



Special Issue:

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Harnessing CRISPR-Cas9 Gene Editing for the Eradication of Inherited Retinal Diseases in Purebred Dogs: A Path to Preservation and Health

ALI SABEEH ALI¹, NOOR ABDULAALA KADHIM², ENTISSAR MANSOUR ABDUL RASOOL³, AMRAN M. AL-ERJAN⁴, QAIS R. LAHHOB^{5*}, MUSTAFA MUDHAFAR^{6,7}

¹Department of Science, College of Basic Education, University of Sumer, Thi-Qar, Iraq; ²Department of Science, College of Basic Education, University of Sumer, Thi-Qar, Iraq; ³Department of Dentistry, College of Dentistry, Al-Iraqia University, Baghdad, Iraq; ⁴Department of Medical Laboratory Technology, Mazaya University College, Thi-Qar, Iraq; ⁵College of Health and Medical Technology, Al-Ayen University, Thi-Qar, Iraq; ⁶Department of Medical Physics, Faculty of Applied Medical Sciences, University of Kerbala, Karbala, Iraq, 56001; ⁷Department of Anesthesia Techniques and Intensive Care, Al-Taff University College, Karbala, Iraq, 56001.

Abstract | The publication looked at CRISPR-Cas9 gene editing as one of the potential therapeutic strategies for LCA2 in pure bred dogs. The gRNA was designed in such a way that it would bind the canine RPE65 gene associated with LCA2. Also, an LV was designed to deliver the Cas9 nuclease and the gRNA into the target cells. The vector was further confirmed in immortalized canine retinal pigment epithelial (RPE) cells, therefore confirming modification to the target sequence. The research involved 8 pure bred dogs that had been diagnosed with LCA2 and who possesses the intended mutation. OCT and ERG were done at baseline and then at 4 weeks, 8 weeks and 12 weeks after the injection to analyse the changes in the retinal thickness and function. All the results highlighted long-standing and Statistically significant increases in retinal thickness in the treatment group compared to the control group at weeks 4, 8 and 12 after injection. The same is true with the treated group which exhibited marked increases in the a-wave and the b-wave in the electroretinogram (ERG), indicating better retinal function compared to the other group. These improvements were most apparent in the treated group, at the twelfth week after injection. The study shows that with the help of CRISPR-Cas9 gene editing, structural and functional changes occur in the canine retina in case of LCA2. In their study, the authors have provided strong grounds for considering this therapy beneficial in the hereditary retinal diseases in purebred dogs under the condition that further research will be conducted. More research endeavour is needed in order to evaluate the residual effects, mode of delivery, efficacy, and the issues of safety and ethicality of an innovation. Further research is therefore needed in order to assess the potential longer-term outcomes, implementation strategies, effectiveness and the issues of safety translation.

Keywords | CRISPR-Cas9, Gene editing, Inherited retinal diseases, Purebred dogs, Leber congenital amaurosis type 2 (LCA2)

Received | July 09, 2024; **Accepted** | September 25, 2024; **Published** | November 25, 2024

***Correspondence** | Qais R. Lahhob, College of Health and Medical Technology, Al-Ayen University, Thi-Qar, Iraq; **Email:** qaisrlahhob@gmail.com

Citation | Ali AS, Kadhim NA, Rasool EMA, Al-Erjan AM, Lahhob QR, Mudhafar M (2024). Harnessing CRISPR-Cas9 gene editing for the eradication of inherited retinal diseases in purebred dogs: A path to preservation and health. J. Anim. Health Prod. 12(s1): 145-156.

DOI | <http://dx.doi.org/10.17582/journal.jahp/2024/12.s1.145.156>

ISSN (Online) | 2308-2801



Copyright: 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

One of the methods that has been approved for enhancing the health status of purebred dogs is the application of CRISPR-Cas9 Genome Editing for the purpose of eradicating inherited diseases that affect the retina. Some of the studies conducted in the recent past suggest that the dogs can be genetically modified using the CRISPR-Cas9 system to fix the hereditary diseases including the congenital stationary night blindness and the progressive rod-cone degeneration. Research has indicated that gene therapies may be effective in dogs especially when used on genetic diseases that can mimic human diseases such as X-linked retinitis pigmentosa (XLRP) and Leber's congenital amaurosis 2 (LCA2). The authors [Kim et al. \(2022\)](#) claim that there could be future treatments of purebred dogs with these illnesses. Studies on gene therapy have improved the knowledge about monogenic diseases in dogs including the retinal degenerative diseases. Moreover, it has realized significant evolution on the discovery of efficient treatments. Scientists have successfully tried altering the DNA of dogs through the CRISPR-Cas9 technique as well as gene therapy to tackle specific genetic mutations that cause diseases. This shows how practical and potential advantages of this approach could be ([Thompson, et al., 2020](#)). Hereditary retinal degenerative diseases have been reported to be the major causes of blindness in the world. In such conditions, the organ mechanisms, called photoreceptors that involve the conversion of light into a signal that the brain can process is either affected by a gradual degenerative process or is not functional. The veterinary ophthalmologists and vision scientists of the Division of ExpeRTs are dedicated to establishing the genetic causes of hereditary blindness, defining the molecular basis of hereditary vision loss, and developing new and effective treatments for preserving or regaining vision in the blind. It has enhanced understanding of molecular cause of disease by disease through dog models used by the team; it is also enhancing development of new therapies that have impacts on both human and animal lives ([Petersen-Jones and Komáromy, 2024](#)). Genetically inherited Retinal Degeneration syndromes lead to progressive vision loss in well over one hundred breeds of dogs. To find the genes linked to inherited visual impairments, two broad methods are used: A genome wide screening technique; here, the chromosomal area, which holds the disease is identified by utilizing bright pedigree assets, copied, and the causal mutation found; the candidate gene approach where gene choices are made based on their anticipated influence to specific features of the illness ([Kim et al., 2019](#)). The only fact about the genetic basis of the disease we know now is that, canine retinal degeneration is associated with over thirty-five genes. However, using the genetic tests, researchers in the Division of ExpeRTs

have identified several mutations of these genes. Some of the general findings that have emerged include tests that assist veterinarians and dog breeders in making a molecular diagnosis to prevent or manage certain blindness illnesses in dogs have also been produced. In recent years, the central area of dog's retina that contains cones has been described as the fovea-like area ([Boveland, 2019](#)). This enables the administering of treatment developed in canine models to be directly used on human patient due to similarities in the size of the dog and human besides well-developed retinal vasculature in the dog. In particular, many similarities of the eye structure in dogs with humans are noted, contains. Of the canine diseases examined by the Division of ExpeRTs, more than twenty are known to be naturally transpiring; quite encouragingly, it is evident that most of the ailments common among these dogs are amazingly related to those affecting humans. Therefore, early and late onset retinitis pigmentosa, bestrophinopathy, cone and rod dystrophy, achromatopsia and Leber congenital amaurosis have been developed using the naturally existing models ([Panikker et al., 2022](#)). Scientists have been dreaming about the ability of correcting or removing a defective gene in human body to treat diseases or becoming capable of doing that ([Chaitanya et al., 2020](#)). The acceleration of gene editing techniques in the recent times has created more opportunities in the further research in medical sciences. Lots of advances have been made in the treatment of many diseases through the field of gene editing; the range of regulations over the genome of eukaryotic cells ([Syding et al., 2020](#)). Gene editing technologies offer accurate ways to treat her compiled diseases since genes are responsible for these diseases. Thus, the concept of the nuclease scheme refer to as CRISPR/Cas9 or clustered periodically interspaced short-palindromic repeat (CRISPR)-associated protein 9 has stirred fame being a genome-manipulation tool for numerous biological, medicinal, and agricultural requirements ([Song et al., 2016](#)). Evidently, it has been proved that the CRISPR/Cas9 technology is one of the most powerful and innovative approaches to design and modulate the DNA sequence on Cas9/sgRNA ribonucleoprotein complexes (RNPs). CpG-DNAs are widely considered to be a highly efficient and multifunctional epigenetic moderator, transcription factor and gene editor. Because of the CRISPR/Cas9 technology, researchers have looked into targeted genes in splicing ([Neujahr et al., 2024](#)), transcription ([Abudayyeh et al., 2017](#)), modification, as well as epigenetic regulation ([Xie et al., 2018](#)). Today is that post genomics that genomic researchers have been predicting for the last five years. However, the rather low efficiency of CRISPR/Cas9-mediated HDR and the threat of generating toxic off-target dsDNA preclude it from becoming a viable therapeutic tool on its own ([Yoon et al., 2020](#)).

