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Applications of Mouse Gene Editing Technology in the Treatment of Hereditary Blindness

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Abstract This study explores the applications of mouse gene editing techniques in the research and treatment of hereditary blindness. Mouse models serve as ideal tools for biomedical research and possess significant advantages such as genetic similarity to humans, rapid growth and reproduction, abundant genetic tools, and controllable laboratory environments. Gene editing techniques, particularly CRISPR/Cas9, have made significant breakthroughs, enabling scientists to simulate genetic mutations related to inherited blindness, validate treatment strategies, conduct drug screening, and explore disease mechanisms. Implemented cases of mouse gene editing treatments, such as the restoration of Leber's hereditary optic neuropathy (LHON) and the regeneration of retinal cells, offer new hope for the treatment of hereditary blindness. While mouse gene editing treatments still face challenges, including ensuring safety and therapeutic efficacy, it holds enormous potential for clinical translation. Ethical considerations, targeting accuracy, cell toxicity, immune responses, and post-treatment effects are also crucial factors that need careful consideration. In conclusion, mouse gene editing techniques provide powerful tools for research in the treatment of hereditary blindness, offering new hope for future therapies.

Keywords Mouse models; Gene editing techniques; Hereditary blindness; CRISPR/Cas9; Ethical considerations

Hereditary blindness is a severe visual impairment typically caused by mutations in disease-associated genes, impacting the quality of life for millions of individuals globally. These genetic mutations can affect various aspects, including visual signal transduction, retinal structure and function, and optic nerve development, resulting in different types of hereditary blindness (Cremers et al., 2018). Patients may face the risk of blindness from birth or experience the onset of blindness in their youth or adulthood. Hereditary blindness imposes significant physical and psychological burdens on patients and their families, underscoring the crucial need for research and the development of therapeutic approaches.

In recent years, the rapid advancement of gene editing technologies has provided new hope for the treatment of hereditary blindness. In this context, mouse models have become essential tools for studying the mechanisms and therapeutic strategies for hereditary blindness. Mice share a similar visual system structure and function with humans while having a shorter lifespan, facilitating experimental research (Liu et al., 2022). Through gene editing technologies, researchers can simulate pathogenic mutations causing hereditary blindness and test different treatment approaches, offering valuable insights for future therapeutic strategies.

The objective of this study is to explore the application of mouse gene editing technology in the treatment of hereditary blindness. By reviewing the molecular basis of hereditary blindness, discussing the importance of mouse models, providing a detailed overview of the development and application of gene editing technology, examining the application of different gene editing techniques, and highlighting specific applications of mouse gene editing technology in the treatment of hereditary blindness (Ma and Liu, 2022). Finally, this study will pay attention to the ethical and safety considerations of gene editing technology, emphasizing the safety and potential risks of treatment. This research aims to provide readers with a comprehensive understanding of the latest developments and prospects of mouse gene editing technology in the treatment of hereditary blindness, offering robust support for future research and clinical applications.

1 Molecular Basis of Hereditary Blindness

1.1 Common genetic mutations and pathogenic genes in hereditary blindness

Hereditary blindness comprises a group of rare diseases caused by genetic mutations, and its incidence varies with different types of genetic alterations. In the investigation of the molecular basis of hereditary blindness, some common genetic mutations and pathogenic genes have been identified.

A prevalent form of hereditary blindness is cone-rod dystrophy (CRD). This condition is often associated with mutations in the *RHO* gene, which encodes opsin and plays a crucial role in the visual transduction process. Similar diseases include retinitis pigmentosa (RP) (Figure 1), caused by mutations in various genes such as *RHO*, *RPGR*, and *RPI* (Böhm et al., 2020). Mutations in these genes result in damage to rod and cone cells, ultimately leading to blindness.



Figure 1 BR clinical manifestations (Source: Wikipedia)

Leber's hereditary optic neuropathy (LHON) is another hereditary blindness disease typically caused by mutations in mitochondrial genes such as *MT-ND1*, *MT-ND4*, and *MT-ND6*. These genes encode subunits of the mitochondrial respiratory chain complex, and their mutations impair mitochondrial function, triggering degenerative damage to the optic nerve.

