Clinical Ophthalmology

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Gene Therapy Using Recombinant Adeno-Associated Virus for Leber Congenital Amaurosis Induced by RPE65 Mutation

Iman Owliaee[1], Ali Teimoori[1], Mohammad Shoushtari[2], Ali Shojaeian[3]

[1]Department of Medical Virology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; [2]Department of Virology, Pasteur Institute of Iran, Tehran, Iran; [3]Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran

Correspondence: Ali Shojaeian, Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran, Tel +989397533875, Email ali.shojaeian65@gmail.com; Mohammad Shoushtari, Department of Virology, Pasteur Institute of Iran, Tehran, Iran, Tel +98-21-66953311 Ext. No.2251, Email mb.shoushtari@gmail.com

Abstract: Gene therapy using recombinant adeno-associated virus (AAV) vectors has emerged as a promising approach for treating genetic disorders, including Leber congenital amaurosis 2 (LCA-2) induced by RPE65 mutation, a severe form of inherited retinal dystrophies (IRDs). This review provides recent advancements, methodological strategies, and therapeutic aims related to AAV vector-mediated retinal gene therapy for LCA-2 induced by RPE65 mutation. The literature search was performed using the PubMed, Scopus, and Web of Science databases, focusing on studies that examine gene therapy as a potential therapeutic strategy for LCA-2 by introducing functional copies of the RPE65 gene into affected cells. Due to their ability to efficiently deliver therapeutic genes without significant immune responses or mutagenesis events, AAV vectors have shown efficacy in restoring retinal and visual functions in animal models of LCA-2. Advancements in molecular biology and retinal surgery have enabled clinical studies and trials for gene therapy in LCA-2, providing a foundation for further research and improving treatment outcomes. While there is currently no known cure for IRDs, treatments such as vitamin supplementation, gene therapy, and assistive devices can help manage symptoms and slow disease progression. Ongoing clinical trials are investigating novel therapies, including stem cell therapy and gene editing technologies, to expand treatment options for IRDs. The favorable safety profile and proven efficacy of AAV vectors, combined with their capacity for sustained transgene expression, position them as ideal vehicles for ocular gene therapy applications. However, immune responses and off-target effects must be addressed carefully.

Keywords: RPE65, AAV, IRDs, gene therapy, gene editing

Introduction

Gene therapy is a promising field of medicine that aims to treat genetic disorders by modifying or replacing defective genes. This approach has the potential to cure diseases that were previously considered untreatable, such as cancer, cystic fibrosis, heart disease, diabetes, hemophilia, AIDS, and muscular dystrophy.[1] As a durable treatment strategy, gene therapy corrects disease-causing mutations at their source, enabling long-term restoration of cellular function via precise insertion of therapeutic transgenes into affected tissues. While still in its early stages, gene therapy has already shown remarkable success in clinical trials and offers hope for millions of people around the world who suffer from genetic disorders.[2] However, gene therapy has its challenges. One major concern is the potential for unintended consequences, such as triggering an immune response or causing mutations in other genes. Furthermore, the substantial cost of gene therapy treatments and the complexity of delivering genes to specific cells in the body continue to be significant hurdles.[3] Despite these challenges, researchers are persistently making progress in developing safer and more effective gene therapies. Rapid progress in genetic and molecular sciences has positioned gene therapy as a transformative medical paradigm, offering unprecedented opportunities to address previously untreatable hereditary disorders.[4] Gene therapy encompasses three main aspects: gene silencing through the use of siRNA, shRNA, and miRNA; gene replacement,

https://doi.org/10.2147/OPTH.S593854
Received: 5 January 2026
Accepted: 24 April 2026
Published: 29 April 2026

where the desired gene is directly administered in the form of plasmids (Non-viral delivery) and viral vectors; and gene editing-based therapy, which involves modifying mutations using specific nucleases such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspaced short tandem repeats (CRISPR)/CRISPR-associated protein (Cas)-associated nucleases.[5] One common approach is viral vectors, modified viruses that can enter cells and deliver the therapeutic gene. Viral delivery involves using a modified virus to carry the desired gene into the patient's cells. While this method is highly efficient, it can also be risky since viruses can trigger an immune response or cause unintended mutations.[6] Non-viral delivery involves using other types of carriers, such as liposomes, electroporation, and nanoparticles, to deliver the gene into the patient's cells. While this method is generally safer than viral delivery, it is also less efficient and may require multiple treatments. Another promising approach is CRISPR-Cas9 gene editing, which allows for precise modifications to the genome.[7,8] However, each method has limitations and challenges, such as immune responses to viral vectors or off-target effects with gene editing. Therefore, it is important to carefully consider the most appropriate method for each patient and disease before proceeding with gene therapy.[9] There are two main types of gene therapy: somatic and germline. Somatic gene therapy involves modifying the genes in a patient's body cells, such as skin or muscle cells, to treat a specific disease. This type of therapy does not affect the patient's offspring since it only modifies the patient's cells.[10] Germline genetic engineering, encompassing modifications to gametic cells that become inheritable by progeny, occupies an ethically contentious position in biomedical research, given the permanence of interventions and their unpredictable phenotypical manifestations across generational lineages. Gene therapy is a promising field that has the potential to revolutionize the way we treat diseases.[11] Gene therapy has shown great promise in treating various genetic disorders and diseases. One of the most successful applications of gene therapy is treating inherited retinal diseases, such as Leber congenital amaurosis 2 (LCA-2), which can cause blindness. In these cases, gene therapy involves delivering a functional copy of the mutated gene to the patient's retina using a viral vector.[12] This approach has resulted in significant improvements in vision for some patients. Clinical applications of gene therapy now include the treatment of select cancers through engineered immune cells with enhanced tumor-targeting capabilities. While there are still challenges to overcome in developing safe and effective gene therapies, the potential benefits for patients with genetic diseases are immense.[13] This review aims to provide an overview of the current advancements, strategies, and goals in ocular gene therapy using AAV as a vector for treating LCA-2 induced by RPE65 mutation. Our research methodology included a systematic search of PubMed, Google Scholar, and Scopus using keywords such as RPE65, LCA-2, AAV, IRDs, gene therapy, and gene editing.

Inherited Retinal Dystrophies: A Group of Genetic Disorders

Inherited retinal dystrophies (IRDs) are a group of genetic disorders that impact the retina, leading to progressive vision loss and eventually blindness. There are over 260 genes associated with IRDs, with each mutation resulting in a unique variant of the disorder. Symptoms often include night blindness, diminished peripheral vision, loss of central vision, issues with color perception, and an increased sensitivity to bright light. The progression of IRDs can vary widely, even among family members carrying the same genetic mutation.[14] IRDs cause blindness in approximately 1 out of every 3000 individuals. These disorders exhibit significant genotypic and phenotypic heterogeneity, with roughly 300 genes and loci involved. IRDs can be inherited through autosomal recessive, autosomal dominant, or X-linked patterns, making them one of the most genetically diverse groups of inherited disorders.[15] LCA-2 and severe early childhood onset retinal degeneration (SECORD) are inherited, genetically heterogeneous forms of retinal dystrophy that initially present as blindness or severe vision impairment.[16] Retinal cells deteriorate progressively in childhood, which continues over the following few decades.[17] Numerous eye tissues directly influence how well the eye's visual acuity and are frequently associated with both inherited and acquired ocular diseases.[18] Approximately 4000 diseases have already been identified as mutated-related, with a documented case-cause association with the mutation.[19] In the context of LCA-2 management, viral vector-mediated RPE65 gene correction targets the underlying mutation with high precision. AAV vectors are small DNA viruses that efficiently deliver therapeutic genes into the target cells without causing significant immune responses or mutagenesis events. Their ability to infect both dividing and non-dividing cells makes them suitable candidates for long-term transgene expression.[20] Recent research suggests that gene therapy may now be able to treat several heritable and non-heritable ocular disorders, including age-related macular degeneration, color blindness, optic neuropathies, and

corneal diseases.[21] Preclinical studies have demonstrated the efficacy of AAV-mediated gene therapy in animal models of LCA-2 disease. These studies have shown that AAV vectors carrying the RPE65 gene can restore retinal and visual functions, improving mobility and light sensitivity.[12] One of the most notable advancements in gene therapy has been treating the retinal condition known as LCA-2, one of the most severe forms of inherited retinal degeneration. Clinical studies for gene therapy have become feasible due to advancements in molecular biology and retinal surgery. These studies serve as a foundation for further research and testing, which could improve LCA-RPE65 gene therapy and explore the potential of gene therapy in treating other retinal diseases.[22] Currently, there is no known cure for IRDs, but there are available treatments that can slow the disease's progression and help manage its symptoms. These treatments may include vitamin supplements, gene therapy, and assistive devices such as magnifiers or telescopes.[23] Clinical trials are ongoing to test new therapies for IRDs, including stem cell therapy and gene editing technologies. Genetic testing and counselling are also important aspects of managing IRDs. They can help patients and their families understand their risk for the disease and make informed decisions about family planning.[24] Gene therapy stands at the forefront of biomedical innovation, employing precise genetic modifications to directly intervene in disease-causing DNA sequences, thereby offering curative solutions for inherited disorders. Gene therapy has shown promise in treating various genetic diseases, including LCA-2, an inherited retinal disease. AAV vectors have also gained attention due to their safety and efficacy.[25]

