



Review Article

The Emerging Role of Crispr in the Treatment of Rare Genetic Disorders

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ABSTRACT

Although each individual rare genetic disorder is uncommon, collectively they represent a significant global health challenge. The clustered regularly interspaced short palindromic repeats (CRISPR) genome-editing technology has attracted substantial attention in medicine due to its adaptability, simple design, high efficiency, and precision, driving rapid progress in the field. It supports scientific research by facilitating disease modelling, enhancing crop strains, and creating treatments for genetic conditions. In the medical field, it has been used or studied for disorders such as sickle cell disease and other inherited conditions. Unlike earlier gene-editing methods, it offers greater speed, lower costs, and higher precision. The technology functions by identifying specific DNA sequences using specialized proteins, allowing not just gene editing but also the control of gene expression.

KEYWORDS: Rare genetic disorder, CRISPR, gene editing, genome, DNA, RNA, Endonucleases

INTRODUCTION

Rare disease or Orphan disease represents a significant impact on health, it is a disease where mutations in DNA effects the gene function or chromosome structure, many rare genetic diseases are inherited or arise as a new-mutations. Without warning, certain individuals carry unusual changes in their genetic code - small alterations in DNA that disrupt cell function or bend chromosome structure abnormally. These traits do not appear in all people; only isolated cases occur. Though often inherited from either parent, on rare occasions the change arises spontaneously, absent in any family history. Rare mutations emerge where none existed before. There are over 8000 rare genetic disorders identified till date and many more are still discovered. New techniques are developed to diagnose the genetic disorders and depending upon the disease treatment plan is generated, which is suitable for each disorder.^[1-3]

A 2019 study using data from Orphanet estimated that rare diseases affect between 3.5% and 5.9% of the global population, equivalent to approximately 263 million to 446 million people worldwide. Around 71.9% of the rare diseases listed in Orphanet are genetic in origin. This estimate relies on epidemiological information from Orphanet rather than GLOBOCAN, as GLOBOCAN functions primarily as a cancer registry and is not designed to assess the prevalence of non-cancer rare genetic disorders.^[4,5]

Genetic damage may arise from various alterations in DNA or genes, including single gene variants, mutations such as point mutations and changes like gene deletions, nonsense or missense substitutions. It can also result from structural chromosome abnormalities, large-scale deletions, translocations, irregular DNA methylation patterns, or errors in genomic imprinting. These genetic disruptions can lead to disorders or impaired biological functions.

Different rare genetic disorders are; Cystic fibrosis, Duchenne muscular dystrophy, Spinal muscular dystrophy, Prader-Willi and Angelman syndrome, Huntington syndrome, Menkes disease, Ribose-5 phosphate isomerase deficiency, Sickle cell and Thalassemia etc are the few diseases caused due to changes in the DNA.

CONVENTIONAL THERAPIES

Various diagnosis techniques are used like Mass spectroscopy, southern blot method, Bisulfite sequencing, Chromosomal microarray, RNA and DNA sequencing, Whole genome sequencing, gene panels, Polymerase chain reaction, Triplet repeat primed PCR technique etc are used for to diagnose the particular genetic disorder, which helps in better treatment plan.

Conventional therapy or Supportive therapy is given to decrease the severity and intensity of disorder and helps control the disease effects and fix the underlying DNA effects. Each treatment is chosen depending upon the organ and type of defect in the gene.

Supportive medicines like pain relief, antiseizure, bronchodilators, corticosteroids are common, Dietary and nutritional support is essential, Blood transfusions if any, bone marrow or hematopoietic stem cell transplant, surgery if required. ^[6]

CRISPR TECHNOLOGY

The clustered regularly interspaced short palindromic repeats (CRISPR) system is a gene-editing tool that has significantly transformed biomedical research. CRISPR originated as a natural bacterial defense mechanism but was transformed into a powerful gene-editing tool when scientists demonstrated its ability to be programmed to target specific DNA sequences. The key advancement occurred with the adaptation of the CRISPR-Cas9 system for accurate DNA cleavage within cells, making gene editing far more straightforward than previous techniques. In bacteria, the system provides immunity by retaining fragments of viral DNA and using them to identify and destroy matching viruses upon reinfection. Researchers harnessed this biological process for genome engineering, turning it into one of the most prevalent tools in contemporary genetic research.

At its core, CRISPR relies on two key components: a guide RNA designed to locate a specific target gene, and the CRISPR-associated protein 9 (Cas9), an enzyme that cuts both strands of DNA. This break in the DNA enables precise alterations to the genome.

Beyond their role in identifying or confirming drug targets, CRISPR-based platforms can also function as diagnostic instruments, offering accurate detection that facilitates early action in cases of both infectious and noninfectious conditions. SHERLOCKv2, for example: it can detect several DNA and RNA sequences at once, which makes it well-suited for use as a rapid, portable, and quantitative method to identify pathogens responsible for diseases like dengue, Zika, and pneumonia.