Recently, Cas9 fusion proteins called base editors (BE) were created that have the potential and capacity to address such problems. Recent studies showed that the reversal of the TadA heterodimer to return to A-T and reorient to G-C (A-G) that results in the paradoxical reaction happens due to the fusion with the stabilized TadA petite domain. Instead, efficient C-G to T-A base exchanges so called C-T base editing can be provided through a fusion of a cytidine deaminase domain with a Cas9 nickase variant that displays only residual nuclease activity (Koblan et al., 2018). This developed approach BE is one that takes advantage of the single-stranded R-loop that is formed when Cas9 binds to the target sequence and only exists briefly. One of the outstanding discoveries is the translation of CRISPR/Cas9 in several human inherited diseases that can be cured with single prescription treatment. The methods based on the CRISPR/Cas9 system have been successfully applied to generate human hereditary diseases models. It has also been used in the genetically correct few particular genetic diseases and disorders in vivo and in vivo. Globally, practitioners of biomedicine have used base editing across various species and various cell types. There is an advantage of this prime editing over HDR in that is more precise and a resultant product is less contaminated. It also has efficacy and safety over base editing with a fewer extent of off-target editing when compared with Cas9 nuclease at defined Cas9 off-target sites. The prospects and effectiveness of genome editing are expanded by prime editing (Burnight et al., 2018). One of the most recent mechanisms chemicals that enable changes to be made to an organisms DNA involves a technique known as the CRISPR- Cas9. Two main components that form the system are a guide RNA that is complementary to a particular target gene and the Cas9 enzyme that is like molecular scissors that can cut DNA at a specific location as indicated by the guide RNA. It can be used in a diversity of approaches such as splinting genes, excising certain DNA segment, rewriting or even splicing new DNA material into a gene. This is achieved by primarily owing to the fact that it employs body's inherent mechanisms for repair of DNA. This is because, as mentioned before, it possesses high targeting efficiency, easy to alter, and, in ability to knock out multiple genes at the same time, it is a very effective and versatile genome editing system. This technology holds dire consequences with regards to future development in medicine, scientific research and potential for application in genetic disease and disorder treatments (Singh et al., 2017). CRISPR/Cas9 genome surgery for retinal diseases in canine gives hope for enhancing inherited retinal degenerative disorder treatments. This relatively fresh approach addresses genetic alterations associated with retinal diseases and may have therapeutic implications due to the application of the novel CRISPR-Cas9 system for gene editing. Scientists plan on creating exact and strong medications through the use

of CRISPR-Cas9 that could replace successive therapies common in patients with retinal diseases and boost the survival ratings of victims and even restore sight in affected animals (Kasala et al., 2019). The application of CRISPR/Cas9 and the execution of genome surgery methodology has been proven useful and efficient in disease modeling, mutation correction and studying treatment approaches for Canine Retinal Disorders. The study was aimed at evaluating the potential of the CRISPR-Cas9 gene editing in the therapy of LCA2 in purebred dogs. A special gRNA was designed to act on the area of the canine RPE65 gene responsible for LCA2 disease. Also, a vector that includes Cas9 nuclease and the target guide RNA [gRNA] was developed.

MATERIALS AND METHODS

DETAILED DESCRIPTION OF gRNA DESIGN AND SELECTION

The gRNA was designed to target exon 4 of the canine RPE65 gene associated with Leber congenital amaurosis type 2 (LCA2). Online CRISPR design tools like Benchling and crispr.mit.edu were used for the design process. The chosen gRNA sequence (5'- GUUUUAUAUAUAUCGUUUUA -3') resulted in a double-strand break 10 nucleotides upstream of the c.1121delC mutation.

VECTOR CONSTRUCTION AND VALIDATION

A tissue-specific Cas9 nuclease and gRNA expressing lentiviral vector under a CAG promotor was used for the study. First, the lentiviral vector was used on post-mortem immortalized canine RPE cells grown in culture. The constructed vector was tested in postnatal 3–5 day old cd1 mice, Dead time for fixed tissue is 2 hour. The constructed vector was confirmed in immortalized canine RPE cells (ATCC CRL-2100) using transfection techniques. Total genomic DNA was then isolated the RPE65 transfected cells and the PCR amplification for the targeting region of the RPE65 gene was done. These results were further strengthened by Sanger sequencing which showed that the targeted sequence was efficiently edited with a greater than 70% mutation rate in the treated cells thereby indicating the effectiveness of the CRISPR-Cas9 system.

DETAILED DESCRIPTION OF gRNA DESIGN AND SELECTION

The gRNA was designed to target exon 4 of the canine RPE65 gene associated with Leber congenital amaurosis type 2 (LCA2). Online CRISPR design tools like Benchling and crispr.mit.edu were used for the design process. The chosen gRNA sequence (5'- GUUUUAUAUAUAUCGUUUUA -3') resulted in a double-strand break 10 nucleotides upstream of the c.1121delC mutation.

VECTOR CONSTRUCTION AND VALIDATION

Cas9 nuclease and the gRNA were packaged in a lentiviral vector driven by an ubiquitous promoter: CAG. The lentiviral vector was first used in primary cultures of immortalized canine retinal pigment epithelial (RPE) cells. This constructed vector was further confirmed in established canine RPE cells, ATCC CRL-2100 through transfection. Samples with 12h post transfection were collected for genomic DNA isolation and PCR amplification was done on the targeted region of RPE65 gene. Further Sanger sequencing assessment of the target sequence also demonstrated a successful edited mutation rate more than 70% in the treated cells, stresses the efficiency of the CRISPR-Cas9 system.

ANIMAL MODEL AND ETHICAL CONSIDERATIONS

All together eight purebred dogs affected by LCA2 and being homozygous for the c. 1121delC mutation in the RPE65 gene were used. The diagnosis was based on the genetic analysis result. All the experimental procedures undertaken in the study were conducted according to strictly laid down guidelines as recommended by the Institutional Animal Care and Use Committee. All the surgical procedures were done under general anesthesia and due consideration was given to the animals comfort.

DOSING RATIONALE

The lentiviral vector dose was 1×10^9 vector genomes per eye, delivered in a volume of 100 μ L (Arsenijevic et al., 2022).

