Drug Discovery and Molecular Docking (DDMD)
ISSN: 3107-6688 | Vol. 01, Issue 03, Sep-Dec. 2025 | pp. 60-74

CRISPR Base-Editing for Correcting Monogenic Mutations: Optimization and Off-Target Profiling

Dhanush Ram Turkane[1], Rupendra Kumar Sahu[1], S. Nandini[1], Komal Awasthi[1], Alisha Banafar[2]*

[1]Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Bhilai, Durg, CG, Pin - 490040

[2*]Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India

***Corresponding Author E-mail: banafaralisha83@gmail.com**

Abstract:

CRISPR base-editing has now emerged as a fast, accurate, and least disruptive technology to correct monogenic mutations in animal models, demonstrating direct A-G and C-T base conversions, without formation of two-stranded breaks. This review summarizes recent advances in optimization of base-editing systems, delivery, and optimization of off-target profiling of mice, zebrafish, rabbits, and livestock. The major results are reported on the development of better versions of the EsCs: BE4max, ABE8e, and high-fidelity Cas9 enzymes can be used to obtain better on-target specificity, whereas AAV vectors, LNPs, and mRNA-based delivery are important in enabling tissue-specific editing. Species-specific variability in off-target studies due to chromatin context and deaminase behavior raises the importance of standardized profiling pipelines. Although it has not been used in therapeutic contexts, despite considerable demonstrated capabilities in metabolic, ocular, and neuromuscular disease models, there are still challenges, including limitations in the use of PAM by bystanders, mosaicism, and long-term safety. All in all, this review provides basis on the possibility of further refinement of editors, safer methods of delivery, and ethical biosafety systems to develop CRISPR base-editing into effective preclinical and translational practices.

Keywords: CRISPR Base Editing, Monogenic Mutations, Animal Models, Adenine Base Editors, Cytosine Base Editors, Off-Target Profiling, AAV Delivery, Genome Engineering.

Received: Sep. 01, 2025 **Revised: Oct. 21, 2025** **Accepted: Nov. 28, 2025** **Published: Dec. 18, 2025**

DOI: https://doi.org/10.64474/3107-6688.Vol1.Issue3.5

https://ddmd.nknpub.com/1/issue/archive

1. INTRODUCTION

CRISPR base-editing has become one of the most revolutionary applications of today genome engineering, and it has the capability of repairing single-nucleotide mutations with unprecedented precision and lesser genomic disturbance[1]. In contrast to paradigmatic CRISPR Cas9 editing, there are two non-catalytic approaches where a base-editing enzyme with a published Cas variant is used to convert one nucleotide to another without breaking through the nucleic acid template backbone, unlike classical editing methods which induce the formation of two DNA strands (or a rearrangement) called awareness of the targeted DNA region. This sets them uniquely apart in studying the monogenic diseases- especially in animal models where the ability to use controlled, hereditary and somatic genome modification allows the researcher to study the pathogenesis, responses to therapy and the stability of the long-term remedy. As more than half of known genetic diseases are associated with point mutations, base editing has rapidly become an indispensable technique to create very precise disease models, as well as to test possible genetic interventions in vivo[2].

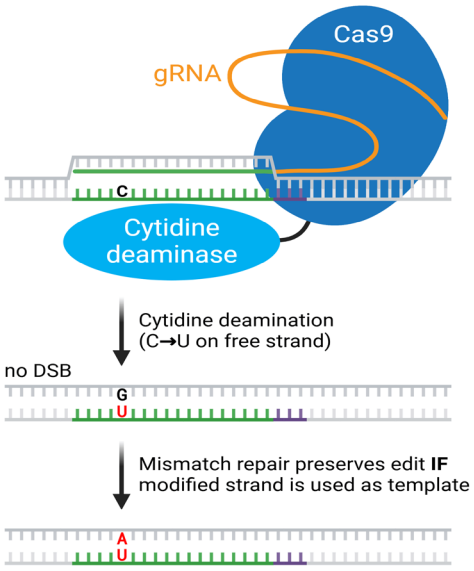

Figure 1: CRISPR[3]