LCA-2 Genetic Deficiency

As previously mentioned, LCA-2 is a rare ocular disorder typically inherited through an autosomal-recessive genetic pathway. The condition affects approximately 1 in 81,000 people, with symptoms typically manifesting within the initial months of life.[26] LCA-2/SECORD is a genetic disorder involving over 20 unique genes or loci caused by a mutation in the RPE65 gene on chromosome 1p31. The RPE65 gene is mutated in 6–16% of cases and 1–2% of other cases. The RPE65 gene is crucial in the retinoid cycle, which converts all-trans-retinol into 11-cis-retinol. In the absence of RPE65, retinyl esters build up in the retina, leading to a decrease in 11-cis-retinal levels. Despite its importance, RPE65, a 65-kDa membrane-associated protein, has exhibited little alteration over time.[27,28] The visual cycle, also known as the recycling process, is vital for vision because photoreceptors need it to convert light photons into brain messages. The RPE cells remain viable, making them a potential target for gene therapy. Interestingly, the lack of normal RPE65 causes the adjacent photoreceptors to deteriorate.[19] These mutations are believed to result from the RPE65 gene, which produces a protein required for the isomer hydrolase activity of the retinal pigment epithelium and is associated with the LCA-2 type of disease.[29,30]

In 1869, the German ophthalmologist Theodor Leber discovered LCA for the first time. He said the disorder is marked by retinitis pigmentosa, nystagmus, and amaurotic pupils and causes substantial vision impairment that begins at or before one month after birth. Typically, visual impairments are identified by the age of six months.[2,31] The clinical diagnosis is supported by diminished pupillary light reflexes and flat or undetectable responses on the electroretinogram.[32] Understanding the function of RPE65 in the visual cycle is crucial for comprehending LCA-2 gene therapy. A summary of the retinal system is provided in Figure 1.[18] Retinal gene therapy is being increasingly recognized as a novel molecular therapy with great promise for treating common causes of blindness, the majority of which have hereditary roots.[33] Numerous once-incurable diseases are now successfully treated by ocular gene therapy, a growing discipline.[18,26] Based on outstanding results in multiple animal models, rAAV has demonstrated significant potential as a DNA-delivery vector to treat serious human diseases.[34] Successful RPE65 gene replacement has increased visual acuity, restored cone and rod sensitivity, and improved vision fields.[16,28]

Currently, the treatment options for LCA-2 are limited. One approach is gene therapy, which involves replacing the mutated gene with a healthy one. However, this treatment is still experimental and has only been successful in a few patients.[35] Another alternative is retinal implants, which can help restore some vision by bypassing the damaged cells in the retina. However, these devices are costly and not widely accessible.[36]

Additionally, they need to provide a comprehensive solution as they can only restore a limited amount of vision and require extensive training for effective use. Researchers continue to work towards finding new therapies that can provide more effective solutions for individuals with this condition.[37] Moreover, these treatments only offer partial vision restoration and require extensive training to be used effectively. Therefore, researchers must continue their efforts to

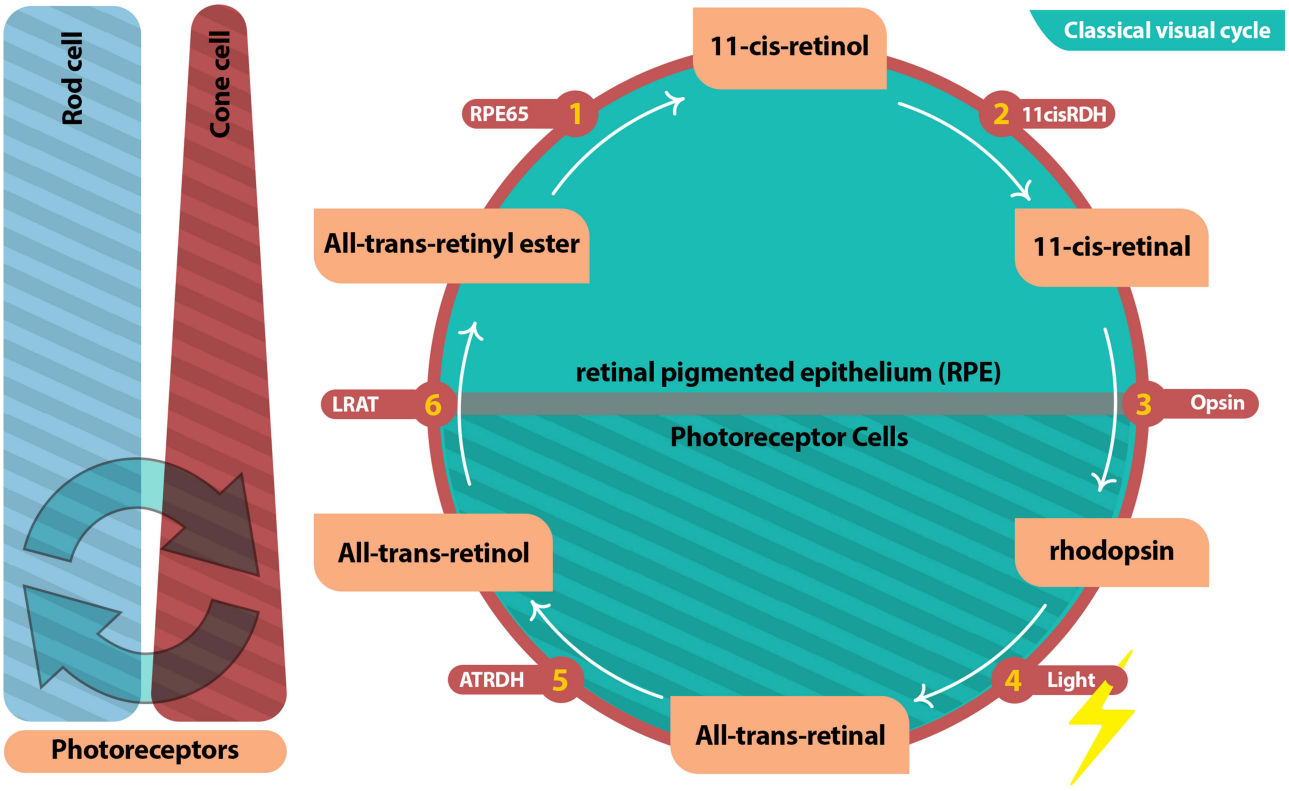

Figure 1 A schematic representation of the visual cycle in the vertebrate retina. This enzymatic cascade, primarily driven by retinoid dehydrogenase/reductase (RDH) activity in both photoreceptors and retinal pigment epithelium (RPE), ensures continuous renewal of visual pigments after light-induced degradation. The process begins when 11-cis-retinal (11cRAL) transports from the RPE to rod outer segments, conjugating with opsin to form rhodopsin (Reaction 3). Subsequent light absorption triggers photoisomerization of the 11-cis-retinal chromophore to its all-trans-retinal configuration (Reaction 4). The resulting all-trans-retinal then undergoes NADPH-dependent reduction to all-trans-retinol, catalyzed by all-trans-retinal dehydrogenase (ATRDH; Reaction 5). Following its formation, all-trans-retinol (atROL) traverses to the retinal pigment epithelium (RPE), where lecithin: retinol acyltransferase (LRAT; Reaction 6) mediates its esterification into all-trans-retinyl esters (atRE). The subsequent conversion of atRE to 11-cis-retinol (11cROL) represents the rate-limiting step of the cycle, catalyzed by the RPE-specific 65 kDa protein (RPE65; Reaction 1). Finally, 11-cis-retinol dehydrogenase (RDH5; Reaction 2) oxidizes 11cROL to regenerate 11-cis-retinal (11cRAL), thus completing the retinoid visual cycle.

find more effective therapies that can overcome the limitations of current treatments. With the progress of research and technological advancements, there is optimism that more accessible and comprehensive solutions will be developed for individuals with LCA-2.[38] In the meantime, it's crucial to raise awareness about this condition and advocate for increased funding for research initiatives.

