodegenerative Diseases*. London: InTech; 2013:55–76. doi:10.5772/54487.
- [161] LaFerla FM, Oddo S. Alzheimer's disease: Abeta, tau and synaptic dysfunction. *Trends Mol Med* 2005;11(4):170–176. doi:10.1016/j.molmed.2005.02.009, PMID:15823755.
- [162] Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat Rev Neurol* 2019;15(9):501–518. doi:10.1038/s41582-019-0228-7, PMID:31367008.
- [163] Raulin AC, Doss SV, Trottier ZA, Ikezu TC, Bu G, Liu CC. ApoE in Alzheimer's disease: pathophysiology and therapeutic strategies. *Mol Neurodegener* 2022;17(1):72. doi:10.1186/s13024-022-00574-4, PMID:36348357.
- [164] Park H, Oh J, Shim G, Cho B, Chang Y, Kim S, *et al.* In vivo neuronal gene editing via CRISPR-Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. *Nat Neurosci* 2019;22(4):524–528. doi:10.1038/s41593-019-0352-0, PMID:30858603.
- [165] Guo J, Wan T, Li B, Pan Q, Xin H, Qiu Y, *et al.* Rational Design of Poly(disulfide)s as a Universal Platform for Delivery of CRISPR-Cas9 Machineries toward Therapeutic Genome Editing. *ACS Cent Sci* 2021;7(6):990–1000. doi:10.1021/acscentsci.0c01648, PMID:34235260.
- [166] Hayes MT. Parkinson's Disease and Parkinsonism. *Am J Med* 2019; 132(7):802–807. doi:10.1016/j.amjmed.2019.03.001, PMID:308904 25.
- [167] Jwoaed A, Schmitt I, Kaut O, Wüllner U. Methylation regulates alpha-synuclein expression and is decreased in Parkinson's disease patients' brains. *J Neurosci* 2010;30(18):6355–6359. doi:10.1523/JNEUROSCI.6119-09.2010, PMID:20445061.
- [168] Guhathakurta S, Kim J, Adams L, Basu S, Song MK, Adler E, *et al.* Targeted attenuation of elevated histone marks at SNCA alleviates α -synuclein in Parkinson's disease. *EMBO Mol Med* 2021;13(2):e12188. doi:10.15252/emmm.202012188, PMID:33428332.
- [169] Yoon HH, Ye S, Lim S, Jo A, Lee H, Hong F, *et al.* CRISPR-Cas9 Gene Editing Protects from the A53T-SNCA Overexpression-Induced Pathology of Parkinson's Disease In Vivo. *CRISPR J* 2022;5(1):95–108. doi:10.1089/crispr.2021.0025, PMID:35191750.
- [170] Li H, Wu S, Ma X, Li X, Cheng T, Chen Z, *et al.* Co-editing PINK1 and DJ-1 Genes Via Adeno-Associated Virus-Delivered CRISPR/Cas9 System in Adult Monkey Brain Elicits Classical Parkinsonian Phenotype. *Neurosci Bull* 2021;37(9):1271–1288. doi:10.1007/s12264-021-00732-6, PMID:34165772.
- [171] Ahfeldt T, Ordureau A, Bell C, Sarrafha L, Sun C, Piccinotti S, *et al.* Pathogenic Pathways in Early-Onset Autosomal Recessive Parkinson's Disease Discovered Using Isogenic Human Dopaminergic Neurons. *Stem Cell Reports* 2020;14(1):75–90. doi:10.1016/j.stemcr.2019.12.005, PMID:31902706.
- [172] Deng HX, Zhai H, Shi Y, Liu G, Lowry J, Liu B, *et al.* Efficacy and long-term safety of CRISPR/Cas9 genome editing in the SOD1-linked mouse models of ALS. *Commun Biol* 2021;4(1):396. doi:10.1038/s42003-021-01942-4, PMID:33767386.
- [173] Chen CX, Abdian N, Maussion G, Thomas RA, Demirova I, Cai E, *et al.* A Multistep Workflow to Evaluate Newly Generated iPSCs and Their Ability to Generate Different Cell Types. *Methods Protoc* 2021;4(3):50. doi:10.3390/mps4030050, PMID:34287353.
