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REVIEW ARTICLE

The Potential of Gene Editing Technologies in the Treatment of Hereditary Retinal Diseases

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ABSTRACT

Hereditary retinal diseases (HRDs) are a group of disorders caused by genetic mutations leading to vision impairment and blindness. Recent advancements in gene editing technologies, particularly CRISPR-Cas9, have opened new therapeutic possibilities for these diseases. This review explores the potential of gene editing in the treatment of HRDs, discussing the various gene editing methods, including CRISPR-Cas9, TALENs, and ZFNs. We examine the successes achieved in experimental models and the translation of these methods to clinical practice. Key issues such as efficacy, delivery methods, off-target effects, and ethical considerations are also addressed, the article also addresses the prospects for gene editing in ophthalmology and how it may impact the way HRDs are treated by providing unique and efficient therapy choices. This study highlights the transformational potential of gene editing technologies in the management and treatment of inherited retinal disorders by offering a comprehensive overview.

Keywords: *Gene editing, Hereditary retinal diseases, CRISPR-Cas9, Genetic therapies, Retinal dystrophies, Vision restoration, Molecular genetics, Retinitis pigmentosa, Leber congenital amaurosis, Retinal gene delivery.*

1. Introduction

Hereditary retinal diseases are a group of diseases that are genetically inherited and manifest in progressive vision decline and in some cases loss of vision at night. Contemporary studies indicate promising results in the last two decades concerning the discovery of genes responsible for I Retinal diseases like retinitis pigmentosa. A direct outcome of this development is that the present relationship between genes, mutations and clinical presentation can best be described as complex. The positive outcomes of searching for the origins of IRDs are numerous; among them, the improved understanding of both the developmental mechanisms and changes that underlie vision, as well as the pathophysiologic processes of diseases of the retina[23,4,5,6.]

Among hereditary retinal diseases, retinitis pigmentosa and Leber congenital amaurosis are well known; these diseases are also accompanied by the loss of photoreceptor cells, which results in a decrease in visual acuity. For instance, retinitis pigmentosa is an extremely diverse disease and is associated with more than eighty genes through which it reveals quite diverse clinical pattern. Likewise, Leber congenital amaurosis is a disabling disease that affects vision in the early childhood due to mutations in at least twenty seven genes[78,9].

Gene augmentation and gene editing are now considered viable options in the management of monogenic inherited retinal diseases. While there are gene augmentation treatments for inherited retinal diseases that are on the commercial market, there are a lot of limitations to them such as progressive retinal degeneration and waning effectiveness in the long run. This article also reviews the uses, advantages, and limitations of traditional gene supplementation or gene addition strategies, new precise genomes in the retina editing tech-nologies, and potential preclinical research to enhance the genome editing therapy in human patients[11,12.].

These diseases are due to genetic changes in the components participating in phototransduction, cellular metabolism, and cytoskeletal structures of retinal cells. For example, mutation in the RPE65 gene affects visual cycle leading to Leber congenital amaurosis and defects in the RPGR gene, which is involved in ciliogenesis are a cause of X-linked retinitis pigmentosa[13], New developments in genetic sequencing techniques have made research into these disorder more productive as we have been able to discover new disease causing genes and a little more about the underlying pathogenesis of these diseases14, With emergence of new technologies in gene editing it appears that in the future doctors will be able to create therapies and curates to stop or reverse vision loss. This review shall review the potentials as well as future obstacles in genome editing for hereditary retinal diseases with a focus on lately published data.

1.1 OVERVIEW OF COMMON HEREDITARY RETINAL DISEASES

High is the potential of gene editing technologies, including those based on the CRISPR/Cas9 system, to treat the disorders resulting from inherited mutations that, until recent years, never had a treatment at all. This is because it may be possible for the above technologies to fix the gene that leads to the genetic disease15 Still, in the present-day scenario, one of the most employed utilizations deals with modalities regarding the insertion of corrected transgenes alone, and these practices have been proving rather effective16. Thus, therapeutic success at the medium and long-term might entail the use of induced pluripotent stem cell technology alongside gene editing at the donor stage with the subsequent ophthalmic application of corrected retinal cells or their by-product[17]. Further progresses in basic science research as well basic or clinical research confirmation are needed to offer the scientific evidence with regard to the safety and efficacy of the utilization of gene editing for the treatment of hereditary retinal diseases18, It is useful to assess the maximum therapeutic effectiveness of gene therapy strategies, where the development of a number of optical examination methods is useful for the evaluation of

the retinofugal system as a whole19. Therapeutic success will go hand in hand with the potential of affordably medicinal treatment in several countries and especially, in low-income areas[21].

1.1.1. Retinitis Pigmentosa (RP)

Retinitis Pigmentosa is one of the most common hereditary diseases affecting the retina is a disease charac- terized by the gradual degeneration of the photoreceptor layer of the retina, but mostly rods, which causes a decrease in the ability to see at night and a gradual loss of peripheral vision. Cone photoreceptors are affected over time and patients develop central vision loss. RP is rather heterogeneous, as more than 80 genes are associated with the corresponding pathology[10].

1.1.2. Leber Congenital Amaurosis (LCA)

Leber Congenital Amaurosis is considered to be retinal dystrophy of the most severe degree, which develops at birth or during the first few months of the baby's life. LCA has features of very poor vision, inability to fixate on objects and light, nystagmus in infants. LCA is caused by disease-causing variants in at least 38 different genes: that shows the genetic diversity of LCA[20,21].

1.2 CURRENT UNDERSTANDING OF GENETIC CAUSES AND THEIR IMPACT ON VISION

Indeed, it was not until the identification of a gene responsible for this disease in 1993, namely the RPE65 gene in autosomal recessive Leber's congenital amaurosis type 2 (LCA2), the number of genetic lesions associated with sight-limiting disease has risen considerably[22], Furthermore, LCA2 is frequently employed as a model disease to assess the effectiveness of the prospective genetic treatments. Specific to the estimations of individual retinal gene disorders the problems relating to the scope regarding mutation which essentially causes identical visual phenotypes cannot be overstated. In addition, a large proportion of the pathogenic cause variations contributing to RP are localized in the coding region. Following the reported finding of RPE65 as a cause of LCA2, more than 23 genes have been confirmed regarding their impact on the onset of LCA phenotype and the severity of the disease23.

Hereditary retinal dystrophies is a class of genetically transmitted diseases that are usually diagnosed after chronic signs making references to photoreceptor degeneration and low vision beginning from juvenile amaurosis to congenital blindness24, Due to the recent development in ophthalmic genetic test and deep phenotyping, inherited genes as well as mutations contributing to IRs have been increasingly being identified. These causative genes vary with respect to the incidence and relation with symptoms of the retinal disease. With further utilization of NGS technologies, the number of known genetic roots of the retinal dystrophies has grown to over 300 genetic loci at present, and viewing the issue from the present, it is considered that no more than 45% of all monogenic retinal diseases are multigenic25.

