Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2025, 16(5), 190-206
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

CRISPR-Cas9 and beyond: Expanding the Frontiers of Gene Editing

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How to cite this paper: Pang, S.Q. and Qu, J.R. (2025) CRISPR-Cas9 and beyond: Expanding the Frontiers of Gene Editing. *Advances in Bioscience and Biotechnology*, **16**, 190-206.
https://doi.org/10.4236/abb.2025.165012

Received: April 15, 2025
Accepted: May 27, 2025
Published: May 30, 2025

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Abstract

Gene editing technology, which allows for precise modifications to an organism's genome, has emerged as a transformative tool in genetic engineering. This review introduces the fundamental concepts and mechanisms of gene editing, with a particular emphasis on CRISPR-Cas systems. The principles and methods used in the development and optimization of gene editing tools, including base editing and prime editing, are discussed. The review also summarizes the applications of gene editing in medicine, agriculture, and biotechnology, highlighting its potential to address complex biological challenges. Finally, the review outlines the current challenges and ethical considerations in the field of gene editing research.

Keywords

Gene Editing, CRISPR-Cas Systems, Medical Applications, Ethical Considerations

1. A Brief Overview of Gene Editing Technology

Gene editing is the process of modifying specific targets of an organism's genome through gene editing technology. The insertion, deletion or substitution of genes is efficiently and precisely realized, thus altering their genetic information and expression characteristics.

Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) were pivotal early tools in gene editing. These nucleases are composed of the Fok I nuclease domain in conjunction with the DNA-binding module derived from TALE proteins. TALE proteins are comprised of repetitive 33 - 35 amino acid units, enabling them to discern individual base pairs [1]. ZFNs, developed in the 1990s, use zinc finger proteins to bind DNA and FokI nuclease

to cut target sites. While effective, their design was complex and costly. TALENs, introduced later, simplified targeting with modular DNA-binding domains, offering improved specificity.

Despite their contributions, both were overshadowed by CRISPR-Cas9 due to its unparalleled flexibility and ease of use (**Table 1**). Discovered in the early 1990s and first used in biochemistry experiments seven years later, CRISPR technology has quickly become the most popular gene editing tool in fields such as human biology, agriculture, and microbiology. In 2007, it was demonstrated that the prokaryotic CRISPR-Cas system functions as an adaptive genome defense mechanism, identifying and targeting exogenous nucleic acids associated with viruses and other mobile genetic elements [15].

Table 1. Comparative analysis of major gene editing technologies.

Technology	Efficiency	Specificity	Off-Target Risk	Key Advantages	Major Limitations	References
ZFN	Low-Moderate	High (protein-DNA binding)	Moderate (homodimer requirement)	First programmable nuclease	Complex design, low scalability	Pacesa *et al.* [1] Chehelgerdi *et al.* [2]
TALEN	Moderate	Very high (1:1 base recognition)	Low	Flexible targeting, lower off-targets	Large plasmid size, labor-intensive	Christian *et al.* [3] Joung & Sander. [4]
CRISPR-Cas9	High	Moderate (PAM-dependent)	Moderate (gRNA-dependent)	Easy design, multiplex editing	Off-target effects, DSB-induced toxicity	Jinek *et al.* [5] Kim *et al.* [6]
Cas12a	Moderate	High (staggered cuts)	Low (TTTV PAM constraint)	Self-processing crRNA, better DNA insertion	Lower efficiency in mammalian cells	Zetsche *et al.* [7] Kleinstiver *et al.* [8]
Cas13a	High (RNA)	Low (trans-cleavage activity)	High (collateral RNA degradation)	RNA editing, viral RNA detection	Unsuitable for therapeutic RNA editing	Abudayyeh *et al.* [9] Kaminski *et al.* [10]
Base Editor	High (single-base)	Very high (no DSBs)	Minimal	Precision C > T or A > G conversions	Limited to specific base changes	Komor *et al.* [11] Gaudelli *et al.* [12]
Prime Editor	Moderate	Extremely high (no DSBs)	Very low	All 12 base edits, small insertions/deletions	Low efficiency in primary cells	Anzalone *et al.* [13] Yu *et al.* [14]

Among the various genome-editing tools available, the CRISPR-Cas9 system has emerged as the most widely adopted and versatile platform. The operational mechanism of CRISPR-Cas9 can be systematically categorized into three distinct phases: target recognition, DNA cleavage, and cellular repair.