Preclinical/Clinical Studies of AAV-Mediated Gene Therapy for LCA-2

Preclinical Studies in Animal Models

Extensive preclinical research has been conducted using animal models to evaluate the potential of AAV-mediated gene therapy for LCA-2. Initial studies in RPE65-deficient dogs and mice demonstrated the therapeutic potential of this approach. Acland et al showed that subretinal delivery of rAAV2/2-RPE65 vectors effectively restored visual function in canines with RPE65-associated LCA.[39] Similarly, Le Meur et al used an AAV serotype 4 (rAAV4-RPE65) vector to target the RPE in RPE65-deficient Briard dogs, restoring vision.[40] Several clinical trials have evaluated the safety and efficacy of AAV-mediated gene therapy for LCA-2 in human patients.

Clinical Trials

The development of AAV-mediated gene therapy for LCA-2 has progressed to several clinical trials, which have provided valuable insights into the efficacy and safety of this approach. One of the landmark trials was the Phase 3 clinical trial of voretigene neparvovec (AAV2-hRPE65v2) conducted by Russell et al In this randomized, controlled, open-label study, 31 patients with RPE65-mediated inherited retinal dystrophy, including those with LCA-2, received a subretinal injection

of the AAV vector carrying the RPE65 gene. The results showed a significant improvement in the primary endpoint of mobility testing, with a mean change of 1.6 points on a 5-point scale, compared to the control group. Additionally, the treated group demonstrated improvements in visual acuity, visual field sensitivity, and pupillary light reflex. The treatment was generally well-tolerated, with no serious adverse events related to the gene therapy.[41] Another notable clinical trial was the study by Jacobson et al, which enrolled 12 patients with LCA-2 and evaluated the safety and efficacy of subretinal injection of an AAV2 vector carrying the RPE65 gene. The results showed that the treatment was safe and well-tolerated, with no serious adverse events. Notably, the study demonstrated improved visual function in the treated eyes, with some patients reporting improved mobility and navigation in daily life.[42]

Further clinical trials are ongoing, such as the Phase 1/2 study by Spark Therapeutics (NCT00999609) and the Phase 1/2 trial by MeiraGTx (NCT03252847), which are exploring different AAV vector designs, delivery methods, and patient populations to optimize the gene therapy for LCA-2. Table 1 meticulously documents all relevant clinical trials published on the clinicaltrials.gov website, and some of the human studies mentioned in the articles have been summarized in Table 2.

Comparing AAV Gene Therapy with Alternative Approaches

Stem Cell Therapy

An alternative approach to LCA-2 treatment is the use of stem cell-based therapies. Researchers have explored the potential of using patient-derived induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) to generate RPE cells for transplantation.[45] This approach aims to replace the dysfunctional RPE cells with healthy, functional cells. While stem cell therapy holds promise, there are challenges related to cell engraftment, immune rejection, and the risk of tumor formation.[46]

Photoreceptor transplantation is a potential strategy for treating broad-spectrum blinding retinal conditions and may be the first transplanted stem cell-derived neuron. The photoreceptor, which does not divide, makes only one synaptic connection and is located in the subretinal space, making it accessible surgically. The retina is relatively devoid of myelin proteins, which inhibit neuronal regeneration. Early investigations focused on transplanting photoreceptor sheets or dissociated cells into *rd* mice with retinal degeneration similar to human retinopathy (RP).[47]

A systematic review and meta-analysis of 21 prospective studies on stem cell therapy for IRDs found that it may be an effective and relatively safe treatment approach. The study found that for retinitis pigmentosa (RP) patients, the best-corrected visual acuity improvement rate was 49% and 30% at 6 and 12 months, respectively. However, the improvement was not significant at 12 months. The authors suggest that suprachoroidal space injection of stem cells may be more efficient for RP patients. The study also indicates that the long-term efficacy for RP patients remains uncertain and suggests exploring suprachoroidal space injection as a promising administration route.[48]

Pharmacological Approaches

Pharmacological interventions, such as retinoid replacement therapy, have also been investigated for LCA-2 treatment. This approach aims to restore the visual cycle by providing an alternative source of retinoids, which are essential for visual function.[49] While pharmacological approaches can be less invasive than gene or cell-based therapies, their efficacy may be limited and may not address the underlying genetic defect.

In summary, AAV gene therapy, stem cell-based therapies, and pharmacological approaches represent different strategies being explored for treating LCA-2. Each approach has its advantages and disadvantages, and the current state of research suggests that AAV gene therapy may be the most advanced and promising option, with ongoing clinical trials demonstrating its potential to restore visual function. However, further research is needed to optimize these treatment modalities and address the remaining challenges to provide effective and durable therapies for patients with this debilitating condition.

Genome Editing Technologies

CRISPR/Cas

Clustered, regularly interspaced short palindromic repeats associated protein (CRISPR) is a technique designed for targeted modification of specific DNA or RNA sequences, often producing unwanted or unexpected changes. In bacteria

Table 1 Summarized Clinical Trials in AAV Gene Therapy

Row	NCT Number	Status	Interventions	Characteristics	Population	Sponsor/ Collaborators	Dates	Country
1	NCT00999609	Active, not recruiting	•Biological: AAV2-hRPE65v2, voretigene neparvovec-rzyl	**Study Type**: Interventional **Phase**: Phase 3	**Enrollment**: 31 **Age**: 3 years and older (Child, Adult, Older Adult) **Sex**: All	•Spark Therapeutics •Children's Hospital of Philadelphia •University of Iowa	**Study Start**: October 2012 **Primary Completion**: July 2015 **Study Completion**: July 2029	United States
2	NCT01208389	Active, not recruiting	•Biological: voretigene neparvovec-rzyl	**Study Type**: Interventional **Phase**: •Phase 1 •Phase 2	**Enrollment**:12 **Age**: 8 years and older (Child, Adult, Older Adult) **Sex**: All	•Spark Therapeutics	**Study Start**: November 2010 **Primary Completion**: March 2030 **Study Completion**: June 2030	United States
3	NCT00516477	Completed	•Biological: voretigene neparvovec-rzyl	**Study Type**: Interventional **Phase**: Phase 1	**Enrollment**: 12 **Age**: 8 years and older (Child, Adult, Older Adult) **Sex**: All	•Spark Therapeutics	**Study Start**: September 2007 **Primary Completion**: March 20, 2018 **Study Completion**: March 20, 2018	United States
4	NCT02946879	Active, not recruiting	•Biological: AAV OPTIRPE65	**Study Type**: Observational	**Enrollment**: 27 **Age**: 3 Years to 100 Years (Child, Adult, Older Adult) **Sex**: All	•MeiraGTx UK II Ltd •Syne Qua Non-Limited	**Study Start**: November 2016 **Primary Completion**: April 2023 **Study Completion**: April 2023	United Kingdom
5	NCT02781480	Completed	•Biological: AAV RPE65	**Study Type**: Interventional **Phase**: •Phase 1 •Phase 2	**Enrollment**: 15 **Age**: 3 years and older (Child, Adult, Older Adult) **Sex**: All	•MeiraGTx UK II Ltd	**Study Start**: April 2016 **Primary Completion**: December 2018 **Study Completion**: December 2018	United States, United Kingdom
6	NCT00643747	Completed	•Biological: tgAAG76 (rAAV 2/2.hRPE65p. hRPE65)	**Study Type**: Interventional **Phase**: •Phase 1 •Phase 2	**Enrollment**: 12 **Age**: 5 Years to 30 Years (Child, Adult) **Sex**: All	•University College, London •Moorfields Eye Hospital NHS Foundation Trust •Targeted Genetics Corporation	**Study Start**: January 2007 **Primary Completion**: December 2014 **Study Completion**: December 2014	United Kingdom

https://doi.org/10.2147/OPTH.S593854

7	NCT03602820	Active, not recruiting	•Biological: AAV2-hRPE65v2	**Study Type**: Observational	**Enrollment**: 41 **Age**: Child, Adult, Older Adult **Sex**: All	•Spark Therapeutics	**Study Start**: June 2015 **Primary Completion**: March 2030 **Study Completion**: June 2030	United States
8	NCT03597399	Active, not recruiting	•Biological: AAV2-hRPE65v2, voretigene neparvovec-rzyl	**Study Type**: Observational	**Enrollment**:87 **Age**: 12 Months and older (Child, Adult, Older Adult) **Sex**: All	•Spark Therapeutics	**Study Start**: January 10, 2019 **Primary Completion**: June 2025 **Study Completion**: June 2025	United States
9	NCT03252847	Completed	Genetic: AAV2/5-RPGR	**Study Type**: Interventional **Phase**: Phase 1 Phase 2	**Enrollment**: 49 **Ages**: 5 Years and older (Child, Adult, Older Adult) **Sex**: Male	•MeiraGTx UK II Ltd •Syne Qua Non-Limited •Bionical Emas	**Study Start**: 2017–07-14 **Primary Completion**: 2021–11-18 **Study Completion**: 2021–11-18	United States

