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Review

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Gene therapy applications in ophthalmology

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ABSTRACT

Gene therapy has gained significant progress in recent years. The success of these therapies is based on efficient gene transfer and stable transgene expression. With the US Food and Drug Administration's (FDA) approval of gene therapy for Leber congenital amaurosis, the use of gene therapies has increased. Studies have provided new information about the benefits and limitations of using gene transfer routes to different regions of the eye. However, there are many old and new difficulties in the long-term effects, immunogenicity, targeting, and production of treatments. The eye's susceptibility to inflammation and immune and inflammatory reactions encountered in gene therapy can render the treatment ineffective or harmful. Although many strategies have some requirements in common, it is not possible for a single gene therapy system to be used for all applications. Moreover, genome-engineering tools based on new delivery methods facilitate more effective and targeted applications to eye compartments. This review presents data on various approaches to gene therapy, their applications to various eye diseases from the anterior segment to the posterior segment, clinical limitations of current systems, future prospects, research aimed at alternative solutions, and advantages and disadvantages of gene transfer routes.

Keywords: Ocular diseases, drug development, ocular gene therapy, gene editing, gene therapy vectors

INTRODUCTION

Ocular diseases comprise a variety of conditions that affect vision and quality of life. Traditional treatments aim to relieve symptoms or delay the progression of disease. Surgery is necessary in only special cases. These approaches are not always curative, and many patients may experience vision loss.[1] Gene therapy aims to cure diseases by transferring genetic material. Although currently offered in limited options, it is promising in ocular treatment.[2] Applications consist of strategies such as changing the gene structure and stopping, increasing, and regulating gene expression.[3] Treatments are administered through subretinal, intravitreal, and suprachoroidal injections.[4] Among the studies on this topic, adeno-associated virus (AAV) vectors for gene transfer and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) systems for gene editing stand out.[5]

The eye is a special organ that is suitable for gene therapy applications due to its immune privilege, small size, and segmented structure. The risk of systemic exposure is quite low when therapeutic agents are applied.[6] In addition, therapy studies can be easily evaluated using noninvasive imaging techniques such as optical coherence tomography, fundoscopy, angiography, and new-generation two-photon microscopy. Thus, the real-time monitoring of therapeutic results and safety becomes easier.[7] Clinical research on gene therapy is currently being applied to various ocular diseases.[8] The efficacy of ocular gene therapy has been continuously improved in animal experiments and clinical studies. In the transition to clinical studies, gene transfer methods, uncertainty of disease molecular mechanisms, therapeutic delivery efficiency at the target site, and differences in disease models pose problems.[9] In this review, we present data on various gene therapy approaches, gene transfer methods to ocular tissues, their applications to various eye diseases from the anterior segment to the posterior segment, clinical limitations of current systems, future prospects, research aimed at alternative solutions, and advantages and disadvantages of gene transfer routes.

OPHTHALMOLOGIC GENE THERAPIES

Inherited retinal diseases (IRDs) are an important focus in ophthalmologic gene therapy research.[10] Currently, more than 300 genes have been associated with IRD.[6] The vast majority of variants lead to the dysfunction and degeneration of photoreceptors and/or retinal pigment epithelium (RPE). Autosomal dominant (AD), autosomal recessive (AR), X-linked

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(XL), polygenic, and mitochondrial inheritances have been reported.[11] IRDs show a high rate of clinical heterogeneity. For example, diseases such as LCA can be seen congenitally or at an early stage, whereas diseases such as Retinitis pigmentosa (RP) and cone-rod dystrophy can be seen in adulthood.[12,13] Gene therapy applications aim to reduce the negative effects of these diseases and improve quality of life. Approaches to gene therapy vary according to genetic variants and heredity characteristics. These can be grouped as alteration, silencing, regulation, and modification of the target gene.[14]

APPLICATION METHODS OF GENE THERAPIES

Viral Vectors

Commercial Luxturna®, Glybera®, Zolgensma®, and Hemgenix® products exert AAVs as a gene therapy system.[14] Recent strategies utilize binary/multivectors to address genetic data transfer size limits in AAVs, employing extein residual and intein analogs to convert peptides into large functional proteins in target cells.[15,16]

