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Genome-edited rabbits: Unleashing the potential of a promising experimental animal model across diverse diseases

Yang Han[1], Jiale Zhou[1], Renquan Zhang[1], Yuru Liang[1], Liangxue Lai[1,2,3,4,*], Zhanjun Li[1,*]

[1] *Key Laboratory of Zoonosis Research*, *Ministry of Education*, *College of Animal Science*, *Jilin University*, *Changchun*, *Jilin* 130062, *China*
[2] *CAS Key Laboratory of Regenerative Biology*, *Guangdong Provincial Key Laboratory of Stem Cell and Regenerative Medicine*, *South China Institute for Stem Cell Biology and Regenerative Medicine*, *Guangzhou Institutes of Biomedicine and Health*, *Chinese Academy of Sciences*, *Guangzhou*, *Guangdong* 510530, *China*
[3] *Guangzhou Regenerative Medicine and Health Guang Dong Laboratory (GRMH-GDL)*, *Guangzhou*, *Guangdong* 510005, *China*
[4] *Institute for Stem Cell and Regeneration*, *Chinese Academy of Sciences*, *Beijing* 100101, *China*

ABSTRACT

Animal models are extensively used in all aspects of biomedical research, with substantial contributions to our understanding of diseases, the development of pharmaceuticals, and the exploration of gene functions. The field of genome modification in rabbits has progressed slowly. However, recent advancements, particularly in CRISPR/Cas9-related technologies, have catalyzed the successful development of various genome-edited rabbit models to mimic diverse diseases, including cardiovascular disorders, immunodeficiencies, aging-related ailments, neurological diseases, and ophthalmic pathologies. These models hold great promise in advancing biomedical research due to their closer physiological and biochemical resemblance to humans compared to mice. This review aims to summarize the novel gene-editing approaches currently available for rabbits and present the applications and prospects of such models in biomedicine, underscoring their impact and future potential in translational medicine.

Keywords: Genome editing; Animal model; Rabbit; CRISPR/Cas9; Genetic diseases

INTRODUCTION

Biomedical research has long relied on animal models to study the pathophysiology of diseases, test the efficacy of therapeutic interventions, and understand the complex interplay of genetic and environmental factors contributing to health and disease. These models are crucial tools, providing researchers with invaluable insights that would be impossible to gain through *in vitro* studies alone (Dutta & Sengupta, 2016). Among the various animal models used in biomedical research, rodents, particularly mice, have been the most commonly used due to their small size, short reproductive cycle, and availability of well-established genetic manipulation techniques (Mukherjee et al., 2022).

However, despite their widespread use, mouse models have limitations. The genetic, physiological, and anatomical differences between mice and humans can lead to discrepancies in disease presentation and response to treatment. Moreover, the small size of mice can pose challenges for certain types of studies, such as those requiring surgical interventions or detailed imaging studies (Dutta & Sengupta, 2016; Rahman et al., 2023). These limitations have led researchers to explore other animal models that might offer a closer approximation of human physiology and disease. Large animal models, such as primates and pigs, exhibit closer genetic and phylogenetical similarities to humans. Nonetheless, their application in biomedical research is significantly constrained due to high maintenance costs, challenges in handling, and stringent ethical regulations (Yin et al., 2022b).

Rabbits offer numerous benefits as a preferred model in biomedical research. They are a cost-effective and practical alternative to pigs and non-human primates, adapting well to laboratory environments. The straightforward nature of their breeding and handling, combined with their recognition by the scientific and regulatory communities as a reliable laboratory model species, further enhances their suitability. Moreover, their larger size compared to mice simplifies surgical procedures, the collection of sequential blood samples, and the execution of tissue and organ biopsies. The longer lifespan of rabbits is also more conducive to observing

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*Corresponding authors, E-mail: lizj_1998@jlu.edu.cn; lai_liangxue@gibh.ac.cn

disease progression and testing therapeutic safety. Hence, rabbits represent a promising alternative animal model (Fan et al., 2021; Hornyik et al., 2022).

Embryonic stem cells (ESCs) play a crucial role in the creation of genetically modified animals, facilitating genetic modifications in the germline (Gossler et al., 1986). To date, however, no germline-transmitting rabbit ESC lines have been developed and the efficiency of somatic cell nuclear transfer is still relatively low, posing significant barriers to the widespread use of rabbits in scientific research. The emergence of novel genome editing based on zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and clustered regularly interspaced short palindromic repeats-associated protein-9 nuclease (CRISPR/Cas9) has revolutionized rabbit modeling (Matsuhisa et al., 2020). These innovative tools enable direct modification of the genome in somatic cells and embryos, bypassing the dependency on ESCs. Leveraging these technologies, genetically modified rabbits can be efficiently generated for different biomedical research purposes (Honda & Ogura, 2017), holding great potential for advancing our understanding of human diseases and developing effective treatments.

In this review, we introduce the novel techniques used for genome editing in rabbits, summarize the applications of genome-edited rabbits in biomedical research, and consider the future uses of these promising experimental models. Overall, we aim to highlight the unique advantages of genome-edited rabbits and their potential in translational biomedical research.