2. Current Treatment Landscape

Although numerous therapeutic approaches have been developed in the last 25 years to treat many de- generative and inherited congenital retinal diseases. It consists of dietary supplements, Non-steroidal anti- inflammatory drugs, anti-VEGF agents, stem cell grafts, Gene transfer. Gene transfer is the rehabilitation approach used for RPE65-linked diseases with a corrective vector for the augmentation of gene or gene expression regulation. Post-surgery, patients do not undergo any therapy but undergo only ophthalmic tests and certain immunologic treatment checkups, because, with the grafting of DNA-modified cells into the host retina and the minimum necessity of further DNA adjustments. The mostly common possible risk is related to the standard procedure of phacoemulsification with lens implants where it could contribute to the degeneration of the retina26. Data collected from a number of recent studies conducted in models of old dry AMD and GA-like diseases enabled the identification of new potential therapeutic targets as well as new alternative treatments like the use of mitochondria-targeting molecules or RCD inhibitors for AMD. These results give reason to believe that in the future

a range of gene transfer therapeutic strategies can be proposed as alternatives to or combinations with Alda-protective or mitochondria-channel targeting drugs. With regard to PTSs induced by ET, the deletion of cell-specific genes instead of overall inhibiting a cell cycle pathway, or performing gene modifications with the use of PT-targeting gene editing techniques seems to be promising therapeutic tactics. Although the use of restricted CRISPR/Cas9 in vivo is still not as clinically advanced as the methods listed above, gene therapy trends indicate that gene manipulation and precision modifications of the mutated genes or mutated regulators responsible for the occurrence of selected progressive RTS diseases will soon become standards in the treatment of various clinical symptoms[27,28].

2.1 TRADITIONAL APPROACHES AND THEIR LIMITATIONS

The experiences of other coalitions and associations and the problems of the traditional way of working.

In their inheritance form, some diseases such as retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) are difficult to treat because of their genes. The previous methods of addressing the illness have en- tailed more of a focus on treating the symptoms and reducing the growth of the disease instead of correcting the genetic abnormalities that are the root cause of the disease.

The first attempts to prevent photoreceptors' degeneration concerned high-dose vitamin A; it emerged that this technique could slow down the decline in retinal function in some RP patients. However, vitamin A does not arrest the disease and is associated with side effects such as hepatotoxic effects in the long-term use of the supplement. Other treatments have also been used in retinal inflammations and prevention of degeneration and such include corticosteroids and neuroprotective agents. Although they help control inflammation and halt exacerbation of the vision decline, these treatments do not alter the genetic fundamentals and come with a range of side effects; corticosteroids, which are widely administered in OA treatment regimens, lead to the development of cataracts and glaucoma[2930].

Thus, present retinal prosthesis systems including Argus II are significant advances in the field of ophthal- mology as the patient with end-stage retinal degeneration is capable of seeing partially again. These devices work through electrical impulses to the remaining part of the retina. But they have a poor visual resolution and are very expensive and are inserted surgically[3132].

Gene augmentation therapy including the use of voretigene neparvovec (Luxturna) in LCA due RPE65 mutation has been found to be very promising. This therapy puts a functional copy of the RPE65 gene into retinal cells with an adeno-associated virus, or AAV, providing great improvements in vision for many patients. However, this approach is only for people with known changes in their DNA and does not reverse the mutation. It only creates an additional functional gene copy; it can be helpful when the mutated gene produces a dominant negative effect[33].

However, traditional approaches have the following demerits: Instantiation of my per- thesisis as fol- lows: However, traditional approaches have the following disadvantages: Many are confined to dealing with the manifestations of the sickness rather than the sickness itself. For gene modifications like augmentation, they can only be administered to particular mutations and thus a majority of people with the diseases receive no treatment.

Whether administered for short-term or for long-term, the treatment therapies like high-dose vitamin A or corticosteroids are seen to have serious complications. In addition, current therapy can only possibly result in a partial or temporary recovery of visual acuity and does not ensure the patient's vision is normalized or the progression of the disease is stopped.

New technologies including CRISPR/Cas9 has an unusually promising potential to eliminate these restric- tions since they use gene editing to treat the source of the hereditary retinal diseases. It may also lead to new "cures", and more targeted and longer lasting ones that can correct more than just a small

sample of mutated genes without harming the patient[15,18].

2.2 EMERGING THERAPIES AND RECENT ADVANCEMENTS

The new approaches towards the management of hereditary diseases of the retina have presented the novel forms of treatment which are aimed at the genetic level. By doing so, the molecular compounds, especially the gene-editing tools such as CRISPR/Cas9 possess possibilities by rectifying the genetic malaise that causes RP and LCA. 15 They are under research for their efficacy for use and the side effects.

Yes, optogenetics involves the use of light-sensitive proteins to reconstruct vision by converting retinal cells into light-sensitive cells as highlighted by the research finding with the partial vision restoration in patients with advanced RP Stem cell therapy targeting the replacement of retinal cells lost to the disease by using iPSCs; early trials are encouraging[3435].

Pharmacological approaches are aimed at the certain pathways which are implicated in the pathogenesis of retinal degeneration, and new therapeutic strategies can be based on neuroprotective agents and anti- inflammatory drugs36. Gene augmentation much as in the case of Luxturna for RPE65-related LCA delivers the functional copy of the gene and has been marked by an improvement37. They are also involved in adapting RNA based therapies for altering gene expression levels with a view to rectifying abnormal working of genes[37].

Such progress gives promise of better treatments pointing to a larger number of genetic mutations, and fewer side effects with which hereditary retinal diseases will be cared for in the future.

3.Overview of Gene Editing Technologies

Gene editing is described as the process by which specific strand of DNA is altered within a cell. Most of the early methods targeted the disease-causing mutation at the DNA level that has drastically changed with improvements in technologies. These new technologies make it possible to directly alter the patients' DNA, with some application of therapeutic nature. They are rigid, economical, not very intricate and can eliminate both autosomal recessive and autosomal dominant mutations. Therefore, gene editing tools are fascinating when undertaking the correction or alteration of the genome within the cellular as well as the organismal levels. All the above tools are being a subject of extensive research in regenerative fields; specifically; they have been applied in ophthalmology. Gene replacement therapy which has been applied in clinical trials for inherited retinal diseases has led to the development of gene editing favourable over gene addition[3839].

3.1 EXPLANATION OF CRISPR-CAS9, TALENS, AND OTHER GENE-EDITING TOOLS

Genetic engineering tools are now rapidly developing at a very high rate and can be used to very accurately manipulate the DNA. Among these, CRISPR-Cas9 and TALENs are more widely used while other popular scRNA-seq techniques are ZFNs and base editors.

CRISPR-Cas9

CRISPR-Cas9 is revolutionary gene editing technology taken from the bacterial immune system. In this approach, a guide RNA (gRNA) helps Cas9 to locate a target DNA sequence and Cas9 then cuts the DNA at the targeted place. This break can be repaired by the cell's non homologous end joining (NHEJ) which typically results in small insertions or deletions or homology directed repair (HDR) which requires a template to be used for repair. CRISPR-Cas9 is highly efficient, versatile and comparatively uncomplicated in design and therefore widely used for genome engineering in several organisms including human[1540].