The efficacy of Luxturna® has been demonstrated in clinical trials, showing improvements in light sensitivity and peripheral vision.[17] However, the link between these objective enhancements and patients' daily experiences remains unclear. AEs include eye inflammation, redness, and pain from injections, alongside ongoing long-term safety studies.[18] Reichel et al.[19] evaluated retinal atrophy in eight patients 6 to 24 months after Luxturna® treatment. The study indicated that subretinal Luxturna® may induce RPE atrophy and photoreceptor loss, despite maintaining treatment benefits. Lorenz et al.[20] assessed the impact of baseline data on psychophysical and morphological outcomes from subretinal Luxturna® in 19 patients over a median 15-month follow-up. The therapy showed consistent primary outcomes, but 50% of treated eyes exhibited new chorioretinal atrophy. Effects were notably stronger in the pediatric subgroup. Daruich et al.[21] studied Luxturna® outcomes in 12 eyes from six pediatric patients over 12 months, noting no intraoperative issues. While macular and outer nuclear layer thickness remained stable, a parafoveal hole emerged at three months, with atrophy at the injection site. One patient faced elevated intraocular pressure. Most showed significant visual function improvements at 12 months with no complications affecting these gains. Fischer et al.[22] conducted a study with 103 patients, the largest assessment of Luxturna®'s long-term safety and effectiveness within the PERCEIVE framework. AEs occurred in 35 patients, or 34% of the group, mainly linked to chorioretinal atrophy. Changes in visual acuity from baseline were not significant, matching the pivotal trials' results. Yang et al.[23] conducted a phase 1/2 study on subretinal AAV5 gene therapy in 15 patients with biallelic guanylate cyclase 2D variants. Over 2.5 years, most AEs were surgical, with no serious incidents. Mild ocular inflammation was noted, treatable with steroids. High-dose patients showed significant retinal sensitivity improvements from day 28, lasting over 12 months. Audo et al.[24] assessed the efficacy and safety of subretinal Luxturna® in six pediatric and six adult patients. All participants had at least one ocular AE. A child experienced retinal detachment, and chorioretinal atrophy was observed in four adults and one child. Improvements in low-light functional vision were noted, with safety results consistent with prior data.

Nonviral Vectors

Physical and chemical methods have been developed for the transfer of genetic material. However, because of the inability of nonviral systems to achieve continuity of gene expression, high transduction efficiency, and proper therapeutic levels, they are less preferred.[16] Plasmid DNA, siRNA, mRNA, or micro-RNA administered to the cell for gene therapy purposes are degraded in a short time.[25]

Among the commercial products, Macugen® has been approved for phase III clinical studies. Macugen® is an antagonistic RNA aptamer for VEGF that competitively binds to VEGF receptors on endothelial cells and prevents receptor activation.[26] It has been broadly studied as an intravitreal therapy to suppress angiogenesis, vascular permeability, and inflammation in AMD. Avacincaptad pegol, which has a stronger affinity for VEGF, was also developed by a similar mechanism and approved for phase II clinical studies.[27]

Antisense Oligonucleotides

ASOs target the gene expression according to the region in the RNA strand to which they bind. There are three methods of ASO application.[28] The first ASO system aims to prevent the interaction of splicing factors in deep intronic variants (DIVs) which create a new donor or acceptor site in the intron, producing splicing defects. Thus, during the splicing process, a portion of the intron is incorporated into the mature mRNA, forming a pseudoexon.[29] An example of this situation is the CEP290-related IRDs. The CEP290 pseudoexon leads to nonsense-mediated mRNA decay, resulting in the synthesis of abnormal protein or no protein at all. ASOs manage the splicing process by binding to the variant region, preventing the formation of the pseudoexon, and protecting protein synthesis.[30] The second ASO system prevents splicing factors from interacting with splice sites or regulatory elements containing variants, avoiding their inclusion in mature mRNA.[31] This applies to usherin2A (USH2A) variants, a major cause of AR RP, particularly in exon 13. QR-421a is a modified ASO that binds to USH2A pre-mRNA, inducing exon skipping and producing a truncated functional protein.[32] The third ASO system targets mRNA to reduce mutant protein synthesis, particularly in AD RP, where variant alleles cause the disease despite normal alleles, known as the dominant-negative effect.[31] ASOs identify these alleles at the pre-mRNA stage, removing pathogenic exons through splicing. They ensure allele-specific degradation of variant mRNA by activating the RNase H enzyme, leading to the production of normal allelic protein.[30,33]

The first commercial ASO product approved in ophthalmology is Fomivirsen, which targets viral genetic sequences to treat cytomegalovirus retinitis.[34] Reports highlight the ease of intravitreal transfer, increasing its clinical value, especially for gene therapy needing frequent dosing over decades.[35] Gérard et al.[36] showed that intravitreal splice-switching oligonucleotides effectively modified CEP290 splicing in retinal cells related to LCA. No significant AEs were reported from the injections, which allowed detection of CEP290-skipped mRNA for over a month. These injections bypassed protein truncation, offering a viable solution for large mRNA sizes beyond AAV vector limits, thus improving gene therapy effectiveness. Cideciyan et al.[37] studied intravitreal ASO to restore splicing in LCA patients with the c.2991+1655A>G allele in CEP290. No severe AEs occurred, and mild treatment-emergent AEs were

noted. After three months, retinal changes were absent, but significant effects were observed, with baseline improvements maintained at six months. Slijkerman et al.[38] studied ASOs for exon 13 skipping in USH2A, discovering that QR-421a effectively induced this in photoreceptor precursors from a USH2A variant patient. Intravitreal injections in non-human primates demonstrated that QR-421a penetrates the retina, achieving exon 13 skipping for at least three months, indicating its promise as an RP therapy for USH2A variants.

