Review Article
DOI: https://doi.org/10.70749/ijbr.v3i1.524

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Current Updates, Recent Trends and Future Directions of Gene Therapy on Various Eye Disorders

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ARTICLE INFO

Keywords

Gene Therapy, Eye Disorders, Ocular Diseases, Retinal Gene Therapy, Corneal Gene Therapy, CRISPR-Cas9 in Ophthalmology, Inherited Retinal Diseases (IRDs), Leber Congenital Amaurosis (LCA), Age-related Macular Degeneration (AMD).

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Declaration

Author's Contributions: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

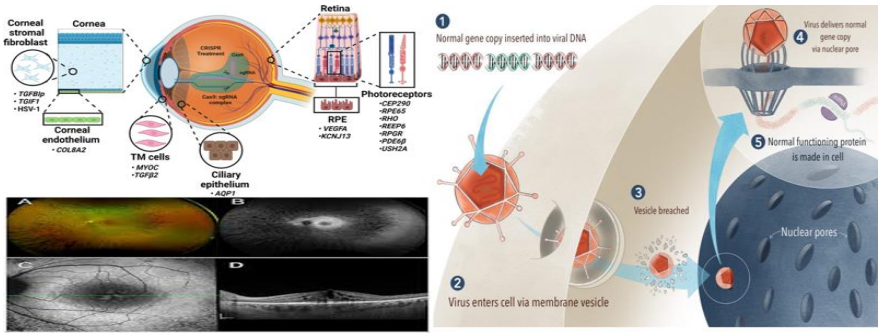
Article History

Received: 02-11-2024
Revised: 01-01-2025
Accepted: 15-01-2025

ABSTRACT

Gene therapy has emerged as a novel strategy in the treatment of eye problems, bringing fresh hope for illnesses previously believed to be untreatable. Recent developments in molecular biology and genetic engineering have enabled the creation of tailored medicines that address the fundamental genetic causes of ocular illnesses rather than just treating symptoms. Gene therapy interventions are showing promise in treating conditions including diabetic retinopathy, age-related macular degeneration, and hereditary retinal dystrophies. The area has undergone a revolution because of methods like CRISPR-Cas9 gene editing and adeno-associated viral (AAV) vectors, which enable precise delivery and change of genetic material within the protected and limited environment of the eye. Patients receiving these treatments have shown notable increases in visual acuity and retinal structural repair in clinical studies. Furthermore, in order to overcome obstacles like immunogenicity and limited payload capacity, next-generation delivery systems like nanoparticles and non-viral vectors are emerging, which could increase the potential of gene therapy. Although the field is still developing quickly, ethical concerns, high expenses, and the requirement for long-term safety assessments are still major problems. This review highlights the latest developments in gene therapy for eye disorders, discussing key breakthroughs, ongoing clinical trials, and future directions to achieve widespread accessibility and efficacy in treating ocular diseases.

Graphical Abstract



INTRODUCTION

Gene therapy is increasing its research powers in different fields. Among those, ophthalmic and eye diseases and their field are gaining much importance owing to gene therapy. Gene therapy used to treat eye disorders is called ocular gene therapy. The eye has three important characteristics, due to which ocular gene therapy has more potential. The eye is a very accessible organ in order to put in injections and perform surgery. Immunologically, the eye is very facilitating when the antigenicity of a viral vector is previewed. The third

important characteristic of eye bares is that it has a very tight and enclosed blood-ocular barrier that will save all other organs from any unnecessary contamination. For different kinds of eye disorders, gene therapy has a huge potential. Gene therapy for ocular diseases not only has the potential to treat monogenic ophthalmic and retinal problems but also gene-determined pharmacotherapies for non-monogenic disorders. Those problems include diabetic retinopathy as well as macular disease that is related to age. (Samiy, N. 2014).

In order to know the genetic pathogenesis of eye disorders, important advancements in this regard have been made. As an effective therapy, gene silencing and replacement have been said to have potential. The methods to transfer ocular genes that are vector-based have gained a lot of recent advancements for a safer and specific transfer. Various experimental models have been prepared for human eye disorders that include vector-based ocular GT. In order to treat Leber congenital amaurosis, initial success has started gaining importance in the 1st phase of clinical trials. This has been made possible after almost 2 decades of research in ocular gene therapy. Retinitis pigmentosa, Stargardt disease, macular degeneration and retinoschisis are the ocular diseases that are going to be treated by gene therapy in the future owing to prospects of gene therapy. Not only retinal diseases but also non-retinal disorders, including glaucoma and uveitis, have also been in the process of treatment by providing experimental models. As gene therapy is going to be the potential treatment for human eye disorders, all the recent advancements in methods are very important to implement this therapy in the future. (Liu, M. 2011)

Why Eye is Suitable for Gene Therapy?