TALENs

TALENs are site specific endonucleases that can be constructed so as to have affinity to specific DNA sequences with a view of causing DSBs. They exist of a DNA binding domain taken from transcription activator-like effector proteins (TALEs) and of a FokI endonuclease domain. TALENs are highly

specific because each target DNA sequence is encoded by the TALE repeats and essentially any sequence can be programmed. The FokI domains ensnarl with the target DNA strand and then undergo a conformation change to cleave the DNA at the site of homology and a second site both of which are recognized by the FokI domains Two parallel cuts on opposite strands of the helix allow the cell repair machinery to process the double strand break. TALENs are highly specific and there have been successful uses of TALENs in different areas such as plants, animals and human cells[4142,43].

Zinc Finger Nucleases (ZFNs)

ZFNs are another type of engineered nucleases that comprise a DNA-binding zinc finger and the Fok I nucalse domain. a zinc finger domain can distinguish a particular triplet in the DNA sequence, and therefore, design and construct unique ZFNs that selectively target required segments of DNA. Likewise to TALENs, FokI domains of TARGEts also form a covalently linked dimer that will cause double-strand breaks at the target site. ZFNs also have been applied in many gene-editing processes although they are relatively more difficult to construct and optimize compared with CRISPR-Cas9 and TALENs[4445].

Base Editors

Base editors are one of the newest tools in the field of gene editing and work by directly changing one base to another without the creation of double-strand breaks. This technology incorporates a deaminase enzyme with a CRISPR-Cas9 or TALE protein which recognises the target DNA sequence for binding but does not cleave it. Base editors have been applied in the treatment of the following point mutation in a variety of genetic diseases, whereby they can be considered more efficient and safer than other methods of gene editing[4647].

3.2 POTENTIAL APPLICATIONS IN MEDICINE

They are another field with great potential for treatment of many genetic diseases through direct modification of the mutated gene. It was used effectively in the treatment of the Duke's muscular dystrophy for example. Recently, applying CRISPR-Cas9 technique researchers have managed to edit the dystrophin gene in animals to regain normal function and it can be speculated that it can be applied to other genetic disorders. This discovery may lead to helping a number of conditions which were believed to have no cure or treatment, In particular, in cancer therapy, gene editing can be used to alter the patient's immune cells, increasing the efficiency of their interaction with cancer cells for their destruction. A classic example of this is the CAR – T cell therapy in which T cells are genetically engineered to express chimeric antigen receptors that directly target cancer cells. Technological advancement, for example, CRISPR functional genomics can increase the rate of making these changes, and enhance the general effectiveness of the cancer therapy[4849].

It also presents fresh tactics to fight diseases that are caused by pathogens; Gene editing also makes it possible to get new approaches of preventing new diseases That are caused by pathogens;. For example, CRISPR-Cas9 has been considered as a tool to guide modifications and destroy the viral DNA in infections such as HIV. It could thus result in finding new cures for viral diseases that for instance, are almost impossible to treat with current treatment modalities giving fresh hope to the patients with chronic viral diseases50.

Some of the advances of gene editing include; gene editing in ophthalmology for the treatment of inherited retinal diseases. Gene editing has been applied to fix mutations impacting on retinitis pigmentosa and Leber congenital amaurosis genes in vitro with the use of CRISPR-Cas9. These research integrations could possibly result in potential cures for many genetic eye diseases, for which there are no current cure also, gene editing has been utilized in the treatment of other ailments through the manipulation of hematopoietic stem cells intended for the treatment of blood diseases like sickle cell disease and beta-thalassemia. This property makes it possible to produce normal red blood cells, and thus treat these disorders, using gene correction in these cells. Clinical trials are being conducted at

this time to determine the effectiveness of these unique methods, giving hope to the patient's diagnosed with these crippling diseases [5152]

4.Gene Editing in Ophthalmology

Some of these gene editing technologies include CRISPR-Cas9, TALENs, and base editors, in inheriting Retinal Diseases Treatment. They allow specific corrections on genetic mutations that cause ailments such as retinitis pigmentosa, Leber congenital amaurosis, and age related macular degeneration.

Retinitis Pigmentosa (RP)

RP which is described as a degenerative process involving the steady decline in the number of photoreceptors. The vectors based on the technology like CRISPR-Cas9 system have been employed in reaching out and fixing errors in the RPGR gene which is more frequent in RP. Avoiding correction of the defective gene is impossible as the goal of the scientific community is to slow down or even reverse the degeneration processes and save vision. Laboratory experiments performed on animals have indicated positive outcomes and current clinical trials which have been conducted on people indicate that these approaches are safe and effective[401540].

Leber Congenital Amaurosis (LCA)

LCA is considered as a severe retinal dystrophy and usually the disease begins in infancy. LCA is known to be often associated with the mutation of the RPE65 gene. With CRISPR-Cas9 systems, animal studies have shown that RPE65 gene mutations could be treated with gene therapy and wanted results have been witnessed on the visual acuity. The only FDA approved gene therapy until date is called Luxturna, where an AAV vector delivers an RPE65 functional gene as the ready reckoner for any future therapy based on CRISPR strategy that aims at rectifying genetic mutations53.

Age-Related Macular Degeneration (AMD)

AMD is known to be the leading cause of blindness in the elderly. The advances in gene editing provide the reprieve of therapeutic intervention since the genes that participate in the pathogenesis of AMD can be manipulated. For instance, gene therapy based on CRISPR-Cas9 can correct the genes that are responsible for inflammation and the formation of new blood vessels which lead to AMD and might reverse retinal dysfunction[5455].

Stargardt Disease

Stargardt disease – this is a condition that is hereditary and characterized by degeneration of the vision. ABCA4 is responsible for its development and is said to involve mutations within the gene. Autosomal dominant disorders are being researched with an intention to delete these mutations with the help of gene editing tools such as cas9- crispr technique as the primary step to revert the protein function to normal and stop the further onset of this ailment. Initial proof exists, although experiments are still in their infancy, and the primary aim is to refine the delivery system and be certain that the areas targeted are specific56.

4.1 SPECIFIC APPLICATIONS OF GENE EDITING IN RETINAL DISEASES

At the present, education on the genetic makeup of inherited retinal degeneration is extensive to a great extent. Diagnosis of inherited retinal dystrophies have enhanced so much in our abilities. Thus gene therapies of today are only the promising figure of a new start in genetic medicine that was potentially possible for the last fifty years at least; Gene therapy however has certain limitations. Not only ichHL is clinically and genetically very variable both between different genes/allelic mutations and between patients who have the same mutation, but also phenotypically ICDs. While biotechnology has continued to grow, the three types of zinc finger nucleases, the TALENs and the CRISPR-Cas9 have been used in both, cell culture and animal experiments on retinal diseases. While these activities are targeting at providing gene replacement through homologous recombination, it also provides one-step point mutation based on specific endogenous gene, activation or inactivation of endogenous gene for tissue-specific, over-

expression or knock-down purpose or directly knock-down on function of transcriptional repressor in cis- or trans- elements. New gene editing technologies expand knowledge about the retinal pathophysiology due to the initial genetic mutations and to allow a real-time evaluation of the impact of certain gene editing approaches as well as to offer more gene correction contexts in the future, Here, we will provide the literature review of the major papers in this field[5758].