The target recognition process originates from the bacterial adaptive immune system, where CRISPR loci function as molecular archives for storing fragments

of invading viral DNA. These sequences undergo transcription into CRISPR RNAs (crRNAs), which, in combination with trans-activating CRISPR RNA (tracrRNA), guide the Cas9 endonuclease to identify and bind to complementary viral DNA sequences [5]. To enhance the efficiency of genome editing applications, researchers have engineered a single-guide RNA (sgRNA) by fusing crRNA and tracrRNA into a single chimeric molecule. This engineered sgRNA incorporates a 20-nucleotide sequence that specifically directs Cas9 to its genomic target, with target site specificity further determined by the presence of a protospacer adjacent motif (PAM) sequence.

Following target recognition, the DNA cleavage process is initiated through the RNA-guided nuclease activity of Cas9, which generates precise double-strand breaks (DSBs) in the target DNA. This process begins with the formation of a ribonucleoprotein complex between Cas9 and sgRNA, which scans the genomic DNA for complementary sequences adjacent to PAM motifs. The cleavage mechanism involves two distinct catalytic domains within the Cas9 protein: the HNH domain specifically cleaves the DNA strand complementary to the sgRNA, while the RuvC domain cleaves the non-complementary strand. Mechanistically, the HNH domain targets and hydrolyzes the phosphodiester backbone of the complementary strand, whereas the RuvC domain performs the same catalytic function on the opposite strand. The coordinated action of these two domains results in the generation of a clean double-strand break at the predetermined genomic locus [16].

The final phase of the CRISPR-Cas9 mechanism involves the cellular DNA repair processes that respond to the induced DSBs. Eukaryotic cells primarily employ two distinct repair pathways to maintain genomic integrity following DSB formation. The non-homologous end joining (NHEJ) pathway initiates with the rapid recognition and binding of Ku heterodimers (comprising Ku70 and Ku80 subunits) to the broken DNA ends. The remarkable affinity and structural flexibility of Ku proteins enable their binding to diverse DNA end structures. Subsequently, DNA-dependent protein kinase catalytic subunits (DNA-PKcs) are recruited to the DNA-Ku complex, where they acquire kinase activity and initiate a signaling cascade through autophosphorylation and phosphorylation of downstream repair factors. Notably, in the absence of canonical NHEJ components such as DNA ligase IV, alternative NHEJ pathways can be activated through the involvement of other DNA ligases (e.g., DNA ligases I or III), albeit with reduced efficiency. This inherent flexibility and redundancy in the NHEJ pathway enable cells to effectively process various DNA end configurations and maintain genomic stability [17].

2. CRISPER-Cas Family of Gene Editing Tools

CRISPR/Cas systems are comprised of Cas genes in operons and CRISPR arrays with genome-targeting sequences, known as "spacers," which alternate with identical repeats. The process of acquiring immunity via CRISPR/Cas involves three

distinct phases. In the adaptive phase, organisms with CRISPR loci integrate foreign sequence fragments (protospacers) into their chromosome at the CRISPR array's proximal end in response to viral or plasmid threats. During the expression and interference phases, the transcription of repeat-spacer elements into pre-crRNA molecules is followed by processing into mature crRNAs. These mature crRNAs then pair with the complementary sequences of invading viruses or plasmids. The subsequent pairing of these crRNAs with Cas proteins facilitates the recognition and subsequent silencing of foreign sequences [5].

