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# *Approaches and Frontiers of CRISPR-Cas9 in the treatment of Huntington's Disease*

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***Abstract:*** Huntington's disease is genetic neurodegenerative disorder that usually appears in individuals between the ages of 30 and 50, causing profound impairments in motor function, cognition, and emotional regulation. The underlying etiology of this condition is linked to mutations in the DNA that lead to the abnormal aggregation of the huntingtin protein, ultimately causing neuronal cell death. In the absence of effective treatment options, pertinent research continues to investigate gene editing technology as a potential therapeutic approach. This paper focuses on the application of CRISPR-Cas9 technology, which aims to precisely edit the mutated huntingtin gene, particularly targeting and disrupting the CAG repeat expansion to mitigate neurodegeneration and restore normal gene function. Through the literature review and case analyses, it evaluates the efficacy of CRISPR-Cas9 in mitigating huntingtin protein aggregation and enhancing patient prognosis. Therefore, the results suggest that CRISPR technology holds promise as an innovative therapeutic approach for Huntington's disease. Nevertheless, challenges such as off-target effects and efficient gene delivery need to be addressed. In short, therapeutic strategies like CRISPR, antisense oligonucleotides (ASOs), and small interfering RNAs (siRNAs) show considerable promise for driving future research advancements.

***Keywords:*** Huntington's disease, CRISPR-Cas9, Small interfering RNAs, Genetic disorder, Antisense oligonucleotides

## 1. Introduction

Huntington's disease (HD) is a progressive, fatal neurodegenerative disorder caused by a genetic mutation that leads to the loss of striatal neurons. This results in a range of symptoms, including motor abnormalities, cognitive decline, and psychiatric disturbances [1]. As the disease advances with age, patients experience worsening symptoms, such as chorea and mental health issues like depression and sleep disturbances. These impairments make it increasingly difficult for individuals to control their movements and, eventually, to carry out daily activities [2]. HD is caused by mutations in the Huntington gene (HTT), which encodes the Huntington protein. The mutated form of this gene (mHTT) contains expanded CAG repeats, leading to the production of a mutant protein with more than 35 repeats. This protein undergoes abnormal post-translational modifications, initiating a cascade of pathogenic events, including disrupted transcriptional regulation, immune system dysfunction, and mitochondrial impairments [3]. The disease follows an autosomal dominant inheritance pattern, typically manifesting in adulthood and progressing over time, with a significant

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impact on the lives of patients and their families. In recent years, CRISPR/Cas9 technology has demonstrated the potential to reduce mHTT levels. This mechanism affects the splicing process by targeting exon-intron boundaries, thereby influencing the translation of mHTT [3]. Despite the discovery of the genetic basis of HD more than two decades ago, there is currently no cure or treatment with significant efficacy. The existing therapies are primarily focused on symptom management rather than addressing the underlying disease [4]. The research aims to explore the potential of CRISPR technology as a treatment for HD, and assess the efficacy of two promising therapeutic approaches, which focuses on the mechanisms of CRISPR/Cas9, particularly its ability to precisely edit the mutated Huntington gene, with the goal of providing fresh perspectives on future treatment strategies for HD. By analyzing both the opportunities and challenges associated with this technology, it seeks to offer valuable insights into the future management of HD and support the development of effective therapeutic interventions..

## 2. Mechanisms of CRISPR-Cas9 in Huntington's Disease Treatment

The CRISPR/Cas9 system has emerged as a promising gene-editing tool for HD, offering the ability to precisely target and modify the CAG repeat expansions in the HTT gene, thereby reducing the production of mHTT. Preclinical studies have shown that CRISPR/Cas9 can lower mHTT levels and alleviate disease symptoms in both cellular and animal models of HD. Nevertheless, the clinical application of CRISPR/Cas9 in humans presents several challenges, such as issues with delivery efficiency, off-target effects, and ethical considerations. Figure 1 outlines the mechanisms behind CRISPR/Cas9 gene editing [5].