Use of CRISPR base-editing systems in animal studies such as mice, zebrafish, rabbits, livestock species, and other animals has increased exponentially in the recent years. Whole-organism phenotyping is a key benefit of animal models, revealing to researchers efficiencies of correction in tissues, mosaicism, immune reactions towards delivery vectors, and the ability to maintain fixed alleles throughout the developmental lifestyles. Simultaneously, to optimally high base editors to work in vivo, it is essential to put affected species-specific chromatin accessibility, delivery limitations, and off-target effects into proper perspective[4]. This has led to an emerging literature which is concerned with the optimization of editing tools, the development of strong pipelines in profiling and showing the utility of this therapeutic

approach in preclinical trials. Not only do these developments aid in the creation of genetically correct animal models, they also offer some words of study in terms of transplantational possibilities of genetically-based therapies in future applications.

1.1 Background Information and Context

CRISPR base-editing technology includes cytosine base editors (CBEs) and adenine base editors (ABEs), respectively, which induce C-mediated and A-mediated C-T and A -G conversions, respectively[5]. These technology variants have been developed to the point of multiple generations of engineered Cas proteins, better-designed deaminases, better guide RNA scaffolds, and novel delivery vehicles including adeno-associated viruses (AAVs), lipid nanoparticles (LNPs), and mRNA-protein complexes. In animal studies, the tools have allowed the correction of a variety of monogenic defects that cause metabolic disorders, retinal degeneration, heart defects and neuromuscular diseases. The animal experiments offer controlled conditions to determine efficacy of editing in diversified tissues, longevity of expression of the fixed genes, and unintentional changes in the genome. Such a contextual base highlights the necessity of base editing in the preclinical research and also in the comprehension of mutation-related diseases at molecular and organismal stages[6].

1.2 Objectives of the Review

The primary objective of this review is to systematically examine how CRISPR base-editing technologies are optimized and applied for correcting monogenic mutations in animal models:

- To evaluate optimized CRISPR base-editing tools, editor variants, and delivery platforms used for correcting monogenic mutations in diverse animal models.
- To analyze on-target efficiency, precision, and phenotypic outcomes achieved through cytosine and adenine base editors across tissues and species.
- To assess genome-wide off-target profiling methods and identify species-specific patterns of unintended edits in animal studies.
- To critically examine the strengths, limitations, and technical challenges of base-editing applications, including PAM constraints, bystander edits, mosaicism, and delivery-related issues.
- To highlight ethical, biosafety, and translational considerations associated with in vivo base editing, and propose future directions to improve safety, accuracy, and applicability.

1.3 Importance of the Topic

The opportunity to optimize and off-target the CRISPR base editors under animal models will help usher in the use of genomics technologies towards safe and productive therapeutic application. Animal research is still the cornerstone in determining the reliability of editing, trying species-specific fixing measures, and determining the species-only challenges that could drive translational possibility[7]. As more sophisticated variants of editors keep being generated,

their practical application in the context of complex life is becoming an even more important issue. Moreover, monogenic mutations also signify a heavy burden to the veterinary science, agriculture, and biomedical research fields- where exact genetic repair may bring better health of the animals, a more realistic model of disease, and better insight into gene activity. Thus, investigations of CRISPR base editing in animals not only provide understanding of scientific value but also prepare the background of new clinical technologies in the future, which is why the subject of this review is topical and scientifically valuable[8].

1. EVALUATION OF BASE-EDITING TOOLS, DELIVERY METHODS, AND OUTCOMES IN ANIMAL STUDIES

CRISPR base-editing in animal models has proven to be very efficient in correcting monogenic mutations and results have shown mice, zebrafish, rabbits and swine exhibit robust phenotypic recovery in cases of metabolic, developmental and retinal diseases[9]. A valuable improvement involves use of optimized editors like BE4max, ABE7.10 and ABE8e, which are transported by AAV, LNPs or mRNA complexes and improve precision and tissue specificity. Although it is highly accurate and validated in vivo, limitations to its use include PAM dependence, interfering with bystanders, mosaic and immune response to viral delivery, with further changes suggested in the design of editors and delivery systems.