GENE THERAPY STUDIES IN OPHTHALMOLOGIC DISEASES

Leber Congenital Amaurosis (LCA)

LCA is a severe retinal dystrophy causing progressive vision loss from birth or early infancy. Over 20 associated genes have been identified. The Luxturna® has been approved by FDA for RPE65 gene related LCA.[10] Genome editing technologies present potential therapies for LCA subtypes linked to CEP290 and RPE65.[17] CEP290 is vital for ciliary structure and photoreceptor trafficking, with its variants associated with LCA type 10.[39] Maeder et al.[13] designed the genome-editing therapeutic EDIT-101, which aims to eliminate a deep-intronic variant responsible for a pseudo-exon in the CEP290, thereby restoring normal CEP290 expression through accurate splicing. Subretinal delivery in animal models proved effective for CEP290 editing. A vector tested in nonhuman primates confirmed the CRISPR/Cas9 system's capacity for in vivo editing, advancing EDIT-101 and similar therapies for LCA10 and other inherited retinal diseases. RPE65 catalyzes compounds for visual chromophore regeneration in the retina. Variants are linked to LCA type 2. Jo et al.[40] used a CRISPR/Cas9 approach in an animal model for an RPE65 variant producing dysfunctional proteins. Their findings showed successful RPE65 correction without histological deterioration or tumors over 7 months. Russell et al.[41] conducted a phase III trial of AAV2 therapy, Luxturna®, in 29 pediatric LCA type 2 patients aged 3 years and older with 20/60 vision or less, showing functional improvement and no serious side effects.

RP

RP is a group of inherited retinal disorders marked by the degeneration of rod and cone photoreceptors. Pathogenic variants in over 80 genes have been linked to RP subtypes.[42] Approximately 70% of cases arise from variants in RP GTPase regulator (RPGR), 25% from RHO, and 20% from USH2A.[12] Current gene therapies have shown poor efficacy and safety issues.[43] Optogenetic therapy is a new method for RP, transferring genes for light-sensitive proteins to nonphotoreceptor retinal neurons like ganglion cells. This could enhance visual perception in RP and other inherited retinal diseases.[44]

The nonhomologous end joining (NHEJ) strategy has been applied in the treatment of AD RP for RHO gene. The S334 variant encodes a premature stop codon, whereas the P23H variant encodes a protein that misfolds and forms aggregates.[45] Bakondi et al.[46] examined the selective knockout of the S334ter variant by subretinal injection with electroporation using the CRISPR/Cas9 system in an animal model. Similarly, Giannelli et al.[47] examined the selective knockout of the P23H variant by intravitreal injection in an animal model and then in a human with the AAV vector using the CRISPR/Cas9 system. In these studies, allele-specific targeting of the variants via NHEJ prevented toxicity and retinal degeneration and improved visual function.

The homology-directed repair (HDR) strategy has been applied for the treatment of AR RP. The CRISPR/Cas9 therapeutic approach was conducted on the phosphodiesterase 6b (PDE6B). The enzyme PDE6B is involved in the visual signal transduction pathway in photoreceptor cells. The method targets specific editing of variants that disrupt enzymatic activity using a donor template. Cai et al.[48] investigated the correction of the Y347X variant using the AAV-based CRISPR/Cas9 system in an animal model. They reported that therapy promoted the survival of rod and cone photoreceptors, PDE6B expression was maintained, and visual function was improved. Similarly, Qin et al.[49] investigated the correction of the R560C variant with the AAV-based CRISPR/Cas9 system in an animal model. They reported significant improvement in optomotor responses driven by visual stimuli and electroretinogram after gene therapy.

The NHEJ strategy has been also applied in the treatment of XL RP. The CRISPR/Cas9 therapeutic approach was applied in the RPGR, which regulates the transfer of various proteins and vesicles required for photoreceptor survival and function. RPGR variants affect the functionality of proteins, disrupt cell homeostasis, and lead to photoreceptor death. Gumerson et al.[50] used an animal model to investigate the correction of the frameshift variant in the OFR15 region with the CRISPR/Cas9-mediated AAV vector. They found that early disease phenotypes such as incorrect localization of cone opsins were not observed in mutant mice due to the intervention. In addition, these authors stated that subretinal injection restored the reading frame and the variant was successfully corrected in adult mice.