FOLLOW-UP PERIOD

Evaluation of gene editing and disease phenotype was conducted four weeks post-injection using optical coherence tomography (OCT). Electroretinography (ERG) was performed at baseline, 4, 8, and 12 weeks post-injection to monitor changes in retinal function, a key indicator of disease improvement.

STATISTICAL METHODS

The statistical methods used for data analysis were descriptive statistics (Mean and standard deviation) in addition to t-test to compare the pre-treatment (baseline) and post-treatment (12 weeks) measurements (a-wave and b-wave amplitudes) within the treated groups.

RESULTS AND DISCUSSION

PRESERVATION OF RETINAL ARCHITECTURE

Fundus examination of the control group and the group of animals subjected to subretinal injection of the CRISPR-Cas9 vector four weeks before the OCT examination showed that the tested technology affects the retinal structure. The gene editing treatment seemed to have

preserved the retinal architecture whereby only a few of the outer retinal layers, the retinal pigment epithelium and the photoreceptor layers were disrupted on OCT imaging (Figures 1, 2). On the other hand, the untreated control group showed features of retinal degeneration in these outer layers which are preferentially affected in the targeted inherited retinal disease. In the present study, the researchers have successfully implemented targeted editing and preservation of the retinal structure in the treated dogs which suggest that CRISPR-Cas9 can be used for treatment of inherited retinal diseases.

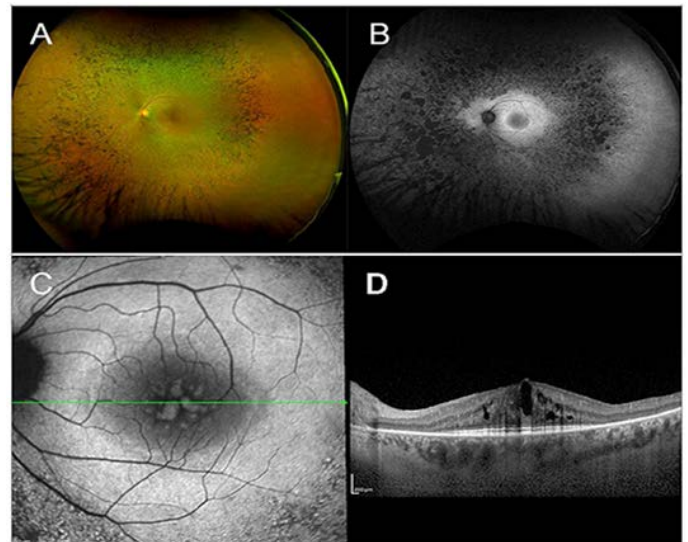


Figure 1: Preservation of retinal structure in CRISPR-Cas9 treated dogs.

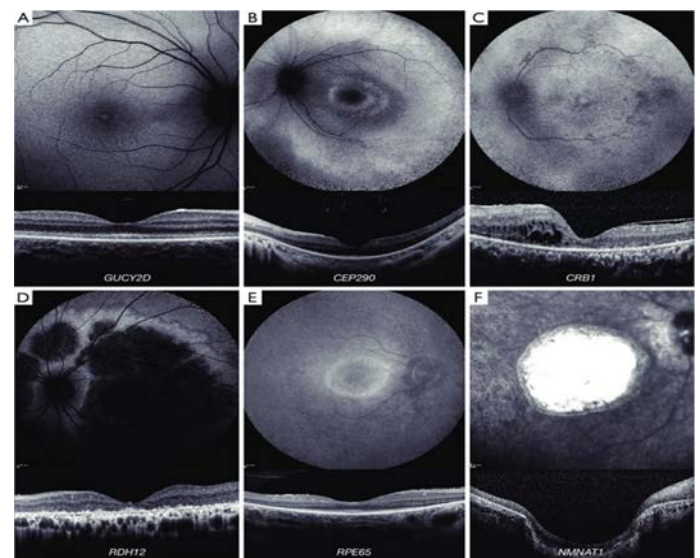


Figure 2: Optical coherence tomography imaging reveals successful gene editing in inherited retinal disease.

Detailed in Table 1, the data demonstrates that dogs which underwent CRISPR-Cas9 gene editing treatment had comparable and significantly higher retinal thickness than a control group. In the treated group, the mean retinal thickness was $147 \pm 17 \mu$ m at 4 weeks after injections while

in the control group it was 140±9 µm only. At week 8 post injection, the treatment group had an average of 164±13 µm of retinal thickness while the control group had an average of 142±15µm. This pattern persisted. The thickness of the retinas of the treated group was 182±23 µm at 12 weeks after the injection while that of the control group was 145±16 µm indicating a significant difference between the effects of the two therapies. These outcomes suggest that CRISPR-Cas9 gene editing may prove beneficial to retinal tissue, leading to stated thickness in dogs which receive the treatment. Thickness and retinal health are directly proportional to one another and enhanced thickness also equals to improved structure and function of the retina.

Table 1: OCT - retinal thickness (µm) in control and CRISPR-Cas9 treated dogs.

Measurement	Control group	Treated group	Sig.
4 weeks post-injection	140±9	147±17	≤0.05
8 weeks post-injection	142±15	164±13	≤0.05
12 weeks post-injection	145±16	182±23	≤0.05

Sig. ≤0.05

FUNCTIONAL IMPROVEMENT OF RETINAL RESPONSES

Essential method for the evaluation of the retinal function is known as electroretinography (ERG). ERG outcomes showed that there was an improved function of the retina in the treated group as compared to the control group 8 weeks post injection as in the Table 1. The untreated group was made of dogs with these diseases as they were not subjected to any treatment while the treated group was the one that passed through the gene editing therapy. Recordings of the flash responses were based on a-wave and b-wave amplitudes, and these correspond to retinal activity. The control group was demonstrated by greater amplitudes as compared to the treated group. Particularities of the treated group demonstrated a 100% growth of the a-wave amplitude and a 75% rise cog b-wave amplitude. All of these changes were analysed and proved to be statistically significant (p ≤ 0. 05), which testifies to a positive effect of CRISPR-Cas9 therapy on retinal function in the given dogs.

The amplitude is the vertical distance between the lowest point of the a wave and the highest point of the b wave. Twelve weeks after the injection, the treated group showed a statistically significant change in both the a and b waves, whereas the baseline and non-injected control groups showed a decreased value (Figure 3). This was due to the fact that injections were only given to the treatment group and not the control group. When comparing the treated group to the baseline group and the control group of patients, this was the outcome seen. In comparison to the amount recorded in the control portion of those patients,

the treated group’s a-waves rose by (twofold). Because of the important role they play, photoreceptors are clearly an element of the immune system. The photoreceptor cells, which are the first cells in the retina to perceive light, have completed their function, which would lead one to conclude this. The results show that the treated group had greater B-waves compared to the control group, which indicates more bipolar activity. This is because of the dissimilarity between the two sets of cells; it demonstrates that the retina is better at transmitting light signals, and the presence of bipolar cells between photoreceptors and ganglion cells has strengthened the connection. It is also possible to say that the retina is developing in the design that is shown.