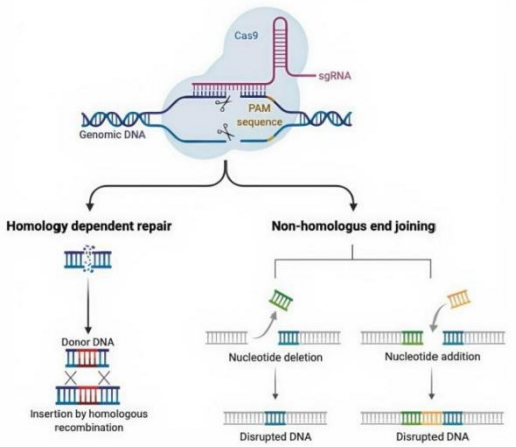


Figure 1: Mechanism of CRISPR-Cas9 Gene Editing

### 2.1. Basic Principles of Gene Editing

The gene editing technologies, particularly the CRISPR/Cas9 system, have become essential tools for precise genome modification. CRISPR is a bacterial immune defense mechanism that uses RNA molecules to direct the Cas9 enzyme to specific DNA sequences. The guide RNA (gRNA) binds to the complementary DNA sequence, guiding Cas9 to induce double-strand breaks at the target locus. The spacer sequence of the gRNA typically consists of 20 nucleotides. The system's high precision and adaptability make it a revolutionary tool in gene editing. However, its clinical application still faces challenges such as off-target effects and delivery efficiency. Therefore, understanding the basic mechanisms of CRISPR is crucial for assessing its potential and limitations in precise genome modification. After the double-strand break, cells repair the damage via non-homologous end joining (NHEJ) or homologous directed repair (HDR). By introducing a donor DNA template, the CRISPR

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system can achieve precise gene modification. HD is caused by the expansion of the CAG repeat sequence in the first exon of the HTT gene, leading to the production of mHTT protein containing 36 or more glutamine residues [6,7]. This mutant protein aggregates in cells, forming inclusions that lead to neuronal cell death, particularly in brain regions like the cortex and striatum, which are highly vulnerable to mHTT-induced toxicity. Currently, diagnostic and therapeutic strategies for HD are under active investigation, aiming to develop more effective interventions, as shown in Figure 2 [5].

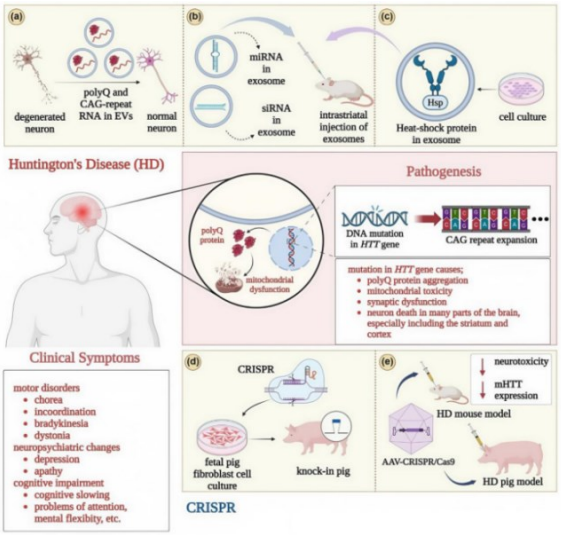


Figure 2: Different Diagnoses and Treatment Types for HD

The CRISPR-Cas system can be divided into two main classes: Class 1 (Types I, III, and IV) and Class 2 (Types II, V, and VI). Class 1 systems are predominantly found in bacteria and archaea, involving multiple Cas proteins, and account for approximately 90% of all known CRISPR-Cas loci. In contrast, Class 2 systems are found only in bacteria, are mediated by a single Cas protein, and account for the remaining 10% of known CRISPR-Cas loci [9,10]. Both classes consist of a ribonucleoprotein complex made up of CRISPR RNA (crRNA) and Cas proteins [11]. The primary distinction is that the Class 2 system, especially Type II, can achieve targeted gene editing using a single Cas9 protein and a sgRNA, making it more suitable for precise genetic modification[12,13].

## 2.2. Targeting Pathogenic Genes in Huntington's Disease

The CRISPR/Cas9 genome editing technology provides a powerful instrument for targeting the causative gene of HD, particularly in the precise correction of mutated genes. The core mechanism of this technology involves three key steps: recognition, cleavage, and repair[14]. Single-guide RNA (sgRNA) binds to the complementary DNA sequence through its 5' crRNA component, guiding the Cas9 protein to the target gene. Cas9 remains inactive until it binds to sgRNA, at which point it is activated and locates the target DNA, specifically at sites near the protospacer adjacent motif (PAM) sequence [15]. Once bound, Cas9 cleaves the DNA, with the HNH domain cleaving the complementary strand and the RuvC domain cleaving the non-complementary strand, resulting in a double-strand break [16]. These double-strand breaks are repaired primarily through two pathways: NHEJ and HDR. NHEJ is a fast but error-prone repair mechanism that directly joins DNA ends, often introducing insertions or deletions (indels), which can lead to frame-shift mutations. In contrast, HDR relies on a homologous DNA template for precise repair, and is most active during the S and G2 phases of the cell cycle. By using a donor DNA template complementary to the target sequence, HDR enables precise gene insertion or replacement, ensuring accuracy in gene editing. In the treatment of

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HD, precise correction of the mutated gene is critical for successful intervention. The selective use of NHEJ and HDR has a significant impact on the treatment outcome. Efficient utilization of HDR can prevent errors caused by NHEJ, achieving more accurate gene repair. Therefore, optimizing the CRISPR/Cas9 system's repair pathways to enhance HDR efficiency and minimize unwanted mutations is an important research direction for future gene editing therapies for Huntington's disease.