CRISPR-based methods for preventing and treating diseases represent one of the most promising applications of the CRISPR system. The CRISPR-Cas9 tool is revolutionizing gene therapy, showing tangible advances in addressing conditions like Duchenne muscular dystrophy, α 1-antitrypsin deficiency, hearing impairments, blood disorders, HIV-1 infection, and several forms of cancer. A growing number of clinical trials are now underway to evaluate the safety and effectiveness of these CRISPR-based treatments. ^[7,8]

CAS PROTEINS

Cas proteins are CRISPR-associated enzymes that use guide RNA to recognize and cleave specific DNA or RNA sequences, providing adaptive immunity against foreign genetic material.

TYPES OF CRISPR SYSTEMS

The three main CRISPR systems you referred to—CRISPR-Cas9, CRISPR-Cas12, and CRISPR-Cas13—all rely on RNA for guidance but differ in the types of molecules they target (DNA or RNA), their cutting mechanisms, and their applications. We'll examine each of these features across the three systems.

The CRISPR-Cas system is typically divided into three primary categories.

- Type I systems are defined by the Cas3 protein.
- In Type II, the key component is Cas9, a protein extensively employed in genetic engineering applications.
- Type III systems are distinguished by Cas10 and its related functional components.
- Newly introduced CRISPR tools are Cas9, Cas12, Cas13, base editing and prime editing. ^[9]

1. CRISPR-Cas9

Cas9 is the most widely recognized example of a Class 2, type II CRISPR system, employed for targeted DNA editing. It relies on a single-guide RNA (sgRNA) to locate a roughly 20-nucleotide DNA sequence situated next to a protospacer adjacent motif (PAM), such as 5'-NGG-3' in the case of *S. pyogenes* Cas9. Once bound, Cas9 induces a blunt double-strand break using its two nuclease domains, HNH and RuvC. The cell then repairs this break either through non-homologous end joining (NHEJ), which often results in insertions or deletions and gene knockouts, or through homology-directed repair (HDR), allowing for precise genetic modifications. This mechanism supports a range of applications, including gene knockouts, knock-ins, and base editing.

CRISPR-Cas9 serves as an RNA-directed adaptive immune mechanism in bacteria and archaea. It retains a record of previous encounters with viruses or plasmids by incorporating brief segments of foreign DNA used as spacers into a CRISPR array. This array is then transcribed and cleaved into small CRISPR RNAs (crRNAs), each carrying a spacer sequence capable of binding to a complementary target (protospacer) in incoming DNA. This pairing directs Cas proteins to recognize and degrade matching foreign genetic material upon subsequent infections. ^[10]

Classification of CRISPR–Cas systems and the role of Cas9

CRISPR–Cas systems are categorized into six distinct types (I through VI). In type II systems, a single DNA-cutting enzyme called Cas9 is responsible for cleaving double-stranded DNA. This enzyme contains two functional nuclease domains HNH and RuvC that carry out the cutting. Cas9 only becomes active when its associated guide RNA aligns with a 20-nucleotide target sequence located next to a short DNA sequence known as the protospacer-adjacent motif (PAM), which helps ensure precise targeting.

In type II CRISPR systems, the crRNA associates with a trans-activating RNA known as tracrRNA, creating a dual-RNA complex. This structure not only guides Cas9 to the specific DNA sequence but also supports the processing of crRNA. By fusing crRNA and tracrRNA into a single guide RNA (sgRNA), the system is streamlined into two essential components Cas9 and sgRNA while still enabling precise double-strand DNA cleavage.^[11]

Genome Editing and Repair

Cas9 creates a double-strand break in DNA, which cells typically repair through error-prone nonhomologous end joining often resulting in small insertions or deletions (indels) that disable the gene or through homology-directed repair, which allows precise modifications when a donor DNA template is provided. Since target specificity is determined by the guide RNA sequence rather than customized proteins, CRISPR–Cas9 can be easily reprogrammed, making it highly efficient and broadly adopted for genome editing and large-scale genetic screening.^[12]

CAS9 Enzyme

Cas9 from *S. pyogenes* is a large, multidomain enzyme with multiple functions, capable of cutting double-stranded DNA. It makes a cut three base pairs upstream of the PAM site using two separate nuclease domains. The HNH-like domain cleaves the DNA strand that is complementary to the guide RNA, the so-called target strand, while the RuvC-like domain cuts the opposite, non-complementary strand.

MECHANISMS OF CRISPR/CAS-9 GENOME EDITING

CRISPR/Cas9 genome editing operates through three main stages: recognition, cleavage, and repair. First, a designed single-guide RNA (sgRNA) guides the Cas9 protein to the target gene sequence by means of its 5' crRNA segment, which base-pairs with the complementary DNA sequence. Cas9 remains inactive without the sgRNA.

Once bound, the Cas9 nuclease induces a double-stranded break (DSB) in the DNA, located three base pairs upstream of the PAM site. The PAM (protospacer adjacent motif) is a short, conserved DNA sequence—typically 2 to 5 base pairs long found downstream of the cut site, with its exact length varying across bacterial species. In most genome-editing applications, the Cas9 protein recognizes a PAM sequence with the pattern 5'-NGG-3', where "N" represents any nucleotide.

