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APPLYING THE CRISPR/CAS9 FOR TREATING HUMAN AND ANIMAL DISEASES – COMPREHENSIVE REVIEW

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Abstract

Recently, genome editing tools have been extensively used in many biomedical sciences. The gene editing system is applied to modify the DNA sequences in the cellular system to comprehend their physiological response. A developing genome editing technology like clustered regularly short palindromic repeats (CRISPR) is widely used in medical sciences. CRISPR and CRISPR-associated protein 9 (CRISPR/Cas9) system is being exploited to edit any DNA mutations related to inherited ailments to investigate in animals (*in vivo*) and cell lines (*in vitro*). Remarkably, CRISPR/Cas9 could be employed to examine treatments of many human genetic diseases such as cystic fibrosis, tyrosinemia, phenylketonuria, muscular dystrophy, Parkinson's disease, retinoschisis, hemophilia, β-thalassemia and atherosclerosis. Moreover, CRISPR/Cas9 was used for disease resistance such as tuberculosis, Johne's diseases, chronic enteritis, and brucellosis in animals. Finally, this review discusses existing progress in treating hereditary diseases using CRISPR/Cas9 technology and the high points accompanying obstacles.

Key words: CRISPR/Cas9, genetic diseases, DNA sequences, disease-resistant animals

In this era of genomics, discovering the hereditary disorder conditions ascending owing to a mutation in multiple genes (polygenic) or a single gene (monogenic) has been extensively explored (O'Connor and Crystal, 2006; Grisch-Chan et al., 2019). In parallel, researchers focused on disease remedy through genomic modification or conquering the competence to restore any portion of the human and animal genome (Hekselman et al., 2022). Recently, genomic editing tools are robust approaches that have been applied in the biological sciences to enhance our life and welfare (Cong et al., 2013; Cobb et al., 2015). Additionally, the capacity to accurately and purposefully modify the eukaryotic cell genome has significantly augmented thanks to the versatility of gene editing as a remediation tool for many diseases (Christian et al., 2010). For treating numerous inherited diseases, studies have demonstrated that the high-accuracy gene depends on several factors.

More recently, CRISPR/Cas9 has been a powerful approach as a persuasive genome manipulating development for numerous fields, including medicine, biological, environmental, agricultural and biomedical (Li et al., 2019). The Cas9/sgRNA ribonucleoprotein complexes (RNPs) authenticated by the CRISPR/Cas9 technology allow for the desired and managed DNA sequences. This strategy can be considered one of the most powerful and versatile tools for epigenetic modification, gene editing, and transcriptional manipulation. Moreover, CRISPR/Cas9 innovative tool technology has been hired to reconnoiter envisioned genes in modification, genome splicing, transcription, and epigenetic regulation (Cox et al., 2015). In the previous study, they discovered that the CRISPR/Cas9-mediated HDR might induce unfavorable off-target double-stranded breaks (dsDNA), impeding its conceivable uses in clinical investigations (Kosicki et al., 2018; Bischoff et al., 2020; Geurts et al., 2020). So, scientists have continually established a new type of CRISPR/Cas9 for diminishing or managing this previous negative effect through developed Cas9 fusion proteins and may be called base editors (BE) (Liu et al., 2020). Recent research has presented that the fusion of a cytidine deaminase to a partially inactive Cas9 protein allows for competent C-G to T-A base alterations (C-T base editing), whereas the opposite is true when the TadA het-

erodimer is modified (A-G) (Irion et al., 2014; Bischoff et al., 2020). This advanced policy BE can represent a small space of the single-stranded R-loop twisted when Cas9 joins with the target sequence. With astonishing developments in genomic tools, CRISPR/Cas9 can offer sustainable treatment after a single medication in many human inherited diseases. In this paper, we summarized the application of CRISPR/Cas9 as a versatile technology in treating certain human hereditary and animal diseases based on *in vitro* and *in vivo* studies (Jiang and Doudna, 2015; Liu et al., 2020; Fu et al., 2022). This criticism aims to present the machinery of the CRISPR/Cas9 technique as a robust gene editing tool and certain methods of enhancing the efficacy of its application. Further, we highlighted the potency of using CRISPR/Cas9 to remedy many human genetic diseases and disease-resistant animals according to the *in vitro* and *in vivo* trials.

Mechanism of CRISPR/Cas9

According to the reports, CRISPR/Cas9 was originally detected as a bacterial adaptive immune scheme that permits promoting the defense system against assaulting viruses (Jinek et al., 2012). When the fragments of assaulting virus DNA are joined with the bacterial genomic sequence, it produces maturation of RNA sequences that assemble with the associated Cas proteins to the degradation of the foreign DNA, creating a CRISPR/Cas9 immune response (Jiang and Doudna, 2015). Figure 1 demonstrates the production of CRISPR/Cas9 form bacteria after attaching to the virus. This formed protein (Cas9 protein) links with a trans-activating CRISPR/RNA (tracrRNA) and CRISPR RNA (crRNA), which shape a rudder for the credit of identical DNA sequences. As discovered, Cas9 is a multi-domain protein consisting of a recognition lobe (REC), which intercedes the nucleic acid binding through a nuclease lobe (NUC) and three domains (REC1, REC2 and REC3) (Anders et al., 2014).

Consequently, this distinctive characteristic of the Cas9 protein makes its robust efficiency in producing DNA breaks at a precise site in target genomic DNA *in vivo*. According to previous documents, the Cas9 formed by *E. coli* bacteria is the utmost discovered and generally oppressed amidst the many Cas nucleases that have been discovered (Jiang and Doudna, 2015). This might be associated with the high flexibility and specificity of the DNA-sequence, which reported that the CRISPR/Cas9 approach had been discovered in bulks of experimental examinations (Makarova et al., 2006), the Cas9 protein can cleave the DNA sequence at specific segments. The same authors suggested that there were resemblances between the function of RNA interference and the CRISPR/Cas9 scheme. Hereafter, Cas9 and Cas13 silence the gene by cleaving RNA, whereas Cas9 cleaves the DNA.

CRISPR/Cas9 scheme necessitates PAMs (protospacer adjacent motifs) for shorting the 2–6 base-pair sequences in the virus genome joined to Cas nucleases targeted sequences (Shah et al., 2013). As described by some authors, the CRISPR/Cas9 scheme can effectively detach or paste through programming gRNA for a detailed position in the terminus genome, to brand genome amendments (Jinek et al., 2012). Hence, CRISPR/Cas9 can manage, edit, remove or deliver new genes and knock out or knock in depending on the target gene, as reported in Figure 2.

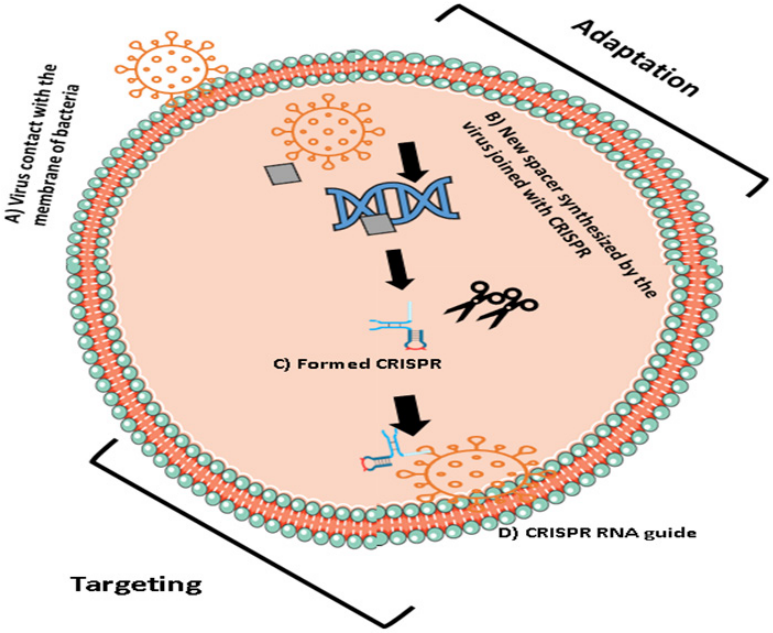

Figure 1. The machinery of CRISPR formed from the bacteria via adaptation and targeting during the natural process

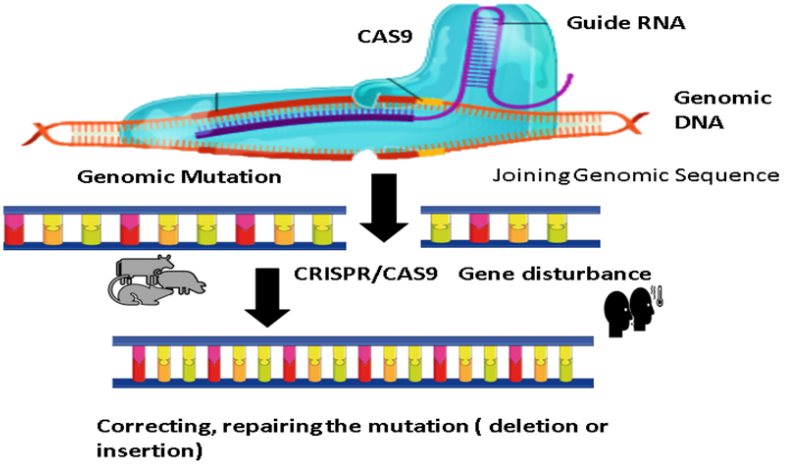

Figure 2. The mode of action of CRISPR/Cas9 in targeting the gene mutation triggering the disease in animal or human