2.1. CRISPER-Cas9

The CRISPR-Cas systems present in various bacterial and archaeal species exhibit distinct compositions and mechanisms. CRISPR-Cas systems are mainly divided into two classes: Class I CRISPR-Cas system effector protein complexes exist with multiple subunits, while Class II functions through a single Cas protein [18]. The Streptococcus pyogenes-derived CRISPR/Cas9 system, a Type II CRISPR platform, has become the most prevalent tool for genome editing to date.

The Cas9 enzyme family relies on the formation of a base-paired structure between the activating tracrRNA and the targeting crRNA to facilitate the cleavage of the double-stranded DNA (dsDNA) at the target site [5]. After recognizing its DNA target, SpCas9 typically induces a blunt double-strand break (DSB). SpCas9 directs DNA targeting through a 20-nucleotide guide sequence and requires a 5'-NGG PAM sequence adjacent to the target, with "N" indicating any nucleotide. Cas9 systems are guided by dual RNAs: the CRISPR RNA (crRNA), which directs DNA targeting and hybridizes with the transactivating crRNA (tracrRNA). The tracrRNA facilitates the assembly of the Cas9 complex. The functions of both the crRNA and tracrRNA can be integrated into a synthetic guide RNA (sgRNA) [19]. Compared to alternative gene editing instruments, Cas9 is an RNA-guided nuclease whose sequence specificity is contingent upon the Watson-Crick base pairing between the target DNA sequence and its guide RNA (gRNA), in addition to the direct interaction between Cas9 and the protospacer-adjacent motif (PAM). The Cas9 protein itself remains unchanged during different DNA targeting; only the short sequence of the gRNA (guide RNA) needs to be altered to re-target the Cas9 complex to a specific DNA sequence [20]. This design makes the CRISPR/Cas9 system highly flexible and versatile. Conformational changes in the Cas9 protein are critical for its DNA shearing activity. The conformational state of the HNH nuclease structural domain directly controls DNA shearing activity. The relative orientation of the Cas9 catalytic structural domain upon binding to DNA was examined by Förster resonance energy transfer (FRET) experiments, and it was found that the DNA shearing efficiency is directly proportional to the degree of sampling of the activated conformation of the HNH structural domain. Cas9 proteins exhibit different conformational states in the absence of RNA, when sgRNA is bound, and when sgRNA/DNA is bound, and these conformational changes are critical for understanding the DNA shearing mechanism of Cas9. The conforma-

tional dynamics of the HNH structural domain may affect the shearing specificity of the Cas9-sgRNAase complex [21].

2.2. CRISPER-Cas12a

Cas12a is a nuclease in the CRISPR-Cas system, which belongs to the Class 2 type V system. Cas12a (also known as Cpf1) is the first member of the Cas12 family to be discovered. It is a single-component RNA-guided DNA nuclease that is capable of crRNA processing and DNA targeting independently of tracrRNA cleavage, this is because Cas12a does not require additional transcriptionally activated crRNA (tracrRNA) to process the crRNA arrays [7]. Cas12a generates staggered cuts at DNA target sites with prominent 5' ends and does not utilise transcriptionally activated crRNAs. The staggered ends generated by Cas12a may prove more advantageous for applications such as precisely targeted integration of DNA sequences in comparison to the flat ends generated by Cas9. Furthermore, Cas12a is capable of cleaving crRNA arrays to generate its own crRNAs, thereby enabling the simplified editing of multiple genomes using a single customised crRNA array [19]. Recently, engineered variants of Cas12a have been developed to expand its targeting range and improve its genome editing activity. These variants, such as enAsCas12a, exhibit two-fold higher genome editing activity on sites with canonical TTTV PAMs compared to wild-type AsCas12a and can target many previously inaccessible PAMs. This advancement allows for more efficient multiplex gene editing, endogenous gene activation, and C-to-T base editing. Additionally, a high-fidelity version of enAsCas12a (enAsCas12a-HF1) has been engineered to reduce off-target effects, which is particularly important for therapeutic applications where specificity is crucial. These engineered variants of Cas12a not only broaden the applicability of the CRISPR-Cas system but also demonstrate the potential for further optimization of CRISPR nucleases for various genetic and epigenetic editing applications [8].