Stargardt Disease (STGD1)

STGD1 is a common inherited retinal disorder leading to juvenile progressive central vision loss, caused by AR variants of ATP Binding Cassette Subfamily A Member 4 (ABCA4). Stargardt-like symptoms may arise from rare AD variants in ELOVL fatty acid elongase 4 or prominin 1, which are not suitable for gene therapy.[51]

ABCA4 is a crucial retinal protein for retinoid transport, found in the outer segment discs of rods and cones. Variants hinder the clearance of N-retinylidene-phosphatidylethanolamine, leading to toxic phosphatidylpyridinium cisretinoic levels and RPE lipofuscin buildup, resulting in degeneration of RPE and photoreceptors. STGD1 poses challenges for gene therapy due to the large size of the ABCA4 gene and the lack of approved treatments.[52] Various DIVs in the ABCA4 cause abnormal splicing outside exons, activating critical splice regions and affecting regulatory units.[53] Gene therapy studies have targeted these DIVs. DeAngeli et al.[54] assessed gene correction therapy for the c.5197-557G>T, causing premature termination, by adding a 188-bp intronic sequence to the ABCA4 mRNA using CRISPR-Cas9 in photoreceptor precursor cells. They achieved an 83% correction in splicing and a 1.8-fold increase in correctly spliced transcripts with *S. pyogenes* Cas9, highlighting the potential of CRISPR/Cas9 in correcting ABCA4 splicing defects. Approximately 25% of STGD1-associated ABCA4 variants disrupt pre-mRNA

splicing, emerging as the main pathological RNA processing events. This has led to interest in ASOs for modulating splicing, as an alternative to traditional gene knock-out methods.[55] Kaltak et al.[56] investigated ASOs designed to skip exon 17 in the ABCA4 gene, achieving 59% exon 17 skipping in retinal organoids. Exon 17 deletion does not fully eliminate protein activity or cause severe STGD1 phenotypes. The authors suggest ASOs could mitigate severe variant effects, restoring partial ABCA4 activity in STGD1 patients. Kaltak et al.[57] used ASOs to restore RNA splicing of the ABCA4 variant linked to severe disease. They tested ASO effectiveness in an ABCA4 midigene model, using 3D human retinal organoids. QR-1011 significantly increased correctly spliced transcripts, confirming ABCA4 protein restoration and marking it as a promising splice-correcting ASO. Kaltak et al.[58] investigated QR-1011 ASO and three other ASOs to address splicing errors in non-canonical splice site variants near exon 39 of the ABCA4 gene. QR-1011 showed the greatest effect, enhancing full-length ABCA4 transcript production for the c.5461-8T>G and c.5584+6T>C variants, indicating a potential treatment for STGD1 patients with various severe ABCA4 variants. There is an alternative approach to the large size of the ABCA4. Li et al.[59] devised dual split intein ABCA4 vectors to improve dual AAV gene therapy efficacy. They engineered a dual AAV8-ABCA4 vector, achieving considerable full-length ABCA4 expression. This reduced bisretinoid build-up and restored vision in ABCA4-knockout mice, highlighting the treatment potential for STGD1. Recently, Yang et al.[60] initiated the SB-007 study for STGD1, utilizing a dual AAV vector to replace ABCA4 and restore the full-length protein. It has orphan drug status in E.U. and the U.S., with dosing for the first trial patient now ongoing.

Cataract

Cataract is lens opacity due to high-molecular-weight protein aggregates in lens cells or deterioration of lens microarchitecture, leading to light scattering. Its development rate is linked to protein accumulation and specific pathogenic gene variants.[61] Patients were evaluated in two groups by age of onset; age-related and congenital cataract. Age-related cataracts are influenced by genetic, environmental, and stochastic factors, leading to variability in onset age. In contrast, congenital cataracts are primarily driven by genetic factors, resulting in consistent onset age. Over 50 loci linked to congenital cataract have been reported, yet many cataract genetics remain unclear.[62] Genetic analyses have identified variants linked to crystalline, connexins, membrane proteins, and intermediate filament proteins that are vital for lens homeostasis. AD inheritance appeared in 44% of bilateral cataract families. Congenital cataracts were isolated hereditary cases in 70% and in association with other ocular abnormalities or syndromes in 15% of instances.[61]

Designing vectors is crucial to limit lens-specific target areas and minimize tissue interaction.[63] Lin et al.[64] developed a surgical technique for lens intervention in gene therapy, removing the cataractous lens while preserving the capsule and epithelial cells. This method aims to maintain endogenous epithelial stem/progenitor cells and their environment for functional lens regeneration, showcasing an effective strategy for tissue regeneration. However, CRISPR/Cas9 models may enable gene therapy combined with lens regeneration surgery. Wu et al.[65] studied gene correction for the dominant crystallin gamma C (CRYGC) variant causing nuclear cataract at the zygote stage. They found repair in mutant embryos via the HDR pathway, with healthy alleles passed to offspring. Wu et al.[66] studied correcting the CRYGC in spermatogonial stem cells and its effects on spermatogenesis and offspring after oocyte injection. They found the CRYGC variant could be repaired by NHEJ or HDR efficiently, preventing cataract phenotypes in offspring with 100% success. Yuan et al.[67] developed a cataract animal model by disrupting gap junction protein alpha 8 function, crucial for lens opacity. The system effectively generated a hereditary cataract model with 98.7% and 100% gene variant efficiency in embryos and offspring, respectively. Similarly, the researchers aimed to create a new animal model targeting αA-crystallin at the zygote stage for studying congenital cataracts. Findings revealed all F0 generation offspring had indel-mediated variants in αA-crystallin without off-target effects.[68] Recently, Zhao and colleagues developed an F0 animal model to identify genes linked to cataract formation and refine treatments, detailing design, microinjection, efficacy, evaluation, dose optimization, and CRISPR-Cas9 methods.[69]