## 3. Applications of CRISPR-Cas9 in Huntington's Disease Treatment

### 3.1. Pre-clinical Studies Application

In 2022, a preclinical study on CRISPR treatment for HIV in the United States designed guide RNAs to direct the Cas9 protein to cut at two specific sites in the HIV genome, effectively removing most of the viral DNA from host cells. This treatment utilized an AAV9 viral vector for a single infusion, aiming to surgically remove the viral genetic material and eliminate HIV from infected cells [17].

### 3.2. Progress in Clinical Trials

With the continuous maturation of gene editing technologies, the application of the CRISPR/Cas9 system in clinical trials has made significant progress, marking a step toward the practical implementation of gene therapies. At the end of 2023, the CRISPR-based gene therapy Casgevy was approved by regulatory agencies for the treatment of sickle cell disease (SCD) and transfusion-dependent β-thalassemia (TDT), representing a major milestone in the field of gene therapy. This approval not only validates the breakthroughs CRISPR technology has achieved in both scientific and clinical applications, but also demonstrates its potential for the precise treatment of complex genetic diseases. The approvals by the UK Medicines and Healthcare products Regulatory Agency (MHRA) and the US Food and Drug Administration (FDA) have provided strong support for the safety and efficacy of this therapy. These regulatory validations indicate that CRISPR has become a viable option for treating genetic disorders and is driving a shift in therapeutic paradigms. Subsequent approvals in the EU and Bahrain further confirm the global recognition of this therapy, reflecting a growing consensus internationally on its value. Meanwhile, the ongoing reviews in Saudi Arabia and Canada highlight the potential for broader market applications, underscoring the increasing acceptance and regulatory consistency regarding gene editing technologies across different healthcare systems. These developments not only expand the reach of gene therapies but also signal a transformation in personalized medicine and clinical practices in the future [17]. Recent studies further underscore the transformative role of the CRISPR/Cas9 system in genome editing, particularly in cancer treatment and rare disease therapy. Compared to traditional gene therapies, CRISPR offers higher precision and adaptability, showing marked improvements in targeting specific mutations and pathological processes, leading to better therapeutic outcomes. In cardiovascular disease treatment, the potential of CRISPR is increasingly recognized, with innovations such as base editing and prime editing significantly improving the accuracy of genetic modifications. This enhanced precision is crucial for optimizing patient outcomes. Also, the development of advanced CRISPR tools, particularly for both in vivo and in vitro applications, opens up new possibilities for cardiovascular research and treatment. Overall, these advancements reinforce the central role of CRISPR technology in gene therapy, offering new methods and perspectives for disease management and treatment [18].

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## 4. Other Therapeutic Strategies Combined with CRISPR-Cas9

### 4.1. Antisense Oligonucleotides (ASOs)

ASOs represent a promising strategy for treating genetic disorders, including HD, by targeting and degrading specific mRNA molecules. They are short, synthetic single-stranded DNA molecules designed to bind specifically to target mRNA, facilitating its degradation. Based on their mechanism of action, ASOs can be classified into allele-specific and non-allele-specific categories. Non-allele-specific ASOs can target both wild-type and mHTT mRNA, broadening the therapeutic scope. Preclinical studies have demonstrated their potential to reverse HD pathology and clinical features, paving the way for clinical trials. For example, the first non-allele-specific ASO in clinical trials, Tominersen (RG6042/IIONIS-HTTRx), reduced mHTT levels in cerebrospinal fluid (CSF) by nearly 40%. Similarly, early clinical trials from Wave Life Sciences showed a reduction of about 12.5% in mHTT mRNA levels, further supporting the therapeutic potential of ASOs for HD.