https://doi.org/10.2147/OPTH.S593854

Table 2 Summary of Relevant Clinical Studies

Study	Population	Intervention	Outcomes	Limitations
Bainbridge et al 2008,[43]	3 patients with RPE65-associated LCA	Single subretinal injection of rAAV2/2-RPE65	Improved visual function, no serious adverse events	Small sample size, short follow-up period
Jacobson et al 2012,[42]	12 patients with RPE65-associated LCA	Single subretinal injection of rAAV2-PR1.7-hRPE65v2	Improved visual function, no serious adverse events	Lack of control group, variable treatment response
Weleber et al 2016,[28]	29 patients with RPE65-associated LCA	Single subretinal injection of rAAV2-CB-hRPE65	Improved visual function, generally well-tolerated	Heterogeneity in baseline characteristics and longer-term safety data needed
Spark Therapeutics 2020,[44]	37 patients with RPE65-associated LCA	Single subretinal injection of voretigene neparvovec-rzyl (Luxturna™)	Improved visual function, manageable safety profile	Lack of long-term follow-up data, potential for immune responses

and archaebacteria, it works as an adaptive defense against invading nucleic acids. The most well-known CRISPR system is CRISPR/streptococcus pyogenes CRISPR associated protein 9 (spCas9), derived from the Streptococcus pyogenes bacterium.[50] The system consists of two primary components: an RNA (containing two distinct RNAs named CRISPR RNA (crRNA) and trans-activator RNA (tracrRNA)) and the Cas9 endonuclease protein, which targets crRNA and tracrRNA. Bioinformatics methods were used to create the functional system-related RNA of this bacterium, which has become a single guide RNA (sgRNA). The Cas9 protein identifies the region after the 5′ sgRNA ends of 20 nucleotides bind to the target site, making a blunt-end double-strand break in DNA. The NHEJ and HDR mechanisms are the two main processes for repairing double-stranded breaks in DNA. The knock-out method has been widely used to assess the genes' function in stem cell stemness and differentiation. CRISPR/Cas12 is another form of this system, first discovered in Acidaminococcus and Lachnospiraceae bacteria. Base editing (BE) and prime editing (PE) represent the next generation of CRISPR technology, with the latter having high potential for personalized medicine if efficiency, precision, and specificity are improved. PE technology has been applied to create changes in DNA with the highest efficiency and least off-target.[51,52] Gene therapy approaches depend on the genetic defect and disease phenotype. Ophthalmic diseases that preserve morphological structure are more suitable for in vivo gene therapy, including exogenous neurotrophic factors, supplementation with the missing gene product, or gene editing. Diseases requiring cell replacement face unique challenges, which stem cells are addressing through research and clinical trials. CRISPR/Cas9 is used for adult retinal disease treatment but is limited to recessive and null genetic diseases. For example, RPE65 insufficiency in LCA 2 patients can be restored by providing exogenous wild-type RPE65. However, patients treated with AAV vectors with the RPE65 gene have not maintained vision over long periods.[53]

Protein-DNA interface systems such as zinc finger nuclease (ZFN) and transcription activator-like effector nucleases (TALEN) are protein-dependent, which complicates target engineering.[54] Gene editing tools like ZFN and TALEN have been developed for genetic disease treatment. However, precision genome editing agents, like base editors and prime editors, have enabled precise target gene correction in various therapeutic settings, including mouse models of IRDs. This expansion of therapeutic applications is crucial, as most genetic disorders cannot be treated by gene disruption. This article discusses progress in using genome editing to treat IRDs and emphasizes the importance of robust clinical translation[55] (Figure 2).

Overview of AAV and Its Characteristics

AAV belongs to the family of parvoviruses. Parvoviruses are among the smallest DNA animal viruses, with a size of about 25 nm in diameter, and are entirely composed of protein and DNA. AAV is categorised as a dependovirus because it requires co-infection with helper viruses, such as adenovirus.[56] This essay will provide an overview of AAV as a vector

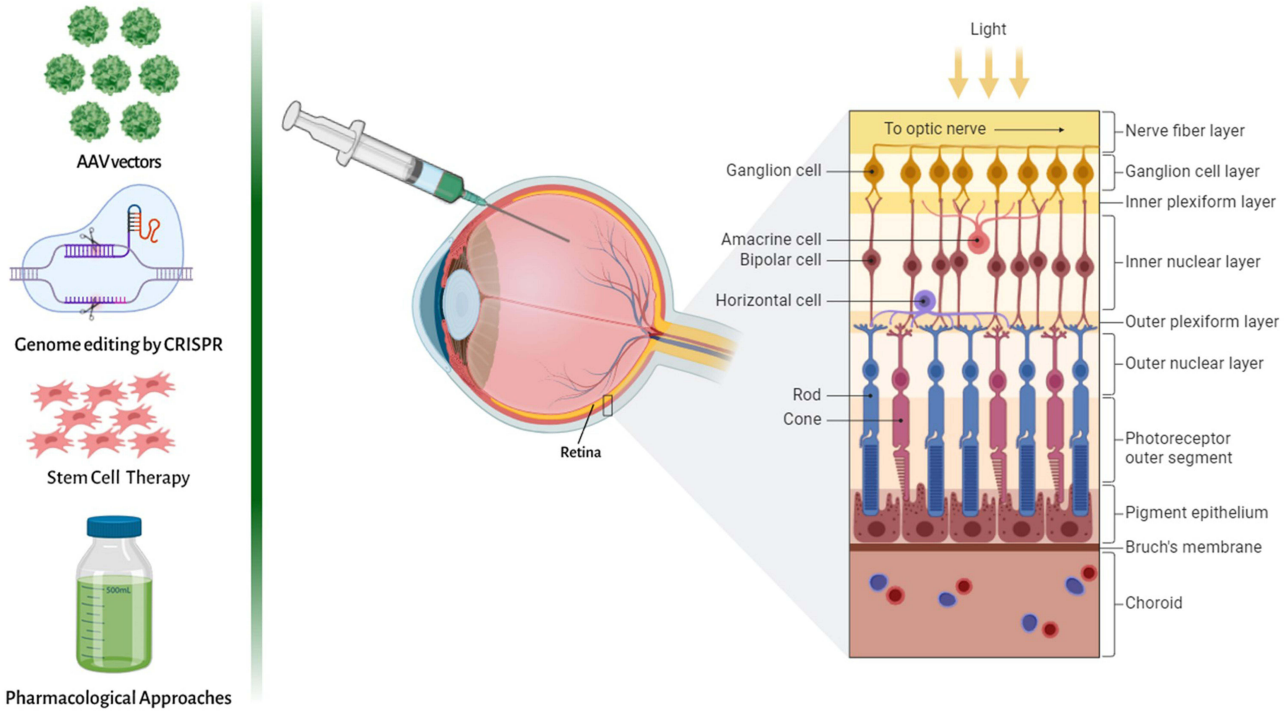

Figure 2 Emerging Therapeutic Strategies in Ocular Medicine: A Comparative Analysis of Gene Therapy, Stem Cell Therapy, Gene editing and Pharmacological Approaches.

for gene therapy in LCA-2 disease. It will discuss its potential use, preclinical studies, clinical trials, safety concerns, and future directions. Recently, gene therapy has been researched to cure previously incurable inherited retinal illnesses in animal and human models.[34] Recombinant AAV (rAAV) vectors for ocular gene therapy have shown promise in several in vitro, in vivo, and ex vivo preclinical models. Extensive preclinical research led to the first successful ocular gene therapy clinical study.[18,26] Recombinant viruses are the most effective method for introducing genes into living cells. The rAAV, safe in rats, dogs, primates, and humans, is one of the most secure and efficient gene delivery techniques.[19] Sensitivity in cone and rod has been restored, the vision fields have been improved, and the RPE65 gene has been successfully substituted.[16,28] Gene transfer technology relies on and seeks to exploit the initial replication stage while simultaneously constructing barriers to prevent the production of infectious viruses. Transduction is a non-replicative or terminal infection that facilitates the delivery of heterologous (non-viral) genetic information to a specific cell. As described below, the viral genome undergoes significant rearrangement to eliminate replication and pathogenicity genes, making room for heterologous genes. Following this transformation, the parental virus becomes a mere carrier of genetic information, hence the viral vector.[57]