- [174] Ghasemi M, Keyhanian K, Douthwright C. Glial Cell Dysfunction in C9orf72-Related Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Cells* 2021;10(2):249. doi:10.3390/cells10020249, PMID:33525344.
- [175] Dianov GL, Hübscher U. Mammalian base excision repair: the forgotten archangel. *Nucleic Acids Res* 2013;41(6):3483–3490.

- doi:10.1093/nar/gkt076, PMID:23408852.
- [176] Shin JW, Kim KH, Chao MJ, Atwal RS, Gillis T, MacDonald ME, *et al*. Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. *Hum Mol Genet* 2016;25(20):4566–4576. doi:10.1093/hmg/ddw286, PMID:28172889.
- [177] Xie G, Meng T, Luo Y, Liu Z. SKF-LDA: Similarity Kernel Fusion for Predicting lncRNA-Disease Association. *Mol Ther Nucleic Acids* 2019;18:45–55. doi:10.1016/j.omtn.2019.07.022, PMID:31514111.
- [178] Manghwar H, Lindsey K, Zhang X, Jin S. CRISPR/Cas System: Recent Advances and Future Prospects for Genome Editing. *Trends Plant Sci* 2019;24(12):1102–1125. doi:10.1016/j.tplants.2019.09.006, PMID:31727474.
- [179] Jinek M, Jiang F, Taylor DW, Sternberg SH, Kaya E, Ma E, *et al*. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science* 2014;343(6176):1247997. doi:10.1126/science.1247997, PMID:24505130.
- [180] Anders C, Niewoehner O, Duerst A, Jinek M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature* 2014;513(7519):569–573. doi:10.1038/nature13579, PMID:25079318.
- [181] Jiang F, Zhou K, Ma L, Gressel S, Doudna JA. STRUCTURAL BIOLOGY. A Cas9-guide RNA complex preorganized for target DNA recognition. *Science* 2015;348(6242):1477–1481. doi:10.1126/science.aab1452, PMID:26113724.
- [182] Wang H, La Russa M, Qi LS. CRISPR/Cas9 in Genome Editing and Beyond. *Annu Rev Biochem* 2016;85:227–264. doi:10.1146/annurev-biochem-060815-014607, PMID:27145843.
- [183] Wang X, Cao C, Huang J, Yao J, Hai T, Zheng Q, *et al*. One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system. *Sci Rep* 2016;6:20620. doi:10.1038/srep20620, PMID:26857844.
- [184] Miyaoka Y, Berman JR, Cooper SB, Mayerl SJ, Chan AH, Zhang B, *et al*. Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing. *Sci Rep* 2016;6:23549. doi:10.1038/srep23549, PMID:27030102.
- [185] Safari F, Zare K, Negahdaripour M, Barekati-Mowahed M, Ghasemi Y. CRISPR Cpf1 proteins: structure, function and implications for genome editing. *Cell Biosci* 2019;9:36. doi:10.1186/s13578-019-0298-7, PMID:31086658.
- [186] Dabrowska M, Juzwa W, Krzyzosiak WJ, Olejniczak M. Precise Excision of the CAG Tract from the Huntingtin Gene by Cas9 Nickases. *Front Neurosci* 2018;12:75. doi:10.3389/fnins.2018.00075, PMID:29535594.
- [187] Vetrivel KS, Zhang YW, Xu H, Thinakaran G. Pathological and physiological functions of presenilins. *Mol Neurodegener* 2006;1:4. doi:10.1186/1750-1326-1-4, PMID:16930451.
- [188] Ortiz-Virumbrales M, Moreno CL, Moreno I, Marazuela P, Sproul A, Jacob S, *et al*. CRISPR/Cas9-Correctable mutation-related molecular and physiological phenotypes in iPSC-derived Alzheimer's PSEN2 (N141I) neurons. *Acta Neuropathol Commun* 2017;5(1):77. doi:10.1186/s40478-017-0475-z, PMID:29078805.
- [189] Wang Y, Wang Y, Bharti V, Zhou H, Hoi V, Tan H, *et al*. Upregulation of Thioredoxin-Interacting Protein in Brain of Amyloid- β Protein Precursor/Presenilin 1 Transgenic Mice and Amyloid- β Treated Neuronal Cells. *J Alzheimers Dis* 2019;72(1):139–150. doi:10.3233/JAD-190223, PMID:31561358.