ASOs represent a promising treatment strategy for HD by targeting the mRNA that encodes the mutant huntingtin protein. By hybridizing with specific mRNA sequences, ASOs induce degradation or inhibit translation. In several clinical trials, the application of ASOs, such as Tominersen, has proven capable of significantly reducing mHTT levels in CSF, but Phase III trials were terminated due to insufficient safety and efficacy. To date, the FDA has approved a total of 17 RNA oligonucleotide therapies, including 6 siRNA and 11 antisense oligonucleotides, further confirming the potential of ASOs in clinical applications [19]. The combination of ASO therapy with CRISPR-Cas9 technology may provide greater therapeutic advantages. With the gene-editing capabilities of CRISPR, ASOs can more precisely target and eliminate specific mutant mRNA, potentially achieving more effective treatment outcomes. The research led by Tabrizi demonstrated that a novel ASO drug could effectively reduce levels of the mutant huntingtin protein in the cerebrospinal fluid of HD patients. This study involved 46 participants and assessed the drug's safety and tolerability through four intrathecal injections, collecting CSF samples before and after treatment to evaluate pharmacokinetics and its impact on mutant HTT levels [20]. Despite the promising future in the treatment of HD, ASOs still need to be combined with other therapeutic strategies to optimize treatment efficacy. Potential treatment options include gene replacement therapy, gene editing techniques such as CRISPR/Cas9, mRNA therapies, and enzyme replacement therapies, to further clarify which strategies are most beneficial for patient health [21].

### 4.2. Small Interfering RNAs (siRNAs)

Small non-coding RNAs, typically 20-30 nucleotides in length, are key regulators of gene expression and genomic stability. Among them, siRNAs have proven to be powerful tools for dissecting gene function in both in vitro and in vivo models. siRNAs also present considerable potential as a novel therapeutic class, particularly for targeting regions previously considered "undruggable," making them highly promising for treating complex diseases, including cancer and other challenging conditions [22]. The RNAi process, where double-stranded RNA (dsRNA) triggers gene silencing by promoting the degradation of complementary mRNA, has greatly advanced gene function research and holds substantial therapeutic potential across a broad spectrum of diseases [23]. By leveraging the cell's inherent gene regulation mechanisms, RNAi can selectively suppress gene expression, and its application has been explored as a therapeutic strategy for neurodegenerative diseases like HD. RNAi-based therapies, such as siRNA and short hairpin RNA (shRNA), target and degrade mHTT mRNA, leading to a reduction in mHTT protein levels in preclinical models. AMT-130, a candidate currently in clinical trials, highlights RNAi's ability to specifically silence mHTT while preserving the wild-type allele, improving therapeutic specificity and minimizing off-target effects.

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Despite these promising developments, the delivery of RNAi molecules across the blood-brain barrier (BBB) remains a significant challenge, limiting their therapeutic potential for central nervous system (CNS) disorders. Current research is focused on overcoming this barrier through the development of advanced delivery systems and optimization of administration protocols to achieve sufficient therapeutic concentrations within the CNS. Furthermore, combining RNAi with CRISPR-Cas9 genome-editing technology holds the potential to enhance the precision and efficacy of targeting mutant alleles, thereby offering a more tailored approach to the treatment of HD and other genetic disorders. Although clinical applications of RNAi therapies have made significant strides, the scope of ongoing investigations remains relatively limited, underscoring the need for further research to fully realize their potential in clinical settings.

## 5. Advantages and Challenges of CRISPR-Cas9 Technology

### 5.1. Advantages of CRISPR-Cas9

The CRISPR-Cas9 technology, as an efficient gene-editing tool, has several distinct advantages, making it widely applicable in genome editing and clinical treatments. The system consists of Cas nucleases and gRNA, where crRNA and tracrRNA can function independently or together to form a ribonucleoprotein (RNP) complex. In this complex, gRNA can specifically recognize and edit target DNA sequences. Studies have shown that a sgRNA can simultaneously target multiple genetic loci, significantly increasing the efficiency and versatility of gene editing, making it a powerful tool for manipulating various genomic regions. Besides, CRISPR-Cas9's cost-effectiveness further enhances its wide application in both research and clinical settings [24]. In comparison to traditional gene-editing technologies, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 offers a significant economic advantage by drastically reducing the overall cost of gene editing, which improves its accessibility for both research and clinical use. The price comparison in Figure 3 further highlights the cost-effectiveness of CRISPR-Cas9, supporting its widespread adoption globally. In addition, the high efficiency of CRISPR-Cas9 has been validated in multiple applications. For instance, CRISPR-based interventions, particularly those that minimize DNA double-strand breaks, have been shown to effectively slow the progression of HD in mouse models. Notably, CRISPR interference (CRISPRi) has been used to reduce the expression of mHTT, while preserving wild-type huntingtin (wtHTT) in human HD fibroblasts. This approach not only delays behavioral decline but also protects neurons in the striatum of HD mice by preventing cell death. Although the effect of CRISPRi is less pronounced compared to traditional CRISPR/Cas9 gene editing methods, it still provides a viable intervention strategy for HD treatment [3].