Retinal pigment epithelium dystrophy (RPED) is a group of diseases often involving mutations in the *RPE65* gene. *RPE65* encodes a crucial enzyme in the retinal pigment epithelial cells, maintaining the balance of vitamin A metabolism in the visual cycle. Mutations in the *RPE65* gene result in the loss of function in retinal pigment epithelial cells, affecting visual function.

1.2 Exploring the biological mechanisms of hereditary blindness

Understanding the molecular basis of hereditary blindness requires an in-depth study of the biological mechanisms related to visual transduction. Visual transduction is a highly complex process involving multiple cell types and signaling pathways.

The visual process begins with rod and cone cells in the retina, containing visual pigments that can sense light and convert it into neural signals. Mutations in visual pigments affect the quality of light perception; therefore, CRD and RP caused by *RHO* gene mutations, as well as RPED caused by *RPE65* gene mutations, are associated with visual pigment dysfunction. The optic nerve is a critical site for transmitting light signals to the brain, and LHON is linked to the loss of mitochondrial function. Mitochondria play a vital role in cellular energy production, crucial for optic nerve conduction. Mutations in *MT-ND* genes affect the function of mitochondrial complexes, resulting in reduced energy production and damage to optic nerve cells.

On the other hand, retinal pigment epithelial cells is also crucial for the maintenance of visual function. The enzyme encoded by the *RPE65* gene plays a critical role in the metabolism of retinaldehyde in the retina, helping the retina maintain the homeostasis of vitamin A. Mutations in *RPE65* disrupt vitamin A metabolism, ultimately leading to RPED. Therefore, a profound understanding of these biological mechanisms is crucial for the treatment of hereditary blindness.

The molecular basis research of hereditary blindness lays the foundation for the development of gene editing therapies, allowing interventions specific to particular genetic mutations. It is hoped that this therapeutic approach will bring new hope to individuals affected by blindness.

2 Importance of Mouse Models

2.1 Introduction to the advantages of mice as biomedical research models

Mice serve as widely utilized models in the scientific community for biomedical research, owing to their numerous advantages. Mice share a significant genetic resemblance with humans, with many genes exhibiting similar functions and structures in both species. This genetic similarity makes mice an ideal model for studying human diseases. In the investigation of hereditary blindness, the genetic similarity of mice plays a crucial role in understanding the mechanisms behind relevant genetic mutations (Gurumurthy and Lloyd, 2019). Mice exhibit rapid growth and have a short reproductive cycle, enabling scientists to conduct large-scale experiments and breed a substantial number of experimental animals within a relatively short timeframe. This is particularly important for the study of hereditary blindness, as it involves the analysis of gene mutations and the validation of therapeutic approaches.

Research on mouse models benefits from a rich array of genetic tools, including various gene-editing technologies, mutant mouse libraries, and tools for analyzing genetic alterations (Figure 2). These tools empower researchers to precisely manipulate mouse genes, facilitating the study of genetic variations related to blindness. Mouse experiments can be easily conducted in controlled laboratory environments, allowing researchers to adjust factors such as temperature, light exposure, diet, and other variables to simulate different physiological conditions. This is crucial for studying the influencing factors of hereditary blindness.

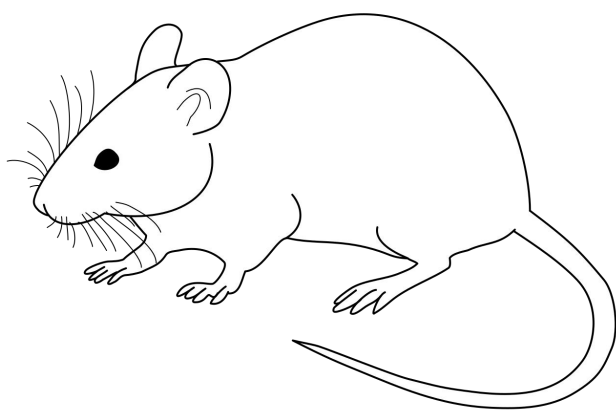


Figure 2 Mouse model (Source: Wikipedia)

2.2 Discussion on the application of mouse models in hereditary blindness research

Mouse models play a crucial role in the study of hereditary blindness, providing valuable information and opportunities to understand disease mechanisms and develop treatment methods. By introducing specific genetic mutations or mutant mouse models, scientists can simulate different types of hereditary blindness, such as retinitis pigmentosa degeneration and hereditary cataracts. These models contribute to the study of blindness mechanisms, identification of key factors leading to blindness, and exploration of potential therapeutic targets. Mouse models are also employed to validate the effectiveness of gene therapy strategies. Scientists can use gene-editing

techniques to repair or replace critical genes, restoring visual function in mice. This provides a robust foundation for the development of potential genetic therapeutic approaches.