After Cas9 identifies a target site with the correct PAM sequence, it initiates localized DNA unwinding and facilitates the formation of an RNA-DNA hybrid. However, the precise mechanism by which the Cas9 enzyme separates the DNA strands remains unclear. Once activated, Cas9 proceeds to cleave the DNA: the HNH domain cuts the complementary strand, while the RuvC domain cuts the non-complementary strand, typically resulting in blunt-ended double-strand breaks. These breaks are subsequently repaired by the cell's own repair machinery.^[13]

Double-Stranded Break Repair Mechanisms

In the CRISPR/Cas9 system, double-stranded breaks (DSBs) are repaired primarily through two pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ repairs DNA breaks by directly ligating the broken ends without requiring a homologous DNA template. This mechanism operates throughout all stages of the cell cycle and is the most common and efficient repair pathway in cells.

However, it is prone to errors, often introducing small insertions or deletions (indels) at the cut site, which can lead to frameshift mutations or premature stop codons. In contrast, HDR is a more accurate repair process that relies on a homologous DNA sequence as a template. It is predominantly active during the late S and G2 phases of the cell cycle. For CRISPR-based gene editing, HDR necessitates the introduction of an exogenous donor DNA template containing the desired sequence. This allows for precise gene insertion or replacement by aligning homologous regions at the targeted DSB site.^[14]

2. CRISPR-Cas12

Cas12 is part of Class 2, type V nucleases and functions as a DNA-targeting enzyme with unique characteristics. It binds to a distinct PAM sequence typically rich in thymine relies on a single guide RNA for targeting, and generates double-strand breaks through staggered nicks, resulting in sticky ends that can aid in accurate DNA insertions.

Similar to Cas9, the double-strand breaks created by Cas12 are repaired via either non-homologous end joining (NHEJ) or homology-directed repair (HDR). However, Cas12 has gained significant use in diagnostic applications, such as in the DETECTR system, due to certain variants exhibiting robust collateral cleavage of single-stranded DNA once activated by their target.^[15]

3. CRISPR-Cas13

Cas13 belongs to Class 2, type VI CRISPR systems and specifically targets single-stranded RNA (ssRNA) rather than DNA. It relies on a guide RNA to recognize and bind complementary sequences in mRNA or viral RNA, subsequently cutting the target to temporarily suppress gene expression without modifying the underlying genome.

Several Cas13 variants also exhibit collateral activity, cleaving nearby non-target ssRNA molecules—a feature leveraged in ultrasensitive diagnostic platforms like SHERLOCK for detecting RNA viruses. These characteristics position Cas13 as a promising tool for RNA-based therapies, antiviral approaches, and molecular diagnostics.^[16]

MECHANISM OF GENOME EDITING

i. DNA recognition

In CRISPR-based editing, DNA recognition is guided by an RNA molecule either a single guide RNA (sgRNA) or a dual system of crRNA and tracrRNA that pairs with a roughly 20-nucleotide sequence in the genome. For Cas9 to act, this target must also be adjacent to a specific short DNA sequence known as the protospacer-adjacent motif (PAM), such as 5'-NGG-3' in the case of *Streptococcus pyogenes* Cas9. Stable binding of the Cas9–guide complex to DNA occurs only when both the RNA–DNA complementarity and the presence of the correct PAM are fulfilled.

ii. DNA cleavage

When the Cas9 guide complex finds and attaches to the target site, the Cas9 enzyme cuts both strands of DNA at that location, creating a blunt double-strand break. This cleavage is carried out by two separate nuclease domains within Cas9, the HNH and RuvC-like domains. While other genome editing tools like ZFNs, TALENs, and Cpf1/Cas12a also generate double-strand breaks at specific, user-defined sites, CRISPR–Cas9 stands out because its targeting is directed by RNA rather than relying on engineered proteins to recognize DNA sequences.^[17]

iii. Repair pathways

Double-strand breaks (DSBs) are repaired by the cell's own natural mechanisms, mainly through two pathways:

- **NON-HOMOLOGOUS END JOINING (NHEJ):**

This is a rapid repair method that reconnects broken DNA ends directly. However, it is prone to errors, often resulting in small insertions or deletions (indels). These changes can disrupt the gene's coding sequence causing frameshifts or premature stop codons—and are commonly used to generate gene knockouts.^[18]

- **HOMOLOGY-DIRECTED REPAIR (HDR):**

This pathway provides a more precise form of repair by using a homologous DNA template, typically introduced experimentally. It enables targeted modifications such as gene corrections, insertions, or replacements. HDR is less common than NHEJ and is primarily active during the S and G2 phases of the cell cycle.^[19]

ADVANCED CRISPR TECHNOLOGIES

- **Base editing**

Base editing, prime editing, and epigenome editing represent advanced versions of standard CRISPR–Cas9 that reduce reliance on double-strand breaks (DSBs) and allow for more accurate or reversible modulation of gene activity. Base editors are CRISPR-based tools that alter DNA bases directly without causing DSBs.