- [190] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012;337(6096):816–821. doi:10.1126/science.1225829, PMID:22745249.
- [191] Cox DB, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. *Nat Med* 2015;21(2):121–131. doi:10.1038/nm.3793, PMID:25654603.
- [192] Malankhanova TB, Malakhova AA, Medvedev SP, Zakian SM. Modern Genome Editing Technologies in Huntington's Disease Research. *J Huntingtons Dis* 2017;6(1):19–31. doi:10.3233/JHD-160222, PMID:28128770.
- [193] Abad-Sojos S, Torres O, Ordóñez KL. Application of the technique CRISPR/Cas9 in the reduction and permanent inactivation of Huntington's disease. *News Views* 2018;3:689–691. doi:10.21931/RB/2018.03.03.11.
- [194] Tavakoli K, Pour-Aboughadareh A, Kianersi F, Poczai P, Etminan A, Shoostari L. Applications of CRISPR-Cas9 as an Advanced Genome Editing System in Life Sciences. *BioTech (Basel)* 2021;10(3):14. doi:10.3390/biotech10030014, PMID:35822768.
- [195] Fields E, Vaughan E, Tripu D, Lim I, Shrout K, Conway J, *et al*. Gene targeting techniques for Huntington's disease. *Ageing Res Rev* 2021;70:101385. doi:10.1016/j.arr.2021.101385, PMID:34098113.
- [196] Alkanli SS, Alkanli N, Ay A, Albeniz I. CRISPR/Cas9 Mediated Therapeutic Approach in Huntington's Disease. *Mol Neurobiol* 2023;60(3):1486–1498. doi:10.1007/s12035-022-03150-5, PMID:36482283.
- [197] Dabrowska M, Ciolak A, Kozłowska E, Fiszer A, Olejniczak M. Generation of New Isogenic Models of Huntington's Disease Using CRISPR-Cas9 Technology. *Int J Mol Sci* 2020;21(5):1854. doi:10.3390/ijms21051854, PMID:32182692.
- [198] Ekman FK, Ojala DS, Adil MM, Lopez PA, Schaffer DV, Gaj T. CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model. *Mol Ther Nucleic Acids* 2019;17:829–839. doi:10.1016/j.omtn.2019.07.009, PMID:31465962.
- [199] Kenjo E, Hozumi H, Makita Y, Iwabuchi KA, Fujimoto N, Matsumoto S, *et al*. Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice. *Nat Commun* 2021;12(1):7101. doi:10.1038/s41467-021-26714-w, PMID:34880218.
- [200] Ling S, Yang S, Hu X, Yin D, Dai Y, Qian X, *et al*. Lentiviral delivery of co-packaged Cas9 mRNA and a Vegfa-targeting guide RNA prevents wet age-related macular degeneration in mice. *Nat Biomed Eng* 2021;5(2):144–156. doi:10.1038/s41551-020-00656-y, PMID:33398131.
- [201] Nahmad AD, Reuveni E, Goldschmidt E, Tenne T, Liberman M, Horovitz-Fried M, *et al*. Frequent aneuploidy in primary human T cells after CRISPR-Cas9 cleavage. *Nat Biotechnol* 2022;40(12):1807–1813. doi:10.1038/s41587-022-01377-0, PMID:35773341.
- [202] Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, *et al*. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature* 2018;556(7699):57–63. doi:10.1038/nature26155, PMID:29512652.
- [203] Lee J, Chung JH, Kim HM, Kim DW, Kim H. Designed nucleases for targeted genome editing. *Plant Biotechnol J* 2016;14(2):448–462. doi:10.1111/pbi.12465, PMID:26369767.
- [204] Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* 2013;8(11):2281–2308. doi:10.1038/nprot.2013.143, PMID:24157548.
- [205] Zhao D, Jiang G, Li J, Chen X, Li S, Wang J, *et al*. Imperfect guide-RNA (igRNA) enables CRISPR single-base editing with ABE and CBE. *Nucleic Acids Res* 2022;50(7):4161–4170. doi:10.1093/nar/gkac201, PMID:35349689.
- [206] Caso F, Davies B. Base editing and prime editing in laboratory animals. *Lab Anim* 2022;56(1):35–49. doi:10.1177/0023677221993895, PMID:33596731.