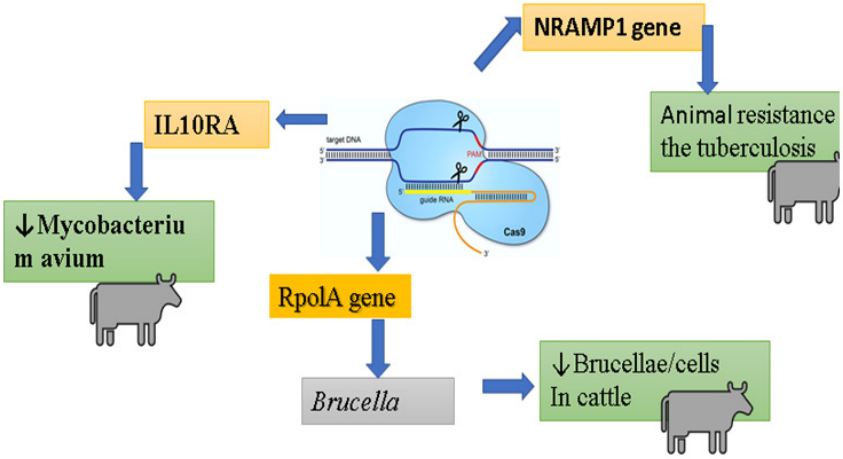

Figure 3. The potential role of CRISPR/Cas9 in treating some diseases in animals

Historically, HDR (homology-directed repair) and NHEJ (non-homologous end joining) can repair the genomic DNA produced via mutations occurrence, disturbing the open reading border and generating an inactivation of the precise gene. Moreover, CRISPR/Cas9 has become a new versatile and humble RNA-focused scheme for genome editing in a varied range of various cell kinds, organisms, humans and animals (Cong et al., 2013). These studies proved that CRISPR/Cas9 is a competent tool to genetically amend genomic DNAs in gametes without establishing embryonic stem cells for genomic modulation (Bevacqua et al., 2016; Han et al., 2022). TALENs (transcription activator-like effector nucleases) have also been lately employed for gene therapy. TALENs are chimeric proteins containing a programmable DNA binding domain attached to the Fok1 nuclease domain, permitting detaching DNAs (double strand) at any anticipated sequences (Christian et al., 2010). According to Wei et al. (2013), it was assumed that TALEN could bind with targeted DNA depending on its ability to bind to a DNA binding domain consisting of numerous repeat parts, each repeat comprising amino acids (mostly 33–35 amino acids). In this regard, TALENs, as a tactic of gene editing, is thought to have inferior off-target result. In spite of assembling TALEN gene tool involves much more attempts than creating CRISPR/Cas9, getting the CRISPR/Cas9 a more extensively employed tactic for genomic editing.

Some human inherited diseases

Atherosclerosis

Atherosclerosis (ATS) is the main cause of cardiovascular diseases and, subsequently, a high death rate worldwide (Kizilay Mancini et al., 2021). The ATS is considered by the attendance of fibro-fatty injuries in the artery barrier owing to lifelong subject to superior levels of LDL (low-density lipoprotein) (Gidding and Allen, 2019). Moreover, patients with hypercholester-

olemia (HCL) have a higher generation risk of untimely CVDs (cardiovascular diseases) such as atherosclerosis. Consequently, lipid-lowering treatments are the main therapeutic approach for managing ATS. According to many previous works (Kizilay Mancini et al., 2021), it was established that HCL is an inherited ailment caused by the mutation of some genes and the vastly prevalent kind of genetics recognized as HCH (hypercholesterolemia). The genomic mutations are well documented involving this disease (ATS), such as the *PCSK9,* or LDL receptor (*LDLR*), and apolipoprotein B (*APOB*), were in accounting for 1%, 90%, and 5% of HCL cases, respectively (Kizilay Mancini et al., 2021; Zha et al., 2021). Cho et al. (2020) reported an improvement in the survival of transplanted rats using CRISPR/Cas9 for targeting the *LEF1* gene (lymphoid enhancer binding factor 1). They also cardio-defend impact of the *LEF1* gene and can join the stem cell-based therapy with gene editing method as a beneficial approach for considering cardiovascular ailments (Cho et al., 2020). In another investigation, Kizilay Mancini et al. (2021) used MSCs (mesenchymal stem cells) isolated from diabetes patients (type 2) and ATS was utilized to explore the impact of *IKKB* manipulation on the immuno-strength of MSCs. The results of previous work demonstrated that the knockout of the *IKKB* gene via applying CRISPR/Cas9 confirmed a significant reduction in pro-inflammatory secretions (e.g., *IKKB* and *NF-κB*), which, in turn, augmented immuno-strength and survival in ATS and diabetes patients (type 2).

For the *LDLR* mutation, this cellular surface protein which is extremely considered in hepatic tissues and the chief regulatory function is to abolish high levels of LDL from the circulatory system (Defesche et al., 2017). This mutation also might support the development and progress of ATS clots in ATS patients. Defesche et al. (2017) clarified the potentiality of using AAV joined with CRISPR/Cas9. They showed a considerable ameliorative effect of ATS caused by *LDLR* gene in mice. With those significant results, gene editing via an AAV-CRISPR/Cas9 tactic (*in vivo* model) may present a fortunate strategy for treating ATS disease. At the same time, it probably augments the efficacy of prevailing lipid-lowering drugs. Moreover, studies have found that *APOC3* (apolipoprotein C3) mutation involves HCL, which increases the risk of cardiac ailment (Yuan et al., 2019). The study was conducted by Zha et al. (2021) using a rabbit embryo and treated with CRISPR/Cas9 system via inserting a sgRNA anchored to exon 2 of *APOC3*. They found that the CRISPR/Cas9 tactic can ameliorate the *APOC3* expression and reduce ATS plaque formation. The anti-atherosclerotic properties of *APOC3* suppression therapy exhibited a robust gene editing strategy and could provide a considerable fact for developing new therapy and further clinical uses (Zha et al., 2021). Still, there are no trials applying the CRISPR/Cas9 tool for treating the HCL or ATS diseases in humans or animals (as models). So further studies are required for discovering all mutations involving this disease which might provide us the potential therapeutic method via CRISPR/Cas9.

β-thalassemia

β-thalassemia (TDT) is an inherited autosomal disease and is thought to be the utmost serious monogenic ailment with severe and probably life-threatening signs. Several types of TDT are identified, such as β-thalassemia, α-thalassemia, and synchronized α- and β-thalassemia. Based on the reports, the annual estimated TDT is in roughly 60,000 patients (Fu et al., 2022; Li et al., 2022). This disease occurs through the mutation of the *HBB* gene (hemoglobin *β* subunit), an induced disorder in the β-globin synthesis in children (Li et al., 2022). Efforts have formed substantial experimental indications that reactivation of *HbF* by gene interference of precise transcription regulators and features could propose possible therapeutic sustenance for TDT disease (Fu et al., 2022). Studies have revealed that the *KLF1* (Krueppel-like factor 1) and *BCL11A* (B-cell lymphoma/leukemia 11A) are principal mediators occupied in the process of *γ*- to *β*-globin fluctuating and the destruction of these genes resulted in *HbF* rehabilitation (Khan et al., 2018). The previous investigations were designed for knocking out the HbF gene, which involved HbF destruction (*KLF1* and *BCL11A*) or interposing the accessory locations of several transcription mediators in the *HBG1/2* gene (*γ*-globin gene) (Khan et al., 2018; Li et al., 2022). Recently, the beneficial applications of CRISPR/Cas9 in β-thalassemia disorders have been studied by several authors (Fu et al., 2022; Li et al., 2022), while the efficacy of this technique still needs further clarification. There are some limitations in gene editing such as lentiviral gene moving of *β*-globin (Negre et al., 2016), which proved beneficial features in observed TDT patients. Furthermore, the lentiviral method may induce a high level of semi-random integration spots leading to the transactivation of the *HMGA2* gene forming the foremost safety worries for using this method (Negre et al., 2016; Khan et al., 2018). The limitation above put scientists to search for a novel gene editing method by applying the CRISPR/Cas9-mediated gene to repair, edit or manage associated mutations genes for TDT disease. In this sense, a bulk of published papers have clarified the efficacy of CRISPR/Cas9 to reactivate HbF transcript, leading to a significant impression after promoters' genetic interference of *BCL11A, HBG1/2,* and *LF1* (Yang et al., 2021; Fu et al., 2022; Li et al., 2022).

Targeting the *KLF1, BCL11A,* and *HBG1/2* via CRISPR/Cas9 to introduce fetal hemoglobin pattern (Yang et al., 2021) revealed that the *BCL11A* gene is the most effective treated gene for supporting the efficacy of CRISPR/Cas9 of β-hemoglobinopathies. Moreover, the same study displayed that the *BCL11A* gene is the chief clinically relevant defiance while *HBG1/2* could indicate a positive decision for the gene therapy of TDT. Recently, CRISPR/Cas9 can target the *BCL11A* erythroid-specific enhancer. Genetically and after treating the patients

with TDT, higher allelic percentages in bone marrow and blood, boosting fetal hemoglobin in SCD patients. Although both patients in the present study successfully induced robust γ-globin expression and got rid of transfusion dependence, longer follow-up of a larger cohort of patients will provide further evidence of the long-term efficacy and safety of this gene-editing strategy, confirming whether CRISPR/Cas9-mediated gene editing of the BCL11A +58 enhancer can cure pediatric TDT patients (Fu et al., 2022). Overall, applying CRISPR/Cas9 may describe a fortunate therapeutic feature to the medicine of TDT; more clinical and preclinical trials should indicate the security and competence of CRISPR/Cas9 when applied for the long term.