They consist of a modified Cas9 rendered as a nickase linked to a deaminase enzyme, which can be either cytosine deaminase for converting C to T or adenine deaminase for changing A to G. This complex is directed to a target site by an sgRNA.

The deaminase alters the target base through a chemical change for instance, converting cytosine to uracil, which cells interpret as thymine, or adenine to inosine, read as guanine. This modified base is then processed by the cell's repair mechanisms, resulting in a permanent single-base mutation. This process allows for precise correction of single-nucleotide variants via base transitions.^[20]

- **Prime editing**

Prime editing is a precise genome editing technique derived from CRISPR, frequently likened to a "search-and-replace" tool. It allows targeted, minor genetic modifications such as adding or removing nucleotides or changing single DNA bases without creating double-strand breaks in the DNA or requiring external donor DNA templates. The method uses a fusion protein created by linking a modified version of the Cas9 enzyme, which cuts only one strand of DNA, to a reverse transcriptase enzyme.^[21]

- **Epigenome editing**

Epigenome editing leverages CRISPR technology to regulate gene expression without altering the DNA sequence itself, usually by introducing or removing epigenetic modifications like DNA methylation or histone changes.

This approach employs a deactivated form of Cas9 (dCas9) or another catalytically inactive Cas protein, which is linked to functional epigenetic effector domains such as DNA methyltransferases (writers), demethylases, histone acetyltransferases (HATs), or histone deacetylases (HDACs) to target specific genomic regions and modulate their activity.

Guided by sgRNA, these dCas9-effector complexes are directed to specific promoters or enhancers to either boost or suppress the activity of target genes. The effect is usually reversible and can be fine-tuned, which makes this method especially promising for addressing diseases driven by irregular gene expression rather than mutations in the coding regions themselves.^[22]

- **CRISPR Activation (CRISPRa)**

CRISPR activation (CRISPRa) is a sophisticated method employed to identify functional immune enhancers involved in regulating gene expression when immune cells become activated. The researchers used a catalytically inactive version of Cas9 linked to a transcriptional activator, which allowed them to uncover previously unidentified enhancers that influence key immune genes, including IL2RA and CD69.^[23]

- **CRISPR Interference (CRISPRi)**

CRISPR interference (CRISPRi) serves as a highly precise and adaptable method for targeting specific genes and repressing their expression without modifying the underlying DNA sequence. It relies on a catalytically dead Cas9 protein (dCas9) directed by single-guide RNAs (sgRNAs) to interfere with transcription, allowing reversible and customizable control over gene activity. The approach has proven to be specific, straightforward, and easily adjustable for use across various genes and organisms, positioning it as a powerful tool for functional genomics, investigations into gene regulation, and large-scale genetic screens. This work has been instrumental in establishing CRISPRi as a widely adopted technique in fundamental research, biotechnology applications, and the exploration of potential therapeutic interventions.^[24]

THERAPEUTIC APPLICATIONS OF CRISPR IN RARE GENETIC DISORDERS

Genetic disorders are commonly understood to result from changes in DNA sequences that disrupt normal biological functions. In the development of such diseases, several genes have already been recognized as playing essential roles. Typically, faulty genes in affected cells can be repaired through two main approaches: ex vivo and in vivo. With the ex vivo method, cells are taken out of the body, genetically modified as needed using programmable nucleases, and then reintroduced into the original organism.

1. DUCHENNE MUSCULAR DYSTROPHY (DMD)

- X-linked inheritance marks Duchenne muscular dystrophy as a serious condition, usually arising when deletions disrupt the reading frame of the dystrophin gene. This particular gene provides instructions for building a protein critical to muscle integrity. In its absence, tissue degradation begins, followed by steady loss of strength across muscle groups. Over time, such decline results in significantly shortened lifespan.
- Despite ongoing efforts, medical science has yet to deliver a treatment that stops or reverses disease progression. Among approaches tested, removal of specific exons stands out, along with integration of complementary DNA via diverse molecular instruments. Instead of standard fusion, altered splicing emerged in lab-grown muscle precursor cells after applying zinc finger nucleases, enabling proper protein translation.
- Targeted correction within mutation-prone areas occurred through precise template-guided fixes; mega-nucleases enabled editing across segments 45 to 52 in long-lived human cell lines. Restoration of working gene sequences appeared in reprogrammed patient stem cells missing key regions - exons 44 and 45 - when either TALEN or CRISPR-Cas9 was applied. Lately, cardiac and regenerative muscle cell types showed renewed gene function following viral delivery of CRISPR components by teams worldwide.
- Recently, Cpf1-(CRISPR from Prevotella and Francisella-1), a new RNA-guided endonuclease, mediated correction of DMD mutations in human cells and in animal disease model was demonstrated that represent a noteworthy step toward therapeutic translation of gene editing for genetic diseases.
- These encouraging results suggest that CRISPR/Cas9 tool possess the therapeutic potential to cure human genetic diseases including DMD. Further, large animal models may serve to augment our understanding about DMD at large level and may useful to develop therapeutic interventions. However, these approaches and strategies have some concerns like efficiency, applied methodology and tool, cell survival and proliferation, toxicity of injected chemicals and so on and should investigate thoroughly before clinical application.^[25,26]

2. SICKLE CELL DISEASE

- Sickle cell anemia is a genetic blood disorder inherited in an autosomal recessive pattern, caused by a mutation in the β -globin (HBB) gene. This mutation leads to the production of an abnormal form of hemoglobin called HbS, which causes red blood cells to become rigid and take on a crescent shape under low-oxygen conditions.