- [207] Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat Biotechnol* 2020;38(7):824–844. doi:10.1038/s41587-020-0561-9, PMID:32572269.
- [208] Karimian A, Gorjizadeh N, Alemi F, Asemi Z, Azizian K, Soleimanpour J, *et al*. CRISPR/Cas9 novel therapeutic road for the treatment of neurodegenerative diseases. *Life Sci* 2020;259:118165. doi:10.1016/j.lfs.2020.118165, PMID:32735884.
- [209] Duan W, Urani E, Mattson MP. The potential of gene editing for Huntington's disease. *Trends Neurosci* 2023;46(5):365–376. doi:10.1016/j.tins.2023.02.005, PMID:36907678.
- [210] Peggion C, Scalcon V, Massimino ML, Nies K, Lopreiato R, Rigobello MP, *et al*. SOD1 in ALS: Taking Stock in Pathogenic Mechanisms and the Role of Glial and Muscle Cells. *Antioxidants (Basel)* 2022;11(4):614. doi:10.3390/antiox11040614, PMID:35453299.
- [211] Bhardwaj S, Kesari KK, Rachamalla M, Mani S, Ashraf GM, Jha SK, *et al*. CRISPR/Cas9 gene editing: New hope for Alzheimer's disease therapeutics. *J Adv Res* 2022;40:207–221. doi:10.1016/j.jare.2021.07.001, PMID:36100328.
- [212] Jurcau A, Jurcau MC. Therapeutic Strategies in Huntington's Disease: From Genetic Defect to Gene Therapy. *Biomedicines* 2022;10(8):1895.

- doi:10.3390/biomedicines10081895, PMID:36009443.
- [213] Ioannou A, Fontana M, Gillmore JD. RNA Targeting and Gene Editing Strategies for Transthyretin Amyloidosis. *BioDrugs* 2023;37(2):127–142. doi:10.1007/s40259-023-00577-7, PMID:36795354.
- [214] de Rus Jacquet A, Denis HL, Cicchetti F, Alpaugh M. Current and future applications of induced pluripotent stem cell-based models to study pathological proteins in neurodegenerative disorders. *Mol Psychiatry* 2021;26(7):2685–2706. doi:10.1038/s41380-020-00999-7, PMID:33495544.
- [215] Tabassum T, Pietrogrande G, Healy M, Wolvetang EJ. CRISPR-Cas9 Direct Fusions for Improved Genome Editing via Enhanced Homologous Recombination. *Int J Mol Sci* 2023;24(19):14701. doi:10.3390/ijms241914701, PMID:37834150.
- [216] Sun W, Liu H, Yin W, Qiao J, Zhao X, Liu Y. Strategies for Enhancing the Homology-Directed Repair Efficiency of CRISPR-Cas Systems. *CRISPR J* 2022;5(1):7–18. doi:10.1089/crispr.2021.0039, PMID:35076280.
- [217] Ali Z, Shami A, Sedeek K, Kamel R, Alhabsi A, Tehseen M, *et al.* Fusion of the Cas9 endonuclease and the VirD2 relaxase facilitates homology-directed repair for precise genome engineering in rice. *Commun Biol* 2020;3(1):44. doi:10.1038/s42003-020-0768-9, PMID:31974493.
- [218] Liang F, Han M, Romanienko PJ, Jasin M. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proc Natl Acad Sci U S A* 1998;95(9):5172–5177. doi:10.1073/pnas.95.9.5172, PMID:9560248.
- [219] Patabhi S, Lotti SN, Berger MP, Singh S, Lux CT, Jacoby K, *et al.* In Vivo Outcome of Homology-Directed Repair at the HBB Gene in HSC Using Alternative Donor Template Delivery Methods. *Mol Ther Nucleic Acids* 2019;17:277–288. doi:10.1016/j.omtn.2019.05.025, PMID:31279229.
- [220] Zhao Y, Zhang C, Liu W, Gao W, Liu C, Song G, *et al.* An alternative strategy for targeted gene replacement in plants using a dual-sgRNA/Cas9 design. *Sci Rep* 2016;6:23890. doi:10.1038/srep23890, PMID:27033976.
- [221] Yeh CD, Richardson CD, Corn JE. Advances in genome editing through control of DNA repair pathways. *Nat Cell Biol* 2019;21(12):1468–1478. doi:10.1038/s41556-019-0425-z, PMID:31792376.