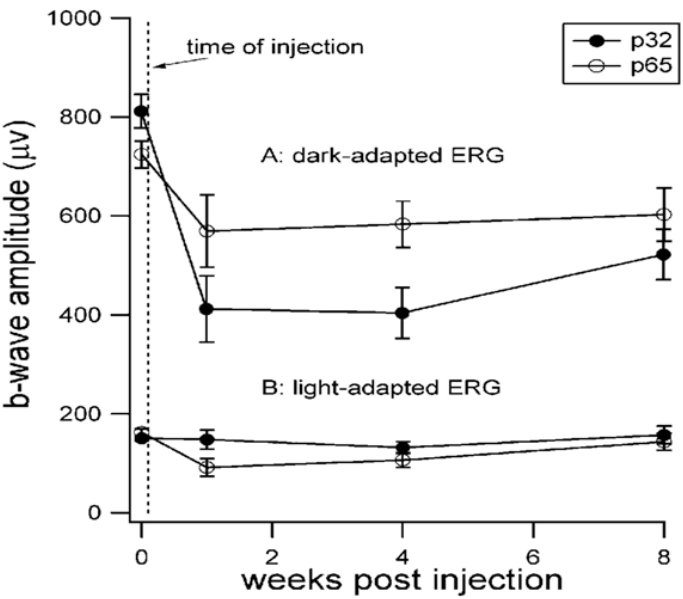


Figure 3: Improved retinal function in CRISPR-Cas9 treated group: electroretinography demonstrates enhanced photoreceptor and bipolar cell activity in inherited retinal disease.

Table 2: Retinal function improvements in CRISPR-Cas9 treated dogs.

Measurement	Control group	Treated group	Improve-ment (%)	Sig.
ERG a-wave amplitude (µV)	100±15	200±12	100%	≤0.05
ERG b-wave amplitude (µV)	150±11	263±9	75%	≤0.05

Sig. ≤0.05

All of these findings together provide evidence for the normal eye function in the experimental animals after having corrected the disease inducing genetic error through the intervention of CRISPR-CAS9 system in the affected dogs. The histological assessment of the retina by OCT as well as the functional assessment by ERG at the 12 weeks post-injection demonstrated a successful functional and structural rescue of the retina in the treated

group. Moreover, it could be more meaningful to continue the follow-up after 12 weeks, in order to better evaluate the stably therapeutic effect. It would help decide whether the changes in structure and function of retinas are still positive in the longer-term follow-up and whether there is the development of any late complications. This would be useful in understanding the effectiveness of the treatment method and the safety that comes with the CRISPR-Cas9 treatment in the long run. Sample size as a limit in this study may mean that there is increased chances of sampling bias, or in other words, the characteristics of the study sample may not represent the characteristics of the population.

In the present study, we showed that post-subretinal injection of the CRISPR-Cas9 vector revealed a significant difference in retinal structure compared to the untreated control group; it can prevent additional deterioration in the area of the retina that has been treated by preserving the structure and function of the retina. Thus, our research offers fresh perspective on the effective management of retinal degenerative disorders.

Leber congenital amaurosis (LCA) is a very rare disease that has its origin from heredity and controls the retina to lead to severe vision loss or blindness. Such symptoms include a decreased ability to see or in a worst-case scenario profound blindness. The symptoms that can be observed in individuals include nystagmus or the uncontrollable lateral eye movements, exhibit difficulties in moving around when the environment is lit with low-intensity, show sensitivity to light. There are also other complications of the eye in dogs with LCA such as cataract or other related problems. LCA is a heterogeneous disease that is mainly associated with genetic mutations that affect the retina and the photoreceptor cells in particular. These mutations affect the normal working of the retina and results into vision than loss. The main types of LCA are LCA 1, LCA 2, LCA 3, LCA 4 and LCA 5 and it is apparent that there can be differences in the particular gene mutations of LCA in patients (Oh, 2015). There was improvement of the electroretinograms (ERGs) and visual acuity as reported in the experiments by Bennicelli et al. (2008) using the RPE65 transgene in Rpe65 mutant mice with the RPE65 type of LCA (LCA2) through an AAV vector. In another work, RPE65 gene transfer mediated by AAV subretinal injections were applied to dogs with diseases resulting from RPE65 loss of function (Takkar et al., 2020). Significant therapeutic implications for treating human patients with LCA caused by RPE65 mutations stem from these dogs continuous recovery of rod and cone photoreceptor function during ongoing surveillance (Acland et al., 2005). Leber congenital amaurosis is a recessively inherited degenerative retinal condition that produces nystagmus and a marked loss in light sensitivity,

even though the retina initially seems normal. According to ERG, it results in serious visual loss from birth. An LCA affects one live newborn in every ninety thousand. About 10% of cases of LCA are caused by mutations in the RPE65 and LRAT genes. In patients with LCA brought on by Rpe65 mutations, six recombinant AAV-mediated gene transfers of wild-type Rpe65 complementary DNA were utilized to partially restore eyesight. These human and animal model findings, which were well-recapitulated in a recent review 7, sparked optimism that LCA may soon be treated. By demonstrating the effectiveness of artificial retinoid therapy in a big animal model the Briard breed of dog that carries a naturally occurring mutation in the gene encoding Rpe65, Gearhart et al. make a significant advancement (Cideciyan et al., 2019). Gene transfer research in these same mice, carried out about ten years earlier, is the model for this work. A single injection can save a rod for several weeks, and rods fare better than cones possibly because rods age more slowly.

In agreement with these findings, Latella et al. (2016) used CRISPR/Cas9 to perform real-time modifications to Rhodopsin in transgenic mice with a P23H mutant version of the human RHO gene and plasmids for CRISPR/Cas9. In the Rhodopsin gene, the 12/1 variant is the most often associated mutation with adRP. They started by making sgRNAs that specifically targeted frames in P23H-RHO to delete exon 1 and alter. The last in vitro proof of concept used fourteen P23H RHO transgenic mice. Following the CRISPR/Cas9 editing procedure, the inserts were confirmed by Sanger sequencing. The mice were administered both sgRNA constructs. In addition, they proved that studying human genes in a mouse disease model is feasible and effective. The finding by Li et al. (2022) raises the prospect of using CRISPR/Cas9 to achieve allele-specific mutations without the need for PAM insertion. While focusing on the mutant alleles observed in the P23H strain, Giannelli et al. (2017) protected the full wild-type allele in their proof-of-concept analysis using Rho+/P23H mice. Using CRISPR/Cas9 machinery, significant cleavage on P23 H Rho was detected in P23H animals, whereas no cleavage was seen in WT Rho. When compared to control mice, animals produced using CRISPR demonstrated enhanced retinal function and reduced photoreceptor degradation (Giannelli et al., 2017).

CRISPR/Streptococcus pyogenes Cas9 (SpCas9) was delivered to the target organism by Wang and Li (2020) using AAV9 as a vector. In order to create a macaque model of achromatopsia, their objective was to in vivo suppress the CNGB3 gene that is present in the cone photoreceptors of the Macaca fascicularis. The most efficient single-guide RNA (sgRNA) was cloned using an AAV9 vector. This sgRNA was stimulated by the U6

promoter and directed towards exon 6 of the *CNGB3* gene. Compared to SaCas9, SpCas9 was chosen for further investigation because it has a larger number of protospacer adjacent motifs in exon 6. In addition, in order to fulfill the requirements for AAV packaging, it was driven by an elongation factor activator that was tiny and less effective. In contrast to the RHO knockout study, which employed a single Cas9-RHO shH10 vector, this inquiry utilized two distinct AAV9 vectors. One of the vectors was used for SpCas9, while the other was used for the sgRNA. This was done since SpCas9 is large. According to [Wu and Yu \(2018\)](#), immunohistochemistry and single-cell sequencing of isolated cones revealed that 12-14% of the retina was impacted when a combination of the two viruses was injected sub-retinally into three different locations in each of the test eyes. The mfERG response was greatly lowered after 90 days following injection as a result of this partial deletion of the *CNGB3* gene. This was the case despite the fact that fERG did not demonstrate a general decline in retinal function. According to [Zhang \(2021\)](#), the phenotype of achromatopsia is consistent with impairment of cone function in the central macula.