The eye has three important characteristics that will make it a very suitable organ for gene therapy. The human eye is easily reachable as well as an immunologically better-suited organ. There is another important point that it is highly channelized and separates all contaminants from other organs of the body. The eye is a much better organ that is accessible to the viral vectors and antigenicity due to its immune-facilitated characteristic. There is less contamination with other body organs due to the tight barriers of eyes in comparison to other parts of the body. The complete disease can be treated if we can treat the eye because many monogenic diseases are truly ophthalmic. Hence, a simple intravitreal injection or common surgical operation, for example, vitrectomy, can be used to deliver the gene to the eye due to its location as well as accessibility. (Sengillo, J. D, 2016)

Current Updates on Gene Therapy for Treating Different Eye Disorders

Dominant Optic Atrophy (DOA)

DOA is an eye disorder that causes problems in adults at an early age, and symptoms appear. DOA is defined as an ocular disorder in which the optic nerve is damaged or degenerated. Vision loss and some other defects of color vision are typical symptoms. The severity of the symptoms can vary and the disease sometimes causes complete blindness. DOA has not yet been cured, and there are no ways currently available, but gene therapy will be a treatment for DOA in the future. (Daniel et, al 2020)

OPA1 Gene

OPA1 is a gene that carries instructions for synthesizing a protein present in different tissues and cells of the body. That protein is very important for balancing the proper functioning of mitochondria.

A mutation occurs in the OPA1 gene in people who have dominant optic atrophy. Due to mutation in this gene, the protein is not manufactured properly, and the dysfunction of mitochondria occurs. This dysfunction is the reason for the continuity of DOA. If this OPA1 gene does not make the protein, mitochondrial functions are lost, and the network in the normal cells is damaged. Hence, mitochondrial functions are pretty much sub-optimal in DOA due to mutations in the OPA1 gene. (Daniel M. Maloney, 2020)

The New Gene Therapy for DOA

A new gene therapy has been developed for DOA that is initiated by two scientists including Professor Jane Farrar and Dr Daniel Maloney. They used this gene therapy on mice and were successful in curing the vision functions of the mice. Both scientists have assured that this gene therapy can also be the solution to treat DOA in human beings and will enhance mitochondrial functions. This treatment has offered hope that it may be possible in the future to treat DOA in human beings while improving mitochondrial performance. (Daniel M. Maloney, 2020).

Their findings are particularly exciting because they show that this OPA1-based gene therapy may support diseases like DOA, which are caused by OPA1 mutations, as well as a broader range of diseases involving mitochondrial dysfunction. Importantly, mitochondrial dysfunction is linked to a number of other neurological conditions, including Alzheimer's and Parkinson's disease. OPA1 mutations cause DOA, so this OPA1-based treatment method is applicable to DOA. However, cell death has been related to a slew of neurological diseases that cause millions of people around the world. It is believed that this form of therapeutic approach addressing mitochondrial dysfunction has a lot of potential to help people and thus have a big societal effect. (Trinity College Dublin. 2020).

Table 1
The New Gene Therapy For DOA

Aspect	Details	Key Insights	Potential Outcomes	Challenges
Therapy Name	OPA1-based Gene Therapy	Targets the OPA1 gene mutations causing DOA	Restoration of vision and enhancement of mitochondrial function	Requires extensive clinical trials for human application
Developers	Professor Jane Farrar and Dr. Daniel Maloney	Scientists leading the research and development of this therapy	Key contributors to advancing gene therapy for mitochondrial dysfunction	Translating results from animal models to humans
Animal Model	Mice	Gene therapy successfully restored vision function in mice	Proof-of-concept for the effectiveness of this approach	Potential differences in human physiology and gene expression
Mechanism of Action	OPA1 Gene Delivery	Enhances mitochondrial functions by targeting and correcting OPA1 mutations	Improvement of mitochondrial health, preventing cell death	Ensuring targeted delivery and avoiding off-target effects
Broader Implications	Diseases with mitochondrial dysfunction (e.g., Alzheimer's, Parkinson's)	OPA1-based therapy may have applications beyond DOA	Potential to address multiple neurological conditions	Validation needed for broader disease contexts
Key Findings	Successful restoration of vision functions in animal models	Demonstrates the potential of OPA1-based gene therapy to treat DOA and related diseases	Opens pathways for clinical applications targeting mitochondrial dysfunction	Bridging the gap between preclinical success and human trials
Ethical Considerations	Gene therapy in humans	Balancing innovation with ethical research practices	Ensuring informed consent and equitable access to advanced treatments	Managing ethical concerns in genetic modifications
Long-term Prospects	Broader application to other mitochondrial diseases	Expanding scope beyond DOA	Revolutionizing mitochondrial disease treatment	Requires deepened understanding of diverse mitochondrial dysfunctions
Therapeutic Goal	Vision restoration and mitochondrial enhancement	Tackles the root cause of DOA at the genetic and cellular levels	Holistic improvements in patient health and quality of life	Addressing potential long-term side effects
Future Directions	Clinical trials in humans	Focus on safety, efficacy, and long-term outcomes	Establishing the therapy as a standard treatment for DOA and possibly other conditions	Extensive resources and collaboration are required for global application