- Haemoglobin, the oxygen-carrying protein in red blood cells composed of two β -globin and two α -globin subunits, is affected by a mutation that substitutes negatively charged glutamate with a neutral, hydrophobic valine. This change creates sticky regions on the protein's surface.
- HBB gene mutation - The HBB-like gene cluster haplotypes are distinct genetic profiles associated with the sickle cell (HbS) mutation and are conventionally classified into five primary categories: Senegal (SEN), Benin (BEN), Cameroon (CAM), Central African Republic/Bantu (CAR), and Arab-India (AI). Typically, individuals with the AI, SEN, or BEN haplotypes experience milder symptoms, while those with the CAM or CAR haplotypes tend to have more severe clinical manifestations. However, recent whole-genome sequencing research has uncovered additional genetic subgroups, indicating that the connection between haplotypes and disease severity is more intricate than earlier models suggested.
- CRISPR-Cas9 gene editing has been used to treat transfusion-dependent β -thalassemia and sickle cell disease by targeting the erythroid-specific enhancer of the BCL11A gene. This gene plays a key role in suppressing fetalhemoglobin (HbF) production, and altering its activity allows for increased HbF levels, potentially alleviating disease symptoms.
- Editing CD34+ hematopoietic stem cells in patients achieved approximately 80% efficiency without any signs of off-target genetic alterations. Following transplantation, both patients showed sustained increases in fetalhemoglobin. The individual with β -thalassemia became transfusion-independent, and the patient with sickle cell disease experienced no additional vaso-occlusive crises.
- CRISPR-Cas9 induces a double-strand break specifically at the mutant HBB gene locus. The normal DNA sequence can be restored via homology-directed repair (HDR), although this process is limited by low efficiency in dormant stem cells. Challenges include potential off-target mutations and the necessity for donor DNA templates to guide repair.^[27,28]

3. β -THALASSEMIA

- β -Thalassemia is among the most prevalent inherited disorders affecting hemoglobin globally, stemming from mutations in the HBB (β -globin) gene on chromosome 11. These genetic alterations lead to either diminished (β^+) or completely absent (β^0) production of β -globin chains, disrupting the normal balance between α - and β -globin synthesis. As a result, excess α -globin chains accumulate and aggregate in maturing erythroid cells, triggering ineffective red blood cell formation, persistent hemolytic anemia, and varying levels of tissue oxygen deprivation. Inheritance follows an autosomal recessive pattern, with disease severity influenced by the specific nature and combination of β -globin gene mutations present.
- CRISPR/Cas9 genome editing combined with induced pluripotent stem cell (iPSC) technology offers a promising approach for gene therapy in β -thalassemia. Scientists derived iPSCs from skin fibroblasts of patients suffering from the disease and applied the CRISPR/Cas9 system to accurately correct the pathogenic mutation in the HBB (β -globin) gene. After correction, iPSC clones were rigorously evaluated to verify precise gene repair and ensure minimal off-target effects. The genetically corrected iPSCs retained full pluripotency and exhibited stable chromosomal integrity.
- The genetically corrected iPSCs were then guided to develop into hematopoietic progenitor cells and introduced into irradiated mice. After transplantation, these cells effectively engrafted, persisted, and gave rise to various blood cell types, confirming their functional capacity in a living organism. Crucially, the corrected cells produced normal β -globin, showing that the mutation causing β -thalassemia had been successfully repaired.
- A gene editing therapy for β -thalassemia is presented as a groundbreaking approach with the potential to revolutionize the management of this inherited blood disorder. The genetic mechanisms and disease processes underlying β -thalassemia, underscoring that current standard treatments such as chronic blood transfusions and iron chelation are burdened by serious long-term complications. Although allogeneic hematopoietic stem cell transplantation offers a cure in some cases, its broader application is hindered by the scarcity of suitable donors and the risks tied to the procedure. In contrast, gene editing stands out as a highly promising alternative, enabling a personalized therapeutic strategy that could provide a definitive cure.
- The three primary nuclease-based genome editing systems applied in β -thalassemia treatment are zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9. Of these, CRISPR/Cas9 has become the preferred approach, favored for its straightforward design, high precision, strong editing efficiency, and cost advantages in targeting disease-related mutations. In treating β -thalassemia, gene editing primarily follows two strategies: directly repairing mutations in the HBB gene to restore normal β -globin synthesis, or reactivating fetalhemoglobin (HbF) by inactivating key regulatory genes like BCL11A. Elevating HbF levels helps offset the lack of functional adult β -globin, alleviating clinical symptoms and enhancing red blood cell performance.^[29,30]

4. CYSTIC FIBROSIS

Cystic fibrosis is a chronic, progressive genetic condition inherited in an autosomal recessive pattern, resulting from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene located on the long arm of chromosome 7. This gene is responsible for producing a chloride channel found in epithelial cells and is expressed in multiple organs, such as the pancreas, kidneys, liver, lungs, gastrointestinal system, and reproductive organs.