2.1 Summary of Key Research Studies in Animal Models

- **Murine Models:** ABEs have also been extensively used in murine models to rescue single-nucleotide defect that cause inherited disorders of metabolism including phenylketonuria. It has been stated that the ABE variants can reach over 3060% mutation correction in hepatic tissue under optimization, which is enough to recover the major enzyme activities and reverse diseases phenotypes. As well, CBE-mediated editing has demonstrated robust therapeutic potential in mouse models of retinal degeneration, in which targeted base conversion of malignant cytosine bases was able to restore the photoreceptor architecture and performance[10].
- **Zebrafish Models:** To these end Zebrafish embryos were identified as being strong and quick platforms to test CRISPR base-editing tools thanks to their transparent embryos, rapid development, and retained gene paths. ABE and CBE Microinjection of embryo Microinjection of embryos with ABE or CBE mRNA has demonstrated high on target editing efficiencies of more than 70 percent, allowing developmental monogenic mutations to be efficiently modeled and corrected. Though zebrafish usually exhibit low off-target activity, it has been shown that the results of editing differ based on local genomic context, chromatin accessibility, and mRNA gRNA sequences.
- **Rabbit and Livestock Models:** Rabbit models have been more used in order to recreate human-like lipid metabolism disorders by precise base-to-T base conversion which interferes with the essential metabolic genes. Base editing in rabbits has the benefits of larger size of organs and similarity to humans in physiologically relevant aspects (when studying the cardiovascular and metabolic system) and size. Base editors have been

used in livestock species (especially swine), to assess somatic editing in liver, heart, and muscle tissue.

2.2 Methodologies and Findings

- **Editor Variants Used:** BE3 and BE4max are cytosine base editors with popular C-to-T conversions, but have moderate bystander activity because the editors have larger editing windows. ABE7.10 and ABE8e in their turn have much greater A-to-G conversion efficiency and good mutation correction in vivo, and ABE8e is among the most commonly optimized versions that are used in animal experiments.
- **Delivery Systems:** There are different delivery methods of various animal models, AAV vectors are the most widely used in targeted delivery methods of mouse liver, muscle, and retinal tissues because they are stable to express and show tissue specificity. Lipid nanoparticles (LNPs) have been put forward as a viable and successful non-viral competitor, in terms of providing transient expression of editors and lowering the chances of prolonged immunogenicity in murine models. The use of mRNA-protein complexes in zebrafish embryos facilitates high-efficiency and rapid on-target editing, being particularly useful in the study of early developmental biology in which on-target editing is only needed to provide transient expression levels.
- **Key Findings:** It has been found that efficient gRNA optimization with spacer length optimization and mismatch avoidance can greatly increase the efficiency of base-editing with minimal bystander conversions. Specificity of editing can be further enhanced by incorporation of tissue-specific promoters like the liver-targeted TBG promoter in murine models so that editing is targeted to tissues of interest only[11].

2.3 Critical Evaluation of Strengths and Weaknesses

- **Strengths**

CRISPR base-editing has a number of advantages, which render it very appropriate in correcting monogenic mutations in animal systems. The outstanding feature is that it has high precision and single-nucleotide variants can be corrected without creating a double-stranded DNA break (DSB) which reduces the possibility of chromosomal rearrangements or a massively sizable deletion. Due to their excellent phenotypic validation capabilities, animal models such as mice, zebrafish, rabbits, and livestock offer a great platform to researchers to directly visualize physiological, developmental, and metabolic benefits of specific base correction. All of these strengths illustrate that CRISPR base-editing tools have strong potential in the study and correction of single-nucleotide mutations in controlled and multi-tissue animal systems[12].

- **Weaknesses**

In spite of the significant advances, there are still multiple pitfalls to the wider use of CRISPR base-editing in animal studies. One of the most significant limitations is that it requires particular PAM sequences, limiting the population of genomic sites that can be efficiently hit particularly in species with a more diverse or compact genome. The bystander editing is another

major problem, especially in cytosine base editors (CBEs), in which various cytosines inside the editing window can change by mistake, making it difficult to fix specifically. There is still also a concern on the long-term stability and mosaic edits in animal tissues because in a case of editing efficiencies can differ between cell types and developmental phases. These shortcomings indicate that the editor design needs to be enhanced, that the PAM compatibility needs to be expanded, and that there should be alternative strategies in delivering them to provide better and consistent results.