In mouse models, researchers can conduct drug screening experiments to identify potential drug treatment options. These trials help evaluate the efficacy of different drugs in alleviating blindness symptoms, providing crucial information for clinical treatments. Optogenetics, a method based on light-sensitive proteins, is used to control neuronal activity. Mouse models play a vital role in optogenetic research, aiding in the understanding of the visual system's functionality and the development of new therapeutic approaches. Blindness involves not only damage to retinal cells but also requires neuroprotective strategies. Mouse models are employed to study the effectiveness of neuroprotective methods to slow down or halt the progression of blindness.

In conclusion, mouse models play a crucial role in hereditary blindness research, offering researchers opportunities to gain in-depth insights into blindness mechanisms and test potential therapeutic methods. Combined with advanced tools such as gene-editing technologies, mouse models hold the promise of bringing new breakthroughs in the treatment of hereditary blindness.

3 Development and Application of Gene Editing Technologies

3.1 Overview of CRISPR/Cas9 gene editing technology and its principles

Since its inception, the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/Cas9) gene editing technology has garnered widespread attention and sparked a revolution in the scientific community. This technology allows scientists to precisely modify gene sequences, providing a powerful tool for researching genetic diseases and exploring gene therapy. The principle of CRISPR/Cas9 is based on a bacterial defense mechanism found in the natural immune system, which can identify and cut the genome of invading viruses. CRISPR represents a series of short DNA sequences, while Cas9 is an enzyme capable of cutting DNA.

During the gene editing process, researchers design a CRISPR guide RNA that can recognize specific gene sequences. Once the CRISPR guide RNA matches the target gene, the Cas9 enzyme is activated to cut the target DNA. Cells then attempt to repair the cut DNA, usually through non-homologous end joining (NHEJ) or homology-directed repair (HDR). These repair processes may result in gene mutations, achieving the goal of gene editing (Figure 3).

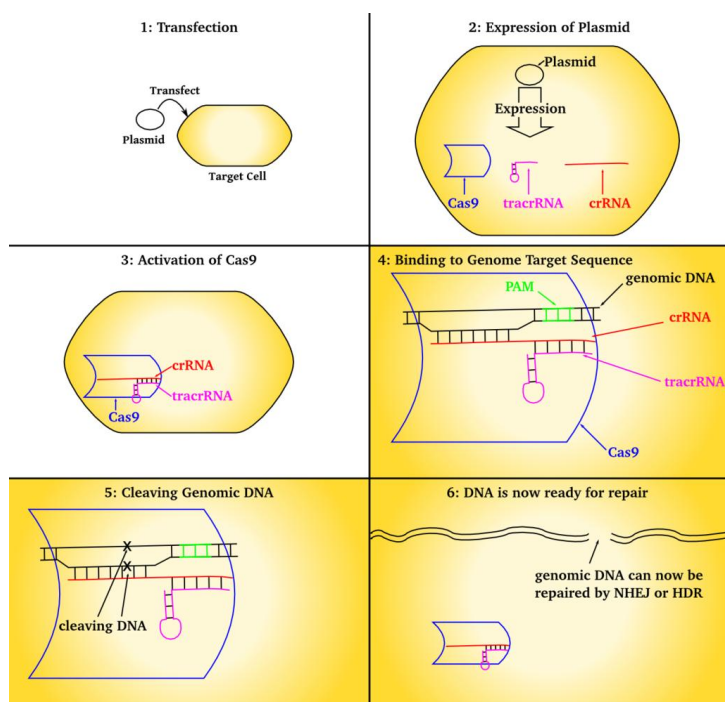


Figure 3 Gene editing process (Source: Wikipedia)

The advantages of CRISPR/Cas9 lie in its simplicity and efficiency, enabling researchers to precisely edit mouse genes to simulate and study gene mutations associated with hereditary blindness.