NOVEL GENOME-EDITING APPROACHES FOR RABBIT MODELING

Since the creation of the first genetically modified rabbit in 1985, pronuclear microinjection has been the primary technique of genetic manipulation in rabbits (Hammer et al., 1985). However, despite its long-term application, transplanted embryo efficiency remains low, at approximately 1% (Bősze et al., 2016; Hirabayashi et al., 2006). Consequently, various optimization strategies have been utilized to enhance efficiency, including somatic cell nuclear transfer (SCNT), transposon-mediated gene transfer, and lentivirus vector approaches (Hiripi et al., 2010; Ivics et al., 2014; Li et al., 2006), although they have failed to achieve precise genome manipulation.

The advent of new nuclease-mediated gene-editing systems —ZFN, TALEN, and CRISPR/Cas9 —has enabled precision gene editing in rabbits (Honda & Ogura, 2017). These engineered nucleases exhibit notable proficiency in inducing double-strand breaks within predetermined genomic loci. The cellular repair machinery then initiates either error-prone non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways. In NHEJ, imprecise repair often results in insertions or deletions (indels) that disrupt gene functionality, leading to gene knockout Alternatively, HDR employs exogenous or endogenous DNA templates as repair templates, enabling precise modifications like point mutations and gene knock-in (Cong et al., 2013; Renaud et al., 2016). Following the microinjection of designed nuclease systems into fertilized embryos at the pronuclear stage, genetically modified animals can be successfully generated without germline-competent pluripotent stem cells (PSCs) or SCNT (Song et al., 2021b).

In 2011, ZFN was first employed in rabbits to knock out the immunoglobulin M gene (Flisikowska et al., 2011). In 2013, TALEN was employed for the first time to construct recombination-activating gene 1 (RAG1) and RAG2 knockout rabbits (Song et al., 2013). Although these gene-editing technologies exhibit relatively high efficiencies, their reliance on protein-DNA recognition necessitates considerable time for redesign and reassembly when targeting different genes, thus constraining their widespread use (Joung & Sander, 2013; Kim & Kim, 2014).

The discovery of the CRISPR/Cas9 system catalyzed rapid advancement in rabbit gene-editing research (Table 1) (Li et al., 2022; Shao et al., 2016). Unlike ZFN and TALEN, CRISPR/Cas9 ingeniously amalgamates the nuclease Cas9 with guide RNA (gRNA). Under the assistance of gRNA, which is complementary to the target DNA sequence, Cas9 induces specific double-strand breaks in the target DNA to accomplish gene editing. As opposed to ZFN and TALEN, CRISPR/Cas9 simplifies the design process and only requires pre-designed single-guide RNAs (sgRNAs) targeting specific sites (Doudna & Charpentier, 2014). This system and its variants can efficiently mediate knock-in and knockout of targeted genes and are thus widely used in the genome-editing of different species (Ma et al., 2018; Savić & Schwank, 2016; Zhao et al., 2019). In 2014, CRISPR/Cas9 technology was successfully used to create the first genome-edited rabbit model (Figure 1) (Yang et al., 2014), with more than 50 genome-edited rabbit models developed since (Xu et al., 2021b).

As many as 50 000 human diseases are caused by single-base mutations, highlighting the urgent need for novel gene-editing systems capable of mediating point mutations (Abbasi, 2017). By fusing adenine or cytosine deaminase to dead- or nick-Cas9, base editors can generate single-base substitutions, such as A-G or C-T, without generating double-

Table 1 Comparison of different CRISPR-related tools in the development of genome-edited rabbit models

	CRISPR/Cas9	Base editors	Prime editors
Preferred editing type	● Gene KO ● Gene KI	● Single-base substitutions ● Gene KO ● Precise single-base substitutions	● Single-base substitutions ● Fragment insertions or deletions
Advantages	● Versatile editing type ● High editing efficiency ● Relatively easy to design and operate with a wealth of tutorials and tools available.	● Precise single-base substitutions ● Reduced risk of off-target effects and indels	● Reduced risk of off-target effects and indels ● Larger editing window compared to base editors
Limitations	● Risk of indels ● Risk of off-target effects	● Limited to certain types of single-base edits (mostly A/T to G/C conversions) ● Possibility of bystander mutations	● Relatively lower editing efficiency than Cas9 and base editors.

Indels: Insertion or deletions; KO: Knockout; KI: Knock-in.