Table 1: Summary of CRISPR Base Editing Studies for Monogenic and Cardiovascular Disorders[13]

Author(s) & Year	Study Focus	Focus Area	Methodology	Key Findings
Jin et al. (2024)[14]	Correction of human nonsense mutations in Duchenne muscular dystrophy	Monogenic muscular disorders	Adenine base editing in mouse model	Restored functional dystrophin expression; demonstrated therapeutic potential of precise base editing for DMD
Kabra et al. (2023)[15]	Nonviral base editing of KCNJ13 mutation	Inherited retinal channelopathy	Nonviral base editing in animal model	Preserved vision and retinal function; demonstrated safe and efficient genome editing in ocular disease
Kabra et al. (2022)[16]	Nonviral base editing of KCNJ13 mutation	Inherited retinal channelopathy	Nonviral base editing	Reinforced effectiveness in preventing vision loss; provided insights into cell-specific editing efficiency and safety
Konishi, C. T., & Long, C. (2020)[17]	Progress and challenges in CRISPR-mediated therapeutic genome editing	Monogenic diseases	Review of CRISPR, base, and prime editing technologies	Summarized advances, delivery strategies, and challenges such as off-target effects and immunogenicity; emphasized need for optimization
Lebek et al. (2023)[18]	Ablation of CaMKIIδ	Cardiac disease	CRISPR-Cas9 base editing in animal model	Prevented pathological cardiac remodeling and improved cardiac

	oxidation by base editing			function; demonstrated expanding applications of base editing beyond genetic diseases

2. ADVANCES IN BASE-EDITING OPTIMIZATION, OFF-TARGET PROFILING, AND THERAPEUTIC APPLICATIONS IN ANIMAL MODELS

Efforts of enhancing base-editing systems in animal models have been directed at improving upon efficiency, specificity as well as delivery of editing in tissues. Better variant forms of an editor, such as codon-optimized BE4max and high-fidelity versions of Cas9 (SpCas9-HF1, eSpCas9) are more accurate and stable in rodents (mice), zebrafish, and more. More accuracy has been achieved through shortening gRNAs and artificially selecting deaminases like TadA-8e that reduce background edits. The innovations in delivery like dual-AAV split-intein and transient lipid nanoparticle (LNP) systems facilitate effective and less harmful delivery in vivo, particularly in tissue with the complicated targeting needs[19].

Unintended edits in animals have been found to exhibit species-dependent patterns with techniques such as Digenome-seq, EndoV-seq, Circle-seq, as well as deep sequencing. AT-rich regions exhibit increased off-targets in zebrafish, but super low off-target with high-fidelity versions of ABE in mice. Such streamlined systems have been used to successfully correct monogenic mutations in a number of disease models such as restored metabolic enzyme's activity in the liver of mice, improved retinal degeneration with ABE editing in eye tissues and partial restore the function of dystrophin in neuromuscular disease models[20].

3.1 Optimization of Base-Editing Systems

The optimization of base-editing systems in animal research has been aimed at enhancing efficiency, precision, and delivery in a wide range of different tissues. Improved versions of the editors like codon-optimized BE4max have demonstrated much better protein stability and catalytic activity in mouse tissues, resulting in increased rate of editing in vivo. On the same note, improved Cas9 variants such as SpCas9-HF1 and eSpCas9 have been used to enhance specificity of the Cas9 in zebrafish and rodent models, by minimizing off-target tolerance during DNA binding. Reducing off-targeted edits can also be enhanced by working with shorter gRNAs, which reduce the recognition of mismatches, and engineered deaminases TadA-8e, which have reduced background activity[21]. Innovations in delivery are also important, and split systems of dual-AAV split-intein systems allow efficient delivery in vivo to small animals in which full-length editors are constrained by vector packaging. In the meantime, lipid nanoparticles (LNPs) can deliver in a non-integrating manner, lowering the systemic and tissue-targeted editing exposure to nuclease, and offering enhanced systemic biosafety.