CHALLENGES OF GENE THERAPY

The unique sensitivity of the eye necessitates specialized interventions. Challenges include identifying disease genes, ensuring precise delivery, application methods, clinical implementation, and immune responses.[70] Many products and trials aim to effectively target ocular tissues.[71] Ocular methods targeting eye tissues directly reduce immunogenicity but need expertise for implementation.[72] Invasive methods enhance bioavailability and delivery precision but risk complications like infection, retinal detachment, and hemorrhage.[73] Thus, selecting the right method for efficient transduction and minimal immune response is crucial.

CONCLUSION

The eye is a prime target for gene therapy due to its size and accessibility. Genome engineering can effectively treat ocular disorders by precisely targeting genes, facilitating therapy development. Future research will emphasize on new vectors, molecular targets, and standardized manufacturing to enhance safety and efficacy. Merging non-viral methods with viral efficiency is crucial. Next-generation therapies will address diseases lacking genetic diagnosis, targeting multiple variants simultaneously to lower costs and improve access, enabling objective assessment of transgene expression and clinical responses.

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Referee Evaluation Process

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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REFERENCES

1. Choi EH, Suh S, Sears AE, et al. Genome editing in the treatment of ocular diseases. *Exp Mol Med*. 2023;55(8):1678-1690. doi:10.1038/s12276-023-01057-2
2. Suh S, Choi EH, Raguram A, Liu DR, Palczewski K. Precision genome editing in the eye. *Proc Natl Acad Sci*. 2022;119(39):e2210104119. doi:10.1073/pnas.2210104119
3. Tang R, Xu Z. Gene therapy: a double-edged sword with great powers. *Mol Cell Biochem*. 2020;474(1-2):73-81. doi:10.1007/s11010-020-03834-3
4. Berkowitz ST, Finn AP. Gene therapy for age-related macular degeneration: potential, feasibility, and pitfalls. *Curr Opin Ophthalmol*. 2024;35(3):170-177. doi:10.1097/ICU.0000000000001043
5. Li B, Tan W, Wang Z, et al. Progress and prospects of gene therapy in ophthalmology from 2000 to 2022: a bibliometric analysis. *Heliyon*. 2023;9(7):e18228. doi:10.1016/j.heliyon.2023.e18228
6. Hu ML, Edwards TL, O'Hare F, et al. Gene therapy for inherited retinal diseases: progress and possibilities. *Clin Exp Optom*. 2021;104(4):444-454. doi:10.1080/08164622.2021.1880863
7. Palczewska G, Wojtkowski M, Palczewski K. From mouse to human: accessing the biochemistry of vision in vivo by two-photon excitation. *Prog Retin Eye Res*. 2023;93:101170. doi:10.1016/j.preteyeres.2023.101170
8. Sudhakar V, Richardson RM. Gene therapy for neurodegenerative diseases. *Neurotherapeutics*. 2019;16(1):166-175. doi:10.1007/s13311-018-00694-0
9. Kim Y, Zharkinbekov Z, Sarsenova M, Yeltay G, Saparov A. Recent advances in gene therapy for cardiac tissue regeneration. *Int J Mol Sci*. 2021;22(17):9206. doi:10.3390/ijms22179206
10. Sinim Kahraman N, Öner A, Özkul Y, Dündar M. Frequency of RPE65 gene mutation in patients with hereditary retinal dystrophy. *Turk J Ophthalmol*. 2022;52(4):270-275. doi:10.4274/tjo.galenos.2021.74944
11. Sahu B, Chug I, Khanna H. The ocular gene delivery landscape. *Biomolecules*. 2021;11(8):1135. doi:10.3390/biom11081135
12. Ghoraba HH, Akhavanrezayat A, Karaca I, et al. Ocular gene therapy: a literature review with special focus on immune and inflammatory responses. *Clin Ophthalmol*. 2022;16:1753-1771. doi:10.2147/OPTH.S364200
13. Maeder ML, Stefanidakis M, Wilson CJ, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nat Med*. 2019;25(2):229-233. doi:10.1038/s41591-018-0327-9
14. Drag S, Dotiwala F, Upadhyay AK. Gene Therapy for retinal degenerative diseases: progress, challenges, and future directions. *Investig Opthalmology Vis Sci*. 2023;64(7):39. doi:10.1167/iovs.64.7.39
15. Zhi S, Chen Y, Wu G, et al. Dual-AAV delivering split prime editor system for in vivo genome editing. *Mol Ther*. 2022;30(1):283-294. doi:10.1016/j.ymthe.2021.07.011
16. Öner A. Recent advancements in gene therapy for hereditary retinal dystrophies. *Turk Oftalmol Derg*. 2017;47(6):338-343. doi:10.4274/tjo.41017
17. Altay HY, Ozdemir F, Afghah F, et al. Gene regulatory and gene editing tools and their applications for retinal diseases and neuroprotection: from proof-of-concept to clinical trial. *Front Neurosci*. 2022;16:924917. doi:10.3389/fnins.2022.924917
18. Maguire AM, Russell S, Chung DC, et al. Durability of voretigene neparvovec for biallelic RPE65-mediated inherited retinal disease. *Ophthalmology*. 2021;128(10):1460-1468. doi:10.1016/j.ophtha.2021.03.031
19. Reichel FF, Seitz I, Wozar F, et al. Development of retinal atrophy after subretinal gene therapy with voretigene neparvovec. *Br J Ophthalmol*. 2023;107(9):1331-1335. doi:10.1136/bjophthalmol-2021-321023