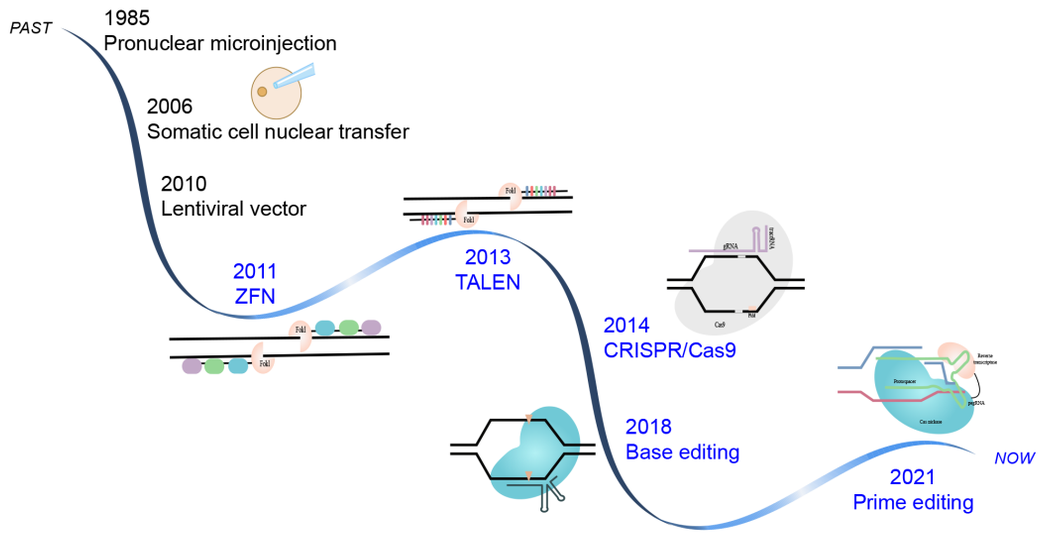

Figure 1 Key millstones in the development of genome-editing techniques

Events in black occurred in the pre-genome-editing era, events in blue represent the genome-editing era.

strand DNA breaks, the most harmful type of lesions to the genome (Rees & Liu, 2018). In 2018, the cytosine base editor was first utilized to generate diverse genome-edited rabbit models, including those for albinism, muscle hypertrophy, and progeria, successfully mimicking phenotypes of human diseases (Liu et al., 2018). Subsequently, optimized base editors, such as A3G-BE, YFE-BE4max, Spy mac-BE, and Cpf1-BE, have been applied to model disease-associated gene editing in rabbits, enabling continual improvement in the efficiency and precision of rabbit gene editing (Chen et al., 2020; Liu et al., 2019b, 2020a, 2020b).

Despite their exceptional efficiency in point mutation editing, base editors have several limitations, including an inability to perform substitutions for all base combinations or mediate small fragment insertions or deletions, and a propensity to generate bystander mutations near multiple C or A editing windows (Anzalone et al., 2020). Qian et al. (2021) successfully demonstrated the potential of the prime editing (PE) system to mediate base insertions, deletions, and transformations in a rabbit model.

Current CRISPR/Cas9-based gene-editing systems still encounter challenges, such as indels caused by CRISPR/Cas or deaminase-dependent DNA or RNA off-target effects, low efficiency of adeno-associated virus (AAV) delivery, as well as restrictions in editing sites for deaminase and protospacer adjacent motif (PAM), all of which significantly impede their application (Anzalone et al., 2020). Hence, developing relevant off-target analysis tools to carefully assess genome-editing risks is warranted. Strategies such as protein engineering have also been employed to introduce mutations or deletions in the protein domains of the editing tools, thereby enhancing their safety and efficacy (Doman et al., 2020; Qian et al., 2023; Zhao et al., 2023; Zuo et al., 2019). Notably, Liu et al. (2022) developed the virus-derived anti-deaminase (Ade) system and demonstrated its capacity to inhibit both Cas9- and deaminase-dependent off-target effects. Moreover, low efficiency in the context of large gene insertions is a significant barrier to the application of CRISPR. This limitation hampers the effective integration of large DNA segments into the rabbit genome, thereby impeding introduction of specific gene modifications during precise modeling of complex human diseases (Maruyama et al., 2015). The recently developed bacterial-based Type I-F CRISPR-associated transposases (CAST) system can catalyze the integration of large DNA segments into the genome without inducing double-strand breaks. This system can achieve more efficient and safer knock-in of large DNA fragment in mammalian cells, although its reliability in rabbit models remains to be verified (Lampe et al., 2023). Collectively, genome-editing technologies have already made tremendous contributions to the development of genome-editing rabbit models, although substantial optimizations are still required in future investigations.

GENERATION OF GENOME-EDITED RABBITS IN BIOMEDICAL RESEARCH

The advent of novel genome-editing tools, particularly the CRISPR/Cas9-related system, has significantly advanced the previously challenging study of gene modification in rabbits. Compared to mice, rabbits have a longer lifespan and are better suited to long-term follow-up studies. Notably, investigations into immune system genes have shown that the genetic diversity between rabbits and humans is markedly lower than that between mice and humans (Neves et al., 2015; Soares et al., 2022). As such, rabbits exhibit considerable advantages as suitable animal models for biomedical research (Table 2) (Esteves et al., 2018).

Given the above species-specific advantages, genome-edited rabbits are superior models for mimicking the pathogenic characteristics of diverse human genetic diseases. In recent years, researchers have successfully established genome-edited rabbit models for a growing number of human diseases, including cardiovascular diseases (CVD), immune system-related diseases, ocular diseases, and neurological disorders (Table 3) (Fan et al., 2021; Song et al., 2020a; Xu et al., 2021b; Zhang et al., 2022b), leading to an improved understanding of disease pathophysiology and ability to develop therapeutic interventions (Figure 2).