Originally, the CRISPR-Cas9 system was identified as bacteria's immune defence mechanism against viruses. In 2012, three groups with independency stressed that the type of the CRISPR array can be adopted as sgRNA to guide the nuclease. Additionally, Cas9 nuclesae from Streptococcus pyogenes is programmable to carry out a double strand break at a particular locus. SSO sequence preferences can be created as well as the sgRNA to be used for the editing of various organisms in the genome. After that, public approval and utilization of the CRISPR-Cas9 system have grown very quickly and are now present in many aspects of research and development in numerous scientific disciplines. This is a case where there has been a great emphasis on the use of CRISPR, lots of progresses made and even successful efforts to achieve the goal of disease modeling and treatment. It is difficult in such investigations to get a precise estimation of the sgRNA sequence along with the most suitable Cas9 on/off-target, Currently, editing either with the geminivirus derived system (BE3), or nuclease deficient dCas9 fusions with Pol II or Catalytically Inactive dCas9 Effector fusions that have TET or APOBEC cytidine deaminase activity is used. Versatile fusion constructs have been developed to enhance laminated targeting to genetic components, with and without the use of a separate linker. Recent large-scale knockout studies conducted in mice also indicated the experimental delivery of the CRISPR has a high either mutation, or unintended insertion/deletion and target-trapping and editing studies have indicated targeted chromosomal translocations with limited genotoxicity. Compared to the more traditional zinc finger nucleases and the pair of frequently truncated TALE proteins or lentiviruses, the CRISPR-Cas9 system is of particularly significant benefit due to the lower cost and relatively fast throughput, enabling potentially easier scale-up[5960,61].

4.2 CASE STUDIES AND EARLY-STAGE CLINICAL TRIALS

The genome-invading technologies such as CRISPR-Cas9 are considered to have great potentials in treating the inherited eye diseases. Here are detailed explanations of some significant case studies and early-stage clinical trials:Here are detailed explanations of some significant case studies and early-stage clinical trials: QR-1123 for LCA10: The first human trials involving CRISPR was conducted on animals back in 2017 and the first in vivo human CRISPR study on Leber Congenital Amaurosis type 10 (LCA10) begun in 2020. The company known as ProQR Therapeutics brought into the market QR-1123 which is an investigational therapeutic with focus on CEP290 LCA10 mutation. This trial means that the participants are divided randomly into different categories with different levels of doses, and the study involves about 18 patients. The major purpose is to determine whether QR-1123 delivered to the subretinal space in a single or double cycle is safe and well-tolerated. That is, the trial will also compare the degree of decrease in CEP290 mRNA that will occur in the treated eyes with the untreated ones. Primary outcomes will be assessed for patients six months after receiving the treatment and main outcome measures will be measured at one, two, and five years after the last session62.

EDIT-101 for LCA10: Another significant trial that uses CRISPR is EDIT-101 derived from Editas Medicine in partnership with Allergan. This intravitreal trial started in November 2019 and is for the CEP290 mutation. In EDIT-101, there are two guide RNAs that target the mutation and ensure modification of the particular gene within the area of the retina. The trial aims for the FDA approval, with the human dosing started during the earlier part of 2020. The objectives of this study are to

evaluate the safety profile, tolerability, and the initial therapeutic efficacy of EDIT-101 which will help to set the stage for gene editing alternative treatment to inherited retinal dystrophies63.

Clinical trials in this area are being carried out with the aim of applying CRISPR-Cas9 to counter genetic disorders that involve mutations in the RPGR gene in X-linked RP. These trials are at the primary stage where safety has been tested; the CRISPR components are administered via subretinal injections to target the RPGR mutation within the retinal cells. The main goals include assessment of the risks associated with this approach and its ability to stop or even revert part of photoreceptor loss and save vision. These clinical trials are important for evaluating how practicable and beneficial the Genetic modification is in treating RP64.

In Age-related macular degeneration (AMD) the gene editing trials have focused on the genes that have roles to play in inflammation and angiogenesis these are most procession in the development of AMD. These genes are being targeted with CRISPR-Cas9, in a bid to decrease pathological neovascularization and inflammation in the retina. These trials are still at the phase 1/2, where the emphasis is on safety, and the first signs of efficacy in delivering intravitreal CRISPR injections. If such studies are successful, then they could help develop new treatment methodologies for AMD, which affects a significant number of elderly persons. Currently, there are three clinical trials of early phases for the treatment of AMD using CRISPR-based therapies. One of the experimental studies is the application of Cas9 ribonucleoprotein to reduce VEGF-A and HIF-1 in a murine model; this result suppressed choroidal neovascularization. This is the promising evidence for AMD treatments that was also followed by human trials to treat or, at least, stop the progression of the disease and consequently, vision loss[6566,67].

Some of the most targeted mutations for gene editing are the ones causing Stargardt disease associated with ABCA4 gene. Stargardt disease is an autosomal recessive inherited disease which affects the retina and begins in childhood with gradual worsening of vision. Innovations are worked out to treat the genetic basis of ABM with CRISPR-Cas9 in the late stage experimental trials. Such studies hope to retain normal protein functioning and decrease the formation of toxic metabolites which cause photoreceptor cell death68, Scientists are also fine-tuning the delivery systems including viral vector and nanoparticle to get the best result of gene editing in the retinal cells. For instance, a submitted study plan was to use CRISPR/Cas9 delivered with an adeno-associated viral (AAV) vector for subretinal delivery in a Stargardt disease mouse model. This strategy seeks to prove the efficiency of the CRISPR/Cas9 system in the gene-editing process of ABCA4 mutation and the reduction of the disease manifestations69.

Also, there is Ascidian Therapeutics which recently revealed that the agency has given the green light to its investigational new drug application for ACDN-01, an RNA-editing drug for Stargardt disease. This trial will serve to determine if ACDN-01 is safe and effective at editing ABCA4 RNA to generate mutation-free, functional protein and could have remarkable therapeutic advantage over other hereditary disorders that permanently alter the patient's DNA without the danger of doing so in the ABCA4 gene70.

The condition that has been targeted by the gene editing of the USH2A gene is uproar being a combined disorder of hearing and vision impairment. Researchers in earlier experiments have demonstrated that USH2A can be repaired by CRISPR-Cas9 in animal models. Such protocols are now being designed to determine the safety of the procedures in human patients for therapeutic efficacy. These trials will seek to bring in CRISPR components to the needed location of the retinal tissue and evaluate the return to normal of hearing and vision in the care of this intricate syndrome[7172].

5. Delivery Methods

Transducing gene-editing tools into retinal cells has its problem because of the sensitivity and

structure of the retina. This is due to difficulties in addressing the desired cell type in the retina like the photoreceptor or the retinal pigment epithelial cells without affecting the other parts of the eye. Another issue is the immune response, for example, the body may react to the delivery vectors or gene editing tools as a form of infection and react negatively to it. This immune response is capable of lowering the efficiency of the therapy offered and leading to the potential destruction of the retinal tissue. Furthermore, the delivery and expression of the gene-editing tools are also important because of the histological structure of the eye; it is small, well- circumscribed, and surrounded by sclera. Another important factor that governs the outcome of the therapy is that the dose of the therapeutic agent, which is taken to the target cells, should be adequate. Reversibility is also an issue, because when using the gene-editing tools, there could be an issue with off-target effects where other unwanted parts of the genome can be edited leading to negative consequences that must be avoided at all costs[37].