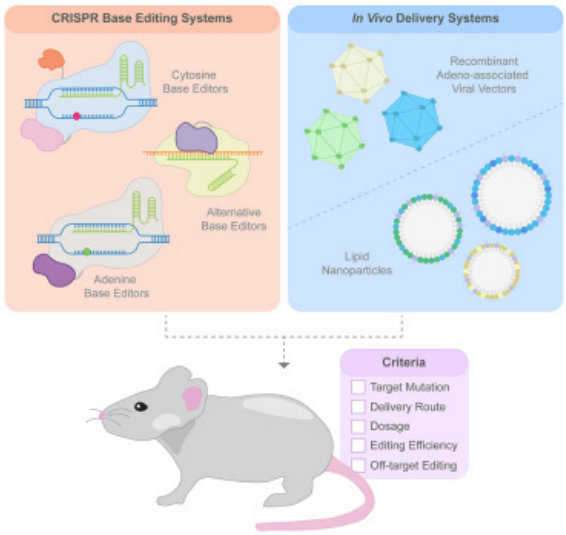

Figure 2: Base-Editing Systems to Rescue Mouse Model[22]

3.2 Off-Target Profiling in Animal Studies

The off-target profiling is critical in explaining the accuracy and safety of base editors in various animal models. Several detection platforms have been used all of which are adapted to different forms of base-editing activity. Digenome-seq The technique is commonly applied to mice to map Genome-wide Cas9 activity and identify off-target cleavage or editing windows. EndoV-seq is capable of identifying A→I editing by ABEs, therefore, it would be useful to analyze inosine sites in off-target sites[23]. Cirdle-seq and amplicon deep sequencing is often used on zebrafish and rodent embryos, and used to identify low-frequency off-target events in a highly sensitive manner. Major evidence shows that the off-target patterns differ among species, in part, because of chromatin accessibility and sequence composition differences. In the example of zebrafish, it is found that off-target rates in AT-rich regions are higher in the case of CBEs; conversely, with high fidelity ABE variants, extremely low off-target rates, frequently less than 0.1% are observed in murine models, reflecting high species- and tool-dependent variability[24].

3.3 Animal Model Applications for Monogenic Mutation Correction

The base-editing technology has made tremendous progress in repairing monogenic mutations in a variety of animal disease models. In studies of metabolic disorders, as targeted A-to-G or C-to-T mutation of mouse liver successfully replaced enzyme activity and overturned metabolic abnormalities[25]. ABE delivery to pigment epithelium of the retina, in ocular models, has been demonstrated to delay or attenuate degenerative phenotypes caused by relationships between its disease-related point mutation correction. In neuromuscular disease research, it was shown that base editing showed the potential to partially re-express dystrophin in mouse muscle with functional benefits in mouse models of muscular dystrophy. These applications help

reflect the versatility of CRISPR base editors in targeting tissues of the soma and indicate the potential of modeling and restoring various single genes disorders in preclinical animal models[26].

3. ETHICAL AND BIOSAFETY CONSIDERATIONS IN ANIMAL-BASED BASE-EDITING RESEARCH

The ethical aspects surrounding animal based research in base-editing revolve around the minimization of animal suffering, necessity to ensure the practicability of scientific research, and overall animal welfare. Base editing is frequently done to create or fix disease-causing mutations in animals including mice, zebrafish, rabbits, and livestock to recreate human-based monogenic disorders[27]. Such processes may cause physiological stress, developmental abnormalities or unintended phenotypes. Ethical principles like the 3Rs including Replacement, Reduction, and Refining direct researchers to ensure the use of animals is justified when there is no other model such as an alternative, the number of animals required is minimal and the experimental designs are refined to minimize the incidence of pain and distress in animals. Instrumental animal care committees are very important in scrutinizing the protocols, evaluating risk-benefit quotient, and making sure that the treatment conducted in the study is humane[28].