Hemophilia

Hemophilia (HEM) is documented as a global X-linked hereditary ailment due to a lack of clotting factors. In addition, HEM is spontaneous bleeding produced by a gene dysfunction connected with the extrinsic, intrinsic, and mutual coagulation path (Han et al., 2022). Studies have revealed that two factors, including hemophilia A (*VIII*) and hemophilia B (factor IX), were associated with this disease (Park et al., 2015). The CRISPR/Cas9 scheme has been exploited to manage factor VIII gene accuracy in hemophilia A (HA) patients through iPSCs (induced pluripotent stem cells). The chromosomal inversion is the main reason for the factor *VIII* gene (Park et al., 2015; Rong et al., 2022)**.**

Previously, the study by Park et al.)2015) has effectively regressed these chromosomal segments back to the wild-type condition. Likewise, Wang et al. (2018) demonstrated that the CRISPR/Cas9 system has been exploited to edit the factor *IX* gene for repairing corrected hemostasis in newborn and adult mice for long. Moreover, the same previous results have been found by Morishige et al. (2020), who have effectively corrected the factor *IX* genes of HB patients using iPSCs. Despite many previous studies presenting a significant result regarding CRISPR/Cas9 in treating hemophilia, clinical reports are also needed to validate this gene therapy. The bleeding syndrome HA (hemophilia A) is produced by F8 (a single gene) imperfection, and a small rise in the plasma FVIII factor can considerably enhance its clinical symptom. The previous year, Han et al. (2022) established and augmented lipid nanoparticles to carry Cas9 mRNA along with sgRNA that targeted anti-thrombin in mouse hepatocytes. This clearly shows that the mediated CRISPR/Cas9 delivery was caused by the suppression of AT that led to the development of thrombin creation.

Moreover, the bleeding-connected phenotypes were restored in hemophilia A and B mice. These results confirmed that CRISPR/Cas9 delivery was a safe and competent tactic for hemophilia therapy (Han et al., 2022). Son et al.)2022) used F8-defective human-brought hiPSCs from an HA patient (F8d-HA hiPSCs) and F8-corrected (F8c) HA hiPSCs produced by CRISPR/Cas9. The same investigation found that a high level of ECs noticeably reduced the bleeding time through numerous successive bleeding hurdles in HA mice, representing a robust hemostatic result (90% survival) (Son et al., 2022). Moreover, another study (Rong et al., 2022) established CRISPR/Cas9-based HDR and base editing for rectifying this mutated gene F8c. They utilized nCas9 (Cas9 nickase) intermediated HDR and the innovative base editor ABE8e to correct G20519A and then restrained the levels and action of FIX. Furthermore, the same author reported that the ABE8e base editor restored the mutation proficiently in both Huh7-FIXmut and HEK293-FIXmut stem cells (Rong et al., 2022).

Retinoschisis

Retinoschisis (RT) is a popular X-linked juvenile inherited macular regression comprising visualization throughout the initial phase of life, with a frequency of 1 in 5,000–25,000. This disease is clinically associated with bilateral foveae, retinal disinterest, vitreous hemorrhage, and departure of the inner retinal hide (Molday et al., 2012). The *RS1* (Retinoschisin 1) gene has been found in the retina of mice at the X-linked with six exons. It encodes a protein that consists of 224 amino acids, which is formed by photoreceptors of the retina (inner and outer coats) (Tantri et al., 2004). Previous elucidations have demonstrated that the patients with mutations *RS1,* such as Asp145His, Arg213Gln, Arg209His, and Arg102Gln, demonstrate cute RT features in the scientific characteristics (Tantri et al., 2004). This mutation in the *RS1* gene might induce the misfolding that affects intracellular protein accumulation, ultimately resulting in cystic systems in the retina (Molday et al., 2012). Genomic editing for modulating the *RS1* gene caused the retinoschisis was reported previously using CRISPR/Cas9 in an animal model (Yang et al., 2021).

For establishing *RS1* (c.625 C>T), Yang et al. (2021) utilized the CRISPR/Cas9 scheme combined with carboxylated nano-diamond (ND; as a carrier 3 nm). Based on the previous study, this method has a consistent and biocompatible element for revolutionizing a precise district of DNA in a cell. The ND might introduce an efficiency assumed by retina cells in mice and hiPSCs associated with the *RS1* gene. Genetically RS inherited can be advanced via applying the CRISPR/Cas9 through establishing the causal mutations into hiPSCs. Despite the achievements above in CRISPR/Cas9 for treating *RS1* mutation, Cho et al. (2020) suggested that it is not accurate to progress CRISPR/Cas9 for gene editing to individual patients' mutations due to a large number of 200 mutation spots in the *RS1* gene that have been revealed. Accomplishing CRISPR/Cas9-interfered knock-cutting-edge of the RS1 gene in the retinas of XLRS patients would be an innovative therapeutic policy. Based on the genetic screening, we suggested that there is some limitation in the applying of CRISPR/Cas9 for gene because there is a huge number of mutations associated with this disease.

More genomic studies are further required in XLRS patients to get more information which will help manipu-

late this genetic disease in which precise mutations only function in local tissues.

Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease (ND) that inflicts considerable emotional, medical, and economic problems on humanity (Buhidma et al., 2020). This PD has a specific mutation in SNCA (synuclein alpha) that encrypts the α-synuclein protein (A53T-SNCA) (Axelsen and Woldbye, 2018), a serious issue for familial PD. This mutation (A53T-SNCA) has been found in the familial PD, and CRISPR/Cas9 can delete or repair it through *in vivo* and *in vitro* trials. The results of those studies showed that the adeno-associated virus carrying SaCas9-KKH with a sgRNA effectively targeting A53T-SNCA and significantly decreased the expression of A53T-SNCA in the cell line. Moreover, A53T-SNCA deletion significantly released the α-synuclein up expression, dopaminergic neurodegeneration, reactive microgliosis, and parkinsonian motor indicators (Yoon et al., 2022). According to this outcome, we supposed that the CRISPR/Cas9 scheme can provide a potential inhibition policy for A53T-SNCA-specific in PD. The other study related to this topic (Li et al., 2021) used the AAV9-delivered CRISPR/Cas9 system to edit the *PINK1* (PTEN-induced kinase 1) and *DJ-1* genes in the monkey brain. Through short time (6–10 months of insertion), the former authors found that CRISPR/Cas9 can progress a bulky number of genetically modified PD monkeys, offering an applied transgenic monkey model for upcoming PD studies (Li et al., 2021). Additional investigation is required for more spotlight on the potentiality of applying CRISPR/Cas9 in treating PD diseases in humans.

Muscular dystrophy

Muscular dystrophy (MD) is defined as continually distributing skeletal muscle growth through spontaneous or genetic hereditary mutations. According to the reports, MD is a common human inherited disorder, with an estimated rate of around 1:5,000 newborn males (Ousterout et al., 2015). Deficiency in the expression of dystrophin and dystrophin-glycoprotein complex resulted in generating arrangements of rejuvenation and necrosis, increasing muscle failure and muscle membrane breakability (Bengtsson et al., 2017; Mata López et al., 2020). The use of gene editing in treating MD has been performed for a long time (Ousterout et al., 2015). Still, it is going in progress using the advantage of the proficiency of vectors derived from AAV in delivering or inserting some genes systemically via the vasculature method. With the progress in molecular genetics, scientists revealed that CRISPR/Cas9 had been potentially used in involving AAV vectors for treating MD. In a previous work explored by Ousterout et al. (2015), the mutational hotspot was targeted at exons 45–55, established fluctuations within the entire exons, and eliminated one or more exons via multiplexed sgRNA for repairing the MD mutation in humans. Certain *in vitro* experiments identified the gene-editing tool's ability to correct DMD (Duchenne muscular dystrophy) patient myoblasts by 62% via CRISPR/Cas9 (Ousterout et al., 2015) and the dystrophin gene was effectively restored. Moreover, the dystrophic mutation *mdx*^{4cv} was corrected via dual and single AAV vector/Cas9 and merged with a sgRNA scheme to repair the MD mutation in mice entirely (Bengtsson et al., 2017). Cas9 has developed multi-targeting genes or specific DNA regions for MD remedy (Zhang et al., 2022).