- [222] Chang HHY, Pannunzio NR, Adachi N, Lieber MR. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat Rev Mol Cell Biol* 2017;18(8):495–506. doi:10.1038/nrm.2017.48, PMID:28512351.
- [223] Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, *et al.* Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* 2017;551(7681):464–471. doi:10.1038/nature24644, PMID:29160308.
- [224] Nambiar TS, Baudrier L, Billon P, Ciccio A. CRISPR-based genome editing through the lens of DNA repair. *Mol Cell* 2022;82(2):348–388. doi:10.1016/j.molcel.2021.12.026, PMID:35063100.
- [225] Wienert B, Nguyen DN, Guenther A, Feng SJ, Locke MN, Wyman SK, *et al.* Timed inhibition of CDC7 increases CRISPR-Cas9 mediated templated repair. *Nat Commun* 2020;11(1):2109. doi:10.1038/s41467-020-15845-1, PMID:32355159.
- [226] Shams F, Bayat H, Mohammadian O, Mahboudi S, Vahidnezhad H, Soosanabadi M, *et al.* Advance trends in targeting homology-directed repair for accurate gene editing: An inclusive review of small molecules and modified CRISPR-Cas9 systems. *Bioimpacts* 2022;12(4):371–391. doi:10.34172/bi.2022.23871, PMID:35975201.
- [227] Song J, Yang D, Xu J, Zhu T, Chen YE, Zhang J. RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency. *Nat Commun* 2016;7:10548. doi:10.1038/ncomms10548, PMID:26817820.
- [228] Gutschner T, Haemmerle M, Genovese G, Draetta GF, Chin L. Post-translational Regulation of Cas9 during G1 Enhances Homology-Directed Repair. *Cell Rep* 2016;14(6):1555–1566. doi:10.1016/j.celrep.2016.01.019, PMID:26854237.
- [229] Nguyen DN, Roth TL, Li PJ, Chen PA, Apathy R, Mamedov MR, *et al.* Polymer-stabilized Cas9 nanoparticles and modified repair templates increase genome editing efficiency. *Nat Biotechnol* 2020;38(1):44–49. doi:10.1038/s41587-019-0325-6, PMID:31819258.
- [230] Tong Y, Whitford CM, Robertsen HL, Blin K, Jørgensen TS, Klitgaard AK, *et al.* Highly efficient DSB-free base editing for streptomycetes with CRISPR-BEST. *Proc Natl Acad Sci U S A* 2019;116(41):20366–20375. doi:10.1073/pnas.1913493116, PMID:31548381.
- [231] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet* 2018;19(12):770–788. doi:10.1038/s41576-018-0059-1, PMID:30323312.
- [232] Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, *et al.* Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 2019;576(7785):149–157. doi:10.1038/s41586-019-1711-4, PMID:31634902.
- [233] Gandhi J, Antonelli AC, Afridi A, Vatsia S, Joshi G, Romanov V, *et al.* Protein misfolding and aggregation in neurodegenerative diseases: a review of pathogenesis, novel detection strategies, and potential therapeutics. *Rev Neurosci* 2019;30(4):339–358. doi:10.1515/revneuro-2016-0035, PMID:30742586.
- [234] Wakabayashi K, Tanji K, Odagiri S, Miki Y, Mori F, Takahashi H. The Lewy body in Parkinson's disease and related neurodegenerative disorders. *Mol Neurobiol* 2013;47(2):495–508. doi:10.1007/s12035-012-8280-y, PMID:22622968.
- [235] Tellone E, Galtieri A, Ficarra S. Reviewing Biochemical Implications of Normal and Mutated Huntingtin in Huntington's Disease. *Curr Med Chem* 2020;27(31):5137–5158. doi:10.2174/0929867326666190621101909, PMID:31223078.
- [236] Markou A, Chiamulera C, Geyer MA, Tricklebank M, Steckler T. Removing obstacles in neuroscience drug discovery: the future path for animal models. *Neuropsychopharmacology* 2009;34(1):74–89. doi:10.1038/npp.2008.173, PMID:18830240.