- "CFTR mutation targeting" encompasses a range of therapeutic approaches aimed at either restoring the expression of functional CFTR protein or adjusting its activity based on the specific genetic mutation involved. These strategies are generally grouped into three main categories: small-molecule CFTR modulators, therapies based on gene or RNA manipulation, and advanced genome-editing techniques such as CRISPR-derived methods like base editing, prime editing, and epigenome editing.
- Targeting CFTR mutations starts with genetic testing to determine the specific class of mutation, which informs the selection of appropriate treatment. Small-molecule CFTR modulators comprising correctors and potentiators are employed to improve the protein's folding, cellular trafficking, and channel activity. When these modulators are not effective, alternative strategies such as gene or RNA-based therapies introduce functional CFTR cDNA or corrected mRNA into airway cells, allowing CFTR expression without modifying the genome. More advanced techniques involve CRISPR-based genome editing methods, including base editing, prime editing, or epigenome modulation, to directly repair or regulate the CFTR gene. These interventions are followed by ongoing monitoring of lung function and safety to evaluate their long-term effectiveness and clinical outcomes.^[31,32]

5. HUNTINGTON'S DISEASE

Huntington's disease is a severe neurodegenerative disorder caused by the autosomal dominant mutation in the front exon of the HTT gene encoding huntingtin protein.

- Although Huntington's disease stems from a faulty stretch of DNA - too many CAG repeats in the HTT gene - it triggers creation of a damaging protein called mutant huntingtin. Scientists now explore gene-editing tools such as CRISPR to interfere directly with this flawed genetic code. Instead of accepting the usual path of damage, some interventions shift focus toward silencing or altering the HTT gene itself.
- Through precise molecular adjustments, researchers hope to limit how much toxic protein builds up inside brain cells. Nerve tissue in regions like the striatum and cortex becomes less vulnerable when mHTT levels drop. Because these areas control movement and thinking skills, protecting them may delay symptoms. When editing takes hold in both neurons and glia, disruption spreads more slowly across neural networks. Over time, interference at the gene level might ease pressure on systems doomed to degenerate. Progress here does not restore lost functions - but it could stall their erosion. What begins as a microscopic change may affect whole-body coordination later.
- CRISPR-Cas9 approaches that selectively target the mutant HTT allele can disable it while leaving the wild-type allele intact, effectively reducing levels of mutant huntingtin (mHTT) without compromising normal protein function.
- Alternatively, RNA-targeting CRISPR platforms such as Cas13d degrade the mutant HTT mRNA without modifying DNA, leading to decreased production of toxic proteins and improved motor performance in mouse models of Huntington's disease, with little impact on other RNA transcripts.
- Cas9 or CRISPR effectors delivered via AAV vectors administered either directly into the brain (such as the striatum) or systemically have demonstrated notable reductions in mutant huntingtin (mHTT), slowed neurodegeneration, and improved motor function in both rodent and large-animal models of Huntington's disease.^[33]

6. LEBER CONGENITAL AMAUROSIS

- Leber congenital amaurosis (LCA) is a rare and severe inherited retinal disease that leads to significant vision impairment or complete blindness at birth or during the initial months of life. As one of the most severe and earliest-onset forms of inherited retinal dystrophy, it stems from developmental or functional defects in the retina, especially affecting photoreceptor cells and the retinal pigment epithelium. Infants with LCA typically exhibit weak visual responsiveness, involuntary eye movements (nystagmus), delayed or absent pupillary reactions to light, and the distinctive oculodigital sign—repetitive poking or rubbing of the eyes.
- Leber congenital amaurosis (LCA) is not infectious and cannot be transmitted between individuals via touch, airborne particles, food, or bodily fluids. Rather, it is a hereditary condition caused by genetic mutations passed from parents to offspring. Individuals who carry one copy of the defective gene typically remain asymptomatic but can pass the mutation to their children. When both parents are carriers of a mutation in the same gene, there is a 25% probability with each pregnancy of having a child affected by LCA. Thus, the development of this condition depends entirely on inherited genetics, not on environmental factors or infectious agents.
- Retinal gene editing – CRISPR/Cas9 genome editing shows promise as a treatment for LCA10 by precisely correcting the underlying CEP290 mutation. This approach successfully restored normal gene function with high accuracy and minimal off-target effects, offering robust preclinical support for the long-term potential of CRISPR-based therapies in inherited retinal conditions. The results have paved the way for the first in vivo CRISPR treatments targeting retinal diseases and underscored the broader potential of precision gene editing in addressing genetic forms of blindness.
- In vivo CRISPR trials – The first in vivo CRISPR/Cas9 clinical trial represented a landmark achievement in gene-editing medicine. By precisely targeting the CEP290 mutation linked to LCA10, the study showed that correcting genetic defects directly inside the body is possible. While the treatment was still undergoing early-stage clinical assessment, it signaled significant progress toward creating durable, safe, and effective therapies using CRISPR technology for inherited retinal diseases and other single-gene disorders.^[34-36]