Dogs have been used as animal models for gene editing. Mata López et al. (2020) clarified the use of TALEN and CRISPR to revitalize the transcript of MD via HDR in myoblasts/myotubes and later via intramuscular injection of corrected MD dogs. They were applying a genome-wide CRISPR/Cas9 awning for detecting gene pathways in relationship with DUX4 in muscle, as clarified by Lek et al. (2020). The previous experiment showed that treating hypoxia pathway suppressors produced improved DUX4 protein turnover, decreasing cellular hypoxia activity and forfeiture (Lek et al., 2020). In addition, these elements confirmed viability in depressing FSHD (facioscapulohumeral muscular dystrophy) gene pattern indices in a patient with myogenic lines, further improving the structural and functionality patterns in zebrafish (as an animal FSHD model). The study of Lin et al. (2020) reported enhancing the competence of gene therapy in SMA (spinal muscular atrophy), achievement in splicing rectification of ESS (exonic splicing silencer A and B) of *SMN* (survival motor neuron) gene at exon 7 through base editing. They confirmed that this accomplishment was operative and like A36G conversion. Both A38G and A36G have a considerable effect on SMN protein formation that has a role in apoptotic paths (Lin et al., 2020), while the evidence of its importance functionality in *in vivo* needed further trials. As mentioned in the above section, base editing is a new effective tool in gene ending by CRISPR/Cas9. This erstwhile trial might confirm the application of modern therapeutic tactics for remedying SMA patients via the base editing-intermediated joining modification. Recently, Zhang et al. (2022) used CRISPR/Cas9 via a sgRNA for targeting exon 51 in mice (inserted human genomic sequence; HGS). They found the high capability of CRISPR/Cas9 in restoring the dystrophin expression and alleviated pathologic trademarks of MD, comprising grip strength and histopathological changes. This feature could open the door for using mice with HGS, allow more *in vivo* valuation of clinically pertinent gene therapy policies and other therapeutic tactics, and characterize a noteworthy phase concerning the therapeutic translation of CRISPR/Cas9 gene editing for rectification of MD in human propulsion.

Phenylketonuria

Phenylketonuria (PKU) is a hereditary syndrome (monogenic) that raises the concentrations of metabolic molecules termed phenylalanine in the circulatory system. This disease results in a deficiency in the formation of phenylalanine hydroxylase (PAH) in hepatocytes.

PKU characterizes one of the broadly prevalent newborn burdens of metabolism. Rising levels of PKU in the blood induced neuro-damages and hyperphenylalaninemia in the nerve cells (Blau, 2016). Moreover, serious neurological weakening, hepatic toxicities, and kidney failure were detected in PKU, subsequent in psychological syndromes (Cazzorla et al., 2018), logical incapacity, and seizures. Nutritional interventions have been suggested to reduce PKU disorder's adverse effects via depressing the phenylalanine. While with the progress in gene editing can apply to gene therapy to rectify this disease (Cazzorla et al., 2018). A study used CRISPR-Cas9 to manage/remove some genes, such as deletion of PINK1 (Yang et al., 2021) and G2019S in LRRK2 deletion of SNCA (Chen et al., 2019).

Investigations on gene editing have demonstrated that CRISPR/Cas9 can modify the genomic sequence for PKU treatment in animals or cell lines (Richards et al., 2020). Using CRISPR/Cas9 in a pig as an animal model for studying the gene therapy in PKU disease, Koppes et al. (2020) investigated the changes in histopathological alteration in pigs to get more data helping in understanding the PKU disease and, after that, evaluated innovative therapeutic interventions. The same report indicated that the zygote was injected with 2 sgRNAs joined with Cas9 mRNA and displayed losses in embryos, with embryo transfer to an extra, leading to two originator pigs. This strategy produced one pig with heterozygous for a *PAH* (phenylalanine hydroxylase) gene at exon six and many heterozygous for losses of exon 6 and 7 (Koppes et al., 2020).

Moreover, the CRISPR/Cas9 displayed continuous modification of the Pahenu2 allele in mice hepatocytes with a significant reduction of phenylalanine in the circulatory system (Richards et al., 2020), resilient fractional refurbishment of hepatocyte PAH activity, and ducking of maternal PKU properties through pregnancy. The study of Singh et al.)2021) applied CRISPR/Cas9 for generating mice with *Pah-KO*, efficiently changing GAG (codon 7) in *Pah* gene to a stop TAG codon. This fact clarified that the Pah-KO mice epitomize the uses of PKU in many biological uses, including metabolic disturbance and clinical and biochemical phenotypes. Delivery of *Pah* gene in mice genome via TALENS or CRISPR/Cas9 for more understanding of the hepatic restoration via PAH recognition. According to Singh et al. (2021), this model may present proposals for a substitute preclinical PKU model that had better be supportive of the valuation of several therapeutic approaches for the treatment of PKU. These different CRISPR/Cas-based gene editing studies have demonstrated that they can be used for correcting different associated genes in PD patients. As mentioned in the above lines, bulks of efforts have revealed that CRISPR/Cas9 system possibly uses a robust device for persistent *PKU* gene correction. Gene alteration for PKU patients is a recent process to improve lifetime neurological protection while authorizing unconstrained nutritional phenylalanine feeding by manipulative hepatocytes recombinant AAV2/8 vectors to kind existing CRISPR/Cas9 system for PKU disorder.

Tyrosinemia

Tyrosinemia (TYR) is recognized as a metabolic, genetic ailment with an occurrence rate of about 1 per 100,000 individuals (Paulk et al., 2010). TYR is triggered by the mutation of FAH (fumarylacetoacetate hydrolase), which is imperative for tyrosine degradation in the body (VanLith et al., 2018). This deficiency in FAH-induced insufficient breakdown of tyrosine results in accretion of tyrosine and, thus, poisonous metabolites. Moreover, TYR disease is complicated with different hepatic disorders, for instance, hepatic failure, serious hepatic cirrhosis, and hepatic cancer (Shao et al., 2018). Consequently, TYR is a respectable classic for gene therapy, and numerous methods have been specified to recover TYR symptoms in mammals (Shao et al., 2018). A study by Yin et al. (2014) showed that the FAH mutation gene was corrected using CRISPR/Cas9. It has been documented that HT1 patients have shown this mutation in exon 8, which has an unbalanced FAH protein (Shao et al., 2018). A CRISPR-Cas scheme has exploited the gene editing of hepatocytes for correcting TYR in a mouse model, and sole nucleotide mutation weakened FAH functionality (Rossidis et al., 2018). Moreover, the study of Rossidis et al. (2018) has established the modification of HT1 (hereditary tyrosinemia type 1) before birth using the CRISPR process. In an existing investigation, Song et al. (2020) applied the CRISPR-Cas9 homology in a mouse model for restoration to correct an A>G point of the FAH mutation gene (Shao et al., 2018). Using gene editing, the principal prenatal phase has been utilized to prevent a fatal metabolic ailment (Shao et al., 2018). Likewise, it has industrialized a novel system for gene editing with prodigious consistency and fashioned a *Fah* mutant rat to scrutinize whether Cas9-mediated genome editing can competently remove, edit or change the FAH, next recognized that Cas9n seldom creates indels in various cell lines (Shao et al., 2018). Besides, applying CRISPR/Cas9 in HTI mice as a constructed gene treatment prevents the improvement of hepatic cirrhosis (Shao et al., 2018). Collectively, screening data proposes that nucleotide base editing can be utilized to change genetic syndromes in other mammalian animals.

Cystic fibrosis

Cystic fibrosis (CYF) is a monogenic genetic disorder caused by the misexpression of the CFTR gene located on chromosome 7. This disease has targeted the tissues of the digestive system. The morbidity rate of CYF is around 1 for 4000 newborns, especially in northern Europe. At normal homeostasis, the function of the *CFTR* gene is to participate in the somatic regulation and ion channel in the cellular body, where it can attach the chloride anion channel with ATP (adenosine triphosphate) (Fan et al., 2018). So, this gene can also contribute to mineral homeostasis in the body, especially chloride

and bicarbonate. Using the CRISPR/Cas9 technique as a developed gene therapy tool, several studies have indicated that this process could improve survivability in patients with CYF (Schwank et al., 2013; Firth et al., 2015). Moreover, it has been clarified that the stem cell isolated from CYF patients (lungs) was used for correcting the CFTR gene by CRISPR/Cas9 (Firth et al., 2015). In this sense, the functionality of CFTR protein was re-established via employing the CRISPR/Cas9 in the form of stem cells (intestinal tissues) of pediatric patients with CYF (Schwank et al., 2013).

Another study (Crane et al., 2015) used the iPSCs to repair the CFRT gene through CRISPR/Cas9 in patients with lung CYF. Sheep (Fan et al., 2018) have been used to study the treatment of CYF in humans via CRISPR/Cas9. The previous investigation created a sheep model for animal construction CFTR model. Successfully newborn *CFTR*$^{-/-}$ sheep were reported and similar pathological characteristics (liver, gallbladder damage, pancreatic fibrosis, intestinal inhibition, and lack of veins) with humans were found.

This developed technique in an animal model will open the door for managing early disease progress, which is intractable to explore in humans and could advance innovative ways. Interestingly, the phenotype of *CFTR*$^{-/-}$ sheep produced by CRISPR/Cas9 indicates a valued reserve to the progress of new *CYF* gene editing therapeutics in humans. Studies on other animals, such as rabbits (Sinn et al., 2011), implemented the CRISPR/Cas9-mediated of CYF rabbits. They reported that an achievable animal model might offer the CYF in a community as a midway-sized typically connected to the pathogenesis of humans with CYF.