AAV stands out because it requires a helper virus to replicate, which renders it safe for human use. It also has high stability and can be engineered to target specific cells and tissues. AAV-based gene therapy entails the delivery of therapeutic genes to the appropriate cells using AAV vector.[57] This approach has demonstrated great potential for treating genetic disorders, such as LCA-2, a rare form of blindness resulting from mutations in the RPE65 gene. AAV has revolutionized gene therapy by providing a safe and effective means of delivering therapeutic genes to specific cells and tissues.[25] This review discusses key studies on RPE65-related retinopathy, highlighting the advancements in ocular gene therapy and the safety of administering an AAV recombinant containing RPE65 cDNA into the subretinal space. It focuses on human retinal disease caused by RPE65 mutations, specifically examining the degeneration and functional loss of retinal cells resulting from these mutations.

https://doi.org/10.2147/OPTH.S593854

AAV as a Potential Vector for Gene Therapy in LCA-2

Using viruses to deliver nucleic acid into cells for replication was one of the initial approaches to artificial delivery. Some preclinical and clinical successes have been with other artificial delivery methods, such as nanoparticles. However, these methods have encountered undesirable safety signals that must be properly identified and handled.[20] AAV is a small, non-pathogenic virus that can be engineered to deliver functional copies of the RPE65 gene to the retina. This approach has demonstrated promising results in preliminary clinical trials, with several patients experiencing substantial enhancements in their visual function. Despite the promise of AAV-based gene therapy in treating LCA-2 and other genetic disorders, we still have some hurdles to clear. These include ensuring we can deliver enough therapeutic genes to the correct cells and sidestepping immune responses that might lessen the treatment's effectiveness. But even with these challenges, the potential of this therapy is immense.[58,59] Recently developed non-pathogenic AAV-based vectors can potentially be used in cancer gene therapy.[60] AAV, a member of the parvovirus family with a small, single-stranded DNA genome of about 4.8 kilobases (kb), is encased in a protein shell. AAV cannot replicate independently and requires co-infection with other viruses.[61] AAV, a dependovirus, requires helper viruses for its replicative life cycle. It integrates into the host genome, protecting infected cells from helper viruses and facilitating successful lytic cycles.[60]

The virus has three genes: Rep (Replication), Cap (Capsid), and Aap (Assembly), which produce at least nine gene products. The expression of the Cap gene results in the production of capsid proteins, which protect the viral genome and are involved in cell binding and internalization. The Rep gene encodes four proteins necessary for replication and packaging.[61] AAV5, AAV6, and AAV2 are growth factor receptors for treating various diseases. AAV8 is being studied for gene transfer in hemoglobinopathies and disorders. AAV1 and AAV9 have proven efficient in delivering genes to skeletal and cardiac muscle. An engineered variant of AAV1 is under investigation for its potential use in treating heart failure andlipoprotein lipase deficiency. These genes are currently undergoing testing in clinical trials.[20] After a single subretinal injection, rAAV2 successfully treated patients with LCA-2, which was caused by RPE65 mutations. This treatment maintained the rescue of the retinal pigment epithelium (RPE) and photoreceptor cells.[46] Several clinical trials have recently examined the potential use of gene therapy techniques to treat LCA-2 caused by RPE65 mutations.[2,62] The AAV2 vector has proven particularly effective at targeting outer retinal layers. A single subretinal injection can deliver a functioning copy of the human RPE65 gene into healthy RPE cells. Young people who received AAV-mediated RPE65 therapy reported an improvement in brightness perception. Clinical trials utilizing AAV vectors have commenced for a variety of diseases, including prostate cancer, malignant melanoma, Parkinson's disease, alpha1-antitrypsin deficiency, arthritis, Batten's disease, Canavan's disease, cystic fibrosis, HIV infection, LCA-2.[63]

AAV Viral Life Cycle and How It Relates to Gene Therapy

The replication process within the retinal cell, as depicted in Figure 3, starts with the virus attaching to host receptors and subsequently being internalized by the host cell through clathrin-mediated endocytosis. The host endosomal membrane is permeabilized, after which the virion is transported toward the nucleus via microtubules and enters the cytoplasm. Subsequently, the viral ssDNA genome invades the nucleus. Cellular proteins convert the genome's panhandle ssDNA into dsDNA. The viral genome can occasionally be incorporated into the host chromosome, even without a helper virus.[63]

The AAV viral life cycle is an essential component of gene therapy. The virus enters the host cell via receptor-mediated endocytosis and then follows a series of steps to deliver its genetic material to the nucleus. Once inside the nucleus, the viral DNA integrates into the host genome, which can be expressed as a therapeutic gene.[64] AAV-based gene therapy has several advantages over other methods of gene delivery. It is safe and efficient and can also be engineered to target specific cells, making it an ideal candidate for treating genetic disorders. Its capacity to deliver therapeutic genes directly to the affected cells ensures the treatment is highly effective and minimizes off-target effects. As research advances, we can anticipate more innovative applications of AAV-based gene therapy in treating various genetic diseases.[20]

Vectors have a significant impact on the effectiveness and safety of gene therapy. AAV vectors provide benefits such as the ability to infect both dividing and non-dividing cells, stable integration, low immunogenicity, and long-term

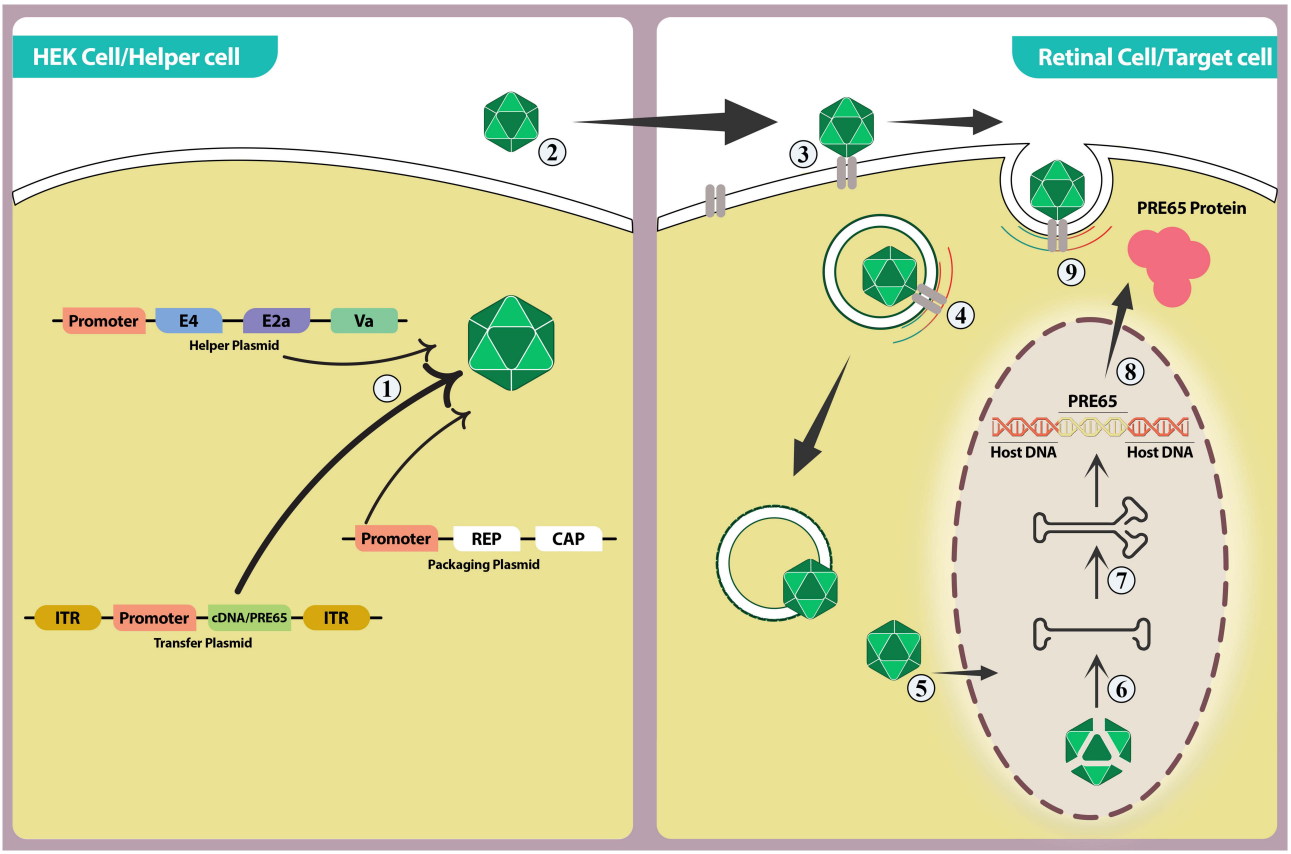

Figure 3 This schematic representation illustrates the process of gene therapy using an AAV vector. 1. Specifically, it depicts the steps in packaging a therapeutic gene (such as the RPE65 gene) into the AAV genome within a HEK (human embryonic kidney) cell. 2. The modified virus is then prepared for delivery to retinal cells. The simplified mechanism of Adeno-associated virus replication includes the following steps: 3. Attachment to host receptors initiates endocytosis of the virion into the host cell via clathrin. 4. The virion enters the cytoplasm using endosomal membrane permeabilization. 5. Transport of the virus towards the nucleus via microtubules. 6. The ssDNA genome of the virus enters the nucleus. 7. Proteins within the cell convert ssDNA into dsDNA. When the host cell enters the S phase, dsDNA transcription produces viral mRNAs (with the RPE65 gene). 8. mRNAs are then translated into viral proteins. 9. The newly formed viruses undergo encapsulation and release upon cell lysis. Notably, this virus can synthesize the RPE65 protein within retinal cells, thereby addressing the RPE65 deficiency.