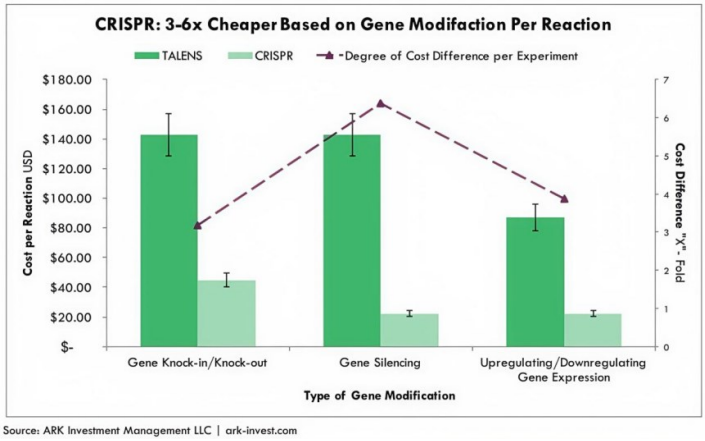


Figure 3: The Prices of Several Gene Editing Technologies

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### 5.2. Challenges of CRISPR-Cas9

Despite the significant potential of CRISPR-Cas9 technology in gene editing [25], its clinical implementation continues to be hindered by numerous challenges. Current therapeutic approaches for diseases such as HD predominantly focus on palliative care, with the development of curative therapies or effective treatments still facing significant obstacles. One major challenge is the difficulty in achieving allele-specific targeting with CRISPR-Cas9, as non-homologous end joining (NHEJ) repair may result in substantial nucleotide deletions, thereby compromising the efficacy of the treatment. Although the genetic mutations underlying such diseases are well-established, the precise pathological mechanisms continue to be the subject of ongoing research. Additionally, while CRISPR-Cas9 offers a more cost-effective alternative to TALEN-based gene editing, it is not without its limitations. CRISPR-Cas9 is more prone to off-target effects, which can lead to unintended modifications of non-target genes. In contrast, TALEN (transcription activator-like effector nucleases) provides greater precision, as it requires dimerization before binding to the FokI nuclease, which induces double-strand breaks (DSBs) at specific genomic sites. CRISPR, by relying on a single guide RNA (gRNA) to direct Cas9 to the target site, simplifies the process but sacrifices some degree of specificity. Moreover, while CRISPR-Cas9 has an estimated 70% success rate in targeting random DNA sequences, TALEN technology theoretically allows for editing any genomic region, making it potentially more versatile in certain applications. Off-target effects in CRISPR-Cas9 remain a significant concern. When Cas9 induces DSBs at unintended genomic loci, it can lead to deleterious consequences. The occurrence of these off-target effects often depends on the design of the gRNA, as Cas9 can tolerate up to three mismatches in its binding. Although computational tools can predict potential off-target sites and estimate the likelihood of such events, some off-target effects are independent of gRNA design. Therefore, unbiased experimental detection methods are essential for accurately identifying and mitigating these risks. This review discusses various strategies for assessing off-target effects, highlighting their respective advantages and limitations, and notes that certain techniques may be applicable to other Cas nucleases, such as Cas12a (Cpf1) [26].

## 6. Conclusion

This study explores various cutting-edge treatment approaches for HD, primarily CRISPR/Cas9 gene editing technology. The results indicate that ASOs show promising preclinical efficacy in reducing levels of mHTT, but face challenges regarding safety and efficacy. CRISPR/Cas9 demonstrates broad potential for application in gene editing, particularly in multi-gene targeting. However, issues related to delivery efficiency and off-target effects still need to be addressed. RNAi technology provides a highly specific method for gene expression suppression, but similarly encounters challenges with effective delivery. These findings suggest that while each treatment approach has its advantages and limitations, they offer new hope for the treatment of Huntington's disease. Due to the complexity of HD, a single treatment method may not fully meet clinical needs, necessitating research that integrates multiple strategies. Future research should focus on strengthening clinical trials for each treatment method to validate their safety and efficacy, particularly for ASOs and CRISPR/Cas9 applications. The development of efficient delivery systems to enhance the therapeutic efficacy of RNAi and CRISPR/Cas9 is an important research direction.

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