Other scientists from Iran (Khatibi et al., 2021) have shown the CRISPR/Cas9 (a sgRNA-Cas9) for modification of the *CFTR* gene using PBMC (peripheral blood mononuclear cells). They clarified the feasibility of site-specific gene targeting with the CRISPR/Cas9 system. As shown in Table 1, positive applications of CRISPR/Cas9 exist on CYF and other kinds of hereditary disorders. For enhancing the efficiency, it confirmed that CRISPR-9-based adenine was effective in treating CYF in *in vitro* model (stem cells). Recently, in bronchial cells, Khatibi et al. (2021) elucidated that CRISPR/Cas9 gene editing effectively enhanced the expression of the *CFTR* gene in humans (*in vitro* model). In a comparative investigation for studying the effective tool of Cas9 or Cas12a in enhancing the expression of the *CFTR* gene (Santos et al., 2022), they reported that Cas9 achieved superior rectification of *CFTR* compared to Cas12a, with values of 18% and 8% for Cas9 and Cas12, respectively. According to the above studies related to CYF disease, applying CRISPR/Cas9 may effectively produce a gene device for restoring the *CFTR* mutation in *in vivo* or *in vitro* models and hopeful outcomes for remedying the genetic CYF disorder. Henceforward, it appears that the employment of CRISPR/Cas9 is a potent device in empowering the medication of genetic therapy for roughly all CYF patients.

Disease-resistant animals

In the present era of advanced molecular explorations, CRISPR/Cas9 is a potent technology for application in disease resistance to animals. Many diseases have affected the welfare and productivity and caused a huge loss in the production of dairy cattle or other agricultural animals (Liu et al., 2022). Vicencio et al. (2022) used the CRISPR/Cas9 nickase for generating the tuberculosis-resistant hereditarily adapted animal via suppression of the expression of NRAMP1 in bovine fetal fibroblasts (BFFs). The previous study generated some animals (cows) that can resist tuberculosis (Gao et al., 2017). Additionally, Bevacqua et al. (2016) demonstrated that the CRISPR/Cas9 modified the bovine *PRNP* gene at exon 3 to generate knockouts in both early and fetal embryos fibroblasts in bovine. This *PRNP* mutation gene might be associated with fetal disorder in mammals (Bevacqua et al., 2016).

Brucellosis is one of the primary severe zoonotic maladies worldwide, and this affects its embarrassment to the animal population and causes a strict finance-related problem for stock breeders. It has been reported that the RpolA gene (RNA polymerase subunit A) is associated with the replication of *Brucella* within the cellular system of the host (Ikeda et al., 2017). A study by Karponi et al. (2019) used transduced infected cells with lentiviral vectors comprising the CRISPR/Cas9 to suppress this gene *RpolA* in cattle. Results indicate that the number of internalized brucellae/cells is considerably decreased after transduction with CRISPR/Cas9 vector with a multiplicity degree infection of 60 (Karponi et al., 2019). The MAP (*Mycobacterium avium* subspecies paratuberculosis) is a common microbial pathogen that causes chronic enteritis and Johne's disorder in cattle (Karponi et al., 2019). The *IL10RA* (interleukin-10 receptor alpha) gene is a critical mediator of inflammation and has been discovered to be associated with mastitis and MAP pathogenesis in cows (Liu et al., 2022). Targeting this gene via CRISPR/Cas9 might be useful for protecting dairy cows from this disease. Authors performed that CRISPR/Cas9 can generate an *IL10RA* knockout in cattle's epithelial tissues of mammary glands (Liu et al., 2022). According to Mallikarjunappa et al. (2020), the wide and significant impacts of IL10RA knockout could improve the modification of pro-inflammatory cytokine expression, thus supporting the immune-boosting molecule of *IL10RA* in bringing an anti-inflammatory action, as well as its probable functional interface attachment among immune responses connected with chronic enteritis and Johne's disorder (Mallikarjunappa et al., 2020).

There is a lack of data concerning applying CRISPR/Cas9 in animals resisting disease. In this sense, further investigations are desired to discover new mutations that caused several diseases which affect the welfare and productivity of dairy cattle for developing modified CRISPR/Cas9 for treatment options involving those mutations in animals, helping to reduce the economic losses in dairy farms.

Table 1. The main findings of using CRISPR/Cas9 therapy in treating hereditary disease in human and animal models

References	Model	Target gene	Main findings	Diseases
1	2	3	4	5
Cho et al., 2020	Rats	*LEF1*	An improvement in the survival of transplanted rats using CRISPR/Cas9 for targeting *LEF1* gene.	Atherosclerosis
Kizilay Mancini et al., 2021	MSCs (mesenchymal stem cells) isolated from diabetes patients (type 2)	*IKKB*	The results of previous work demonstrated that the knockout of *IKKB* gene via applying CRISPR/Cas9 confirmed a significant reduction in pro-inflammatory secretions (e.g., *IKKB* and NF-κB), which, in turn, augmented immuno-strength and survival in ATS and diabetes patients (type 2).	
Defesche et al., 2017	Mice	*LDLR*	AAV joined with CRISPR/Cas9 showed a considerable ameliorative effect of ATS caused by *LDLR* gene in mice	
Zha et al., 2021	Rabbit embryo	*APOC3*	CRISPR/Cas9 tactic can ameliorate the *APOC3* expression and reduce ATS plaque formation. The anti-atherosclerotic properties of *APOC3* suppression therapy exhibited a robust gene editing strategy.	
Yang et al., 2021	Rabbit	*KLF1, BCL11A,* and *HBG1/2*	*BCL11A* gene is the chief clinically relevant defiance while *HBG1/2* could indicate a positive decision for the gene therapy of TDT.	β-thalassemia
Fu et al., 2022	Pediatric TDT patients	*BCL11A*	Successfully induced robust γ-globin expression and got rid of transfusion dependence, longer follow-up of a larger cohort of patients will provide further evidence on the long-term efficacy and safety of this gene-editing strategy, confirming whether CRISPR/Cas9-mediated gene editing. Higher allelic percentages in bone marrow and blood boost fetal hemoglobin in SCD patients.	
Wang et al., 2018	Mice	factor IX gene	CRISPR/Cas9 system has been exploited to edit the factor *IX* gene for repairing corrected hemostasis in newborn and adult mice for lifelong.	Hemophilia
Morishige et al., 2020	Patients	IX gene	Effectively corrected the factor *IX* gene of HB patients using iPSCs.	
Han et al., 2022	Hepatic mouse	----	Augmented lipid nanoparticles carry Cas9 mRNA along with sgRNA that targeted antithrombin in hepatocytes of the mouse. This clearly shows that the mediated CRISPR/Cas9 delivery was caused by the suppression of AT that led to the development of thrombin creation. Moreover, the bleeding-connected phenotypes were restored in hemophilia A and B mice.	
Son et al., 2022	hiPSCs from HA patient	*F8-c*	F8-defective humans brought hiPSCs from an HA patient (F8d-HA hiPSCs) and F8-corrected (F8c) HA hiPSCs produced by CRISPR/Cas9. The same investigation found that a high level of ECs noticeably reduced the bleeding time through numerous successive bleeding hurdles in HA mice, representing a robust hemostatic result (90% survival).	
Rong et al., 2022	Stem cell	*F8c* gene	The base editor ABE8e corrected G20519A and then restrained the levels and action of FIX. Furthermore, the same author reported that the ABE8e base editor restored the mutation proficiently in both Huh7-FIXmut and HEK293-FIXmut stem cells.	
Yang et al., 2021	Retina cells in mice and hiPSCs	*RS1* gene	This method has a consistent and biocompatible element for revolutionizing a precise district of DNA in a cell. The carboxylated nanodiamond might introduce efficiently assumed retina cells in mice and hiPSCs, which are associated with the *RS1* gene.	Retinoschisis
Axelsen and Woldbye, 2018	Cell line	*A53T-SNCA*	The results of those studies showed that the adeno-associated virus carrying SaCas9-KKH with a sgRNA effectively targeted A53T-SNCA and significantly decreased the expression of A53T-SNCA in cell line	Parkinson's disease

Table 1 – contd.