20. Lorenz B, Künzel SH, Preising MN, et al. Single center experience with voretigene neparvovec gene augmentation therapy in RPE65 mutation-associated ınherited retinal degeneration in a clinical setting. *Ophthalmology*. 2024;131(2):161-178. doi:10.1016/j.ophtha.2023.09.006
21. Daruich A, Rateaux M, Batté E, et al. 12-month outcomes after voretigene neparvovec gene therapy in paediatric patients with RPE65 -mediated inherited retinal dystrophy. *Br J Ophthalmol*. 2025;109(2):281-285. doi:10.1136/bjo-2024-326221
22. Fischer MD, Simonelli F, Sahni J, et al. Real-world safety and effectiveness of voretigene neparvovec: results up to 2 years from the prospective, registry-based PERCEIVE study. *Biomolecules*. 2024;14(1):122. doi:10.3390/biom14010122
23. Yang P, Pardon LP, Ho AC, et al. Safety and efficacy of ATSN-101 in patients with Leber congenital amaurosis caused by biallelic mutations in GUCY2D: a phase 1/2, multicentre, open-label, unilateral dose escalation study. *Lancet*. 2024;404(10456):962-970. doi:10.1016/S0140-6736(24)01447-8
24. Audo I, Barale PO, Devisme C, et al. Voretigene neparvovec in RPE65-related inherited retinal dystrophy: the 1-year real-world study LIGHT. *Eye*. 2025;39(9):1758-1764. doi:10.1038/s41433-025-03691-8
25. Oliveira AV, Rosa Da Costa AM, Silva GA. Non-viral strategies for ocular gene delivery. *Mater Sci Eng C*. 2017;77:1275-1289. doi:10.1016/j.msec.2017.04.068
26. Zhu G, Chen X. Aptamer-based targeted therapy. *Adv Drug Deliv Rev*. 2018;134:65-78. doi:10.1016/j.addr.2018.08.005
27. Nimjee SM, White RR, Becker RC, Sullenger BA. Aptamers as therapeutics. *Annu Rev Pharmacol Toxicol*. 2017;57(1):61-79. doi:10.1146/annurev-pharmtox-010716-104558
28. Xue K, MacLaren RE. Antisense oligonucleotide therapeutics in clinical trials for the treatment of inherited retinal diseases. *Expert Opin Investig Drugs*. 2020;29(10):1163-1170. doi:10.1080/13543784.2020.1804853
29. Bacchi N, Casarosa S, Denti MA. Splicing-correcting therapeutic approaches for retinal dystrophies: where endogenous gene regulation and specificity matter. *Investig Opthalmology Vis Sci*. 2014;55(5):3285. doi:10.1167/iovs.14-14544
30. Collin RWJ, Garanto A. Applications of antisense oligonucleotides for the treatment of inherited retinal diseases. *Curr Opin Ophthalmol*. 2017;28(3):260-266. doi:10.1097/ICU.0000000000000363
31. Girach A, Audo I, Birch DG, et al. RNA-based therapies in inherited retinal diseases. *Ther Adv Ophthalmol*. 2022;14:25158414221134602. doi:10.1177/25158414221134602
32. Dulla K, Slijkerman R, Van Diepen HC, et al. Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations. *Mol Ther*. 2021;29(8):2441-2455. doi:10.1016/j.ymthe.2021.04.024
33. Kuijper EC, Bergsma AJ, Pijnappel WWMP, Aartsma-Rus A. Opportunities and challenges for antisense oligonucleotide therapies. *J Inherit Metab Dis*. 2021;44(1):72-87. doi:10.1002/jimd.12251
34. Hutcherson SL LR. A randomized controlled clinical trial of intravitreous fomivirsen for treatment of newly diagnosed peripheral cytomegalovirus retinitis in patients with aids. *Am J Ophthalmol*. 2002;133(4):467-474. doi:10.1016/S0002-9394(02)01327-2
35. Kumar S, Fry LE, Wang JH, et al. RNA-targeting strategies as a platform for ocular gene therapy. *Prog Retin Eye Res*. 2023;92:101110. doi:10.1016/j.preteyeres.2022.101110
36. Gérard X, Perrault I, Munnich A, Kaplan J, Rozet JM. Intravitreal injection of splice-switching oligonucleotides to manipulate splicing in retinal cells. *Mol Ther Nucleic Acids*. 2015;4:e250. doi:10.1038/mtna.2015.24
37. Cideciyan AV, Jacobson SG, Drack AV, et al. Effect of an intravitreal antisense oligonucleotide on vision in Leber congenital amaurosis due to a photoreceptor cilium defect. *Nat Med*. 2019;25(2):225-228. doi:10.1038/s41591-018-0295-0
38. Slijkerman R, Van Diepen H, Albert S, et al. Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by mutations in USH2A exon 13. 2020:10. doi:10.1101/2020.10.06.320499
39. Russell SR, Drack AV, Cideciyan AV, et al. Intravitreal antisense oligonucleotide sepofarsen in Leber congenital amaurosis type 10: a phase 1b/2 trial. *Nat Med*. 2022;28(5):1014-1021. doi:10.1038/s41591-022-01755-w
40. Jo DH, Song DW, Cho CS, et al. CRISPR-Cas9-mediated therapeutic editing of Rpe65 ameliorates the disease phenotypes in a mouse model of Leber congenital amaurosis. *Sci Adv*. 2019;5(10):eaax1210. doi:10.1126/sciadv.aax1210
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. Verbakel SK, Van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res*. 2018;66:157-186. doi:10.1016/j.preteyeres.2018.03.005
43. Liu Y, Zong X, Cao W, Zhang W, Zhang N, Yang N. Gene therapy for retinitis pigmentosa: current challenges and new progress. *Biomolecules*. 2024;14(8):903. doi:10.3390/biom14080903
44. Parnami K, Bhattacharyya A. Current approaches to vision restoration using optogenetic therapy. *Front Cell Neurosci*. 2023;17:1236826. doi:10.3389/fncel.2023.1236826