2.3. CRISPER-Cas13a

The CRISPR-Cas13 system (also known as C2c2) belongs to the Type VI CRISPR-Cas system. Unlike the widely used Type II systems (e.g., Cas9), which target DNA, the Cas13 system targets RNA. Cas13's ability to specifically cleave single-stranded RNA (ssRNA) targets has been used in the development of RNA-targeting tools and nucleic acid detection platforms [10]. The system's RNA-guided RNase functionality allows for the specific cleavage of complementary primary spacer sequences, making it an attractive option for sequence-specific RNA manipulation [9]. Additionally, Cas13's "side effect"—non-specific cleavage of nearby RNA after target recognition—has been applied to create sensitive diagnostic tools [10].

The mechanism of action of Cas13 involves two higher eukaryotic and prokaryotic nucleotide-binding (HEPN) structural domains that mediate RNA cleavage upon target RNA recognition. The Cas13 system is guided to the target RNA tran-

script via a CRISPR RNA (crRNA), which is complementarily paired to the target RNA by the spacer sequence of the crRNA. Activation of the HEPN structural domain of Cas13 results in cleavage of the target RNA. This activation of Cas13 leads to the cleavage of target ssRNAs (cis-cleavage) as well as the cleavage of non-specific bystander ssRNAs (trans-cleavage), a property that has been utilized for in situ detection of viral RNAs [22] [23].

The versatility of Cas13 is also reflected in its potential use in RNA editing. By fusing catalytically inactivated Cas13 (dCas13) with adenosine deaminase, researchers have achieved targeted A-to-I (adenine to inosine) RNA editing, which allows codon changes without the need for DNA double-strand breaks. This approach provides a temporary and reversible form of genetic manipulation that is particularly suitable for applications where permanent genetic changes are undesirable [22].

The Cas13 system is characterized by its unique structure and mechanism within the CRISPR-Cas system. Unlike the Cas9 and Cas12 systems, the Cas13 system exhibits substrate RNase activity activated by the target RNA. Through its unique structural and mechanistic principles, the Cas13 system provides a powerful platform for RNA targeting, editing, detection, and imaging, and shows potential for biotechnological and therapeutic applications [23].

CRISPR-Cas9 exhibits variable off-target rates (0.1% to >50%), strongly influenced by gRNA design and chromatin accessibility [24]. While high fidelity variants such as HiFi-Cas9 reduce off-target editing by 100-fold, they incur a ~30% loss in on-target efficiency [25]. For RNA-targeting systems, Cas13a's collateral cleavage of bystander RNAs-useful in diagnostics-poses therapeutic risks. However, recent engineered mutants (e.g. dCas13a-ADAR) achieve 95% suppression of trans-activity while maintaining target specificity, offering a safer alternative [22].

2.4. Base Editing

Base editing signifies a pioneering strategy in the realm of genome editing, allowing for the precise modification of specific DNA bases without the formation of double-strand breaks (DSBs) [10]. At the heart of the Base editing technology lies a variant of the CRISPR-Cas system that combines an inactivated Cas9 enzyme (dCas9) with a cytosine deaminase (e.g. APOBEC1), which makes it possible to convert specific cytosines (C) to thymine (T), or adenine (A) to guanine (G) [12]. Base Editing technology provides an efficient, precise and safe means of genome editing, capable of converting A-T base pairs to G-C directly in human cells without double-stranded DNA breaks, with extremely high product purity and very low insertion or deletion rates. This technique not only extends the scope of gene editing by allowing direct introduction of all four conversion mutations in genomic DNA, but also reduces the risk of potential genotoxicity by reducing non-target site editing [1].

In an early study of Base editing, this technique was first reported by Komor *et al.* in 2016, who showed how the CRISPR-Cas9 system could be used to perform

precise genome editing without relying on the homology-directed repair (HDR) mechanism [10]. As Base editing technology continues to evolve, scientists are exploring ways to improve its specificity and efficiency, as well as how it can be applied to the treatment of a wide range of genetic diseases. For example, Musunuru *et al.* in 2021 demonstrated the potential of Base editing technology in reducing PCSK9 gene expression and durably lowering cholesterol levels in primates [26]. These advances suggest that Base editing technology has great potential for future gene therapy.