- [237] Bhattacharjee G, Gohil N, Khambhati K, Mani I, Maurya R, Karapurkar JK, *et al.* Current approaches in CRISPR-Cas9 mediated gene editing for biomedical and therapeutic applications. *J Control Release* 2022;343:703–723. doi:10.1016/j.jconrel.2022.02.005, PMID:35149141.
- [238] Tu Z, Yang W, Yan S, Guo X, Li XJ. CRISPR/Cas9: a powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. *Mol Neurodegener* 2015;10:35. doi:10.1186/s13024-015-0031-x, PMID:26238861.
- [239] Safari F, Hatam G, Behbahani AB, Rezaei V, Barekati-Mowahed M, Petramfar P, *et al.* CRISPR System: A High-throughput Toolbox for Research and Treatment of Parkinson's Disease. *Cell Mol Neurobiol* 2020;40(4):477–493. doi:10.1007/s10571-019-00761-w, PMID:31773362.
- [240] Chen Y, Zheng Y, Kang Y, Yang W, Niu Y, Guo X, *et al.* Functional disruption of the dystrophin gene in rhesus monkey using CRISPR/Cas9. *Hum Mol Genet* 2015;24(13):3764–3774. doi:10.1093/hmg/ddv120, PMID:25859012.
- [241] Roy B, Zhao J, Yang C, Luo W, Xiong T, Li Y, *et al.* CRISPR/Cascade 9-Mediated Genome Editing-Challenges and Opportunities. *Front Genet* 2018;9:240. doi:10.3389/fgene.2018.00240, PMID:30026755.
- [242] Zhou L, Yao S. Recent advances in therapeutic CRISPR-Cas9 genome editing: mechanisms and applications. *Mol Biomed* 2023;4(1):10. doi:10.1186/s43556-023-00115-5, PMID:37027099.
- [243] Wimberger S, Akrap N, Firth M, Brengdahl J, Engberg S, Schwinn MK, *et al.* Simultaneous inhibition of DNA-PK and Polθ improves integration efficiency and precision of genome editing. *Nat Commun* 2023;14(1):4761. doi:10.1038/s41467-023-40344-4, PMID:37580318.
- [244] Kotagama OW, Jayasinghe CD, Abeyasinghe T. Era of Genomic Medicine: A Narrative Review on CRISPR Technology as a Potential Therapeutic Tool for Human Diseases. *Biomed Res Int* 2019;2019:1369682. doi:10.1155/2019/1369682, PMID:31687377.
- [245] Brokowski C, Adli M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. *J Mol Biol* 2019;431(1):88–101. doi:10.1016/j.jmb.2018.05.044, PMID:29885329.
- [246] Feng S, Wang Z, Li A, Xie X, Liu J, Li S, *et al.* Strategies for High-Efficiency Mutation Using the CRISPR/Cas System. *Front Cell Dev Biol* 2021;9:803252. doi:10.3389/fcell.2021.803252, PMID:35198566.
- [247] Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *J Control Release* 2017;266:17–26. doi:10.1016/j.jconrel.2017.09.012, PMID:28911805.
- [248] Khoshandam M, Soltaninejad H, Mousazadeh M, Hamidieh AA, Hosseinkhani S. Clinical applications of the CRISPR/Cas9 genome-editing system: Delivery options and challenges in precision medicine. *Genes Dis* 2024;11(1):268–282. doi:10.1016/j.gendis.2023.02.027, PMID:37588217.

- [249] Nouri Nojaded J, BildirenEryilmaz NS, Ergüder BI. CRISPR/Cas9 genome editing for neurodegenerative diseases. *EXCLI J* 2023;22:567–582. doi:10.17179/excli2023-6155, PMID:37636024.
- [250] Grimm D, Lee JS, Wang L, Desai T, Akache B, Storm TA, *et al*. In vitro and in vivo gene therapy vector evolution via multispecies interbreeding and retargeting of adeno-associated viruses. *J Virol* 2008;82(12):5887–5911. doi:10.1128/JVI.00254-08, PMID:18400866.
- [251] Mani S, Jindal D, Singh M. Gene Therapy, A Potential Therapeutic Tool for Neurological and Neuropsychiatric Disorders: Applications, Challenges and Future Perspective. *Curr Gene Ther* 2023;23(1):20–40. doi:10.2174/1566523222666220328142427, PMID:35345999.