That is why recent achievements in the delivery methods have been aimed at addressing these challenges for enhanced safety and efficiency of gene-editing drugs for retina disorders. AAVs are the most utilized viral vectors in the present day to deliver gene-editing technologies to the affected retinal cells. AAVs are preferred for their high tropism to the retinal cells and low, if any, immune response in their host. Subsequent research has unpacked AAV vectors to improve their tropism and efficiency of transduction, with AAV8/9 being demonstrated to deliver CRISPR components to photoreceptors and/mixed RPE cells[7374,75,]

That is why non-viral systems, including nanoparticles, are considered to be very hopeful in gene delivery. The gene-editing tools can be embedded in nanoparticles that will help in the delivery of gene therapies to the retinal cells. Such particles can be made in a way that they shield the genetic material from being damaged, help the particles enter the target cells and assist in the controlled release of the therapeutic modules. Besides, lipid-based nanoparticles and gold nanoparticles have been demonstrated to have efficient gene delivery in preclinical studies with acceptable biocompatibility[7576,77].

Another more recent attempted is the use of electroporation to deliver electrical pulses that cause temporary opening of the cell membrane to let in the gene editing tools. It has been studied for the application in retinal gene therapy, which helps deliver the components of CRISPR directly to the target cells. Thus, electroporation seems to be a very effective method of gene delivery with low toxicity on cells, which makes it suitable for the treatment of retina[7879].

6. Safety and Ethical Considerations

The successful use of AAV vectors or other viral and non-viral vectors to deliver CRISPR/Cas9 gene editing reagents is another step forward in treating hereditary diseases affecting the retinal tissue. However, there are several large scale challenges that need to be solved in order for these recombinant viruses to be utilized to deliver gene editing cargoes to human retina. Consequently, there exists a very limited packaging capacity in AAV, which will need efficient cloaking and de-cloaking methods. Non-specific cleavage and on target mutation rates higher than zero or even using two-plasmid, two-step system are still practical issues, as well as the immune response that might occur with repeated doses of the encoded endonuclease[8081].

Several of these off-target points can be dismissed by using the ELVIS (Endonuclease-Liability via In Vivo Screening) assay which can help identify the safest engineered variant relatively easily. Even in the future, other challenges will emerge concerning other vital genetic controlling elements which will be discovered from the rest 70% of the human genome that is not contained in the exome. Despite the fact that effective treatments for blinding retinal diseases are still under development, several translational challenges need to be overcome to allow the application of gene

editing technologies as a routine clinical practice.

These gene-editing techniques are still evolving innovative techniques and diagnostics are going to advance with these as a consequence high throughput assays will be cheaper and quicker. There are several start-up firms trying to find and launch the standard, best-in-class CRISPR/Cas9 gene editing types for clinical use. In the longer term, other new treatments, including the SOMA therapy that restarts the molecular clock or the prevention of the secondary loss of cones by averting this process, can be added to the gene treatment to increase therapeutic time82.

The fact that Tsinghua University SC side recently brought vision back to a large animal model of RPE65-associated Leber's congenital amaurosis shows that the next level of gene-editing advancement would be relatively easy. Though, biosecurity and biosafety of these accelerating technologies must enhance and boost up. They include balancing patients' best interest in these projects, and an assurance that technological dependencies are not necessarily the most rapid, but the surest[83].

6.1 POTENTIAL RISKS AND UNINTENDED CONSEQUENCES OF GENE EDITING

Nevertheless, it is a great advance and has many applications, introducing double-stranded breaks with CRISPR/Cas9 in eukaryotic genomes brings ethical and regulatory concern, including off-target effects which are risk factors for people undergoing gene therapy. The CRISPR guide might have constrained complementarity with a single-nucleotide mismatch recognition, in positions from 12 to 15 in protospacer sequence. However, it should be noted that the most relevant off-target effect is highly dependent on the gRNA design; nevertheless, comprehensive high-throughput screening of genome-wide patient and control cell lines is a key step. Other testable modulator factors of off-target effects are the proteins' expression vectors where too high expression of Cas9 might chock competitive access of the endogenous targeting factor CDCK and DNA repair pathways. The biodistribution of the CRISPR components may also have other effects due to an immune interaction, for example, possible Cas9 T-cell mediated immune responses. To reduce the immune response in the context of gene therapy, it has been tried to engineer organisms that will express the enzyme or vector in one patient dose. Also, using a single AAV serotype with a cell type-specific promoter to transduce the patient's retinal cells while restricting the transgene expression may be useful in enhancing the treatment's safety. Another ethical issue is the subject of the germline interventions which brings question of safety to the clinicians and raises the point on genetics that can be altered on genes the child will pass on to offspring. However, two papers have demonstrated that, indeed, with a specific human oocyte, an off-target effect resulting from CRISPR-induced double-strand breaks is fully healed through two pathways so as to create healthy embryos[8485].

7. Future Prospects

While there are many attractions and possible use of the gene editing technologies, the delivery and introduc- tion of double-stranded breaks in eukaryotic genomes by CRISPR/Cas9 system has some ethical and several regulatory issues such as off-targets that has confronting risks for patients with gene therapies. Thus, the CRISPR guide can have a complementarity with a single-nucleotide mismatch recognition within positions between the 12th and 15th in the protospacer sequence. As already mentioned, the most critical aspect to reduce off-target effects is the proper design of the gRNA; however, high-throughput GWAS in patient and control cell lines is essential. Other factors that influence the possibility of off target effects that can be tested include the vectors used in expressing the protein of interest and the level of protein that is expressed where darn high levels of cas9 may chime out the access of the endogenous targeting factor CDCK and the DNA repair machinery84.

The biodistribution of the CRISPR components is also likely to have side effects because of immune reac- tions, for example, Cas9-specific T-cell

immunity reactions might be experienced. To reduce the immune response in regards to gene therapy regulating the enzyme or vector to be expressed in one patient dose has also been proposed. Furthermore, performing the transduction of the patient's retinal cells with a single Serotype AAV and cell type selective promoter that controls the transgene expression may be useful in raising the safety levels. Another ethical concern is the question of the germline interventions which present questions of clinical efficacy as well as a potential access to tomorrow's generations, changing genes that are inherited from the parents. But there are two studies in a human oocyte that has demonstrated that it can repair a double-stranded break induced by CRISPR and gives healthy embryos through two pathways[8586].