2.5. Prime Editing

Prime editing represents a novel, next-generation genome editing technology that operates on a distinctive mechanism, setting it apart from earlier CRISPR-Cas9 systems. The core of prime editing lies in the use of a fusion protein comprising a nicking version of Cas9 and a reverse transcriptase (RT), along with a prime editing guide RNA (pegRNA) [13]. The pegRNA is a sophisticated construct that extends a standard CRISPR-Cas9 guide RNA with a primer binding site (PBS) and a reverse transcription template (RTT), which carries the desired genetic change [27].

When the prime editor complex, consisting of the fusion protein and pegRNA bind to the target DNA, whereupon the Cas9 nickase introduces a single-strand break at a specific location, generating a 3' flap [28]. This flap then anneals with the PBS on the pegRNA, priming the RT to synthesise the new DNA sequence specified by the RTT, thereby effectively writing the intended edit into the genome [23]. The cell's natural DNA repair mechanisms, such as DNA mismatch repair (M MR) play a pivotal role in integrating the new sequence into the genome.

Prime editing is distinguished by its capacity to perform "search and replace" operations directly on the genome, obviating the necessity for double-strand breaks (DSBs) or external DNA templates [13]. This attribute enables prime editing to surmount certain constraints associated with DSB-based editing, such as the dependence on homology-directed repair (HDR) and the concomitant risk of inducing [29]. Furthermore, prime editing has the potential to make all 12 types of base substitutions, as well as insertions and deletions, thereby expanding the range of editable genetic variations beyond the capabilities of traditional base editors [13].

Prime editing stands out for its innovative mechanism that allows precise genetic modifications without DSBs, offering a promising tool for research and therapeutic applications with its versatility and potential for high fidelity and specificity.

3. Applications of Gene Editing in Different Fields

3.1. *Ex Vivo* Gene Therapy

Ex vivo gene editing has revolutionized the field of biological sciences by offering a controlled and precise method for modifying cells before their reintroduction

into the organism or application in industrial or agricultural settings. This approach significantly reduces risks such as immune responses and off-target effects, making it highly adaptable for various applications [30].

3.1.1. Applications in Medicine

Ex vivo gene editing offers a potentially efficacious solution for the treatment of genetic disorders. For instance, recent studies have demonstrated the efficacy of modifying hematopoietic stem cells (HSCs) in the treatment of sickle cell disease and beta-thalassemia. A pioneering study illustrated the potential of CRISPR-Cas9 in rectifying faulty β-globin genes among individuals with β-thalassemia, leading to the long-term generation of normal hemoglobin [31]. Moreover, gene editing has been demonstrated to correct mutations responsible for cystic fibrosis and Duchenne muscular dystrophy (DMD). For DMD, CRISPR has been employed *ex vivo* to restore the dystrophin gene in patient-derived cells [32].

In the domain of immunotherapy and cancer treatment, CAR-T cell therapy has emerged as a leading application of *ex vivo* gene editing, demonstrating remarkable efficacy in treating cancerous cells [33]. In this technique, T cells are extracted from the patient, genetically engineered to produce chimeric antigen receptors (CARs) designed to recognize specific cancer antigens, and subsequently infused back into the patient. This approach has yielded notable success in the treatment of B-cell malignancies, including leukemia and lymphoma [34]. Furthermore, researchers are exploring the potential of *ex vivo* editing to enhance immune system function. For instance, studies have shown that CRISPR-edited T cells can be engineered to resist immune checkpoint inhibitors, thereby enhancing their efficacy in solid tumors [35].

Ex vivo gene editing also holds potential in the field of regenerative medicine by correcting genetic defects in induced pluripotent stem cells (iPSCs). The edited iPSCs possess the capacity to differentiate into functional cells, which can be utilized in tissue repair, organ transplantation, and the management of chronic diseases [36].