3.2 Discussion on the methods and results of applying CRISPR/Cas9 in mouse models

Significant progress has been made in applying CRISPR/Cas9 technology in mouse models, providing robust support for the study and treatment of hereditary blindness. Mice are commonly used experimental animal models due to their biological similarities to humans, making the application of CRISPR/Cas9 particularly beneficial in mice.

Through CRISPR/Cas9, researchers can accurately simulate gene mutations associated with hereditary blindness in mouse models. They can select target genes, design corresponding CRISPR guide RNAs, and introduce the Cas9 enzyme into mouse embryos. This approach has been successfully used to simulate various hereditary blindness diseases, such as retinitis pigmentosa, familial cataracts, and glaucoma (Liu et al., 2023). By observing the behavior and molecular changes in mice, researchers can gain in-depth insights into the pathogenesis of these diseases.

Furthermore, CRISPR/Cas9 can be used to develop new therapeutic approaches. By repairing or replacing damaged genes, scientists can attempt to correct gene mutations associated with hereditary blindness. In mouse models, these therapeutic strategies have shown potential, although further research and optimization are still required.

3.3 Discussion on the application of other gene editing technologies

In addition to CRISPR/Cas9, there are other gene editing technologies that can be applied to the study of hereditary blindness in mouse models. The most notable ones include zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). These technologies are also targeted, allowing researchers to edit mouse genes.

ZFNs use zinc finger proteins to guide the cutting of specific DNA sequences, while TALENs use engineered proteins to achieve the same goal. Although their principles and application methods differ from CRISPR/Cas9, they still provide effective gene editing tools.

In mouse models, these technologies have been used to simulate hereditary blindness and have demonstrated similar potential to CRISPR/Cas9. The choice of these methods depends on the specific needs and experimental conditions of researchers. Sometimes, different gene editing technologies can complement each other for a more comprehensive study.

It should be noted that different gene editing technologies differ in efficiency, specificity, and complexity. Therefore, when choosing the most suitable technology, researchers need to balance these factors.

In the context of studying hereditary blindness, gene editing technologies such as CRISPR/Cas9, ZFNs, and TALENs provide scientists with various tools to better understand the pathogenesis of hereditary blindness and explore new treatment strategies. The application of these technologies is expected to bring more hope to blind patients. Although further research and clinical validation are still needed, they have paved the way for the future treatment of hereditary blindness.

4 Mouse Gene Editing Technology in the Treatment of Hereditary Blindness

4.1 Introduction to implemented cases of mouse gene editing therapy

In the treatment of hereditary blindness, mouse models have become an ideal choice for studying gene editing technology due to their unique advantages. There have been some exciting cases of mouse gene editing therapy, bringing new hope to the treatment of hereditary blindness.

One notable case is the study on Leber's hereditary optic neuropathy (LHON) (Yu et al., 2018). LHON is a genetic eye disorder associated with mitochondrial dysfunction, leading to acute vision loss. Researchers successfully

used CRISPR/Cas9 technology to repair the mutated genes in a mouse model of LHON. This study demonstrates that gene editing technology can correct genetic eye disorders in mice, providing a promising approach for clinical treatment.

Another important case involves the regeneration of retinal cells. Retinal cells are crucial cells in the visual process, and their damage can lead to blindness. Researchers used gene editing technology to activate regeneration-related genes in the mouse retina, successfully restoring the visual function of blind mice. This case provides strong support for applying gene editing technology to the treatment of hereditary blindness, especially in diseases related to the retina.

4.2 Discussion of treatment methods, results, and potential limitations

Mouse gene editing therapy typically involves using CRISPR/Cas9 technology or other gene editing tools to repair or replace damaged genes in the patient's body. The application of these treatment methods in mouse models usually requires several steps. Researchers first identify the gene mutations causing blindness and then design CRISPR guide RNA to precisely locate and repair these mutations. This often involves in-depth molecular biology research and gene sequencing. Researchers introduce the CRISPR/Cas9 complex into mouse embryos to precisely cut and modify the damaged genes. This may require optimization to ensure efficient editing and minimal non-specific damage. Once the genes are edited, damaged cells can restore normal function through self-repair or regeneration. This may take time, and not all cells can successfully repair. Treatment outcomes typically require long-term tracking and evaluation in mouse models. These outcomes include the restoration of visual function (Figure 4), cellular pathological changes, and the repair of genetic mutations.