8. Conclusion

Over the last 40 years, there has been significant progress in molecular genetics hence depicting comprehen- sive views on the causes of retinal diseases. Molecular genetics of most inherited retinal diseases has been deciphered and there is research work progressing regarding to understanding the mechanisms of retinal degeneration. In the last six years, there has been an enhanced advancement of new technologies for gene editing like CRISPR/cas9 making it possible to treat diseases associated with genes. Several papers published in 2012 are regarded as the beginning of editing by the CRISPR/Cas9 system as a perspective technological approach. After that, this technology has been applied to the germline manipulation of Eukaryotic model organisms. This has drastically changed the course of how perspectives on delivering the gene editing tools in the eye have been viewed due to the simplicity rendered by the CRISPR/Cas9 system. Thus, with regard to gene editing therapies, the therapeutic value is associated with comparatively lesser risks to genomic stability. However, the application of gene editing in the treatment of Hereditary Retinal dystrophies has future problems. From a moral aspect, therefore, tighter regulations are placed on germline editing. The concentration of this review is on the treatment of mutations in the retina via somatic cells and other separate therapies. The risk that exists with CRISPR/Cas9 or another comparable system is off-target effects that have not yet been countered to the fullest. However, the developments of the technique in the recent past have enhanced the use of the in research activities. However, for gene editing to go to trials, there is a need for large-scale, systemic safety assessments other than technological advancements. Future research should try to focus on these and other disadvantages of therapy. As gene editing appears to treat mutations of both monogenic forms of hereditary retinal disease, it is predicted to be a very effective remedy and decrease current limitations[8788,89,90].

Conflict of Interest:

None

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References:

1. Maeder, M.L., & Gersbach, C.A. (2016). Genome-editing Technologies for Gene and Cell Therapy. Molecular Therapy, 24(3), 430-446. doi:10.1038/mt.2016.10

2. Bakondi, B., Lv, W., Lu, B., Jones, M.K., Tsai, Y., Kim, K.J., & Young, M.J. (2016). In Vivo CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa. Molecular Therapy, 24(3), 556-563. doi:10.1038/mt.2015.220

3. Gao, X., Tao, Y., Lamas, V., Huang, M., Yeh, W.H., Pan, B., & Chen, S. (2018). Treatment of Autosomal Dominant Hearing Loss by In Vivo Delivery of Genome Editing Agents. Nature, 553(7687), 217-221. doi:10.1038/nature25164

4. Yanik M, Müller B, Song F, et al. In vivo genome editing as a potential treatment strategy for inherited retinal dystrophies. Prog Retin Eye Res. 2017;56:1-18. doi:10.1016/j.preteyeres.2016.09.001

5. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096. doi:10.1126/science.1258096

6. Carroll D. Genome editing: progress and challenges for medical applications. Genome Med. 2016;8(1):120. Published 2016 Nov 15. doi:10.1186/s13073-016-0378-9

7. Wu WH, Tsai YT, Justus S, et al. CRISPR Repair Reveals Causative Mutation in a Preclinical Model of Retinitis Pigmentosa. Mol Ther. 2016;24(8):1388-1394. doi:10.1038/mt.2016.107

8. Zhang F, Wen Y, Guo X. CRISPR/Cas9 for genome editing: progress, implications and challenges. Hum Mol Genet. 2014;23(R1):R40-R46. doi:10.1093/hmg/ddu125

9. Daiger SP, Bowne SJ, Sullivan LS. Perspective on genes and mutations causing retinitis pigmentosa. Arch Ophthalmol. 2007 Feb;125(2):151-8. doi: 10.1001/archopht.125.2.151. PMID: 17296890; PMCID: PMC2580741.

10. O'Neal TB, Luther EE. Retinitis Pigmentosa. PubMed. Published 2020. https://www.ncbi.nlm.nih.gov/books/NBK519518/

11. Cremers FP, van den Hurk JA, den Hollander AI. Molecular genetics of Leber congenital amaurosis. *Hum Mol Genet*. 2002;11(10):1169-1176. doi:10.1093/hmg/11.10.1169

12. Shieh BH. Molecular genetics of retinal degeneration: A Drosophila perspective. *Fly (Austin)*. 2011;5(4):356-368. doi:10.4161/fly.5.4.17809

13. Sladen P, Naeem A, Toyin Adefila-Ideozu, et al. AAV-RPGR Gene Therapy for RPGR-Associated X-Linked Retinitis Pigmentosa (XLRP): Human retinal organoid vector efficacy data. *Investigative Ophthalmology & Visual Science*. 2022;63(7):4503-F02904503-F0290. Accessed June 28, 2024. https://iovs.arvojournals.org/article.aspx?articleid=2782024

14. Hafler BP. CLINICAL PROGRESS IN INHERITED RETINAL DEGENERATIONS. *Retina*. 2017;37(3):417-423. doi: https://doi.org/10.1097/iae.0000000000001341

15. Doudna JA, Charpentier E. The New Frontier of Genome Engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096-1258096. doi: https://doi.org/10.1126/science.1258096

16. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. *Science*. 2013;339(6121):823-826. doi:10.1126/science.123203

17. Yamanaka S. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell*. 2012;10(6):678-684. doi:10.1016/j.stem.2012.05.005

18. Cox DB, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. *Nat Med*. 2015;21(2):121-131. doi:10.1038/nm.3793

19. Sahel JA, Marazova K, Audo I. Clinical characteristics and current therapies for inherited retinal degenerations. *Cold Spring Harb Perspect Med*. 2014;5(2):a017111. Published 2014 Oct 16. doi:10.1101/cshperspect.a017111

20. Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. *Nat Biotechnol*. 2016;34(9):933-941. doi:10.1038/nbt.3659

21. Huang CH, Yang CM, Yang CH, Hou YC, Chen TC. Leber's Congenital Amaurosis: Current

Concepts of Genotype-Phenotype Correlations. *Genes (Basel)*. 2021;12(8):1261. Published 2021 Aug 19. doi:10.3390/genes12081261

22. Hufnagel RB, Ahmed ZM, Corrêa ZM, Sisk RA. Gene therapy for Leber congenital amaurosis: advances and future directions. *Graefes Arch Clin Exp Ophthalmol*. 2012;250(8):1117-1128. doi:10.1007/s00417-012-2028-2

23. den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res*. 2008;27(4):391-419. doi:10.1016/j.preteyeres.2008.05.003

24. Viswarubhiny S, Anjanamurthy R, Vanniarajan A, Bharanidharan D, Perumalsamy V, Sundaresan P. Clinical exome sequencing facilitates the understanding of genetic heterogeneity in Leber congenital amaurosis patients with variable phenotype in southern India. *Eye Vis (Lond)*. 2021;8(1):20. Published 2021 May 6. doi:10.1186/s40662-021-00243-5

25. Huang CH, Yang CM, Yang CH, Hou YC, Chen TC. Leber's Congenital Amaurosis: Current Concepts of Genotype-Phenotype Correlations. *Genes (Basel)*. 2021; 12(8):1261. Published 2021 Aug 19. doi:10.3390/genes12081261

26. Hu ML, Edwards TL, O'Hare F, et al. Gene therapy for inherited retinal diseases: progress and possibilities. *Clinical & Experimental Optometry*. 2021;104(4):444-454. doi:https://doi.org/10.1080/08164622.2021.1880863