7. SPINAL MUSCULAR ATROPHY

OTHER EMERGING RARE GENETIC DISORDERS

FANCONI ANEMIA – A rare genetic condition marked by chromosomal instability, gradual bone marrow failure, congenital malformations, and a significantly elevated cancer risk. The authors highlight that Fanconi anemia (FA) arises from mutations in genes associated with the Fanconi DNA repair pathway, which plays a key role in fixing DNA interstrand cross-links and preserving genomic integrity. When this pathway is disrupted, it results in increased chromosomal breakage, loss of genomic stability, and compromised function of hematopoietic stem cells.^[37]

FRAGILE X SYNDROME – Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability and ranks among the most common single-gene causes of autism spectrum disorder (ASD). This condition follows an X-linked inheritance pattern and results from a mutation in the FMR1 (Fragile X Messenger Ribonucleoprotein 1) gene, which is situated at position Xq27.3 on the X chromosome. A new treatment idea for Fragile X syndrome uses CRISPRa to target a gene called ASCC3, which plays a key role in checking ribosome health. Rather than correcting the original genetic defect, this method turns on a backup system that helps cells work normally again.^[38]

RETT SYNDROME – Rett syndrome (RTT) is a rare brain disorder that gets worse over time. It mostly affects girls and is one of the main genetic reasons for severe intellectual disability in females. Most cases are caused by new mutations in the MECP2 gene, found on the X chromosome at location Xq28. This gene helps control how other genes work and is vital for brain cell growth, the formation of nerve connections, and keeping the brain functioning properly. Rett syndrome (RTT) is a serious brain development disorder caused mostly by changes in the MECP2 gene. It is linked to the X chromosome. New treatments now focus on genes and molecules to fight the disease. Studies in animals show that fixing MECP2 function can reverse brain problems. Because of this, researchers see gene therapies as the best hope for changing the course of RTT.^[39]

DELIVERY SYSTEMS FOR CRISPR THERAPEUTICS

Getting CRISPR tools into cells safely and effectively is key to successful gene editing. These tools can be delivered in different forms: as DNA (in a plasmid or virus), as messenger RNA (mRNA), or as ready-to-work complexes made of the Cas protein and guide RNA (called RNP). The delivery method affects how well editing works, which tissues are targeted, how long the editing lasts, whether the immune system reacts, and the chance of unintended edits. Delivery platforms are broadly classified into viral and non-viral systems, each with distinct advantages and limitations.

Viral Delivery Systems:

- i. **Adeno-Associated Virus (AAV)** – This vector is the most commonly used because it triggers minimal immune response, can infect both dividing and non-dividing cells, and supports long-lasting gene expression. However, its capacity to carry genetic material is limited around 4.7 kb which poses challenges for delivering the relatively large Cas9 protein. To address this, researchers have developed approaches like employing smaller versions of Cas9 from other species (such as SaCas9) or using two separate AAV vectors in combination.
- ii. **Lentiviral Vectors** - Lentiviruses insert themselves into the host's DNA, allowing for sustained and durable expression of CRISPR elements. Although this is advantageous for ex vivo genome editing applications, it raises safety issues due to the potential for insertional mutagenesis and persistent Cas9 activity, which can elevate the likelihood of off-target effects.
- iii. **Non-Viral Delivery Systems** – A non-viral delivery method based on lipid nanoparticles (LNPs) has been developed to carry CRISPR/Cas9 plasmids specifically to the liver, enabling therapeutic genome editing. In contrast to viral vectors known for risks such as immune reactions, unintended genetic insertions, and sustained Cas9 activity this approach provides a safer alternative with more controlled, short-term expression of the editing components.
- iv. **Lipid Nanoparticles** – lipid nanoparticles (LNPs) as one of the most promising non-viral delivery platforms because of their low immunogenicity, biodegradability, high encapsulation efficiency, and ability to deliver diverse CRISPR cargoes.

Ionizable lipids help encapsulate nucleic acids during nanoparticle formation and facilitate escape from endosomes once inside cells.

Phospholipids contribute to the structural stability of the nanoparticles.

Cholesterol strengthens the integrity of the lipid membrane and supports efficient delivery into cells.