45. Nakazawa M, Hara A, Ishiguro S. Optical coherence tomography of animal models of retinitis pigmentosa: from animal studies to clinical applications. *BioMed Res Int*. 2019;2019:1-16. doi:10.1155/2019/8276140
46. Bakondi B, Lv W, Lu B, et al. In Vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 Rat model of autosomal dominant retinitis pigmentosa. *Mol Ther*. 2016;24(3):556-563. doi:10.1038/mt.2015.220
47. Giannelli SG, Luoni M, Castoldi V, et al. Cas9/sgRNA selective targeting of the P23H Rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9.PHP.B-based delivery. *Hum Mol Genet*. 2018;27(5):761-779. doi:10.1093/hmg/ddx438
48. Cai Y, Cheng T, Yao Y, et al. In vivo genome editing rescues photoreceptor degeneration via a Cas9/RecA-mediated homology-directed repair pathway. *Sci Adv*. 2019;5(4):eaav3335. doi:10.1126/sciadv.aav3335
49. Qin H, Zhang W, Zhang S, et al. Vision rescue via unconstrained in vivo prime editing in degenerating neural retinas. *J Exp Med*. 2023;220(5):e20220776. doi:10.1084/jem.20220776
50. 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
51. Piotter E, McClements ME, MacLaren RE. Therapy approaches for Stargardt disease. *Biomolecules*. 2021;11(8):1179. doi:10.3390/biom11081179
52. Al-Khuzaei S, Broadgate S, Foster CR, et al. An overview of the genetics of ABCA4 retinopathies, an evolving story. *Genes*. 2021;12(8):1241. doi:10.3390/genes12081241
53. Sangermano R, Khan M, Cornelis SS, et al. ABCA4 midigenes reveal the full splice spectrum of all reported noncanonical splice site variants in Stargardt disease. *Genome Res*. 2018;28(1):100-110. doi:10.1101/gr.226621.117
54. De Angeli P, Reuter P, Hauser S, et al. Effective splicing restoration of a deep-intronic ABCA4 variant in cone photoreceptor precursor cells by CRISPR/SpCas9 approaches. *Mol Ther Nucleic Acids*. 2022;29:511-524. doi:10.1016/j.omtn.2022.07.023
55. Huang D, Thompson JA, Chen SC, et al. Characterising splicing defects of ABCA4 variants within exons 13-50 in patient-derived fibroblasts. *Exp Eye Res*. 2022;225:109276. doi:10.1016/j.exer.2022.109276
56. Kaltak M, Blanco-Garavito R, Molday LL, et al. Stargardt disease-associated in-frame ABCA4 exon 17 skipping results in significant ABCA4 function. *J Transl Med*. 2023;21(1):546. doi:10.1186/s12967-023-04406-x
57. Kaltak M, De Bruijn P, Piccolo D, et al. Antisense oligonucleotide therapy corrects splicing in the common Stargardt disease type 1-causing variant ABCA4 c.5461-10T>C. *Mol Ther Nucleic Acids*. 2023;31:674-688. doi:10.1016/j.omtn.2023.02.020
58. Kaltak M, De Bruijn P, Van Leeuwen W, et al. QR-1011 restores defective ABCA4 splicing caused by multiple severe ABCA4 variants underlying Stargardt disease. *Sci Rep*. 2024;14(1):684. doi:10.1038/s41598-024-51203-7