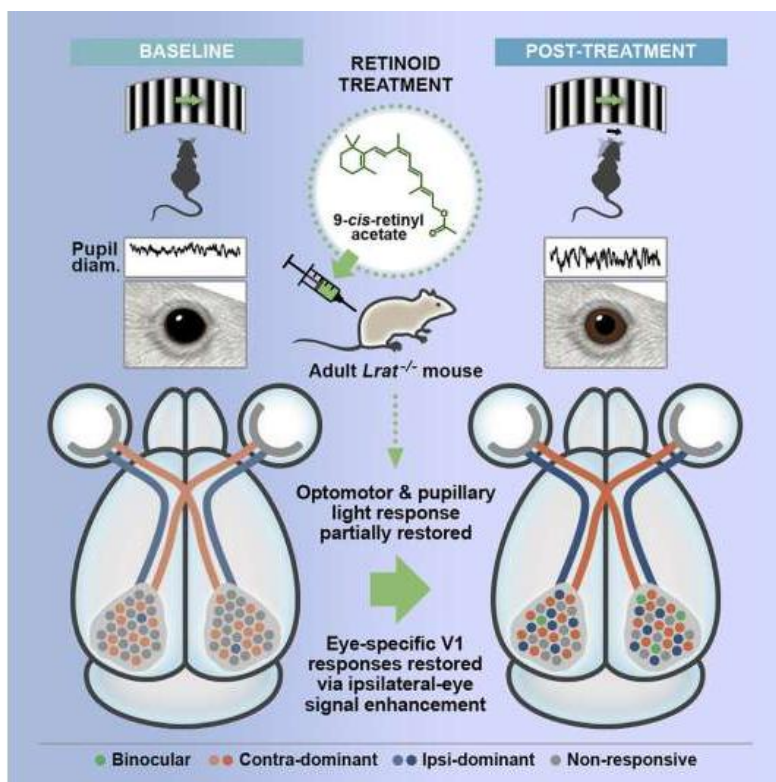


Figure 4 Visual function recovery process

However, mouse gene editing therapy also faces some potential limitations. Firstly, there are biological and genetic differences between mouse models and humans, so the efficacy of treatment needs validation in more suitable animal models before clinical translation. Secondly, the safety and precision of gene editing technology need further research to ensure no adverse consequences. Additionally, the cost and complexity of treatment are potential limitations that need to be addressed for widespread application.

4.3 Emphasis on potential clinical translational value

Although mouse gene editing therapy is still in its early stages, it holds tremendous potential for clinical translation. Mouse gene editing technology provides the possibility of personalized treatment. Since each patient's genetic mutations may differ, treatment plans can be customized to precisely repair their genes. Mouse gene editing therapy is not only used for treatment but also helps researchers better understand the pathological processes of hereditary blindness. This aids in the development of new treatment strategies. By validating treatment methods in mouse models, researchers can lay the foundation for future clinical trials, offering more treatment options for patients with hereditary blindness.

While mouse gene editing therapy still faces multiple challenges before clinical application, these treatment methods open new avenues for the treatment of hereditary blindness, providing hope for patients and the potential for clinical application in the near future. Therefore, mouse gene editing technology holds significant clinical translational potential in the treatment of hereditary blindness.

5 Ethical and Safety Considerations of Gene Editing Technologies

5.1 Discussion on ethical issues of gene editing in the medical field

The development of gene editing technologies in the medical field has raised numerous ethical concerns, particularly in the context of treating hereditary blindness. When employing gene editing therapies, especially using mouse models, there may be a need to select specific genotypes to create a "corrected" model. This raises questions about whether humans should intervene in genetic diversity, especially when blindness is associated with certain genetic traits. Additionally, it raises issues of social equity, questioning whether equal treatment opportunities should be provided to all patients (Khan, 2019).

In the context of gene editing in mouse models, some edits may affect germ cells, leading to the genetic transmission of the edits. This raises an ethical dilemma about whether gene editing in human germ cells is acceptable, allowing edited changes to be passed on to future generations. This also involves the uncertainty of generational effects and long-term impacts.

While gene editing is primarily discussed in the context of medical applications, there are non-therapeutic uses such as enhancing cognitive abilities or appearance. This raises ethical questions about whether humans should use this technology for self-improvement rather than just treating diseases. Ethical scrutiny and regulation of gene editing are crucial. Society needs to ensure that research and treatments are conducted within appropriate ethical frameworks to prevent misuse and potential risks. This requires the establishment of clear ethical guidelines and legal regulations to oversee and manage the application of gene editing technologies.