3.1.2. Applications in Agriculture

Ex vivo gene editing involves isolating plant cells [37] for *in vitro* genetic modification before regenerating them into whole plants. This approach is particularly valuable for introducing precise edits in crops such as rice, wheat and tomato, where stable transformation and regeneration protocols are well established. Key successes include the CRISPR-mediated knockout of the OsERF922 gene in rice, which reduced susceptibility to blast disease by 70% in field trials, and the development of Golden Rice, in which edited Psy and CrtI genes increase β-carotene accumulation to combat vitamin A deficiency. However, *ex vivo* editing faces challenges such as low regeneration efficiency in crops such as soybean, which requires optimised hormone treatments [38], and the risk of chimerism, where only a subset of cells carry the edit, requiring additional screening. Despite these hurdles, *ex vivo* remains the gold standard for generating heritable, stable edits in

plants with established tissue culture systems.

3.1.3. Applications in Industrial Biotechnology

Ex vivo gene editing has become a prevalent technique in microbial engineering for biofuel production. For instance, CRISPR-Cas9 has been employed to enhance yeast strains, thereby facilitating more efficient bioethanol production [39]. Similarly, cyanobacteria have been genetically modified to enhance hydrogen and lipid production, paving the way for renewable energy sources [40].

The pharmaceutical industry has adopted *ex vivo* gene editing to enhance the productivity of microbial production of drugs and biologics. For example, genetically modified *E. coli* and yeast strains have been shown to facilitate the economical production of insulin and monoclonal antibodies [41].

Ex vivo editing is also employed in bioremediation, where genetically modified microorganisms degrade pollutants or recycle waste. For example, engineered bacteria have been developed to break down plastics and remove heavy metals from wastewater [42].

3.2. *In Vivo* Gene Therapy

In vivo gene editing is a revolutionary approach to treating diseases by delivering gene editing tools directly into the patient's body. Compared to *in vitro* gene editing, *in vivo* editing avoids the complex steps of cell isolation, culture and transfusion, and is more suitable for tissue or organ diseases that cannot be treated *in vitro* [43].

Viral vectors, particularly adeno-associated viruses (AAVs), are the leading platform for *in vivo* gene editing due to their tissue-specific tropism and sustained transgene expression. However, their efficacy is limited by pre-existing immunity in ~50% of the population, and high doses can induce hepatotoxicity [44]. While novel capsid engineering approaches (e.g. AAV-SPR for brain targeting) offer improved specificity, further validation is required [45]. In contrast, non-viral methods such as lipid nanoparticles (LNPs) allow transient Cas9 mRNA delivery, but exhibit poor organ selectivity (<5% liver uptake in mice) [46]. *Ex vivo* electroporation achieves high editing efficiency (>80% in T cells) but is inherently invasive [47].

3.3. Applications in Medicine

In medicine, *in vivo* gene editing technology has brought the possibility of treating many previously untreatable diseases. For example, *in vivo* gene editing technology has been widely researched for the treatment of hereditary liver diseases. Tyrosinemia Type 1 (HT1), an inherited liver dysfunction caused by mutations in the FAH gene, has been successfully treated in mouse models using CRISPR-Cas9 delivered via adeno-associated virus (AAV). This approach significantly improved the animals' liver function and prolonged their survival time, suggesting that CRISPR technology could be used to treat inherited metabolic diseases [48].

In vivo gene editing also offers new possibilities for cardiovascular disease treat-

ment. For example, hypercholesterolemia, a disease associated with mutations in the PCSK9 gene, has been addressed using CRISPR-Cas9 technology to knock out the PCSK9 gene in the liver. The safety and long-term efficacy of this strategy were first validated in a Phase I clinical trial in human patients, where patients' LDL-C levels fell by an average of more than 50% within six months of treatment [49].