1	2	3	4	5
Mata López et al., 2020	Dog	------	Have clarified the use of TALEN and CRISPR to revitalize the transcript of MD via HDR in myoblasts /myotubes and later via intramuscular injection of corrected MD dog.	Muscular dystrophy
Lek et al., 2020	Muscle cells	*DUX4*	Treating hypoxia pathway suppressors improved DUX4 protein turnover, decreasing cellular hypoxia activity and forfeiture.	
Lin et al., 2020	Zebrafish	------	Results confirmed viability in depressing *FSHD* (facioscapulohumeral muscular dystrophy) pattern indices in myogenic patients, further improving the structural and functionality patterns in zebrafish (as an animal FSHD model). Enhancing the competence of gene therapy for SMA (spinal muscular atrophy), achievement in splicing rectification of ESS (exonic splicing silencer A and B) of SMN (survival motor neuron) gene at exon 7 through base editing. They confirmed that this accomplishment was operative and like A36G conversion. Both A38G and A36G have a considerable effect on SMN protein formation that has a role in apoptotic paths.	
Zhang et al., 2022	Mice	*Exon 51*	They found the high capability of CRISPR/Cas9 in restoring the dystrophin expression and alleviated pathologic trademarks of MD, comprising grip strength and histopathological changes.	
Koppes et al., 2020	Pigs	*PINK1* gene	The same report indicated that the zygote was injected with 2 sgRNAs joined with Cas9 mRNA and displayed losses in embryos, with embryo transfer to an extra, leading to two originator pigs. This strategy produced one pig heterozygous for a *PAH* gene at exon six and many heterozygous for losses of exon 6 and 7.	Phenylketonuria
Singh et al., 2021	Mice	*PAH* gene	Applied CRISPR/Cas9 for generating mice with Pah-KO, efficiently changing GAG (codon 7) in *Pah* gene to a stop TAG codon.	
Shao et al., 2018	Rats	------	HT1 patients have shown this mutation in exon 8, which has an unbalanced FAH protein. Applying CRISPR/Cas9 in HTI mouse as a constructed gene treating preventing the improvement of hepatic cirrhosis	Tyrosinemia
Schwank et al., 2013	Pediatric patients with CYF	*CFTR*	It was re-established the functionality of *CFTR* protein via employing the CRISPR/Cas9 in the form of stem cells (intestinal tissues) of pediatric patients with CYF	Cystic fibrosis
Fan et al., 2018	Sheep	*CFTR*	The previous investigation created a sheep model for animal construction CFTR model. Successfully newborn *CFTR*$^{-/-}$ sheep were reported, and similar pathological characteristics (liver, gallbladder damage, pancreatic fibrosis, intestinal inhibition, and lack of veins) with humans were found	
Khatibi et al., 2021	PBMC (peripheral blood mononuclear cells)	*CFTR*	CRISPR/Cas9 (a sgRNA-Cas9) for modification of *CFTR* gene using PBMC (peripheral blood mononuclear cells). They clarified the feasibility of site-specific gene targeting with the CRISPR/Cas9 system	
Khatibi et al., 2021	Bronchial cells	*CFTR*	The CRISPR/Cas9 gene editing effectively enhanced the expression of *CFTR* gene in humans (*in vitro* model).	
Santos et al., 2022	CFF-16HBEge W1282X CFTR cell line	------	A comparative investigation for studying the effective tool of Cas9 or Cas12a in enhancing the expression of *CFTR* gene. They reported that Cas9 achieved superior rectification of CFTR compared to Cas12a, with values of 18% and 8% for Cas9 and Cas12, respectively. According to the above studies related to CYF disease, applying CRISPR/Cas9 may effectively produce a gene device for restoring the *CFTR* mutation in *in vivo* or *in vitro* models.	

Improving the efficacy of CRISPR/Cas9

As mentioned in the previous sections, CRISPR/Cas9 permits quick, accurate, and simplified methods to replace or knockout sequences in various animal species. Moreover, using Cas9 protein as a simple method fortified with the sgRNA (a single guide RNA) is widely common in targeting several genes (Statkute et al., 2022). The delivery system can be achieved with adenovirus, mRNA, protein transfection, or lentiviral delivery (Liu et al., 2020). Moreover, studies have reported that the use of lentiviral or plasmid could present antibiotic resistance signs (Hinderer et al., 2018; Statkute et al., 2022), and also animals treated with CRISPR/Cas9 with high AAV ($\geq 1.5 \times 10^{14}$ vg/kg) may activate the severity of liver toxicity. In this regard, Statkute et al. (2022) reported that the use of Cas9 is more effective than the Cas12 method for resorting to some genetic mutations that cause inherited diseases such as CYF. According to the previous studies of Zhang et al. (2019), the effectiveness of applying CRISPR/Cas9 as adaptable epigenetic editing was higher in sgRNA than in AAV. To combat the previous issues, Ad (adenovirus) vectors avoid several of these effects, with high efficiency, no integration, higher packaging limits, transient expression, and safety profile (Statkute et al., 2022).

The era of using DDS (drug delivery systems) in many biomedical uses provided innovative alterations for improving the potential applications of CRISPR/Cas9 in treating many inherited diseases (Ikeda et al., 2017). Studies by Zhang et al. (2019) recommended that recent DDS commonly have superior competence and inferior immunogenicity related to previous conventional methods (Wei et al., 2020). Moreover, the hire of DDS can be eligible to bring the differences of inherited diseases through stuffing the multidrug combination within the equal delivery amenity.

Researchers have recognized respective types of DDS incorporated with CRISPR/Cas9 for enhancing efficacy and safety, such as inorganic nanoparticles (Zhang et al., 2019), lipid-based nanoparticles (Liu et al., 2020), and polymer-based nanoparticles (Chen et al., 2019) to remedy inherited diseases. Another technique was performed to improve the efficacy of CRISPR/Cas9 and its application; Wang et al. (2018) used a base editor (BE) to extricate off-target impacts, scrutinize the landscapes, and remove the restrictions of these off-target valuation schemes.

An earlier trial by Zuo et al. (2019) in China, have confirmed cases of how the off-target properties of BE can be weakened via biological-understanding-directed engineering to develop the competence of these vigorous epigenetic treatments schemes for either treatment or to investigate attainments. According to Komor et al. (2016) studies, they used BE joined Cas9, and APOBEC1 (cytosine deaminase), in the track of sgRNA to improve the exact revolution of a base from C·G to T·A.

Recently, scientists developed many kinds of BE generations to boost the competence of the CRISPR/Cas9 uses in inherited diseases. For instance, it was exploited that BE3 was more effective in enhancing the competence of CRISPR/Cas9 in the genomic editing of embryos of various species (Komor et al., 2016). Interestingly, this privileged exertion for the first time demonstrated that respective *de novo* SNVs are enhanced by BE3. Additionally, Zuo et al. (2019) discovered the genome-wide off-target analysis by two-cell embryo injection (GOTI) to recognize off-target mutations via deletion or modification of DNA sequence in one blastomere of two-cell mouse embryos utilizing either CRISPR-Cas9 or base editors. Comprehensively, GOTI is a newly developed technology that could be a supportive policy for exploring off-target effects of numerous fragments in the DNA without inhibiting single-nucleotide polymorphisms in many individuals. Remarkably, Zuo et al. (2019) mentioned that a good clarification is that our scheme, GOTI, notices the cellular system established from an exact gene-modified blastomere.

Conclusion

In this review, we highlighted the efficacy of using CRISPR/Cas9 technology for treating some inherited diseases in humans. Firstly, we described the development of CRISPR/Cas9 gene editing, then studies reported on using CRISPR/Cas9 in some animal diseases have also been reviewed. Finally, when CRISPR/Cas9 has been established with some vectors showing some obstacles, the method for enhancing the application of CRISPR/Cas9 in different fields is also documented for knowledge of the stop-point in the research related to this topic. Although experts are faithful in decreasing off-target impacts and enhancing targeting competence, there are still some unescapable borders. Hence, it is necessary to increase explorations using animal models, empowering profound comprehension of the assortment among patients to notify personalized treatments.

Author contributions

All authors shared equally in writing this review article.

Conflicts of interest

The authors declare no conflict of interest.

References

Anders C., Niewoehner O., Duerst A., Jinek M. (2014). Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature, 513: 569–573.

Axelsen T.M., Woldbye D.P. (2018). Gene therapy for Parkinson's disease, an update. J. Parkinson's Dis., 8: 195–215.

Bengtsson N.E., Hall J.K., Odom G.L., Phelps M.P., Andrus C.R., Hawkins R.D., Hauschka S.D., Chamberlain J.R., Chamberlain J.S. (2017). Corrigendum: Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nat. Commun., 8: 16007.

Bevacqua R.J., Fernandez-Martín R., Savy V., Canel N.G., Gismondi M.I., Kues W.A., Carlson D.F., Fahrenkrug S., Niemann H., Tabo-