expression. However, they can potentially infect different cell types in varying ways, and the size of the recombinant genome can pose a major disadvantage.[6] Early in vitro experiments have shown that AAV can infect various human and animal cells from different origins. Subsequently, the in vivo efficacy of AAV was demonstrated in murine and nonhuman primate models using several candidate genes and target organs.[60,65] However, despite their substantial benefits, they do have certain drawbacks. These include limited packaging options, which restrict the size of transgenes that can be delivered. There is also a risk of insertional mutagenesis when integrating vector seropositivity. Antibodies against wild-type AAV are common, and humoral immune responses to the vector are inhibited. Furthermore, achieving sufficiently high titers necessary for human clinical studies can be challenging.[66]

Pros and Cons of Gene Therapy by AAV Vectors

Every form of human gene therapy carries numerous complex side effects. This meta-analysis suggests that gene therapy only prevents the long-term loss of visual function for up to two years. Gene therapy only addresses the biochemical chromophore deficiency, assuming some photoreceptor cells survive. A biochemical chromophore deficiency and the progressive degeneration of photoreceptor cells cause the loss of visual function in LCA-2. Despite the initial benefits of gene therapy, the continued loss of visual function due to ongoing retinal degeneration remains a possibility.[67] Despite the promise of AAV gene therapy for LCA-2 and other genetic disorders, several challenges and limitations must be considered. One potential challenge is the body's immune response, which can recognize the viral vector used in AAV

https://doi.org/10.2147/OPTH.S593854

gene therapy as a foreign invader. This immune response can lead to inflammation and damage to the targeted cells, thereby reducing the effectiveness of the treatment.[68]

Additionally, there might be an increase in the amount of genetic material that can be delivered through AAV gene therapy, which could affect its ability to correct genetic mutations fully. Another potential limitation is the long-term safety and efficacy of AAV gene therapy, as we still have a lot to learn about its interactions with our bodies over time.[69] Despite these challenges, continued research and development in this field are promising to improve the lives of individuals with LCA-2 and other genetic disorders. One such limitation is the amount of genetic material delivered through this therapy, which could affect its ability to entirely correct mutations.[70] Despite these challenges, ongoing research and development in this field offer significant potential for enhancing the lives of individuals with LCA-2 and other genetic disorders. One limitation is the quantity of genetic material this therapy can deliver, which may affect its ability to entirely correct mutations.[71] By overcoming these limitations and advancing our understanding of AAV gene therapy, we can strive for a future where genetic disorders no longer hinder pursuing healthy and fulfilling lives. As we delve deeper into AAV gene therapy, we continually uncover novel methods that could benefit individuals with genetic disorders, such as LCA-2.

Challenges, Limitations, and Breakthroughs of Gene Therapy Using AAV Vectors

While the initial clinical trial results have been promising, there are still challenges and limitations associated with AAV-based gene therapy for LCA-2. Immune responses: the potential for immune responses to the AAV vector remains a concern, as it may limit the long-term safety and sustainability of the treatment.[72] The immune response can be directed against the AAV capsid, the transgene product, or both. Strategies to overcome this challenge include using capsid variants with reduced immunogenicity, developing immune-evasive AAV vectors, and exploring immunosuppressive regimens to suppress the immune response.[71,73] Sample size and diversity: the current clinical trial data needs to be more extensive in terms of sample size and the diversity of the patient population, which may affect the generalizability of the findings.[74] Long-term safety and efficacy: Comprehensive longitudinal data collection remains essential for verifying enduring safety profiles and therapeutic efficacy of AAV-mediated gene therapy in LCA-2. This innovative treatment could transform our approach to genetic disorders such as LCA-2.[75] Another concern with gene therapy is the risk of unintended mutations. Integrating the therapeutic gene into the host genome can disrupt endogenous gene expression or the activation of oncogenes, potentially causing adverse effects. In the case of LCA-2, AAV vectors, which typically do not integrate into the host genome, can mitigate this risk. However, the potential for off-target effects or the integration of the therapeutic gene into unintended genomic locations cannot be eliminated.[76] To address this challenge, researchers are exploring strategies such as using site-specific integration approaches, developing self-inactivating AAV vectors, and implementing rigorous safety monitoring protocols.[77,78]

Despite these challenges, AAV gene therapy remains a promising avenue for improving the lives of individuals with LCA-2 and other genetic disorders. In addition to safety and efficacy, accessibility is also a crucial factor in the success of gene therapy.[79] The high cost of treatment and limited availability can prevent many individuals from obtaining the care they require. Efforts must be made to make gene therapy more affordable and accessible to those who need it most. Current methods involve injecting the viral vector directly into the affected tissue or organ, which can be invasive and challenging to target accurately. Researchers are investigating new delivery methods that could be less invasive and more precise, such as inhalation or topical application. A search on the clinicaltrials.gov website reveals four studies, two of which have been completed.[28,80,81] The others are active, not recruiting, and these studies, for up to 15 years after subretinal AAV2-hRPE65v2 administration for each subject (NCT02946879, NCT03602820) that one of them enrolled 41 people in the United States and United Kingdom and the other 27 (Table 1).

Conclusion and Future Perspective

The development of AAV gene therapy marks a critical turning point for inherited genetic disorders, offering tangible hope for severe conditions such as LCA-2. As we learn more about AAV's interactions with our bodies, we can develop

more targeted and effective treatments for genetic disorders. With continued research and development, we hope for a future where individuals with genetic disorders no longer suffer from debilitating symptoms and can live healthy and fulfilling lives. The promise of AAV gene therapy is exciting, and its potential benefits are significant for global health and well-being. While there are still challenges to overcome, such as ensuring the safety and efficacy of AAV gene therapy, the progress made thus far is remarkable. The technology may also treat various conditions, including cancer and neurological disorders like Parkinson's.

AAV-mediated gene therapy has great potential for treating inherited retinal diseases like LCA-2, but there are ethical concerns and questions about its long-term use. The potential for gene therapy to move beyond medical treatment into human enhancement or modification raises concerns about exposing more human subjects to risks and ensuring patients are fully aware of potential outcomes. The high costs of developing and delivering gene therapies also present challenges in equitable access, as those unable to afford new treatments may face worsening health disparities. Patent restrictions on medical innovations can limit wider availability in certain countries and populations. Increased dialogue is necessary to promote fairness and prevent discrimination based on socioeconomic status. Researchers must consider ethical obligations, long-term impacts, and access issues alongside scientific advancements in gene therapy applications like AAV vectors.

AI-Assisted Text Generation

During the manuscript preparation, the authors utilized [ChatGPT/GPT-4] solely for linguistic enhancement and initial structuring of non-technical sections. All AI-processed content was critically evaluated, rewritten, and scientifically validated by the authors to ensure compliance with academic standards and originality.

Data Sharing Statement

Data will not be shared, according to the rules of Hamadan University of Medical Sciences.

Ethics Approval

This study was approved by the ethics committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1404.156). It is worth noting that the scientific code of this project is 140403062050.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors declare no competing interests in this work.