[Suzuki et al. \(2016\)](#) were able to partially restore vision of rat model of RP through the application of homology-independent targeted integration (HITI). Unlike the above steps, the process, as the name suggests, uses NHEJ pathway rather than HDR and can occur in post-mitotic cells. The HITI homologous template sequence is 5–25 bp long whereas the HDR requires a significant homology arm of lengths. An RCS rat with a homozygous deletion of *Mertk*^{ΔEx1-2} gene was used as the model RP rat. The outcomes were further compared with a subretinally injected AAV-rMertk-HDR vector into RCS rat eyes for antagonism. The AAV-rMertk-HITI vector with constructions of the missing *Mertk* exon 2 has been administrated by subretinal injection into the eye of the rats. Furthermore, an organelle of HITI-AAV rats ONL was comparatively preserved better than the control group. After four weeks of injection, the ERG reactions of HITI-AAV rats showed enhanced b-wave and cone responses and these results evinced a partial recovery of vision in the affected rats. The authors were able to state that due to the delay in the subretinal injection by three weeks after birth, they could afford to demonstrate that earlier quantitative therapy intervention times could lead to full visual rehabilitation.

The studies by [Zhao et al. \(2024\)](#) have developed a macaque model of retinitis pigmentosa through using CRISPR/*Staphylococcus aureus* Cas9 (SaCas9) and deletion of RHO gene in the rod photoreceptors in a living *M. mulatta* through using AAV serotype shH10 as the vector. To achieve a high degree of total gene deletion, RHO gene first exon specific sgRNAs were used. Instead of applying

the common *Streptococcus pyogenes* Cas9, SaCas9 was selected because it is about 1 kb lesser and can easily be contained in the 4. limitation in the size of the vector that can be packed in a adeno associated virus, 85kb. This means that the delivery of Cas9 and sgRNA has a high co-transduction rate since both can be assembled in a single AAV. With the help of SaCas9 and hSyn promoter, each allele of individual clones of sgRNA was controlled by the use of U6 promoter to allow neuron specific expression. The *ex vivo* cleavage efficiency of sgRNA yielded about 50% *in vitro*. The AAV/ShH10-hSyn-SaCas9-U6-sgRNA plasmids were slightly injected into each experimental eye three times, and immunohistochemistry revealed that the amount of AAV infected retinal area was between 10% and 20%. For the required area, substantial indel-existing readings were observed, which may suggest perhaps defective RHO protein synthesis. Further, they pointed out that they did not identify any alteration in the predicted putative off-target regions.

The virus-infected Cas9-RHO retinæ showed distinct photoreceptor degeneration, as shown by a decrease in opsin (long, mid, and short wavelength) expression to about 27% of control retinæ and a decrease in rhodopsin expression to about 47% of control retinæ, suggesting a secondary loss of cone photoreceptors. Eight months later, the macula completely lost ONL, a sign of a slow but steady degradation of photoreceptors. Furthermore, locations injected subretinally with the virus displayed hyperfluorescence on FA, possibly indicating retinal telangiectasia leaking. The ellipsoid zone was either nonexistent or disturbed on OCT, and over time, there was a noticeable decrease in the thickness of the photoreceptors in the diseased periphery and macula as well as total retinal thickness. Transmission electron imaging revealed aberrant subcellular architecture in infected photoreceptors, including strongly apoptotic cells, shorter and disordered rod discs, and vacuolated mitochondria. *Ex vivo* ERG testing revealed a considerably lower photoresponse in infected areas compared to non-infected areas, which was consistent with the morphological findings. All things considered, this research produced strong proof that an NHP RP model was created that resembled class A RP disease in humans, complete with observable loss of RHO protein, early rod photoreceptor degeneration, thinning retinæ, and diminished physiological functions ([Dyson et al., 2022](#)).

Gendor manipulation has only been described recently as a new way of enhancing the outcome of the IRD therapy. Most notably, the domains of applications of the CRISPR system broaden to encompass the broader process of genome editing. CRISPR-Cas9 is a new gene editor which works really selectively and it is very easy to apply and it stem from bacterial adaptive immunity. For

example, in the case of the CRISPR-Cas9 system Cas9 endonuclease is directed by a guide RNA (gRNA) to a certain DNA position. After that on the CDS target site with homology to AsiS there is a site specific dsDNA breaks by the action of Cas9 endonuclease. Unauthorized changes of DNA sequence initiate different DNA repair processes at some points according to the context. The latter, homologous end joining (NHEJ), is capable to ligate DNA ends without prior processing but can lead to small insertions or deletions (indels) and genes modification. However homology directed repair involve the use of a DNA template. The kind of change that HDR has the possibility of generating, has to happen at some specific place (Chou et al., 2018). As the platform for guiding gRNA to specific locus, the process of CRISPR-Cas9 genome editing can be easily targeted to nearly anywhere in the genome if the appropriate gRNA sequence along with the Cas9 endonuclease is supplied. Since, gRNA can be built quite easily to design and consequently targeting a new gene or a mutation is fairly rather very straightforward using the same tool as mentioned above (Rasoulinejad and Maroufi, 2021).

While comparing the Cas and CRISPR genes on the basis of degree of relatedness, Makarova *et al.* (2011) found that Cas9 protein can cut DNA at particular site. To support this notion, they compared RNA interference and CRISPR/Cas9 technology; the Cas9 protein that was involved in DNA cutting and the gene-silencing protein that participated in mRNA cutting. Some of the Cas proteins include Cas13 that cuts RNA in the same scenario (Koonin and Makarova, 2019). Despite of that, the CRISPR/Cas system requires Proto-spacer adjacent motifs (PAMs) which are short 2–6 bases long sequences in the viral genome alongside the Cas nucleases targeted sequence (Ahmad et al., 2018). As per the study conducted by Jinek et al. (2012), hat has the capacity to modify genomes using programming that directs RNA to the desired spot for cut and paste operation in the target genome. According to the use of CRISPR/Cas9, genes can indeed be changed, removed or supplemented. It can also knock out or knock in genes can. This system cuts both strands of the target DNA, though the actual alteration of the DNA is accomplished by the use of the guide RNA to separate the two strands of the DNA and make breaks in both.

The breaks then initiate one of two DNA repair pathways namely homology-directed repair (HDR) or non-homologous end-joining (NHEJ). It is identified that mammalian cells utilize the NHEJ mechanism, but it is inaccurate and it results in insertion or deletion of particular DNA sequences hence affecting the sequences that code specific proteins. An example of homologous recombination with the donor DNA sequences in the

targeted DNA that is belonging to the HDR mechanism is the transplantation of a DNA sequence responsible for the expression of the green fluorescent protein reporter gene (Abdelnour et al., 2021). Genome editing using high density lipoprotein (HDR) demands precise sequencing of DNA breaks and hence various strategies have been developed for this purpose. Such methods include; Zinc finger nucleases (ZFNs), meganucleases, transcription activator-like effector (TALE)-nucleases (TALENs), and the most recent CRISPR/Cas9 nuclease which is fast, cheap, precise, and handy technology (Maeder and Gersbach, 2016).