Genome-edited rabbit models of cardiovascular disease

Rabbits can also serve as reliable animal models for investigating lipoprotein metabolism and atherosclerosis-related CVD. This is due, in part, to the susceptibility of wild-type rabbits to hyperlipidemia when fed a high cholesterol diet, leading to the rapid development of aortic and coronary

Table 2 Lifespan, cost, handling, and example phylogenetical differences between primate, rabbit, and mouse models

	Non-human primates	Rabbits	Mice
Lifespan (year)	20–30	5–10	1–3
Cost of care* (dollar/year)	5 000–15 000	1 500–2 000	100–300
Difficulty in handling	High	Moderate	Low
Example features of cardiovascular system	Maximum heart weight: 360–480 g Low heart rate (varied depending on primate species): 90–150/min	Medium heart weight: 9–11 g Medium heart rate: 120–300/min	Small heart weight: around 0.15 g High heart rate: 300–600/min
Example features of neurological structure	Large brain size (1.2% to 2.5% over body volume) Highly convoluted cerebral cortex and neocortical structure	Medium brain size (0.1% to 0.3% over body volume) Less developed neocortical regions primarily involved in basic sensory processing and motor control	Small brain size (0.015% to 0.003% over body volume) Simple brain structure with minimal convolution. Lack of obvious sulcus and gyrus on surface of cerebral cortex
Example features of immune system	Complex and highly adaptable immune system that can respond to a wide range of pathogens Susceptible to HIV	Strong antibody response, commonly used for antibody production HIV infectious after removing several molecular barriers (exp. *CD4* and *CCR5*)	Well-studied for immune system research Not infectable with HIV, numerous molecular barriers such as (exp. *TRIM5*, *APOBEC3*, *Tetherin*, and *SAMHD1*)
Example features of eye structure	Large eye size (diameter 15–20 mm)	Medium eye size (diameter 15–20 mm)	Small eye size (diameter ~3–8 mm)

*: Costs of animal care can vary significantly depending on specific contexts.

atherosclerosis. Rabbits also possess shared attributes with humans in regarding lipid metabolism and blood lipoprotein profiles, which greatly differ from those observed in mice (Fan et al., 2018; Hou et al., 2022). Given these advantages, several CVD-related rabbit models have been produced via genome editing. While *ApoCIII* has been demonstrated to inhibit the hepatic clearance of triglyceride (TG)-rich lipoproteins, its underlying mechanism has remained elusive. To better understand the role of *ApoCIII* in CVD, an *ApoCIII* knockout rabbit model was first established in 2013 using the ZFN technique. Compared with wild-type control rabbits, *ApoCIII* knockout rabbits showed significantly lower plasma total cholesterol and TG levels and less atherosclerosis in the aorta and coronary arteries when fed a cholesterol-rich diet (Yan et al., 2020; Yang et al., 2013). The successful establishment of this model paves the way for the development of hypertriglyceridemia-related CVD drugs targeting *ApoCIII*. CETP, an essential factor regulating high-density lipoprotein (HDL) and cholesterol metabolism, is expressed in both rabbits and humans, but is absent in mice. In 2017, *CETP* knockout rabbits generated using ZFN exhibited higher HDL levels than their wild-type counterparts and evinced protection against atherosclerosis induced by cholesterol-rich diets (Zhang et al., 2017). The successful generation of such a model may lead to the development of new *CETP* inhibitors for hypercholesterolemia and atherosclerosis treatment. ApoE and *LDL* receptor (*LDLR*) knockout rabbit models created using CRISPR/Cas9 technology developed severe hyperlipidemia when exposed to a cholesterol-rich diet, showing importance for studying human hyperlipidemia and familial hypercholesterolemia (Yang et al., 2014; Yuan et al., 2019). Most recently, Chen et al. (2023) established a rabbit model deficient in the CVD risk factor lipoprotein-associated phospholipase A_2 (*Lp-PLA*$_2$) and demonstrated that *Lp-PLA*$_2$ regulated blood lipid homeostasis, protecting against dietary cholesterol-induced atherosclerosis (Chen et al., 2023). Overall, aided by genome editing, rabbits are expected to greatly promote investigations of CVD-related spontaneous hyperlipidemia and atherosclerosis.

Genome-edited rabbit models of immune system-related diseases

Human immunodeficiency virus (HIV) exhibits high infectivity in the human body and can replicate within immune cells. Its invasion leads to a series of immune cell dysfunctions, particularly the depletion of $CD4^+$ T cells, ultimately resulting in a severe and life-threatening immunodeficiency. While mice were the earliest animals used to establish models for studying acquired immunodeficiency syndrome (AIDS), significant differences in the $CD4^+$ molecule sequences between humans and mice, as well as variations in restriction factors such as *TRIM5* and *APOBEC3*, have prevented HIV from replicating and surviving in mouse cells (Hoang et al., 2008). The use of rabbits for HIV research has emerged as a new trend, since they exhibit fewer molecular barriers compared to rodents. $CD4^+$ T cells and CCR5 in rabbits have been shown to be essential molecules for effective HIV-1 infection. The expression of human $CD4^+$ in rabbit cells was successfully reported in 1995 (Dunn et al., 1995). Moreover, transgenic rabbits expressing humanized *CD4* and *CCR5* receptors have been effectively generated, allowing the envelope-specific and co-receptor dependent entry of HIV-1. This model enables the pathological study of HIV-1 infection in a model organism that closely recapitulates the human immune response (Esteves et al., 2018; Tervo & Keppler, 2010). Thus, with the application of novel gene-editing technologies, rabbits have become a valuable model for preclinical AIDS research.