ga O.A. (2016). Efficient edition of the bovine *PRNP* prion gene in somatic cells and IVF embryos using the CRISPR/Cas9 system. Theriogenology, 86: 1886–1896.
Bischoff N., Wimberger S., Maresca M., Brakebusch C. (2020). Improving precise CRISPR genome editing by small molecules: is there a magic potion? Cells, 9: 1318.
Blau N. (2016). Genetics of Phenylketonuria: Then and Now. Hum. Mutat., 37: 508–515.
Buhidma Y., Rukavina K., Chaudhuri K.R., Duty S. (2020). Potential of animal models for advancing the understanding and treatment of pain in Parkinson's disease. NPJ Parkinson's Dis., 6: 1–7.
Cazzorla C., Bensi G., Biasucci G., Leuzzi V., Manti F., Musumeci A., Papadia F., Stoppioni V., Tummolo A., Vendemiale M., Polo G., Burlina A. (2018). Living with phenylketonuria in adulthood: the PKU ATTITUDE study. Mol. Genet. Metab., 16: 39–45.
Chen Y., Dolt K.S., Kriek M., Baker T., Downey P., Drummond N.J., Canham M.A. Natalwala A., Rosser S., Kunath T. (2019). Engineering synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated deletion of the SNCA gene. Eur. J. Neurosci., 49: 510–524.
Cho H.-M., Lee K.-H., Shen Y.M., Shin T.J., Ryu P.D., Choi M.C., Kang K.-S., Cho J.Y. (2020). Transplantation of hMSCs genome edited with *LEF1* improves cardio-protective effects in myocardial infarction. Mol. Ther. Nucleic Acids, 19: 1186–1197.
Christian M., Cermak T., Doyle E.L., Schmidt C., Zhang F., Hummel A., Bogdanove A.J., Voytas D.F. (2010). Targeting DNA double-strand breaks with TAL effector nucleases. Genetics, 186: 757–761.
Cobb R.E., Wang Y., Zhao H. (2015). High-efficiency multiplex genome editing of Streptomyces species using an engineered CRISPR/Cas system. ACS Synth. Biol., 4: 723–728.
Cong L., Ran F.A., Cox D., Lin S., Barretto R., Habib N., Hsu P.D., Wu X., Jiang W., Marraffini L.A. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science, 339: 819–823.
Cox D.B.T., Platt R.J., Zhang F. (2015). Therapeutic genome editing: prospects and challenges. Nat. Med., 21: 121–131.
Crane A.M., Kramer P., Bui J.H., Chung W.J., Li X.S., Gonzalez-Garay M.L., Hawkins F., Liao W., Mora D., Choi S., Wang J., Sun H.C., Paschon D.E., Guschin D.Y., Gregory P.D., Kotton D.N., Holmes M.C., Sorscher E.J., Davis B.R. (2015). Targeted correction and restored function of the CFTR gene in cystic fibrosis induced pluripotent stem cells. Stem Cell Rep., 4: 569–577.
Defesche J.C., Gidding S.S., Harada-Shiba M., Hegele R.A., Santos R.D., Wierzbicki A.S. (2017). Familial hypercholesterolaemia. Nat. Rev. Dis. Primers, 3: 1–20.
Fan Z., Perisse I.V., Cotton C.U., Regouski M., Meng Q., Domb C., Van Wettere A.J., Wang Z., Harris A., White K.L., Polejaeva I.A. (2018). A sheep model of cystic fibrosis generated by CRISPR/Cas9 disruption of the *CFTR* gene. JCI Insight, 3.
Firth A.L., Menon T., Parker G.S., Qualls S.J., Lewis B.M., Ke E., Dargitz C.T., Wright R., Khanna A., Gage F.H., Verma I.M. (2015). Functional gene correction for cystic fibrosis in lung epithelial cells generated from patient iPSCs. Cell Rep., 12: 1385–1390.
Fu B., Liao J., Chen S., Li W., Wang Q., Hu J., Yang F., Hsiao S., Jiang Y., Wang L. (2022). CRISPR–Cas9-mediated gene editing of the *BCL11A* enhancer for pediatric β0/β0 transfusion-dependent β-thalassemia. Nat. Med., 28: 1573–1580.
Gao Y., Wu H., Wang Y., Liu X., Chen L., Li Q., Cui C., Liu X., Zhang J., Zhang Y. (2017). Single Cas9 nickase induced generation of *NRAMP1* knockin cattle with reduced off-target effects. Genome Biol., 18: 13.
Geurts M.H., de Poel E., Amatngalim G.D., Oka R., Meijers F.M., Kruisselbrink E., van Mourik P., Berkers G., de Winter-de Groot K.M., Michel S. (2020). CRISPR-based adenine editors correct nonsense mutations in a cystic fibrosis organoid biobank. Cell Stem Cell, 26: 503–510.
Gidding S.S., Allen N.B. (2019). Cholesterol and atherosclerotic cardiovascular disease: a lifelong problem. Am. Heart Assoc., 012924.
Grisch-Chan H.M., Schwank G., Harding C.O., Thöny B. (2019.) State-of-the-art 2019 on gene therapy for phenylketonuria. Hum. Gene Ther., 30: 1274–1283.
Han J.P., Kim M., Choi B.S., Lee J.H., Lee G.S., Jeong M., Lee Y., Kim E.A., Oh H.-K., Go N., (2022). *In vivo* delivery of CRISPR-Cas9 using lipid nanoparticles enables antithrombin gene editing for sustainable hemophilia A and B therapy. Sci. Adv., 8: eabj6901.
Hekselman I., Kerber L., Ziv M., Gruber G., Yeger-Lotem E. (2022). The organ-disease annotations (ODiseA) database of hereditary diseases and inflicted tissues. J. Mol. Biol., 167619.
Hinderer C., Katz N., Buza E.L., Dyer C., Goode T., Bell P., Richman L.K., Wilson J.M. (2018). Severe toxicity in nonhuman primates and piglets following high-dose intravenous administration of an adeno-associated virus vector expressing human SMN. Hum. Genet. Ther., 29: 285–298.
Ikeda M., Matsuyama S., Akagi, S., Ohkoshi K., Nakamura S., Minabe S., Kimura K., Hosoe M. (2017). Correction of a disease mutation using CRISPR/Cas9-assisted genome editing in Japanese black cattle. Sci. Rep., 7: 1–9.
Irion U., Krauss J., Nüsslein-Volhard C. (2014). Precise and efficient genome editing in zebrafish using the CRISPR/Cas9 system. Development, 141: 4827–4830.
Jiang F., Doudna J.A. (2015). The structural biology of CRISPR-Cas systems. Curr. Opin. Struct. Biol., 30: 100–111.
Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science, 337: 816-821.
Karponi G., Kritas S.K., Papadopoulou G., Akrioti E.K., Papanikolaou E., Petridou E. (2019). Development of a CRISPR/Cas9 system against ruminant animal brucellosis. BMC Vet. Res., 15: 422.
Khan K.N., Robson A., Mahroo O.A., Arno G., Inglehearn C.F., Armengol M., Waseem N., Holder G.E., Carss K.J., Raymond L.F. (2018). A clinical and molecular characterisation of CRB1-associated maculopathy. Eur. J. Hum. Genet., 26: 687–694.
Khatibi S., Modaresi M., Kazemi O.R., Salehi M., Aghaee-Bakhtiari S.H. (2021). Genetic modification of cystic fibrosis with ΔF508 mutation of *CFTR* gene using the CRISPR system in peripheral blood mononuclear cells. Iran. J. Basic Med. Sci., 24: 73–78.
Kizilay Mancini O., Huynh D.N., Menard L., Shum-Tim D., Ong H., Marleau S., Colmegna I., Servant M.J. (2021). Ex *vivo* Ikkβ ablation rescues the immunopotency of mesenchymal stromal cells from diabetics with advanced atherosclerosis. Cardiovasc. Res., 117: 756–766.
Komor A.C., Kim Y.B., Packer M.S., Zuris J.A., Liu D.R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533: 420–424.
Koppes E.A., Redel B.K., Johnson M.A., Skvorak K.J., Ghaloul-Gonzalez L., Yates M.E., Lewis D.W., Gollin S.M., Wu Y.L., Christ S.E., Yerle M., Leshinski A., Spate L.D., Benne J.A., Murphy S.L., Samuel M.S., Walters E.M., Hansen S.A., Wells K.D., Lichter-Konecki U., Wagner R.A., Newsome J.T., Dobrowolski S.F., Vockley J., Prather R.S., Nicholls R.D. (2020). A porcine model of phenylketonuria generated by CRISPR/Cas9 genome editing. JCI Insight, 5.
Kosicki M., Tomberg K., Bradley A. (2018). Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nat. Biotechnol., 36: 765–771.
Lek A., Zhang Y., Woodman K.G., Huang S., DeSimone A.M., Cohen J., Ho V., Conner J., Mead L., Kodani A., Pakula A., Sanjana N., King O.D., Jones P.L., Wagner K.R., Lek M., Kunkel L.M. (2020). Applying genome-wide CRISPR-Cas9 screens for therapeutic discovery in facioscapulohumeral muscular dystrophy. Sci Transl. Med., 12.
Li H., Wu S., Ma X., Li X., Cheng T., Chen Z., Wu J., Lv L., Li L., Xu L. (2021). Co-editing *PINK1* and *DJ-1* genes via adeno-associated virus-delivered CRISPR/Cas9 system in adult monkey brain elicits classical parkinsonian phenotype. Neurosci. Bull., 37: 1271–1288.
Li J., Hong S., Chen W., Zuo E., Yang H. (2019). Advances in detecting and reducing off-target effects generated by CRISPR-mediated genome editing. J. Genet. Genomics, 46: 513–521.
Li L., Yi H., Liu Z., Long P., Pan T., Huang Y., Li Y., Li Q., Ma Y. (2022). Genetic correction of concurrent α- and β-thalassemia patient-derived pluripotent stem cells by the CRISPR-Cas9 technology. Stem Cell Res. Ther., 13: 1–12.

Lin X., Chen H., Lu Y.Q., Hong S., Hu X., Gao Y., Lai L.L., Li J.J., Wang Z., Ying W., Ma L., Wang N., Zuo E., Yang H., Chen W.J. (2020). Base editing-mediated splicing correction therapy for spinal muscular atrophy. Cell Res., 30: 548–550.

Liu Q., Wang C., Zheng Y., Zhao Y., Wang Y., Hao J., Zhao X., Yi K., Shi L., Kang C. (2020). Virus-like nanoparticle as a co-delivery system to enhance efficacy of CRISPR/Cas9-based cancer immunotherapy. Biomaterials, 258: 120275.

Liu Z., Wu T., Xiang G., Wang H., Wang B., Feng Z., Mu Y., Li K. (2022). Enhancing animal disease resistance, production efficiency, and welfare through precise genome editing. Int. J. Mol. Sci., 23: 7331.

Makarova K.S., Grishin N.V., Shabalina S.A., Wolf Y.I., Koonin E.V. (2006). A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol. Direct, 1: 1–26.

Mallikarjunappa S., Shandilya U.K., Sharma A., Lamers K., Bissonnette N., Karrow N.A., Meade K.G. (2020). Functional analysis of bovine interleukin-10 receptor alpha in response to *Mycobacterium avium* subsp. *paratuberculosis* lysate using CRISPR/Cas9. BMC Genet., 21: 121.