5.2 Emphasizing the safety of treatment and potential risks

Beyond discussing the ethical issues of gene editing technology, researchers must also focus on the safety and potential risks of its therapeutic applications. A successful gene editing therapy must have extremely high targeting accuracy to avoid unnecessary interference with normal genes. Inaccurate edits may result in unintended genetic variations, possibly worsening diseases. When conducting gene editing therapy, appropriate carriers are needed to deliver editing tools, such as CRISPR/Cas9. This may lead to cell and tissue toxicity reactions, posing potential risks to the patient's physiological health.

The patient's immune system may react to exogenous editing tools, potentially reducing the effectiveness of the treatment. Additionally, this may lead to the patient's immune system attacking edited cells, yielding undesired outcomes. Gene editing therapy may produce uncertain long-term effects that may not immediately manifest. Long-term monitoring and assessment are critical factors to ensure treatment safety and avoid unknown risks.

If gene editing therapy results in genetic changes in patients, ethical and legal responsibility issues may arise. Clear definitions of responsibility are necessary to resolve disputes in uncertain situations.

In summary, the application of gene editing technology in treating hereditary blindness presents numerous ethical issues and potential risks. A comprehensive ethical framework and regulatory measures must be taken to ensure

that research and treatments in this field are acceptable in terms of safety, ethics, and law. Furthermore, long-term monitoring and assessment are crucial steps to ensure patient safety and treatment effectiveness. While gene editing technology offers new hope for treating hereditary blindness, exploration of its potential must be approached with extreme caution to ensure the maximum benefit for patients.

6 Summary

Gene editing technology has a wide range of applications in the research and treatment of hereditary blindness in mouse models. This study discusses several aspects, including the significance of mouse models, the development and application of gene editing technology, specific applications of mouse gene editing in the treatment of hereditary blindness, and ethical and safety considerations.

Mouse models play an irreplaceable role in the study of hereditary blindness. Mice share a similar genome with humans, grow rapidly, and possess rich genetic tools in a controllable experimental environment. This makes them ideal research subjects, aiding scientists in understanding the mechanisms of blindness and testing therapeutic methods. Gene editing technologies, especially CRISPR/Cas9, have made significant advancements, allowing precise modification of mouse genes to simulate genetic mutations associated with hereditary blindness. This provides powerful tools for disease modeling, validation of gene therapy strategies, drug screening, and efficacy testing. Implemented cases of gene editing therapy in mice bring new hope to the treatment of hereditary blindness. For instance, in LHON research, CRISPR/Cas9 successfully repaired mutated genes in blind mice, offering prospects for clinical treatment. Additionally, gene editing technology shows significant potential in retinal cell regeneration, contributing to the management of retina-related diseases. Despite facing challenges such as ensuring safety and treatment efficacy (Van Haasteren et al., 2020), mouse gene editing therapies hold immense clinical translational potential. These treatments enable personalized therapy, deepen our understanding of hereditary blindness in research, and lay the foundation for future clinical trials.

In the treatment of hereditary blindness, ethical issues encompass genetic diversity, the hereditary transmission of genetic editing, non-therapeutic applications, and ethical review and regulation. Safety and potential risks of treatment involve targeting accuracy, cell and tissue toxicity, immune reactions, post-treatment effects, and the ethical and legal responsibilities of genetic editing. This study emphasizes the importance and potential of mouse gene editing technology in the research and treatment of hereditary blindness. Mouse models provide powerful tools, and gene editing technology brings new hope for treatment. However, caution and ethical considerations must be paramount to ensure that these treatment methods are both safe and ethically acceptable. Through comprehensive ethical frameworks and regulatory measures, it is expected that gene editing technology will soon see clinical applications in the treatment of hereditary blindness, providing more hope for patients (Doudna, 2020).

In conclusion, mouse gene editing technology offers new prospects for the research and treatment of hereditary blindness, demonstrating its potential clinical translational value. Research in this field will continue to deepen, overcoming challenges and driving the application of gene editing technology in the treatment of hereditary blindness. With time, we can anticipate further breakthroughs in assisting blind patients in regaining their vision through these methods.

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