59. Li R, Jing Q, She K, et al. Split AAV8 mediated ABCA4 expression for gene therapy of mouse Stargardt disease (STGD1). *Hum Gene Ther*. 2023;34(13-14):616-628. doi:10.1089/hum.2023.017
60. Gabrielczyk T. SpliceBio lauches Phase I/II study on dual-AAV-based therapy for Stargardt Disease. Accessed March 20, 2025. https://european-biotechnology.com/latest-news/splicebio-lauches-phase-i-ii-study-on-dual-aav-based-therapy-for-stargardt-disease/#top
61. Shiels A, Hejtmancik JF. Inherited cataracts: genetic mechanisms and pathways new and old. *Exp Eye Res*. 2021;209:108662. doi:10.1016/j.exer.2021.108662
62. Ahmad R, Bell S, Moosajee M. Genetics of congenital cataract. *Adv Ophthalmol Optom*. 2022;7(1):89-118. doi:10.1016/j.yaoo.2022.03.004
63. Malecaze F, Lubsen NH, Serre B, et al. Lens cell targetting for gene therapy of prevention of posterior capsule opacification. *Gene Ther*. 2006;13(19):1422-1429. doi:10.1038/sj.gt.3302790
64. Lin H, Ouyang H, Zhu J, et al. Lens regeneration using endogenous stem cells with gain of visual function. *Nature*. 2016;531(7594):323-328. doi:10.1038/nature17181
65. Wu Y, Liang D, Wang Y, et al. Correction of a genetic disease in mouse via use of CRISPR-Cas9. *Cell Stem Cell*. 2013;13(6):659-662. doi:10.1016/j.stem.2013.10.016
66. Wu Y, Zhou H, Fan X, et al. Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells. *Cell Res*. 2015;25(1):67-79. doi:10.1038/cr.2014.160
67. Yuan L, Sui T, Chen M, et al. CRISPR/Cas9-mediated GJA8 knockout in rabbits recapitulates human congenital cataracts. *Sci Rep*. 2016;6(1):22024. doi:10.1038/srep22024
68. Yuan L, Yao H, Xu Y, et al. CRISPR/Cas9-Mediated Mutation of αA-Crystallin gene induces congenital cataracts in rabbits. *Investig Opthalmology Vis Sci*. 2017;58(6):BIO34. doi:10.1167/iovs.16-21287
69. Zhao D, Jones JL, Gasperini RJ, Charlesworth JC, Liu GS, Burdon KP. Rapid and efficient cataract gene evaluation in F0 zebrafish using CRISPR-Cas9 ribonucleoprotein complexes. *Methods*. 2021;194:37-47. doi:10.1016/j.ymeth.2020.12.004
70. Jiang J, Zhang X, Tang Y, Li S, Chen J. Progress on ocular siRNA gene-silencing therapy and drug delivery systems. *Fundam Clin Pharmacol*. 2021;35(1):4-24. doi:10.1111/fcp.12561
71. Wu KY, Fujioka JK, Gholamian T, Zaharia M, Tran SD. Suprachoroidal injection: a novel approach for targeted drug delivery. *Pharmaceuticals*. 2023;16(9):1241. doi:10.3390/ph16091241
72. Huang P, Narendran S, Pereira F, et al. The learning curve of murine subretinal injection among clinically trained ophthalmic surgeons. *Transl Vis Sci Technol*. 2022;11(3):13. doi:10.1167/tvst.11.3.13
73. Awwad S, Mohamed Ahmed AHA, Sharma G, et al. Principles of pharmacology in the eye. *Br J Pharmacol*. 2017;174(23):4205-4223. doi:10.1111/bph.14024