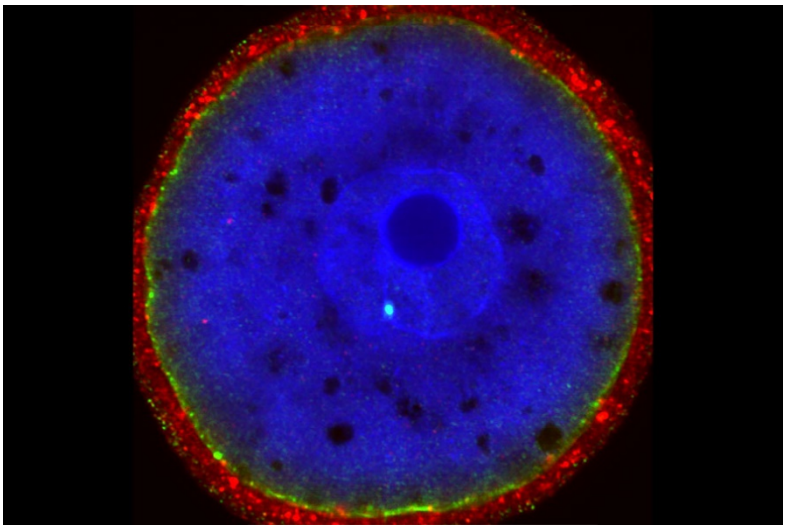

Figure 3: Correcting Disease-Causing Mutations[29]

The aspect of bio safety is also crucial, particularly when using viral vectors, lipid nanoparticles, or mRNA protein complexes to deliver base editors into vivo. Lack of immunogenicity at low levels and the fact that viral vectors such as AAV are likely to integrate prevents their use in other species, making them unsafe to both animals and researchers working with the material[30]. Accidental release or exposure needs to be prevented through proper containment measures that are usually under BSL-1 (or BSL-2) based on the conditions by the control of the organism-carrying vectors as well as the organism itself. Alternatively, genetic modifications are permanent and thus have to be carefully assessed regarding the safety

of the environment. The escaped or poorly managed edited animals can offer engineered alleles to wild populations and can cause ecological issues. Plantations should implement tough housing, waste collection and record keeping procedures to eliminate the possibility of unintentional release[31].

Off-target activity is a major source of biosafety concern since unforeseen edits may result in the appearance of unwanted phenotypes, or the abuse of animals, or the modeling of diseases. This risk is minimized using the high-fidelity base editors, optimized gRNAs, and genome-wide off-target profiling, but it cannot be completely eliminated yet. It is the responsibility of the researchers to ensure that they have validated pipelines which are stringent to confirm the specificity of editing before increasing the breeding colonies or launching long-term research[32]. There are also ethical requirements in regards to the openness in reporting off-target effects, which is essential to reproducibility and the avoidance of overinterpretation of therapeutic potential. There is a need to have sustained monitoring of the edited animals over generations to be able to identify late-onset effects or mosaicism which can affect study outcomes or welfare[33].

There is a wider ethical aspect created by such base-editing of animals, which includes its implications on the translational level. Even though these studies are intended to model or correct monogenic diseases, they also make precedents on their further clinical application. By ensuring that preclinical research meets the highest safety, scientific rigor, and animal welfare standards, people place more trust in preclinical research, and progenitor communications beginning to innovate responsibility, in the direction of therapeutic genome editing. These accruing ethical and biosafety issues underscore the necessity of an Interdisciplinary approach to designing and advancing CRISPR base-editing in animal roles with accountability via collaboration between geneticists, veterinarians, ethicists, and regulators[34].

4. DISCUSSION

CRISPR base editing is highly efficient and precise in animal models, with the assistance of enhanced variants of the editor, improved delivery mechanisms, and enhanced off-target profiling. These developments indicate its immense therapeutic benefits in the correction of monogenic diseases and the production of dependable animal models. Nevertheless, several critical issues, such as PAM limits, bystander edits, mosaicism, tissue-specific differences, and uneven off-target detection, preclude the broader use case. Further advances in the engineering of editors, more secure methods of delivery, and universal profiling are prerequisites, and long-term safety testing and interdisciplinary studies to make base-editing technologies responsible and useful[35].