- v. **Polyethylene glycol (PEG)-lipids** enhance the stability of the particles, extend their circulation time in the body, and minimize clumping.
- vi. **Gold Nanoparticles** - Gold nanoparticles (AuNPs) have become attractive non-viral carriers for CRISPR/Cas genome-editing tools due to their strong biocompatibility, straightforward surface functionalization, substantial cargo capacity, and minimal immune response. These nanoparticles facilitate the effective transport of various CRISPR elements such as plasmid DNA, mRNA, sgRNA, and Cas9 RNP complexes offering a safer alternative to viral delivery systems by reducing associated risks.
- vii. **Electroporation** - Electroporation is a commonly used physical, non-viral technique that delivers CRISPR/Cas genome-editing components into cells through brief electrical pulses. These pulses induce temporary nanopores in the cell membrane, enabling nucleic acids or proteins to enter the cytoplasm. The method has emerged as one of the most efficient approaches for ex vivo genome editing, especially in stem and immune cells relevant to gene therapy applications.^[40-43]

ADVANTAGES OF CRISPR-BASED THERAPEUTICS

- Precision and Specificity – One major benefit of CRISPR-based treatments is how precisely they can edit genes. The system uses a guide RNA that can be programmed to find a specific DNA sequence. It brings the Cas enzyme exactly where it needs to go. This allows scientists to disable, add, fix, or control genes linked to disease. Because it's so accurate, it rarely makes changes in the wrong places.
- Cost-Effectiveness – Compared to older gene-editing tools like ZFNs and TALENs, CRISPR is much cheaper and simpler to set up. To target a new gene, you usually only need to change the guide RNA—the Cas protein stays the same. This makes the process faster and less expensive. It also allows scientists to test many genes at once.
- Potential for Permanent Cure – Most treatments today only ease symptoms of diseases. CRISPR therapies work differently. They go straight to the root cause—faulty genes. By fixing, removing, or replacing these broken genes, CRISPR can offer lasting results, sometimes even a cure, after just one treatment. This makes it especially hopeful for single-gene disorders like Sickle Cell Disease, β -thalassemia, Leber Congenital Amaurosis, and some inherited forms of blindness.
- Personalized Medicine Applications – CRISPR is a flexible tool that helps create personalized treatments based on a person's genes. Doctors can find specific gene errors using DNA testing and fix them with specially designed guide molecules. CRISPR also allows scientists to modify a patient's own cells, like blood stem cells or immune cells, to treat diseases. These modified cells can be used in therapies such as corrected stem cell transplants or engineered immune cells to fight cancer. In addition, CRISPR helps build models of diseases using a patient's cells, which aids in finding precise drug targets. This leads to more effective treatments with fewer side effects.^[44-46]

CHALLENGES AND LIMITATIONS OF CRISPR TECHNOLOGY

- Unintended genetic modifications
 - Difficulties in delivering editing tools to target cells
 - Potential activation of immune reactions
 - Possibility of DNA breaks and disruption to genome integrity
 - Variable or low rates of successful gene editing
 - Hurdles in achieving precise edits through homology-directed repair
 - Ethical questions and evolving regulatory frameworks
 - Complexities in large-scale production and manufacturing
 - Uncertainties regarding long-term safety profiles
 - Potential for mosaic patterns of editing within tissues^[47,48]

DISCUSSION

Rare genetic disorders remain a significant challenge in contemporary medicine due to their diverse genetic origins, lack of effective treatments, frequent delays in diagnosis, and substantial socioeconomic impact. Current therapies are mostly supportive, aiming to manage symptoms rather than address the root genetic defects. The development of CRISPR-based genome editing has shifted this approach, offering a new potential to directly correct the genetic mutations responsible for these conditions.

The CRISPR/Cas system has advanced quickly since the initial Cas9 technology, giving rise to more sophisticated tools such as Cas12, Cas13, base editing, prime editing, CRISPR activation (CRISPRa), CRISPR interference (CRISPRi), and epigenome editing. These developments have enhanced the accuracy of genome editing and minimized unintended genetic changes. Notably, base and prime editing address key drawbacks of traditional double-strand DNA breaks, broadening the scope of mutations that can potentially be corrected.

CONCLUSION

CRISPR-based genome editing has become a major milestone in modern molecular medicine, opening new possibilities for treating rare genetic diseases. While traditional treatments typically focus on alleviating symptoms, CRISPR directly targets the root genetic causes of these conditions. The development of various CRISPR systems such as Cas9, Cas12, Cas13, along with innovations like base editing, prime editing, epigenome editing, CRISPRa, and CRISPRi has significantly enhanced the accuracy, adaptability, and therapeutic reach of genetic interventions. Strong preclinical and clinical data support the efficacy of CRISPR-based therapies for conditions including sickle cell disease, β -thalassemia, Duchenne muscular dystrophy, cystic fibrosis, Huntington's disease, and Leber congenital amaurosis. In parallel, ongoing advances in both viral and non-viral delivery methods are improving the safety and precision of genome editing treatments.

Nonetheless, significant hurdles such as off-target effects, effective delivery to target tissues, immune reactions, ethical concerns, regulatory standards, and long-term safety assessments need to be resolved before broad clinical use can be realized. Ongoing research should prioritize enhancing precision in gene editing, creating delivery systems tailored to specific tissues, reducing potential side effects, and carrying out extended clinical trials to confirm both safety and lasting efficacy.

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