Rabbits have also demonstrated susceptibility to papillomavirus (PV) infections. As early as 2001, the cottontail rabbit papillomavirus (CRPV) was utilized to infect transgenic rabbits carrying the oncogenic *EJRAS* gene (Peng et al., 2001). This pivotal study revealed that CRPV infection effectively reactivated the expression of the *EJRAS* cancer gene, controlled by *CRP*, in the transgenic rabbits and that enhanced *EJRAS* expression promoted the development of CRPV-induced tumors. Transgenic rabbits expressing human *MHC-I* (*HLAA2.1*) were also established to investigate $CD8^+$ T cell responses during virus infections (Hu et al., 2006), with the same research group subsequently applying

Table 3 Representative genome-edited rabbit models across diverse diseases

Disease type	Targeted gene	Modification	Technology	Remarks	References
Cardiovascular diseases	*CETP*	Knockout	ZFN	Protect from CVD	Zhang et al., 2017
	APOCIII	Knockout	ZFN	Protect from CVD	Yang et al., 2013
	APOE	Knockout	CRISPR/Cas9	Hyperlipidemia	Yang et al., 2014; Yuan et al., 2019
	LDLR	Knockout	CRISPR/Cas9	Familial hypercholesterolemia	Yang et al., 2014; Yuan et al., 2019
	Lp-PLA$_2$	Knockout	CRISPR/Cas9	Protect from atherosclerosis	Chen et al., 2023
Immune system-related diseases	*hCD4/hCCR5*	Transgenic	Pronuclear microinjection	HIV	Esteves et al., 2018; Tervo & Keppler, 2010
	EJRAS	Transgenic	Pronuclear microinjection	CRPV	Peng et al., 2001
	hMHC-I	Transgenic	Pronuclear microinjection	PV	Hu et al., 2006
	RAG1; *RAG2*	Knockout	TALEN	Immunodeficiency	Song et al., 2013
	RAG, *TIKI1*, *ALB*, and *IL2RG*	Knockout	CRISPR/Cas9	Immunodeficiency	Yan et al., 2014
	Il2RG	Knockout	CRISPR/Cas9	X-linked severe combined immunodeficiency	Hashikawa et al., 2020
	WAS	Knockout	CRISPR/Cas9	Wiskott-Aldrich syndrome	Zhou et al., 2020
	FOXN1	Mutation	CRISPR/Cas9	Immunodeficiency	Song et al., 2021a
Premature aging	*LMNA*	Knockout	CRISPR/Cas9	Hutchinson Gilford Progeria Syndrome	Sui et al., 2019
	WRN	Knockout	CRISPR/Cas9	Werner Syndrome	Wu et al., 2018
Neurological diseases	*HEXA*	Knockin	CRISPR/Cas9	Tay-Sachs disease	Qian et al., 2021
	YIPF5	Mutation	CRISPR/Cas9	Primary microcephaly	Liu et al., 2023
	SOD1	Mutation	CRISPR/Cas9	ALS	Zhang et al., 2023
	ARPP21	Mutation	CRISPR/Cas9	Amyotrophic lateral sclerosis	Zhang et al., 2023
Ocular diseases	*GJA8*	Knockout	CRISPR/Cas9	Congenital cataracts	Yuan et al., 2016
	αA-LENS	Knockout	CRISPR/Cas9	Congenital cataracts	Yuan et al., 2017
	USH2A	Mutation	CRISPR/Cas9	Usher syndrome	Nguyen et al., 2023
Muscular-related diseases	*ANO5*	Mutation	CRISPR/Cas9	DMD	Liu et al., 2018
	DMD	Mutation	CRISPR/Cas9	DMD	Sui et al., 2018a
	MSTN	Knockout	CRISPR/Cas9	Muscular hypertrophy	Liu et al., 2018; Lv et al., 2016
	CLPG1	Mutation	CRISPR/Cas9	Muscular hypertrophy	Wan et al., 2019
Skeletal-related diseases	*PHEX*	Knockout	CRISPR/Cas9	X-linked hypophosphatemia	Sui et al., 2016
	DMP1	Knockout	CRISPR/Cas9	Hypophosphatemic rickets	Liu et al., 2019a
Other monogenic diseases	*TYR*	Mutation	CRISPR/Cas9	Albinism	Liu et al., 2019b; Song et al., 2018b
	OTC	Mutation	CRISPR/Cas9	Hyperammonemia	Chen et al., 2020
	CFTR	Knockout	CRISPR/Cas9	Cystic Fibrosis	Xu et al., 2021a
	SRY	Mutation	CRISPR/Cas9	Hermaphroditism	Song et al., 2017, 2018a
	XIST P1	Knockout	CRISPR/Cas9	X-chromosome inactivation	Yao et al., 2020
	GCK	Mutation	CRISPR/Cas9	MODY-2	Song et al., 2020b
	HBB2	Knockout	CRISPR/Cas9	β-thalassemia	Yang et al., 2021
	HOXC13	Mutation	CRISPR/Cas9	Hair and nail ectodermal dysplasia	Liu et al., 2020c
	FAM83H	Knockout	CRISPR/Cas9	Amelogenesis imperfecta	Zhang et al., 2022a