References

1. Yazdani A, Alirezaie Z, Motamedi M, Amani J. Gene therapy: a new approach in modern medicine. *Int J Med Rev*. 2018;5(3):106–117. doi:10.29252/IJMR-050304
2. Le Meur G, Lebranchu P, Billaud F, et al. Safety and long-term efficacy of AAV4 gene therapy in patients with RPE65 leber congenital amaurosis. *Mol Ther*. 2018;26(1):256–268. doi:10.1016/j.ymthe.2017.09.014
3. Gonçalves GAR, Paiva RMA. Gene therapy: advances, challenges and perspectives. *Einstein*. 2017;15(3):369–375. doi:10.1590/s1679-45082017rb4024
4. Bulaklak K, Gersbach CA. The once and future gene therapy. *Nat Commun*. 2020;11(1):5820. doi:10.1038/s41467-020-19505-2
5. Sayed N, Allawadhi P, Khurana A, et al. Gene therapy: comprehensive overview and therapeutic applications. *Life Sci*. 2022;294:120375. doi:10.1016/j.lfs.2022.120375

6. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther*. 2021;6(1):53. doi:10.1038/s41392-021-00487-6
7. O'Keeffe AJ, Lara-Sáez I, Zhou D, et al. Non-viral delivery of CRISPR–Cas9 complexes for targeted gene editing via a polymer delivery system. *Genet Ther*. 2022;29(3):157–170. doi:10.1038/s41434-021-00282-6
8. Behr M, Zhou J, Xu B, Zhang H. In vivo delivery of CRISPR-Cas9 therapeutics: progress and challenges. *Acta Pharmaceutica Sinica B*. 2021;11 (8):2150–2171. doi:10.1016/j.apsb.2021.05.020
9. Kazemian P, Yu SY, Thomson SB, Birkenshaw A, Leavitt BR, Ross CJD. Lipid-nanoparticle-based delivery of CRISPR/Cas9 genome-editing components. *Mol Pharm*. 2022;19(6):1669–1686. doi:10.1021/acs.molpharmaceut.1c00916
10. Dwivedi S, Purohit P, Vasudeva A, et al. Gene therapy and gene editing in healthcare. 2022;147–175.
11. Wolf DP, Mitalipov PA, Mitalipov SM. Principles of and strategies for germline gene therapy. *Nat Med*. 2019;25(6):890–897. doi:10.1038/s41591-019-0473-8
12. Miraldi Utz V, Coussa RG, Antaki F, Traboulsi EI. Gene therapy for RPE65-related retinal disease. *Ophthalmic Genet*. 2018;39(6):671–677. doi:10.1080/13816810.2018.1533027
13. Nuzbrokh Y, Ragi SD, Tsang SH. Gene therapy for inherited retinal diseases. *Ann Transl Med*. 2021;9(15):1278. doi:10.21037/atm-20-4726
14. Simonelli F, Sodi A, Falsini B, et al. Care pathway of RPE65-related inherited retinal disorders from early symptoms to genetic counseling: a multicenter narrative medicine project in Italy. *Clin Ophthalmol*. 2021;15:4591–4605. doi:10.2147/OPTH.S331218
15. Chen TC, Huang DS, Lin CW, et al. Genetic characteristics and epidemiology of inherited retinal degeneration in Taiwan. *NPJ Genom Med*. 2021;6 (1):16. doi:10.1038/s41525-021-00180-1
16. Pennesi ME, Weleber RG, Yang P, et al. Results at 5 years after gene therapy for RPE65-deficient retinal dystrophy. *Hum Gene Ther*. 2018;29 (12):1428–1437. doi:10.1089/hum.2018.014
17. Bennett J, Ashtari M, Wellman J, et al. AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Transl Med*. 2012;4 (120):120ra15. doi:10.1126/scitranslmed.3002865
18. Roy K, Stein L, Kaushal S. Ocular gene therapy: an evaluation of recombinant adeno-associated virus-mediated gene therapy interventions for the treatment of ocular disease. *Hum Gene Ther*. 2010;21(8):915–927. doi:10.1089/hum.2010.041
19. Rakoczy EP, Narfström K. Gene therapy for eye as regenerative medicine? Lessons from RPE65 gene therapy for Leber's congenital amaurosis. *Int J Biochem Cell Biol*. 2014;56:153–157. doi:10.1016/j.biocel.2014.09.022
20. Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a vector for gene therapy. *BioDrugs*. 2017;31(4):317–334. doi:10.1007/s40259-017-0234-5
21. Solinís M, Del Pozo-Rodríguez A, Apaolaza PS, Rodríguez-Gascón A. Treatment of ocular disorders by gene therapy. *Eur J Pharm Biopharm*. 2015;95(Pt B):331–342. doi:10.1016/j.ejpb.2014.12.022
22. Stein L, Roy K, Lei L, Kaushal S. Clinical gene therapy for the treatment of RPE65-associated Leber congenital amaurosis. *Expert opin biol ther*. 2011;11(3):429–439. doi:10.1517/14712598.2011.557358
23. Georgiou M, Fujinami K, Michaelides M. Inherited retinal diseases: therapeutics, clinical trials and end points-A review. *Clin Exp Ophthalmol*. 2021;49(3):270–288. doi:10.1111/ceo.13917
24. Ziccardi L, Cordeddu V, Gaddini L, et al. Gene Therapy in Retinal Dystrophies. *Int J Mol Sci*. 2019;20(22):5722. doi:10.3390/ijms20225722
25. Maurya S, Sarangi P, Jayandharan GR. Safety of Adeno-associated virus-based vector-mediated gene therapy—impact of vector dose. *Cancer Genet Ther*. 2022;29(10):1305–1306. doi:10.1038/s41417-021-00413-6
26. Ashtari M, Cyckowski LL, Monroe JF, et al. The human visual cortex responds to gene therapy-mediated recovery of retinal function. *J Clin Invest*. 2011;121(6):2160–2168. doi:10.1172/JCI57377
27. Cai X, Conley SM, Naash MI. RPE65: role in the visual cycle, human retinal disease, and gene therapy. *Ophthalmic Genet*. 2009;30(2):57–62. doi:10.1080/13816810802626399
28. Weleber RG, Pennesi ME, Wilson DJ, et al. Results at 2 years after gene therapy for RPE65-deficient leber congenital amaurosis and severe early-childhood-onset retinal dystrophy. *Ophthalmology*. 2016;123(7):1606–1620. doi:10.1016/j.ophtha.2016.03.003
29. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008;358 (21):2240–2248. doi:10.1056/NEJMoa0802315
30. Bennett J, Wellman J, Marshall KA, et al. Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by RPE65 mutations: a follow-on phase 1 trial. *Lancet*. 2016;388(10045):661–672. doi:10.1016/S0140-6736(16) 30371-3
31. Pierce EA, Bennett J. The status of RPE65 gene therapy trials: safety and efficacy. *Cold Spring Harb Perspect Med*. 2015;5(9):a017285. doi:10.1101/cshperspect.a017285
32. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet*. 2009;374(9701):1597–1605. doi:10.1016/S0140-6736(09)61836-5
33. Xue K, Jolly JK, Barnard AR, et al. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. *Nat Med*. 2018;24 (10):1507–1512. doi:10.1038/s41591-018-0185-5
34. Testa F, Maguire AM, Rossi S, et al. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. *Ophthalmology*. 2013;120(6):1283–1291. doi:10.1016/j.ophtha.2012.11.048
35. Sharif W, Sharif Z. Leber's congenital amaurosis and the role of gene therapy in congenital retinal disorders. *Int J Ophthalmol*. 2017;10 (3):480–484. doi:10.18240/ijo.2017.03.24
36. Wu KY, Mina M, Sahyoun J-Y, Kalevar A, Tran SD. Retinal prostheses: engineering and clinical perspectives for vision restoration. *Sensors*. 2023;23(13):5782. doi:10.3390/s23135782
37. Kondkar AA, Abu-Amero KK. Leber congenital amaurosis: current genetic basis, scope for genetic testing and personalized medicine. *Exp Eye Res*. 2019;189:107834. doi:10.1016/j.exer.2019.107834
38. Sahel J-A, Boulanger-Scemama E, Pagot C, et al. Partial recovery of visual function in a blind patient after optogenetic therapy. *Nature Med*. 2021;27(7):1223–1229. doi:10.1038/s41591-021-01351-4
39. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. 2001;28(1):92–95. doi:10.1038/ng0501-92