27. Pontikos N, Arno G, Jurkute N, et al. Genetic Basis of Inherited Retinal Disease in a Molecularly Characterized Cohort of More Than 3000 Families from the United Kingdom. *Ophthalmology*. 2020;127(10):1384-1394. doi:10.1016/j.ophtha.2020.04.008

28. Arabi F, Mansouri V, Ahmadbeigi N. Gene therapy clinical trials, where do we go? An overview. *Biomed Pharmacother*. 2022;153:113324. doi:10.1016/j.biopha.2022.113324

29. Berson EL, Rosner B, Sandberg MA, et al. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol*. 1993;111(6):761-772. doi:10.1001/archopht.1993.01090060049022

30. Grover S, Apushkin MA, Fishman GA. Topical dorzolamide for the treatment of cystoid macular edema in patients with retinitis pigmentosa. *Am J Ophthalmol*. 2006;141(5):850-858. doi:10.1016/j.ajo.2005.12.030

31. Schaffrath K, Schellhase H, Walter P, et al. One-Year Safety and Performance Assessment of the Argus II Retinal Prosthesis: A Postapproval Study. *JAMA Ophthalmol*. 2019;137(8):896-902. doi:10.1001/jamaophthalmol.2019.1476

32. Retinal Implants for RP: An Update on Argus II and Others. American Academy of Ophthalmology. Published December 2, 2019. https://www.aao.org/eyenet/article/retinal-implants-for-rp

33. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial [published correction appears in Lancet. 2017 Aug 26;390(10097):848. doi: 10.1016/S0140-6736(17)32235-3]. *Lancet*. 2017;390(10097):849-860. doi:10.1016/S0140-6736(17)31868-8

34. Sahel JA, Boulanger-Scemama E, Pagot C, et al. Partial recovery of visual function in a blind patient after optogenetic therapy. *Nat Med*. 2021;27(7):1223-1229. doi:10.1038/s41591-021-01351-4

35. Ferenchak K, Deitch I, Huckfeldt R. Antisense Oligonucleotide Therapy for Ophthalmic Conditions. *Semin Ophthalmol*. 2021;36(5-6):452-457. doi:10.1080/08820538.2021.1914116

36. Gao H, Chen M, Liu Y, et al. Injectable Anti-Inflammatory Supramolecular Nanofiber Hydrogel to Promote Anti-VEGF Therapy in Age-Related Macular Degeneration Treatment. *Adv Mater*. 2023;35(2):e2204994. doi:10.1002/adma.202204994

37. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial [published

correction appears in Lancet. 2017 Aug 26;390(10097):848. doi: 10.1016/S0140-6736(17)32235-3]. *Lancet*. 2017;390(10097):849-860. doi:10.1016/S0140-6736(17)31868-8

38. Girach A, Audo I, Birch DG, et al. RNA-based therapies in inherited retinal diseases. *Ther Adv Ophthalmol*. 2022;14:25158414221134602. Published 2022 Nov 4. doi:10.1177/25158414221134602

39. Ballios BG, Pierce EA, Huckfeldt RM. Gene editing technology: Towards precision medicine in inherited retinal diseases. *Semin Ophthalmol*. 2021;36(4):176-184. doi:10.1080/08820538.2021.1887903

40. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816-821. doi:10.1126/science.1225829

41. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *Journal of Clinical Investigation*. 2014;124(10):4154-4161. doi: https://doi.org/10.1172/jci72992

42. Nemudryi AA, Valetdinova KR, Medvedev SP, Zakian SM. TALEN and CRISPR/Cas Genome Editing Systems: Tools of Discovery. *Acta Naturae*. 2014;6(3):19-40.

43. Siles L, Ruiz-Nogales S, Arnau Navinés-Ferrer, Paula Gaudó Pardo, Pomares E. High efficiency in genome editing of pathogenic variants causing Inherited Retinal Dystrophies: the importance of CRISPR/TALEN guides and repair templates designs. *Investigative Ophthalmology & Visual Science*. 2023;64(8):759-759. Accessed June 29, 2024. https://iovs.arvojournals.org/article.aspx?articleid=2786180

44. Christian M, Cermak T, Doyle EL, et al. Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics*. 2010;186(2):757-761. doi:10.1534/genetics.110.120717

45. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. *Nature Reviews Genetics*. 2010;11(9):636-646. doi: https://doi.org/10.1038/nrg2842

46. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016;533(7603):420-424. doi: https://doi.org/10.1038/nature17946

47. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 2019;576(7785):149-157. doi:10.1038/s41586-019-1711-4

48. Nelson CE, Hakim CH, Ousterout DG, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science*. 2016;351(6271):403-407. doi:10.1126/science.aad5143

49. Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. *Biomark Res*. 2017;5:22. Published 2017 Jun 24. doi:10.1186/s40364-017-0102-y

50. Ebina H, Misawa N, Kanemura Y, Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. *Sci Rep*. 2013;3:2510. doi:10.1038/srep02510

51. Maeder ML, Gersbach CA. Genome-editing Technologies for Gene and Cell Therapy. *Mol Ther*. 2016;24(3):430-446. doi:10.1038/mt.2016.10

52. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature*. 2016;539(7629):384-389. doi: https://doi.org/10.1038/nature20134

53. Cideciyan AV. Leber congenital amaurosis due to RPE65 mutations and its treatment with gene therapy. *Prog Retin Eye Res*. 2010;29(5):398-427. doi:10.1016/j.preteyeres.2010.04.002

54. Khanani AM, Thomas MJ, Aziz AA, et al. Review of gene therapies for age-related macular degeneration. *Eye (Lond)*. 2022;36(2):303-311. doi:10.1038/s41433-021-01842-1

55. Boyer DS, Schmidt-Erfurth U, van Lookeren Campagne M, Henry EC, Brittain C. THE PATHOPHYSIOLOGY OF GEOGRAPHIC ATROPHY SECONDARY TO AGE-RELATED MACULAR DEGENERATION AND THE COMPLEMENT PATHWAY AS A THERAPEUTIC TARGET. *Retina*. 2017;37(5):819-835. doi:10.1097/IAE.0000000000001392

56. Wang L, Shah SM, Mangwani-Mordani S, Gregori NZ. Updates on Emerging Interventions for Autosomal Recessive *ABCA4*-Associated Stargardt Disease. *J Clin Med*. 2023;12(19):6229. Published 2023 Sep 27. doi:10.3390/jcm12196229

57. Rasoulinejad SA, Maroufi F. CRISPR-Based Genome Editing as a New Therapeutic Tool in Retinal Diseases. *Mol Biotechnol*. 2021;63(9):768-779. doi:10.1007/s12033-021-00345-4

58. Quinn J, Musa A, Kantor A, et al. Genome-Editing Strategies for Treating Human Retinal Degenerations. *Hum Gene Ther*. 2021;32(5-6):247-259. doi:10.1089/hum.2020.231

59. Datta A, Sarmah D, Kaur H, et al. Advancement in CRISPR/Cas9 Technology to Better Understand and Treat Neurological Disorders. *Cell Mol Neurobiol*. 2023;43(3):1019-1035. doi:10.1007/s10571-022-01242-3