3.4. Applications in Agriculture

In vivo gene editing bypasses tissue culture by delivering CRISPR tools directly into intact plants, offering a faster and more versatile alternative to *ex vivo* methods. Techniques such as nanoparticle carriers (e.g. lipid-based delivery of Cas9 RNPs to wheat leaves) and viral vectors (e.g. TMV-mediated systemic editing in tomatoes) achieve efficient editing without regeneration. Notable applications include engineering maize for pest resistance via pollen tube delivery of Bt toxin genes, reducing pesticide use by 50%, and enhancing soybean thermotolerance by editing GmHsp90A2 to maintain yield under heat stress. However, *in vivo* editing struggles with delivery barriers in monocots (e.g., maize cell wall impedance) and regulatory fragmentation as policies vary globally (e.g., EU GMO regulations vs. USDA exemptions for nontransgenic edits). Emerging solutions, such as cell-penetrating peptides and tissue-specific promoters, aim to overcome these limitations and position *in vivo* editing as a transformative tool for rapid crop improvement. [33] [50].

3.5. Applications in Environment

On the environmental front, applications include the control of mosquito-borne diseases, the protection of endangered species, and the management of invasive species. These technologies have significantly improved the efficiency of environmental protection by enabling genome modification through direct DNA editing in living organisms.

Mosquito-borne infectious diseases previously posed a major threat to global public health [51]. *In vivo* gene editing technologies, particularly Gene Drive, offer revolutionary solutions for mosquito-borne disease control [52]. Gene drives achieve population suppression or functional modification of specific mosquito species by enhancing the genetic bias of target genes in the population [53].

Kyrou *et al.* developed a gene drive system that targets the "doublesex" gene in the Anopheles gambiae mosquito, preventing females carrying the drive from reproducing, thus achieving complete extinction of the mosquito population in a laboratory setting [54]. Meanwhile, Carballar-Lejarazú and James proposed a strategy to render mosquitoes incapable of transmitting malaria parasites through gene editing, *i.e.*, by inserting anti-parasite genes into mosquitoes, thus effectively blocking disease transmission [55].

In addition, gene editing technology has been used to control other mosquito-borne diseases. For example, Li *et al.* (2020) successfully introduced a gene affecting the replication of the dengue virus into the Aedes aegypti population using

CRISPR technology, which dramatically reduced the transmission ability of the virus [56].

4. The Future Direction of Gene Editing Technology

Gene editing technology, spearheaded by tools like CRISPR-Cas9, has revolutionized genetic research and biomedical applications. Over the last decade, advancements in precision and efficiency have propelled this technology into a critical platform for addressing challenges in medicine, agriculture, and ethical governance.

4.1. Enhancing Precision and Efficiency of Gene Editing

The precision of gene editing represents a fundamental aspect that will continue to shape its future development. Current tools, such as CRISPR-Cas9, are confronted with challenges, including off-target effects, which can result in unintended mutations. Future innovations are directed towards enhancing editing accuracy, reducing error rates, and expanding the toolbox of gene editing methods [57].

One such advancement is the development of base editors, which enable single-nucleotide changes without double-strand breaks. Researchers have refined adenine and cytosine base editors to achieve a degree of precision in altering DNA sequences that are unparalleled in the field [12]. Furthermore, prime editing, a relatively recent technological advancement, enables precise insertion, deletion, and substitution with minimal collateral damage, thereby expanding the range of genetic targets [13].

Additionally, future research is investigating the potential of artificial intelligence (AI) to facilitate the development of more precise guide RNAs. The application of machine learning models enables the prediction of off-target effects and the optimization of CRISPR design, thereby enhancing specificity [5]. Combining gene editing tools with AI-driven prediction models is likely to accelerate the development of safe and effective therapies.

4.2. Medical Applications for Genetic Diseases and Cancer

Among the most promising applications of gene editing is its use in the field of medical science. The advent of genetic therapies holds the potential to address previously intractable genetic conditions, including blood-related disorders such as sickle cell disease and beta-thalassemia. Preliminary clinical trials employing CRISPR-based therapies have demonstrated the capacity to modify hematopoietic stem cells for the purpose of correcting defective genes [31].