- [252] Chavez M, Chen X, Finn PB, Qi LS. Advances in CRISPR therapeutics. *Nat Rev Nephrol* 2023;19(1):9–22. doi:10.1038/s41581-022-00636-2, PMID:36280707.
- [253] Zetsche B, Volz SE, Zhang F. A split-Cas9 architecture for inducible genome editing and transcription modulation. *Nat Biotechnol* 2015;33(2):139–142. doi:10.1038/nbt.3149, PMID:25643054.
- [254] Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L, *et al*. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol* 1998;72(12):9873–9880. doi:10.1128/JVI.72.12.9873-9880.1998, PMID:9811723.
- [255] Gurumoorthy N, Nordin F, Tye GJ, Wan Kamarul Zaman WS, Ng MH. Non-Integrating Lentiviral Vectors in Clinical Applications: A Glance Through. *Biomedicines* 2022;10(1):107. doi:10.3390/biomedicines10010107, PMID:35052787.
- [256] Cota-Coronado A, Díaz-Martínez NF, Padilla-Camberos E, Díaz-Martínez NE. Editing the Central Nervous System Through CRISPR/Cas9 Systems. *Front Mol Neurosci* 2019;12:110. doi:10.3389/fn-mol.2019.00110, PMID:31191241.
- [257] Shah SZ, Rehman A, Nasir H, Asif A, Tufail B, Usama M, *et al*. Advances In Research On Genome Editing Crispr-Cas9 Technology. *J Ayub Med Coll Abbottabad* 2019;31(1):108–122. PMID:30868795.
- [258] Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. *Science* 2018;361(6405):866–869. doi:10.1126/science.aat5011, PMID:30166482.
- [259] Lin Y, Wagner E, Lächelt U. Non-viral delivery of the CRISPR/Cas system: DNA versus RNA versus RNP. *Biomater Sci* 2022;10(5):1166–1192. doi:10.1039/d1bm01658j, PMID:35103261.
- [260] Yip BH. Recent Advances in CRISPR/Cas9 Delivery Strategies. *Biomolecules* 2020;10(6):839. doi:10.3390/biom10060839, PMID:32486234.
- [261] Yang W, Liu Y, Tu Z, Xiao C, Yan S, Ma X, *et al*. CRISPR/Cas9-mediated PINK1 deletion leads to neurodegeneration in rhesus monkeys. *Cell Res* 2019;29(4):334–336. doi:10.1038/s41422-019-0142-y, PMID:30770867.
- [262] Mancuso M, Coppede F, Migliore L, Siciliano G, Murri L. Mitochondrial dysfunction, oxidative stress and neurodegeneration. *J Alzheimers Dis* 2006;10(1):59–73. doi:10.3233/jad-2006-10110, PMID:16988483.
- [263] Mufson EJ, Ikonovic MD, Counts SE, Perez SE, Malek-Ahmadi M, Scheff SW, *et al*. Molecular and cellular pathophysiology of preclinical Alzheimer's disease. *Behav Brain Res* 2016;311:54–69. doi:10.1016/j.bbr.2016.05.030, PMID:27185734.
- [264] Demirci S, Leonard A, Essawi K, Tisdale JF. CRISPR-Cas9 to induce fetal hemoglobin for the treatment of sickle cell disease. *Mol Ther Methods Clin Dev* 2021;23:276–285. doi:10.1016/j.omtm.2021.09.010, PMID:34729375.
- [265] Khiabani A, Kohansal MH, Keshavarzi A, Shahraki H, Kooshesh M, Karimzade M, *et al*. CRISPR/Cas9, a promising approach for the treatment of β -thalassemia: a systematic review. *Mol Genet Genomics* 2023;298(1):1–11. doi:10.1007/s00438-022-01978-z, PMID:36403178.
- [266] Marangi M, Pistritto G. Innovative Therapeutic Strategies for Cystic Fibrosis: Moving Forward to CRISPR Technique. *Front Pharmacol* 2018;9:396. doi:10.3389/fphar.2018.00396, PMID:29731717.
- [267] Bez Batti Angulski A, Hosny N, Cohen H, Martin AA, Hahn D, Bauer J, *et al*. Duchenne muscular dystrophy: disease mechanism and therapeutic strategies. *Front Physiol* 2023;14:1183101. doi:10.3389/fphys.2023.1183101, PMID:37435300.