60. Bhattacharjee G, Gohil N, Khambhati K, et al. Current approaches in CRISPR-Cas9 mediated gene editing for biomedical and therapeutic applications. *J Control Release*. 2022;343:703-723. doi:10.1016/j.jconrel.2022.02.005

61. Akram F, Ikram Ul Haq, Ahmed Z, Khan H, Ali MS. CRISPR-Cas9, A Promising Therapeutic Tool for Cancer Therapy: A Review. *Protein Pept Lett*. 2020;27(10):931-944. doi:10.2174/0929866527666200407112432

62. Harvard.edu. Published 2024. https://hms.harvard.edu/news/gene-editing-improves-vision-some-people-inherited-blindness

63. Eye M, Ear. Gene Editing Breakthrough: CRISPR Improves Vision in Clinical Trial. SciTechDaily. Published May 13, 2024. https://scitechdaily.com/gene-editing-breakthrough-crispr-improves-vision-in-clinical-trial/

64. Gumerson JD, Alsufyani A, Yu W, et al. Restoration of RPGR expression in vivo using CRISPR/Cas9 gene editing. *Gene Ther*. 2022;29(1-2):81-93. doi:10.1038/s41434-021-00258-6

65. Genome surgery with CRISPR-Cas9 to prevent blindness. ScienceDaily. https://www.sciencedaily.com/releases/2017/02/170216133252.htm

66. Finocchio L, Zeppieri M, Gabai A, Giacomo Toneatto, Spadea L, Salati C. Recent Developments in Gene Therapy for Neovascular Age-Related Macular Degeneration: A Review. *Biomedicines*. 2023;11(12):3221-3221. doi:https://doi.org/10.3390/biomedicines11123221

67. Salman A, McClements ME, MacLaren RE. CRISPR Manipulation of Age-Related Macular Degeneration Haplotypes in the Complement System: Potential Future Therapeutic Applications /Avenues. *Int J Mol Sci*. 2024;25(3):1697. Published 2024 Jan 30. doi:10.3390/ijms25031697

68. News: FDA Clears First Clinical Trial of RNA Exon Editor Developed to Treat Stargardt Disease. CRISPR Medicine. Accessed June 29, 2024. https://crisprmedicinenews.com/news/fda-clears-first-clinical-trial-of-rna-exon-editor-developed-to-treat-stargardt-disease/

69. Suh S. CRISPR/Cas9-Mediated Correction of Abca4 Mutations in a Stargardt Mouse Model. *grantomecom*. Accessed June 29, 2024. https://grantome.com/grant/NIH/F30-EY029136-02

70. Fujinami K, Waheed NK, Laich Y, et al. Stargardt macular dystrophy and therapeutic approaches. *British Journal of Ophthalmology*. Published online November 8, 2023:bjo-323071. doi: https://doi.org/10.1136/bjo-2022-323071

71. Major L, McClements ME, MacLaren RE. New CRISPR Tools to Correct Pathogenic Mutations in Usher Syndrome. *International Journal of Molecular Sciences*. 2022;23(19):11669. doi: https://doi.org/10.3390/ijms231911669

72. RePORT ⟩ RePORTER. reporter.nih.gov. Accessed June 29, 2024. https://reporter.nih.gov/project-details/10626831

73. Surace EM, Auricchio A. Versatility of AAV vectors for retinal gene transfer. *Vision Res*. 2008;48(3):353-359. doi:10.1016/j.visres.2007.07.027

74. Maugeri M, Nawaz M, Papadimitriou A, et al. Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells. *Nature Communications*. 2019;10(1):4333. doi:

https://doi.org/10.1038/s41467-019-12275-6

75. News: FDA Clears First Clinical Trial of RNA Exon Editor Developed to Treat Stargardt Disease. CRISPR Medicine. https://crisprmedicinenews.com/news/fda-clears-first-clinical-trial-of-rna-exon-editor-developed-to-treat-stargardt-disease/

76. Suh S. CRISPR/Cas9-Mediated Correction of Abca4 Mutations in a Stargardt Mouse Model. *grantomecom*.https://grantome.com/grant/NIH/F30-EY029136-02

77. RePORT ⟩ RePORTER. reporter.nih.gov. https://reporter.nih.gov/project-details/10626831

78. Young JL, Dean DA. Electroporation-mediated gene delivery. *Adv Genet*. 2015;89:49-88. doi:10.1016/bs.adgen.2014.10.003

79. Manufacturing challenges and rational formulation development for AAV viral vectors. *Journal of Pharmaceutical Sciences*. Published online April 2, 2021. doi: https://doi.org/10.1016/j.xphs.2021.03.024

80. Wörner TP, Snijder J, Friese O, Powers T, Heck AJR. Assessment of genome packaging in AAVs using Orbitrap-based charge-detection mass spectrometry. *Mol Ther Methods Clin Dev*. 2021;24:40-47. Published 2021 Nov 29. doi:10.1016/j.omtm.2021.11.013

81. Synthego | Full Stack Genome Engineering. www.synthego.com . https://www.synthego.com/blog/delivery-crispr-cas9

82. Behr M, Zhou J, Xu B, Zhang H. *In vivo* delivery of CRISPR-Cas9 therapeutics: Progress and challenges. *Acta Pharm Sin B*. 2021;11(8):2150-2171. doi:10.1016/j.apsb.2021.05.020

83. Gostimskaya I. CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Moscow)*. 2022;87(8):777-788. doi: https://doi.org/10.1134/s0006297922080090

84. Shinwari ZK, Tanveer F, Khalil AT. Ethical Issues Regarding CRISPR Mediated Genome Editing. *Curr Issues Mol Biol*. 2018;26:103-110. doi:10.21775/cimb.026.10

85. Doudna JA. The promise and challenge of therapeutic genome editing. *Nature*. 2020;578(7794):229-236. doi:10.1038/s41586-020-1978-5

86. Delhove J, Osenk I, Prichard I, Donnelley M. Public Acceptability of Gene Therapy and Gene Editing for Human Use: A Systematic Review. *Hum Gene Ther*. 2020;31(1-2):20-46. doi:10.1089/hum.2019.197

87. Dasgupta I, Flotte TR, Keeler AM. CRISPR/Cas-Dependent and Nuclease-Free *In Vivo* Therapeutic Gene Editing. *Hum Gene Ther*. 2021;32(5-6):275-293. doi:10.1089/hum.2021.013

88. Cremers FPM, Lee W, Collin RWJ, Allikmets R. Clinical spectrum, genetic complexity and therapeutic approaches for retinal disease caused by ABCA4 mutations. *Prog Retin Eye Res*. 2020;79:100861. doi:10.1016/j.preteyeres.2020.100861

89. da Costa BL, Levi SR, Eulau E, Tsai YT, Quinn PMJ. Prime Editing for Inherited Retinal Diseases. *Front Genome Ed*. 2021;3:775330. Published 2021 Nov 25. doi:10.3389/fgeed.2021.775330

90. Jang H, Jo DH, Cho CS, et al. Application of prime editing to the correction of mutations and phenotypes in adult mice with liver and eye diseases. *Nature Biomedical Engineering*. Published online August 26, 2021. doi: https://doi.org/10.1038/s41551-021-00788-9