Mata López S., Balog-Alvarez C., Vitha S., Bettis A.K., Canessa E.H., Kornegay J.N., Nghiem P.P. (2020). Challenges associated with homologous directed repair using CRISPR-Cas9 and TALEN to edit the DMD genetic mutation in canine Duchenne muscular dystrophy. PloS One, 15: e0228072.

Molday R.S., Kellner U., Weber B.H. (2012). X-linked juvenile retinoschisis: clinical diagnosis, genetic analysis, and molecular mechanisms. Prog. Retin. Eye Res., 31: 195–212.

Morishige S., Mizuno S., Ozawa H., Nakamura T., Mazahery A., Nomura K., Seki R., Mouri F., Osaki K., Yamamura K. (2020). CRISPR/Cas9-mediated gene correction in hemophilia B patient-derived iPSCs. Int. J. Hematol., 111: 225–233.

Negre O., Eggimann A.V., Beuzard Y., Ribeil, J.A., Bourget P., Borwornpinyo S., Hongeng S., Hacein-Bey S., Cavazzana M., Leboulch P. (2016). Gene therapy of the β-hemoglobinopathies by lentiviral transfer of the βA (T87Q)-globin gene. Hum. Gene Ther., 27: 148–165.

O'Connor T.P., Crystal R.G. (2006). Genetic medicines: treatment strategies for hereditary disorders. Nat. Rev. Genet., 7: 261–276.

Ousterout D.G., Kabadi A.M., Thakore P.I., Majoros W.H., Reddy T.E., Gersbach C.A. (2015). Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. Nat. Commun., 6: 6244.

Park C.Y., Kim D.H., Son J.S., Sung J.J., Lee J., Bae S., Kim J.H., Kim D.W., Kim J.S. (2015). Functional correction of large factor *VIII* gene chromosomal inversions in hemophilia A patient-derived iPSCs using CRISPR-Cas9. Cell Stem, 17: 213–220.

Paulk N.K., Wursthorn K., Wang Z., Finegold M.J., Kay M.A., Grompe M. (2010). Adeno-associated virus gene repair corrects a mouse model of hereditary tyrosinemia *in vivo*. Hepatology (Baltimore, Md.), 51: 1200–1208.

Richards D.Y., Winn S.R., Dudley S., Nygaard S., Mighell T.L., Grompe M., Harding C.O., (2020). AAV-mediated CRISPR/Cas9 gene editing in murine phenylketonuria. Mol. Ther. Methods Clin. Dev., 17: 234–245.

Rong L., Chen D., Huang X., Sun L. (2022). Delivery of Cas9-guided ABE8e into stem cells using poly (l-lysine) polypeptides for correction of the hemophilia-associated FIX missense mutation. Biochem. Biophys. Res. Commun., 628: 49–56.

Rossidis A.C., Stratigis J.D., Chadwick A.C., Hartman H.A., Ahn N.J., Li H., Singh K., Coons B.E., Li L., Lv W., Zoltick P.W., Alapati D., Zacharias W., Jain R., Morrisey E.E., Musunuru K., Peranteau W.H. (2018). In utero CRISPR-mediated therapeutic editing of metabolic genes. Nat. Med., 24: 1513–1518.

Santos L., Mention K., Cavusoglu-Doran K., Sanz D.J., Bacalhau M., Lopes-Pacheco M., Harrison P.T., Farinha C.M. (2022). Comparison of Cas9 and Cas12a CRISPR editing methods to correct the W1282X-CFTR mutation. J. Cyst. Fibros, 21: 181–187.

Schwank G., Koo B.K., Sasselli V., Dekkers J.F., Heo I., Demircan T., Sasaki N., Boymans S., Cuppen E., van der Ent C.K., Nieuwenhuis E.E., Beekman J.M., Clevers H. (2013). Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell, 13: 653–658.

Shah S.A., Erdmann S., Mojica F.J., Garrett R.A. (2013). Protospacer recognition motifs: mixed identities and functional diversity. RNA Biol., 10: 891–899.

Shao Y., Wang L., Guo N., Wang S., Yang L., Li Y., Wang M., Yin S., Han H., Zeng L., Zhang L., Hui L., Ding Q., Zhang J., Geng H., Liu M., Li D. (2018). Cas9-nickase-mediated genome editing corrects hereditary tyrosinemia in rats. J. Biol. Chem., 293: 6883–6892.

Singh K., Cornell C.S., Jackson R., Kabiri M., Phipps M., Desai M., et al. (2021). CRISPR/Cas9 generated knockout mice lacking phenylalanine hydroxylase protein as a novel preclinical model for human phenylketonuria. Sci. Rep., 11: 7254.

Sinn P.L., Anthony R.M., McCray P.B. Jr. (2011). Genetic therapies for cystic fibrosis lung disease. Hum. Mol. Genet., 20: 79–86.

Son J.S., Park C.Y., Lee G., Park J.Y., Kim H.J., Kim G., Chi K.Y., Woo D.H., Han C., Kim S.K. (2022). Therapeutic correction of hemophilia A using 2D endothelial cells and multicellular 3D organoids derived from CRISPR/Cas9-engineered patient iPSCs. Biomaterials, 283: 121429.

Song Y., Sohl-Dickstein J., Kingma D.P., Kumar A., Ermon S., Poole B. (2020). Score-based generative modeling through stochastic differential equations. arXiv preprint, arXiv:2011.13456.

Statkute E., Wang E.Y., Stanton R.J. (2022). An optimized CRISPR/Cas9 adenovirus vector (AdZ-CRISPR) for high-throughput cloning of sgRNA, using enhanced sgRNA and Cas9 variants. Hum. Gene Ther., 33: 990–1001.

Tantri A., Vrabec T.R., Cu-Unjieng A., Frost A., Annesley Jr, W.H., Donoso L.A. (2004). X-linked retinoschisis: a clinical and molecular genetic review. Surv. Ophthalmol., 49: 214–230.

VanLith C., Guthman R., Nicolas C.T., Allen K., Du Z., Joo D.J., Nyberg S.L., Lillegard J.B., Hickey R.D. (2018). Curative ex *vivo* hepatocyte-directed gene editing in a mouse model of hereditary tyrosinemia type 1. Hum. Gene Ther., 29: 1315–1326.

Vicencio J., Sánchez-Bolaños C., Moreno-Sánchez I., Brena D., Vejnar C.E., Kukhtar, D. et al., (2022). Genome editing in animals with minimal PAM CRISPR-Cas9 enzymes. Nat. Commun., 13: 1–13.

Wang X., Li J., Wang Y., Yang B., Wei J., Wu J., Wang R., Huang X., Chen J., Yang L. (2018). Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion. Nat. Biotech., 36: 946–949.

Wei C., Liu J., Yu Z., Zhang B., Gao G., Jiao R. (2013). TALEN or Cas9 – rapid, efficient and specific choices for genome modifications. J. Genet. Genom., 40: 281–289.

Wei T., Cheng Q., Min Y.L., Olson E.N., Siegwart D.J. (2020). Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. Nat. Commun., 11.

Yang Y., Kang X., Hu S., Chen B., Xie Y., Song B., Zhang Q., Wu H., Ou Z., Xian Y. (2021). CRISPR/Cas9-mediated β-globin gene knockout in rabbits recapitulates human β-thalassemia. J. Biol. Chem., 296.

Yin H., Xue W., Chen S., Bogorad R.L., Benedetti E., Grompe M., Koteliansky V., Sharp P.A., Jacks T., Anderson D.G. (2014). Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat. Biotechnol., 32: 551–553.

Yoon H.H., Ye S., Lim S., Jo A., Lee H., Hong F., Lee S.E., Oh S.J., Kim N.R., Kim K. (2022). CRISPR-Cas9 gene editing protects from the A53T-SNCA overexpression-induced pathology of Parkinson's disease *in vivo*. CRISPR J., 5: 95–108.

Yuan T., Zhong Y., Wang Y., Zhang T., Lu R., Zhou M., Lu Y., Yan K., Chen Y., Hu Z. (2019). Generation of hyperlipidemic rabbit models using multiple sgRNAs targeted CRISPR/Cas9 gene editing system. Lipids Health Dis., 18: 1–9.

Zha Y., Lu Y., Zhang T., Yan K., Zhuang W., Liang J., Cheng Y., Wang Y. (2021). CRISPR/Cas9-mediated knockout of *APOC3* stabilizes plasma lipids and inhibits atherosclerosis in rabbits. Lipids Health Dis., 20: 1–11.

Zhang L., Wang L., Xie Y., Wang P., Deng S., Qin A., Zhang J., Yu X., Zheng W., Jiang X. (2019). Triple-targeting delivery of CRISPR/

Cas9 to reduce the risk of cardiovascular diseases. Angewandte Chemie Int. Ed., 58: 12404–12408.

Zhang Y., Li H., Nishiyama T., McAnally J.R., Sanchez-Ortiz E., Huang J., Mammen P.P.A., Bassel-Duby R., Olson E.N. (2022). A humanized knockin mouse model of Duchenne muscular dystrophy and its correction by CRISPR-Cas9 therapeutic gene editing. Molecular therapy. Nucleic Acids, 29: 525–537.

Zuo E., Sun Y., Wei W., Yuan T., Ying W., Sun H., Yuan L., Steinmetz L.M., Li Y., Yang H. (2019). Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science, 364: 289–292.

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