CVD: Cardiovascular disease; PV: Papillomavirus; CRPV: Cottontail rabbit papillomavirus; MODY-2: Maturity-onset diabetes of the young 2; DMD: Duchenne muscular dystrophy; ALS: Amyotrophic lateral sclerosis.

computational algorithms to screen and identify multiple human papillomavirus (HPV)-related genes in rabbit models infected with HPV (Hu et al., 2010). Their research revealed significant dysregulation in several immune-related genes, including *APOBEC2* and *IL36γ*, in persistent HPV-associated tumors, consistent with the characteristics of HPV-related cancers. Future construction of *APOBEC2* and *IL36γ* gene knockout rabbits will provide valuable insights into the roles of these genes in HPV-related disease research.

Furthermore, several other immunodeficient genome-edited *RAG*, *Il2RG*, *WAS*, and *FOXN1* knockout rabbits have been separately constructed in different studies (Hashikawa et al., 2020; Song et al., 2013, 2021a; Zhou et al., 2020). Of note, multiplexed gene knockout in rabbits has also been successfully conducted for the *RAG*, *TIKI1*, *ALB*, and *IL2RG* genes, showing great potency for better mimicking clinical phenotypes of immunodeficiency syndrome (Yan et al., 2014). Taken together, genome-edited rabbits are promising models for advancing our understanding of the pathogenesis of high-risk infectious diseases and the development of vaccines targeting related viruses.

Genome-edited rabbit models of neurological diseases

Large animal models are well-suited for mimicking

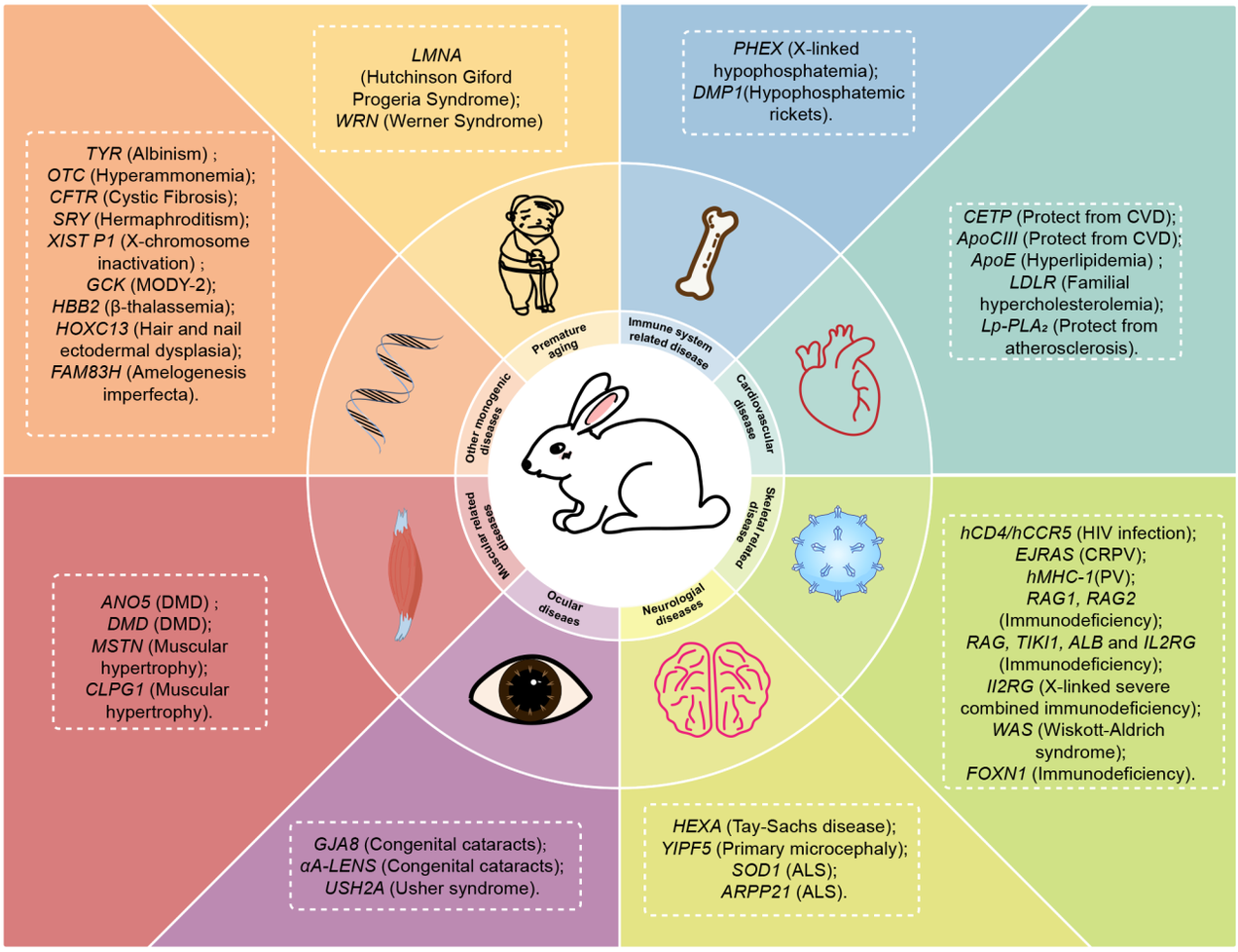

Figure 2 Genome-edited rabbit models established across various biomedical studies