40. Le Meur G, Stieger K, Smith AJ, et al. Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. *Gene Ther*. 2007;14(4):292–303. doi:10.1038/sj.gt.3302861
41. 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. *Lancet*. 2017;390(10097):849–860. doi:10.1016/S0140-6736(17)31868-8
42. Jacobson SG, Cideciyan AV, Ratnakaram R, et al. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. *Arch Ophthalmol*. 2012;130(1):9–24. doi:10.1001/archophthalmol.2011.298
43. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008;358(21):2231–2239. doi:10.1056/NEJMoa0802268
44. Therapeutics S. Voretigene neparvovec-rzyl (Luxturna™) prescribing information [Internet]. Silver Spring, MD: U.S. Food and Drug Administration; 2020. Available from: https://www.fda.gov/media/109906/download. Accessed April 25, 2026.
45. Schwartz SD, Regillo CD, Lam BL, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet*. 2015;385(9967):509–516. doi:10.1016/S0140-6736(14)61376-3
46. Seiler MJ, Aramant RB. Cell replacement and visual restoration by retinal sheet transplants. *Prog Retin Eye Res*. 2012;31(6):661–687. doi:10.1016/j.preteyeres.2012.06.003
47. MacLaren RE, Bennett J, Schwartz SD. Gene therapy and stem cell transplantation in retinal disease: the new frontier. *Ophthalmology*. 2016;123(10s):S98–s106. doi:10.1016/j.ophtha.2016.06.041
48. Chen X, Xu N, Li J, Zhao M, Huang L. Stem cell therapy for inherited retinal diseases: a systematic review and meta-analysis. *Stem Cell Res Ther*. 2023;14(1):286. doi:10.1186/s13287-023-03526-x
49. Koenekoop RK, Sui R, Sallum J, et al. Oral 9-cis retinoid for childhood blindness due to Leber congenital amaurosis caused by RPE65 or LRAT mutations: an open-label phase 1b trial. *Lancet*. 2014;384(9953):1513–1520. doi:10.1016/S0140-6736(14)60153-7
50. Le Rhun A, Escalera-Maurer A, Bratovič M, Charpentier E. CRISPR-Cas in Streptococcus pyogenes. *RNA Biol*. 2019;16(4):380–389. doi:10.1080/15476286.2019.1582974
51. Mollashahi B, Latifi-Navid H, Owliaee I, et al. Research and therapeutic approaches in stem cell genome editing by CRISPR toolkit. *Molecules*. 2023;28(4):1982. doi:10.3390/molecules28041982
52. Kleinstiver BP, Pattanayak V, Prew MS, et al. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*. 2016;529(7587):490–495. doi:10.1038/nature16526
53. Chrenek MA, Nickerson JM, Boatright JH. Clustered regularly interspaced short palindromic repeats: challenges in treating retinal disease. *Asia Pac J Ophthalmol*. 2016;5(4):304–308. doi:10.1097/APO.0000000000000225
54. Lohia A, Sahel DK, Salman M, et al. Delivery strategies for CRISPR/Cas genome editing tool for retinal dystrophies: challenges and opportunities. *Asian J Pharm Sci*. 2022;17(2):153–176. doi:10.1016/j.ajps.2022.02.001
55. Suh S, Choi EH, Raguram A, Liu DR, Palczewski K. Precision genome editing in the eye. *Proc Natl Acad Sci U S A*. 2022;119(39):e2210104119. doi:10.1073/pnas.2210104119
56. Strauss JH, Strauss EG. CHAPTER 7 - DNA-containing viruses. In: Strauss JH, Strauss EG, editors. *Viruses and Human Disease*. Second ed. London: Academic Press; 2008:261–323.
57. Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M. Viral vectors: a look back and ahead on gene transfer technology. *New Microbiol*. 2013;36(1):1–22.
58. Maguire AM, Bennett J, Aleman EM, Leroy BP, Aleman TS. Clinical perspective: treating RPE65-associated retinal dystrophy. *Mol Ther*. 2021;29(2):442–463. doi:10.1016/j.ymthe.2020.11.029
59. Trapani I, Tornabene P, Auricchio A. Large gene delivery to the retina with AAV vectors: are we there yet? *Gene Ther*. 2021;28(5):220–222. doi:10.1038/s41434-020-0174-4
60. Ponnazhagan S, Curiel DT, Shaw DR, Alvarez RD, Siegal GP. Adeno-associated virus for cancer gene therapy. *Cancer Res*. 2001;61(17):6313–6321.
61. Samulski RJ, Muzyczka N. AAV-mediated gene therapy for research and therapeutic purposes. *Ann Rev Virol*. 2014;1(1):427–51. doi:10.1146/annurev-virology-031413-085355
62. Cremers FP, Collin RW. Promises and challenges of genetic therapy for blindness. *Lancet*. 2009;374(9701):1569–1570. doi:10.1016/S0140-6736(09)61869-9
63. Kang C, Scott LJ. Voretigene neparvovec: a review in RPE65 mutation-associated inherited retinal dystrophy. *Mol Diagn Ther*. 2020;24(4):487–495. doi:10.1007/s40291-020-00475-6
64. Wörner TP, Bennett A, Habka S, et al. Adeno-associated virus capsid assembly is divergent and stochastic. *Nat Commun*. 2021;12(1):1642. doi:10.1038/s41467-021-21935-5
65. Qu Y, Liu Y, Noor AF, Tran J, Li R. Characteristics and advantages of adeno-associated virus vector-mediated gene therapy for neurodegenerative diseases. *Neural Regen Res*. 2019;14(6):931–938. doi:10.4103/1673-5374.250570
66. Monahan PE, Samulski RJ. Adeno-associated virus vectors for gene therapy: more pros than cons? *Mol Med Today*. 2000;6(11):433–440. doi:10.1016/S1357-4310(00)01810-4
67. Dhurandhar D, Sahoo NK, Mariappan I, Narayanan R. Gene therapy in retinal diseases: a review. *Indian J Ophthalmol*. 2021;69(9):2257–2265. doi:10.4103/ijo.IJO_3117_20
68. Wang X, Yu C, Tzekov RT, Zhu Y, Li W. The effect of human gene therapy for RPE65-associated Leber's congenital amaurosis on visual function: a systematic review and meta-analysis. *Orphanet J Rare Dis*. 2020;15(1):49. doi:10.1186/s13023-020-1304-1
69. Ertl HCJ. Immunogenicity and toxicity of AAV gene therapy. *Front Immunol*. 2022;13:975803. doi:10.3389/fimmu.2022.975803
70. Chowdhury EA, Meno-Tetang G, Chang HY, et al. Current progress and limitations of AAV mediated delivery of protein therapeutic genes and the importance of developing quantitative pharmacokinetic/pharmacodynamic (PK/PD) models. *Adv Drug Deliv Rev*. 2021;170:214–237. doi:10.1016/j.addr.2021.01.017
71. Colella P, Ronzitti G, Mingozzi F. Emerging issues in AAV-mediated in vivo gene therapy. *Mol Ther Methods Clin Dev*. 2018;8:87–104. doi:10.1016/j.omtm.2017.11.007

72. Bastola P, Song L, Gilger BC, Hirsch ML. Adeno-associated virus mediated gene therapy for corneal diseases. *Pharmaceutics*. 2020;12(8):767. doi:10.3390/pharmaceutics12080767
73. Basner-Tschakarjan E, Mingozzi F. Cell-mediated immunity to AAV vectors, evolving concepts and potential solutions. *Front Immunol*. 2014;5:350. doi:10.3389/fimmu.2014.00350
74. Dalkara D, Goureau O, Marazova K, Sahel JA. Let there be light: gene and cell therapy for blindness. *Hum Gene Ther*. 2016;27(2):134–147. doi:10.1089/hum.2015.147
75. Burdett T, Nuseibeh S. Changing trends in the development of AAV-based gene therapies: a meta-analysis of past and present therapies. *Gene Ther*. 2023;30(3–4):323–335. doi:10.1038/s41434-022-00363-0
76. Holkers M, Maggio I, Henriques SF, Janssen JM, Cathomen T, Gonçalves MA. Adenoviral vector DNA for accurate genome editing with engineered nucleases. *Nat Methods*. 2014;11(10):1051–1057. doi:10.1038/nmeth.3075
77. Senís E, Fatouros C, Große S, et al. CRISPR/Cas9-mediated genome engineering: an adeno-associated viral (AAV) vector toolbox. *Biotechnol J*. 2014;9(11):1402–1412. doi:10.1002/biot.201400046
78. Kay MA. State-of-the-art gene-based therapies: the road ahead. *Nat Rev Genet*. 2011;12(5):316–328. doi:10.1038/nrg2971
79. Au HKE, Isalan M, Mielcarek M. Gene therapy advances: a meta-analysis of AAV usage in clinical settings. *Front Med*. 2021;8:809118.
80. Gardiner KL, Cideciyan AV, Swider M, et al. Long-term structural outcomes of late-stage RPE65 gene therapy. *Mol Ther*. 2020;28(1):266–278. doi:10.1016/j.ymthe.2019.08.013
81. Ameline B, Tshilenge KT, Weber M, et al. Long-term expression of melanopsin and channelrhodopsin causes no gross alterations in the dystrophic dog retina. *Gene Ther*. 2017;24(11):735–741. doi:10.1038/gt.2017.63