5.1 Interpretation and Analysis of Findings

The results of animal studies indicate that the animal CRISPR base-editing technologies have made significant advancements in the high efficiency and precision of monogenic genetic mutations repair. Variants of the editor, including BE4max, ABE8e, and high-fidelity versions

of Cas9 were identified as consistently optimal as far as on-target accuracy is improved and minimizing background activity, so it is clear that molecular optimizations directly scale to improve in vivo behavior[36]. The delivery vectors, particularly the AAV vectors, and lipid nanoparticles turned out to be effective in performing tissue specific editing in mouse liver, retina, muscle and zebrafish embryos. Profiling of off-targeted shows that the range of off-target edits in different species depends on the chromatin architecture, genomic setting, and behavior of deaminases. All of these observations indicate that base-editing systems are generally very effective in the animal model but need optimization by the species to ensure predictable outcomes[37].

5.2 Implications and Significance of the Findings

The improvements witnessed in the study of animal base-editing have significant implications to the field of biomedical and veterinary genetics. The observed success of base editors in mutating disease model mice with metabolic, neuromuscular, and ocular diseases successfully indicates that base editors can be used to treat various single-gene disorders in practice. The ability of base editing to produce highly-accurate disease models with fewer off-target implications than DSB-based CRISPR strategies is also demonstrated in animal studies, enhancing the translational success of preclinical studies[38]. In addition to medicine, base editing may have a substantial contribution to agriculture, evolution and domestication of livestock, and conservation genetics by providing an opportunity to correct an economically or biologically valuable allele of controlled significance. In general, these results support the usefulness of base editing as an accurate, multifaceted and less harmful platform of genome engineering.

5.3 Gaps and Unresolved Challenges

In spite of significant advancements, there are still a number of fatal gaps in the research of animal-based base-editing. The limitation of targets by PAM remains in species where there are genomic regions that have no suitable PAM sequences. Editing in larger editing frames is dangerous to accuracy, especially with CBEs in AT-rich genomes like zebrafish. The safety of complex organisms is not well determined yet as there is a limited long-term analysis of mosaicism, immune reaction to vectors and generation stability of corrections. Also, the majority of research projects target tissues which are relatively easy to work with such as liver or retina, and there are no gaps with extending the research on editing performance to tissues that are more difficult to retrieve, such as the heart or central nervous system. In addition, off-target detection methodologies vary in different studies; hence, causing inconsistencies because of which it is not possible to straightforwardly compare the results[39].

5.4 Future Research Directions

Future studies ought to focus on implementing PAM-pliable or PAM-free base editors to increase the number of genomic sites that can be targeted by animals. Enhancement of

deaminase engineering to decrease engineering windows can greatly minimize the bystander effect and increase precision. This will be necessary in the long terms to conduct whole-organism monitoring (including multi-generational studies) with an eye to assessing edit permanence, mosaicism, and late-onset phenotypes. More development of non-viral delivery vehicles including the LNPs or the virus-like particles could provide safer options to the AAV-based systems. There should be a universal system of off-target detection, where many sequencing systems are unified in order to streamline assessment in different species. Translational relevance should also be enhanced by increasing the investigation into the use of larger and more physiologically relevant animal models like pigs and livestock other than humans. Finally, the development of the base-editing research will be responsible and efficient due to interdisciplinary collaboration across the fields of molecular biology, bioinformatics, ethics, and veterinary science[40].

5. CONCLUSION

CRISPR base-editing has become a potent and very specific genome-engineering platform to fix monogenic mutations in a variety of animal models, with great therapeutic potential and translational applicability. The optimized versions of the editors, the enhancement of the delivery, as well as more advanced off-target profiling, have collectively empowered the reliability and safety of the in vivo editing, which enabled the achievement of phenotypic rescue in the models of metabolic, ocular and neuromuscular diseases. Nonetheless, certain significant difficulties, such as PAM constraints, bystander edits, mosaicism, species-specific variability, and lapses in long-term safety testing still limit wider implementation. To solve these restrictions, it will be necessary to deal with creating next-generation editors, safer non-viral delivery systems, and standardized off-target detection frameworks, to enhance precision and risks reduction. The interdisciplinary approach to work and proper ethical and biosafety control will play a significant role in responsible development as research moves to more complex animal systems. All in all, the existing literature highlights the enormous potential of CRISPR base-editing and underscores the importance of ongoing improvement to enable its safe move to preclinical and therapeutic uses taken to another level.

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