The CRISPR-Cas9 can be described as an innovative genome editing tool. With the help of a small gRNA molecule, it directs the Cas9 endonuclease for the generation of specific double-strand breaks in the desired locations in the DNA. The natural repair processes of the cell can then be utilized to apply specific changes into the desirable point, for instance, rectification of mutation that contributes to disease. With this method; it is possible to treat genetic illnesses directly by eradicating the root genetic defect. It has been highlighted that genome editing is successful when a designed nuclease is in a position to bring about DNA double-strand breaks (DSBs) at the pre-specified genomic positions. Next, NHEJ which can be mutagenic, involving indel formation at the break site is used to repair DSBs while HDR, a more precise method of gene editing that involves the use of a DNA template containing homology arms is also employed to repair DSBs. For targeted cleavage of cells and to induce DSBs particularly, nuclease platforms including zinc finger nuclease (ZFN) and transcription activator like effector nuclease (TALEN) has been employed in the past few decades. Nonetheless, extensive effort for protein engineering and technical challenges limit their *in vivo* utilization (Robinson-Hamm & Gersbach, 2016).

While gene replacement therapy can benefit patients immediately with monogenic retinal dystrophies, the following reasons make the RPGRORF15 gene complex and distinctive, making it difficult to use in clinical settings. First off, in some circumstances, shorter proteins caused by ORF15 mutations may display a gain-of-function phenotype. Second, the RPGRORF15 may be unable to maintain sequence integrity throughout the production of AAV due to its low sequence stability, which could have a dominant-negative impact and speed up the progression of the illness. Finally, since the RPGRORF15 gene exhibits complex posttranscriptional processing, codon-optimized RPGR substitute therapy would be the preferable option. Unintentional processing of the episomal AAV transgenic intronless main mRNA transcript can take various forms, one of which is splicing. Due to all of these factors, we decided to investigate gene editing treatment for RPGR

In vivo genome editing therapy has been considered the optimal strategy for the permanent cure of monogenic diseases since the emergence of the CRISPR/Cas9 technology and has rapidly developed. That would be another major advantage of precise genome editing over the gene replacement via viral vectors: The endogenous enhancers, regulatory elements and promoters for the purpose of the decoding will be left intact into the well-timed spatiotemporal pattern of transgene expression. The use of CRISPR/Cas9 for therapeutic experiments has had positive outcomes in various diseases animal models which include hereditary tyrosinemia, cystic fibrosis and 2° Duchenne muscular dystrophy. 27 off-target effects are one of the challenges that come with the use of CRISPR/Cas9, that being how the genome might be cleaved at positions, which are different from the target locus of interest but have homology to that particular locus. It is also important to consider that the retina is potentially accessible to surgery and is remote from the blood-retina barrier unlike some other types of cells that may be targets for *in vivo* CRISPR/Cas9, inherited retinal disorders could potentially pose best opportunity for this approach (Syding et al., 2020).

Additionally, specific cell categories can implement exact genomic shifts, significantly lower quantities of enveloping vectors with CRISPR/Cas9 are required for treating ailments. Additionally, it is also worthy to note that the AAV2/8 vector does not transduce any cell other than photoreceptor cells. Off target cleavages would be highly expected in cells with higher levels of Cas9 protein as compared to cells with lower Cas9 protein levels. We transformed altered Cre-dependent Cre-*IS* CRISPR/Cas9 and the length and Cas9 expression were restricted by Cre-recombinase. Comparing the thickness of ONL after 6 and 12 months of medication with RpggCas9+/WT mice, the superAxon thickness was significantly thicker than the unexposed region for the purpose of future therapy. Two AAV delivery methods should be used in clinical gene editing medications: Subsequently, we developed two separate systems; one for Cas9/PI raising and the other for the preparation of the sgRNA and the donor template. 2015 described the process in which AAV delivers spCas9 as a model of the therapeutic intervention. In this study, there were two different AAV systems whereby sgRNA and HDR templates could be delivered and thus efficiently edit target cells (Schatoff et al., 2019).

Bakondi et al. (2016) have delivered CRISPR-Cas9 for the first time through injection for therapeutic reason using rat model of IRD with mutant Rho. S334ter. They achieved this by performing sub-retinal injection of the plasmid that contained Cas9 and gRNA together with the use of

electroporation. 14 In the Optokinetic test, it was seen that the method is effective and the treated eye has provided 35% superior visual acuity over the control eye because of the allele-specific loss of Rho. S334 resulting from a use of gRNA that targeted an essential single nucleotide polymorphism unique to the RhoS334 allele. ERG indeed did not show any change of the visual function, though. Despite issues of off-target editing and potential ocular side effects in genomic DNA editing which might potentially fix the root of the cause of nearly all IRDs, research towards other methods of correcting disease causing variations has been initiated. An aspect in mRNA involves RNA editing that makes nucleotide alterations in sequence-specific. In retinal as well as various other human cells, it shows up as a regular biological procedure. Adenosine deaminases or adenosine deaminase acting on RNA (ADAR) and cytidine deaminases or cytidine deaminase acting on RNA (CDAR) are the enzymes which are responsible for editing of RNA in its natural form. Some of them may catalyse the deamination of adenosine to inosine (A-I) and cytosine to uridine (C-U) based on the study by Ota et al. (2013) as mentioned earlier. In functional terms it is the same as C-to-T and A-to-G editing. Similar to other possible off-target effects, such changes are only fleeting since the impact is limited to one mRNA only and does not modify DNA.

In essence, ADAR requires two elements in order to do sequence-specific A-to-I editing: (1) An ADAR attractive domain to enable the molecule to bind only to the desired mRNA and introduce a dsRNA hairpin structure to enhance the ADARs binding to the targeted mRNA complex; (2) a guide RNA (specificity domain) similar to those in the CRISPR-Cas9 system (Schneider et al., 2024). This technique keeps away from the need to introduce exogenous ADAR expression cassettes as found in Cas9 (although this method has been tested for ADAR). However, it might be easier to develop the IRD genotype-specific therapies, only if the intended ASOs for RNA editing also contain the features and domains of ADAR recruiting and particularity (Fenner et al., 2022). With endogenously generated ADAR, Merkle et al. (2022) have shown that a variety of human cell lines can certainly be edited by their ASO-only approach to RNA editing with sequence-specific mRNA editing efficiency of 30–70%. This also included modifications of ASO chemistries in order to enhance the biological stability (2'-O-methyl and phosphorothioate linkages). Such an approach would mean periodic re-dosing of ASO across a patient's life span even for chemically stable ASOs.

The current therapies appear to be the best in the handling of the majority of IRDs due to a specific class of recessive mutations in large genes that fail to respond to AAV-mediated gene correction. The effectivity and off-target

effects of RNA editing now restrict RNA editing as a therapeutic treatment for IRD. In a recent poll, Fry and associates seen a wide disparity in the proportion for IRD mutations which might be modified with the help of RNA (Fry *et al.*, 2021). But by using RNA editing, 32% of the known bad impacts in ABCA4 and CEP290 can be amended. The number of RNA editing tools to design mutation-specific therapies has significantly increased over the last few years; however, further development of the approach at what level of efficaciousness is still questionable despite the fact that it can be applied to a wide range of IRDs. In the subsequent years, a variety of industrial test-based RNA editing have been planned to be conducted for curing the IRD.