In the context of cancer treatment, gene editing has the potential to facilitate the development of personalized immunotherapies, such as the enhancement of T-cells to recognize and attack tumors. The combination of CAR-T cell therapy with CRISPR is currently being investigated with the objective of enhancing the efficacy and reducing the immunogenicity of cancer treatments [47].

In addition to monogenic diseases, future research endeavors to target complex disorders such as Alzheimer's and diabetes by modifying multiple genetic pathways. Nevertheless, issues pertaining to the delivery of gene editors, such as viral vectors or nanoparticles, must be resolved to guarantee the safe and efficacious *in vivo* editing of genes [58].

4.3. Ethical and Societal Considerations

The rapid development of gene-editing technologies, particularly CRISPR-Cas9, has had a profound impact on the field of genetics and biomedical research, marking a revolutionary shift in the way these disciplines are conducted. Globally, regulatory responses remain fragmented, the EU bans germline editing under the Oviedo Convention, while the US and China provide case-by-case oversight, revealing a lack of consensus on permissible boundaries [59] [60]. The controversial case of edited human embryos in 2018, in which twin girls were reportedly modified to resist HIV infection, sparked global outrage and highlighted the urgent need for strict ethical guidelines and international regulation [61]. Such interventions have the potential to permanently alter the genetic make-up of future generations, with unintended consequences for human evolution, genetic equity and social acceptance.

Public perception further complicates acceptance. Surveys show that 62% of Americans support therapeutic editing but oppose enhancement uses [62]. Future research must prioritise ethical governance and public engagement to ensure transparent and responsible use of gene editing. Initiatives such as the Global Observatory for Genome Editing aim to create collaborative platforms for scientists, ethicists and policymakers to discuss standards and risks [63].

Another critical issue is genetic inequality, as the high cost of gene-editing therapies could exacerbate health inequalities between wealthy and underserved populations. Access to these technologies may remain limited to wealthy nations or individuals, creating a genetic divide and undermining principles of global health equity. Treatments such as CRISPR-Cas9-based Casgevy for sickle cell disease cost over $2 million per patient, disproportionately excluding low-income populations [64]. This disparity is exacerbated by the concentration of 95% of clinical trials in high-income countries, leaving diseases prevalent in the Global South (e.g., malaria) understudied. Reducing these inequalities necessitates collaborative actions to promote the just allocation of gene editing interventions, with a focus on low- and middle-income regions [65]. It is evident that international organizations, such as the World Health Organization (WHO), have underscored the significance of establishing ethical and fair frameworks surrounding gene editing. In doing so, they have advocated for the creation of systems that enhance equity and participation among nations.

4.4. Agricultural Innovations and Food Security

The potential for gene editing to revolutionize agriculture is significant. It can enhance crop resilience, improve nutritional content, and ensure food security.

CRISPR-based editing allows for targeted modifications that can accelerate the development of crops resistant to pests, diseases, and climate stress.

For example, researchers have successfully employed gene editing techniques to confer resistance to fungal infections and improve yield in rice and wheat [37]. Furthermore, gene editing has the potential to address malnutrition by fortifying crops with essential vitamins and minerals. This can be achieved through techniques such as biofortification, which has been successfully employed to enhance the nutritional value of crops such as tomatoes and cassava [38].

The future of gene editing in agriculture also entails expanding this technology to livestock. The focus will be on enhancing traits such as disease resistance and productivity while reducing environmental impact. However, regulatory frameworks for genetically edited organisms remain inconsistent on a global scale. To facilitate the adoption of these technologies and address public concerns regarding genetically modified foods, harmonizing policies will be essential [66].

The future of gene editing technology depends on its ability to enhance precision, transform medicine, address ethical challenges, and augment agricultural productivity. As scientists continue to develop innovative tools and applications, collaboration between researchers, policymakers, and society will be vital to ensuring the ethical and equitable use of this transformative technology. Ongoing progress in this domain is set to reveal answers to several of humanity's most urgent issues, from curing genetic disorders to bolstering worldwide food safety.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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