CVD: Cardiovascular disease; PV: Papillomavirus; CRPV: Cottontail rabbit papillomavirus; MODY-2: Maturity-onset diabetes of the young 2; DMD: Duchenne muscular dystrophy; ALS: Amyotrophic lateral sclerosis.

neurological disorders (Zhou et al., 2015), but require substantial maintenance costs and present challenges in manipulation. Importantly, the development of the central nervous system in rabbits closely resembles that in humans, enabling cost-effective and accurate generation of neurological disease models that cannot be effectively recapitulated in mice (De Almeida Da Anunciação et al., 2021). Tay-Sachs disease (TSD), characterized by progressive neurodegeneration, arises from autosomal recessive deficiency of β-hexosaminidase A (HeXA). HeXA-deficient mouse models do not display typical phenotypes of TSD, possibly due to differences in the ganglioside degradation pathways between humans and mice. In contrast, a rabbit model of the disease, displaying neurological abnormalities in the brain akin to the clinical TSD phenotype, has been successfully generated through pilot editing-mediated insertion of TATC bases into the *HEXA* gene (Qian et al., 2021), holding significant promise for advancing research on TSD pathogenesis and drug screening.

Primary microcephaly (PMCPH) is an autosomal recessive neurodevelopmental disorder characterized by a low-volume prenatal and postnatal brain. A homozygous missense mutation in *YIPF5* was previously identified as a causative mutation of severe microcephaly. Using the adenine base editor SpRY-ABEmax, a genome-edited rabbit model harboring the *YIPF5* (p. W218R) mutation was established in 2023 (Liu et al., 2023), better mimicking the clinical features of human primary microcephaly compared to mice. Subsequent research using these rabbit models has revealed that alterations in *YIPF5* function may lead to endoplasmic reticulum stress and neurological developmental disorders. The link between endoplasmic reticulum stress-induced unfolded protein response (UPR) and the development of primary microcephaly with or without pontocerebellar hypoplasia (PMCPH) has also been established, shedding light on the role of *YIPF5* in human brain development and providing a theoretical basis for the differential diagnosis and clinical treatment of PMCPH (Liu et al., 2023). Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects spinal cord and cortical motor neurons. Both p.I151V and p.P529L have been identified as crucial ALS-associated pathogenic mutations in the *Sod1* and *Arpp21* genes, respectively. Thus, Zhang et al. (2023) employed an optimized near-PAMless CRISPR/Cas9 variant to separately generate rabbit models with *Sod1* and *Arpp21* gene mutations. Of note, these models exhibited clear clinical symptoms of ALS, including muscle atrophy and spinal motor neuron loss (Zhang et al., 2023). Collectively, the above evidence underscores the potential of genome-edited rabbits in the study of diverse neurological disorders.

Genome-edited rabbit models of ocular diseases

Rabbit eyes, notably larger than those of mice, exhibit ocular

structures and physiological characteristics closely aligned with human eyes, making them more suitable for research into human ophthalmic diseases (Zhou et al., 2008). Historically, most rabbit models used in ophthalmic research were generated through surgical or pharmacological interventions, limiting their utility in simulating the pathological states and revealing the underlying genetic mechanisms of inherited eye diseases (Zernii et al., 2016). The application of genome-editing techniques in rabbit-based research has significantly improved genetic-level investigations, thus driving progress in understanding the pathogenesis of genetic eye diseases. Cataracts, characterized by the progressive clouding of the crystalline lens, result in diminished visual acuity and impaired visual function, particularly in the elderly. Yuan et al. (2016) utilized CRISPR/Cas9 technology to develop a GJA8 knockout rabbit model, which exhibited prominent clinical manifestations of cataracts. In a subsequent study, Yuan et al. (2017) successfully generated αA-lens protein knockout rabbits to effectively mimic congenital cataracts. The development of these models has helped advance our understanding of congenital cataracts and exploring potential treatments.

Usher syndrome (USH) is an autosomal recessive genetic disorder characterized by sensorineural hearing loss (SNHL), retinitis pigmentosa (RP), and occasional vestibular dysfunction. The main ocular pathological feature in USH is photoreceptor degeneration in the retina, leading to impaired vision in affected individuals. USH2A is one of the most common mutated genes identified in USH patients. By CRISPR/Cas9 targeting, Nguyen et al. (2023) established a genome-edited rabbit model with USH2A mutation on exon 12, exhibiting progressive photoreceptor degeneration and hearing loss, sufficiently mimicking the relevant clinical symptoms of USH. Moving forward, genome-edited rabbits are expected to replicate ophthalmic diseases more accurately, thereby making increasingly significant contributions to clinical treatments and drug development in the field of ophthalmology.