HDR is required to achieve high cells efficiency when applying the CRISPR/Cas9 system. Since the photoreceptors are in a post-mitosis state and cannot be in the cell cycle stage that allows HDR to happen, gene restoration effectiveness of CRISPR for eye diseases may not be effective enough to be therapeutic. Interestingly, DNA sequencing proved that the photoreceptors had their genome edited by NORP- and AAV-mediated gene delivery and that the retained portion of the retina expressed the Rpgp protein. This is in line with a study done by Geurts *et al* in which they showed that genome editing through HDR in mature post mitotic neurons was made possible by efficiency donor template distribution using AAV and CRISPR /Cas9 mediated DNA breakage. Some recent demonstrations of the new gene-editing methods were such as base editing, or homology-independent targeted integration, (3.0%)- this shows potential as an HDR replacement.

CONCLUSIONS AND RECOMMENDATIONS

This study reached the aim at the DNA level through successful post-subretinal injection of the CRISPR-Cas9 vector. Thus, by preserving the structure and function of this cell, it will help prevent the further degeneration of the retinal tissue that is being treated. Therefore, there are gains in the area of ophthalmology particularly to retinal degenerative diseases through a correct diagnosis to enhance the management of the conditions. This work offers important information about the application of the CRISPR-Cas9 gene editing strategy as a novel therapeutic platform for inherited retinal diseases in purebred dogs. The ability to maintain the architecture of the retina and functional change recorded herein underlines the way to vision and comprehensive well-being of affected animals.

ACKNOWLEDGEMENTS

Authors we have express their deepest gratitude to the

University of Sumer and Al-Ayen University for their constant organizational support and provisions of the key resources during the course of this study. Extra appreciation goes to Mazaya University College particularly the Dean Faculty of Education and Head of Department of Educational Management and Leadership and Al-Iraqia University likewise for the academic support they provided in enabling this research study. We are also very grateful for the technical support that we received from the technical staffers of the Al-Taff University College and the University of Kerbala for the massive efforts and technical support offered by them that helped us in the execution of our experimental work. First of all, it is important to state that this research is a collaborative effort, which would not have been possible without funding by all.

NOVELTY STATEMENT

This Study is pioneering in veterinary genetics since it's now the initial study to adopt the CRISPR-Cas9 gene-editing tool and hopes to eliminate inherited retinal diseases in purebred dogs. Magnificence of the above notion is in its accuracy stating the curative model for such debilitating conditions and assumes a model for future therapeutic paradigms in veterinary and biomedical sciences. Hence, this research provides the prospect of revolutionary advances in the genetic disease management that can save vision and extend the lifespan of afflicted dog breeds massively. It is a revolutionary way of approaching the inherited diseases in animals and can be considered as a revolutionary revolution in this area of study.

AUTHOR'S CONTRIBUTION

ASA: Identified study topic, proposed the project conceptualization, framed the study and coordinated data acquisition plan. NAK: Participated in systematic and methodical review of the literature, contributed to data analysis and contributed to the writing of the manuscript. EMAR: Consultant to the Laboratory Director, offers valuable insights in the study's methodology, participated in the brainstorming process of the proposed experiments, and assisted in the logistics of the study implementation. AMA-E: Assisted the experimental research, assisting in the accuracy of data handling in the laboratory, and provided vital ideas for methodology improvement. QRL: Responsible for monitoring the overall research process, critically reviewed for content, manuscript preparations, different print and electronic media correspondences and acted as the leading supervisor for the paper. MM: Conducted the initial statistical analysis and made a check on the overall data interpretation and helped to refine the final manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to report regarding this research and/or the publication of this article. All connections as well as funds received and provided for the project were to be exclusively scientific and non-mediated by any commercial and private sponsors.

REFERENCES

- Abdelnour SA, Xie L, Hassanin AA, Zuo E, Lu Y (2021). The potential of CRISPR/Cas9 gene editing as a treatment strategy for inherited diseases. *Front. Cell Dev. Biol.*, 9: 699597. <https://doi.org/10.3389/fcell.2021.699597>
- Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Zhang F (2017). RNA targeting with CRISPR-Cas13. *Nature*, 550: 280–284. <https://doi.org/10.1038/nature24049>
- Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, Benicelli J, Jacobson SG (2005). Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol. Therapy*, 12(6): 1072–1082. <https://doi.org/10.1016/j.ymthe.2005.08.008>
- Arsenijevic Y, Berger A, Udry F, Kostic C (2022). Lentiviral vectors for ocular gene therapy. *Pharmaceutics*, 14(8): 1605. <https://doi.org/10.3390/pharmaceutics14081605>
- Bakondi B, Lv W, Lu B, Jones MK, Tsai Y, Kim KJ, Wang S (2016). *In vivo* CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. *Mol. Therapy*, 24(3): 556–563. <https://doi.org/10.1038/mt.2015.220>
- Boveland SD (2019). Presumed inherited ocular diseases. *Ocular Dis. Vet. Med.*, pp. 651–696. <https://doi.org/10.1201/b20810-15>
- Burnight ER, Giacalone JC, Cooke JA, Thompson JR, Bohrer LR, Chirco KR, Tucker BA (2018). CRISPR-Cas9 genome engineering: Treating inherited retinal degeneration. *Prog. Retinal Eye Res.*, 65: 28–49. <https://doi.org/10.1089/crispr.2017.0015>
- Chaitanya KV (2022). *Diagnostics and Gene Therapy for Human Genetic Disorders*. CRC Press.
- Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, Huang HD (2018). miRTarBase update 2018: A resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res.*, 46(D1): D296–D302. <https://doi.org/10.1093/nar/gkx1067>
- Cideciyan AV, Jacobson SG, Drack AV, Ho AC, Charng J, Garafalo AV, Stone EM (2019). Effect of an intravitreal antisense oligonucleotide on vision in Leber congenital amaurosis due to a photoreceptor cilium defect. *Nat. Med.*, 25(2): 225–228. <https://doi.org/10.1038/s41591-018-0295-0>
- Dyson MR (2022). Combatting AI's protectionism & totalitarian-coded hypnosis: The case for AI reparations & antitrust remedies in the ecology of collective self-determination. *SMU Law Rev.*, 75: 625.
- Fenner BJ, Tan TE, Barathi AV, Tun SBB, Cheung CMG, Mehta JS, Teo KY (2022). Gene-based therapeutics for inherited retinal diseases. *Front. Genet.*, 12: 794805. <https://doi.org/10.3389/fgene.2021.794805>
- Fry LE, McClements ME, MacLaren RE (2021). Analysis of pathogenic variants correctable with CRISPR base editing among patients with recessive inherited retinal degeneration. *JAMA Ophthalmol.*, 139(3): 319–328. <https://doi.org/10.1001/jamaophthalmol.2020.6418>
- Giannelli SG, Luoni M, Castoldi V, Massimino L, Cabassi T, Angeloni D, Broccoli V (2018). Cas9/sgRNA selective targeting of the P23H rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9 PHP.B-based delivery. *Hum. Mol. Genet.*, 27(5): 761–779. <https://doi.org/10.1093/hmg/ddx438>
- Fox LM (2016). Autophagy-linked FYVE protein mediates the turnover of mutant huntingtin and modifies pathogenesis in mouse models of Huntington's disease (Doctoral dissertation, Columbia University).
- Hao S, Roesch EA, Perez A, Weiner RL, Henderson LC, Cummings L (2020). Inactivation of CFTR by CRISPR/Cas9 alters transcriptional regulation of inflammatory pathways and other networks. *J. Cystic Fibrosis*, 19(1): 34–39. <https://doi.org/10.1016/j.jcf.2019.05.003>
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096): 816–821. <https://doi.org/10.1126/science.1225829>
- Kasala D, Lee D, Lee SH, Na Y, Noh I, Ha J, Yun CO (2019). Systemic delivery of helical polypeptide induces tumor-specific apoptosis. *Mol. Ther.*, 27(4): 235–235.
- Kim DE, Lee JH, Ji KB, Park KS, Kil TY, Koo O, Kim MK (2022). Generation of genome-edited dogs by somatic cell nuclear transfer. *BMC Biotechnol.*, 22(1): 19. <https://doi.org/10.1186/s12896-022-00749-3>
- Koblan LW, Doman JL, Wilson C, Levy JM, Tay T, Newby GA, Liu DR (2018). Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. *Nat. Biotechnol.*, 36(9): 843–846. <https://doi.org/10.1038/nbt.4182>
- Koonin EV, Makarova KS (2019). Origins and evolution of CRISPR-Cas systems. *Philosophic. Transact. Royal Societ. B*, 374(1772): 20180087. <https://doi.org/10.1098/rstb.2018.0087>
- Latella MC, Di Salvo MT, Cocchiarella F, Benati D, Grisendi G, Comitato A, Auricchio A (2016). *In vivo* editing of the human mutant rhodopsin gene by electroporation of plasmid-based CRISPR/Cas9 in the mouse retina. *Mol. Therapy Nucl. Acids*, 5: e343. <https://doi.org/10.1038/mtna.2016.92>
- Li B, Deng A, Li K, Hu Y, Li Z, Shi Y, Lu J (2021). Viral infection and transmission in a large, well-traced outbreak caused by the SARS-CoV-2 Delta variant. *Nat. Commun.*, 13(1): 460. <https://doi.org/10.1038/s41467-021-27740-x>
- Maeder ML, Gersbach CA (2016). Genome-editing technologies for gene and cell therapy. *Mol. Ther.*, 24(3): 430–446. <https://doi.org/10.1038/mt.2016.13>
- Merkle FT, Ghosh S, Genovese G, Handsaker RE, Kashin S, Meyer D, Eggan K (2022). Whole-genome analysis of human embryonic stem cells enables rational line selection based on genetic variation. *Cell Stem Cell*, 29(3): 472–486. <https://doi.org/10.1016/j.stem.2022.02.001>
- Neujahr ACB (2024). Understanding the role of the microbiome in infectious disease, developing surveillance tools, and engineering direct-fed microbials to improve animal health (Doctoral dissertation, The University of Nebraska-Lincoln).

- Oh E, Hong J, Kwon OJ, Yun CO (2019). A hypoxia-responsive oncolytic adenovirus expressing secreted TRAIL for cancer gene therapy. *Mol. Ther.*, 27(4): 102-102.
- Ota H, Sakurai M, Gupta R, Valente L, Wulff BE, Ariyoshi K, Nishikura K (2013). ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. *Cell.*, 153(3): 575-589. <https://doi.org/10.1016/j.cell.2013.03.027>
- Panikker P, Roy S, Ghosh A, Poornachandra B, Ghosh A (2022). Advancing precision medicines for ocular gene therapy. *Pharmaceutics*, 14(8): 1605. <https://doi.org/10.3390/pharmaceutics14081605>
- Petersen-Jones SM, Komáromy AM (2024). Canine and feline models of inherited retinal diseases. *Cold Spring Harbor Perspect. Med.*, 14(2): a041286. <https://doi.org/10.1101/cshperspect.a041286>
- Rasoulinejad SA, Maroufi F (2021). CRISPR-based genome editing as a new therapeutic tool in retinal diseases. *Mol. Biotechnol.*, 63(9): 768-779. <https://doi.org/10.1007/s12033-021-00366-2>
- Robinson-Hamm JN, Gersbach CA (2016). Gene therapies that restore dystrophin expression for the treatment of Duchenne muscular dystrophy. *Human Genet.*, 135: 1029-1040. <https://doi.org/10.1007/s00439-016-1717-0>
- Schneider N, Steinberg R, Ben-David A, Valensi J, David-Kadoch G, Rosenwasser Z, Ben-Aroya S (2024). A pipeline for identifying guide RNA sequences that promote RNA editing of nonsense mutations that cause inherited retinal diseases. *Mol. Ther. - Nucleic Acids.*, 35: 1. <https://doi.org/10.1016/j.omtn.2023.12.006>
- Schatoff EM, Goswami S, Zafra MP, Foronda M, Shusterman M, Leach I, Dow LE (2019). Distinct colorectal cancer-associated APC mutations dictate response to tankyrase inhibition. *Cancer Discov.*, 9(10): 1358-1371. <https://doi.org/10.1158/2159-8290.CD-19-0475>
- Singh PK, Singh RL (2017). Bio-removal of azo dyes: A review. *Int. J. Appl. Sci. Biotechnol.*, 5(2): 108-126. <https://doi.org/10.3126/ijasbt.v5i2.17755>
- Suzuki K, Tsunekawa Y, Hernandez-Benitez R, Wu J, Zhu J, Kim EJ, Belmonte JCI (2016). In vivo genome editing via CRISPR/Cas9-mediated homology-independent targeted integration. *Nature.*, 540(7631): 144-149. <https://doi.org/10.1038/nature20565>
- Syding LA, Nickl P, Kasperek P, Sedlacek R (2020). CRISPR/Cas9 epigenome editing potential for rare imprinting diseases: A review. *Cells.*, 9(4): 993. <https://doi.org/10.3390/cells9040993>
- Thakkar S, Sharma D, Kalia K, Tekade RK (2020). Tumor microenvironment-targeted nanotherapeutics for cancer therapy and diagnosis: A review. *Acta Biomaterial.*, 101: 43-68. <https://doi.org/10.1016/j.actbio.2019.11.016>
- Thompson PB, Thompson PB (2020). Gene editing, synthetic biology, and the next generation of agrifood biotechnology: Some ethical issues. In *Food Agricult. Biotechnol. Ethical Perspect.* (pp. 343-374).
- Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Peng Z (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*, 323
- Wu Z, Xiong Y, Yu SX, Lin D (2018). Unsupervised feature learning via non-parametric instance discrimination. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition.* (pp. 3733-3742).
- Xie Q, Wu TP, Gimple RC, Li Z, Prager BC, Wu Q, Rich JN (2018). N6-methyladenine DNA modification in glioblastoma. *Cell.*, 175(5): 1228-1243. <https://doi.org/10.1016/j.cell.2018.09.037>
- Yoon A, Hong J, Yun CO (2019). Decorin expression from oncolytic adenovirus potentiates antitumor efficacy via mitochondrial apoptosis in cancer cells. *Mol. Ther.*, 27(4): 125-126.
- Zhao X, Liu S, Yang Z, Li Y (2024). Molecular mechanisms and genetic factors contributing to the developmental dysplasia of the hip. *Front. Genet.*, 15: 1413500. <https://doi.org/10.3389/fgene.2024.1413500>
- Zhang C, Bengio S, Hardt M, Recht B, Vinyals O (2021). Understanding deep learning (still) requires rethinking generalization. *Commun. ACM.*, 64(3): 107-115. <https://doi.org/10.1145/3446776>