Genome-edited rabbit models in other genetic diseases

Rabbit models have also shown unique advantages in other genetic diseases. For instance, introducing both *LMNA* mutations and *WRN* gene knockout in rabbits has been shown to induce growth retardation, cardiomyopathy, muscle atrophy, and other premature aging phenotypes of Hutchinson-Gilford progeria syndrome (HGPS) and Werner syndrome (WS) (Sui et al., 2019; Wu et al., 2018). Duchenne muscular dystrophy (DMD), an X-linked recessive genetic disease characterized by severe muscle atrophy, has been successfully replicated in rabbits through gene-editing techniques targeting *Ano5* mutation or *Dmd* missense mutation, resulting in typical pathological phenotypes (Liu et al., 2018; Sui et al., 2018b). Genome-edited rabbits with *TYR* gene mutations have also been generated using CRISPR/Cas9 technology, providing models for various albinism-related diseases (Liu et al., 2019b; Song et al., 2018b). Furthermore, genome-edited rabbits have been generated for various other disease models, including cystic fibrosis (Xu et al., 2021a), type 2 diabetes (Song et al., 2020b), muscular dystrophy (Sui et al., 2018b), and hyperammonemia (Chen et al., 2020). Of note, the wide application of genome-edited rabbit disease models is expected to greatly advance our understanding of disease mechanisms and facilitate the development of novel gene therapies.

FUTURE PERSPECTIVES ON GENOME-EDITED RABBIT MODELS

Genome-edited rabbit models have demonstrated considerable potential in translational medicine. Currently, gene-editing technologies continue to evolve, particularly with the development of novel genome-editing systems via artificial engineering. These systems, in comparison to the natural CRISPR system, exhibit higher DNA cleavage activity, enhanced specificity, and smaller size (Saito et al., 2023; Sun et al., 2023; Wilson et al., 2020; Yin et al., 2022a). They constitute a powerful toolkit for more efficient DNA sequence knockout, replacement, epigenetic editing, and gene expression activation and inhibition. Furthermore, mitochondrial DNA (mtDNA) mutations, which contribute to mitochondrial dysfunction, are implicated in various human diseases (Nissanka & Moraes, 2020). Notably, recent studies have shown that novel systems constructed by fusing deaminase DDA or TadA to TALENs can effectively edit mtDNA (Cho et al., 2022; Mok et al., 2022). The emergence of such innovative tools will assist in the creation of more genome-edited rabbit models relevant to human diseases, thereby laying the foundation for advancing pathological research and the development of novel treatments for various diseases.

In the future, the utility of genome-edited rabbit models is anticipated to extend beyond the field of translational biomedicine. Notably, rabbits have been extensively employed for commercial antibody production due to their robust immune response. By modifying rabbit antibody-related genes via novel genome-editing techniques, rabbit models with highly potent antibody production can be generated, which are anticipated to boost the development of antibody drugs and the advancement of immunotherapies for various diseases (Chen et al., 2018; Cheong et al., 2016; Pinheiro et al., 2016).

The utilization of rabbit models in preclinical research indeed presents numerous advantages. However, it is important to recognize that rabbits may not accurately replicate all pathophysiological processes observed in humans for all diseases. For instance, in humans, type 1 diabetes mellitus (T1DM), a complex autoimmune disease characterized by the destruction of insulin-producing beta cells in the pancreas, typically involves the production of autoantibodies targeting specific pancreatic antigens, which is not consistently observed in rabbit models (Bora et al., 2023; Jing et al., 2018). Moreover, when investigating autoimmune diseases such as systemic lupus erythematosus (SLE), characterized by a complex interplay of genetic and environmental factors, rabbits exhibit a limited genetic predisposition to autoimmune responses compared to certain mouse strains that better model this disease (Ganor et al., 2014; Yang et al., 2009). Additionally, research objectives and available resources play crucial roles in model selection. For studies requiring extended observation periods or extensive experiments, the relatively longer lifespans and larger size of rabbits may be advantageous, albeit at a potentially higher cost. The choice of an appropriate model system for translational medical research requires a comprehensive assessment of multiple factors. Furthermore, the effectiveness of genome-edited animal models still needs further validation. Advancements in multiomics techniques, such as human epigenomics and gut microbiomics, may assist in the identification of more comprehensive disease-causing factors (Cavalli & Heard, 2019; Fan & Pedersen, 2020), with genome-

edited rabbit models potentially offering more precise simulation of the pathogenesis and progression of diseases.

CONCLUSIONS

In conclusion, the unique advantages and versatile applications of genome-edited rabbits represent a powerful tool in translational biomedical research. Their use in modeling various diseases has not only advanced our understanding of disease pathophysiology but also facilitated the development of novel therapeutics. As gene-editing technologies continue to evolve, the use of rabbit models is expected to expand, further accelerating the translation of research findings into clinical applications.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Y.H., Z.L., and L.L. wrote the manuscript. J.Z., R.Z., and Y.L. collected the references and prepared the figures. All authors contributed to the article and read and approved the final version of the manuscript.

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