Leber Congenital Amaurosis (LCA)

LCA stands for Leber congenital amaurosis, categorized by a group of often early-onset retinal disorders with intense clinical results that cause vision loss during childhood. (den Hollander et al., 2008). There are various mutations have been known in almost 25 genes in the case of LCA as it is genetically heterogeneous. Various genes among these 25 are very important in the development and proper functioning of photoreceptors in the eyes. (Kumaran et al., 2017). The inheritance pattern shown by patients having LCA is autosomal recessive. The etiology of LCA can be elaborated by measuring or preparing mouse models. (Veleri et al., 2015)

In order to get clinical trials therapeutic applications, contributing to the first adeno-associated virus (AAV)-based gene therapy for recessive LCA prompted by RPE65 mutations to be licensed by the US Food and Drug Administration (Apte, 2018). Clinical translations are slowed down owing to the complexities involved in human disease due to the animal models currently present that don't fully fit. There is currently no proper treatment available for the various dominant types of LCA.

CRX-A Cone-Rod Homeobox Protein

Cone-rod homeobox protein is a protein found in cone-rods. CRX is needed for photoreceptor production in the retina (Furukawa et al., 1999). Via its association with the bZIP transcription factor NRL (Mitton et al., 2000) or an extraordinary amount of regulatory proteins, CRX controls the activity of most genes involving cone photoreceptors and rod cells. (Hennig et al., 2008). Initial retinal signs of weakness with substantial phenotypic variability are caused by heterozygous CRX mutations, with occasional observations of biallelic variants in LCA (Huang et al., 2012; Hull et al., 2014; Ibrahim et al., 2018; Rivolta et al., 2001; Swaroop et al., 1999). CRX is responsible for the majority of recorded dominant LCA. CRX mutations that cause disease may decrease transcriptional activity or cause a benefit of feature effect (Tran et al., 2014). When comparing dominant frameshift mutations in CRX to loss-of-function alleles, experiments in animal models showed an extreme phenotype. (Roger et al., 2014; Tran et al., 2014).

First Gene Therapy for Eye Disease

When new approaches are considered for treating eye disorders, gene therapy has earned the most coverage. In 2017, the United States (US) approved Luxturna®, the first gene therapy for any eye condition, for people with RPE65 mutations that were the reason for LCA or retinitis pigmentosa. Several gene therapy clinical trials are currently in progress, with still more gene therapies being studied and established. Continue reading to learn

about the various forms of gene therapy, who may be a candidate and a list of current gene therapy medical testing. In 2017, Luxturna became the first gene therapy for hereditary blindness to be approved by the FDA. The therapy was accepted in Europe a year later. Luxturna was the first in vivo gene therapy, a procedure that is administered directly into the patient's cells rather than removing and manipulating the cells before reinjecting them. This was a breakthrough for innovation in the treatment of blindness as well as gene therapy. (Fernández, C. R. 2019)

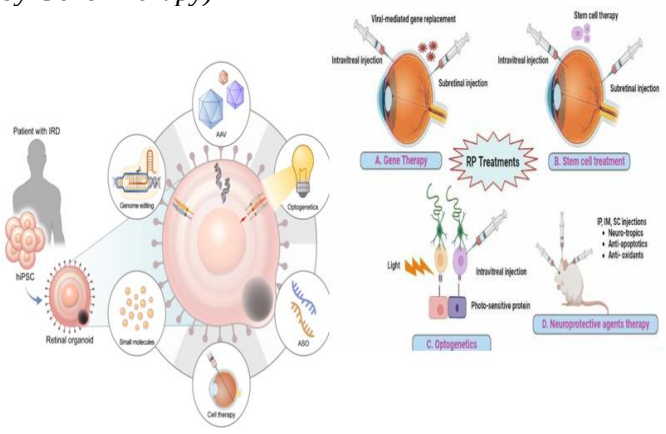
Novel Gene Therapy for Treating LCA

To the classification of iPSCs into 3D retinal organoids (Kruczek and Swaroop, 2020), the simulation of retinopathies in a patient-specific genetic context has been made possible. Next-generation sequencing technologies have allowed for a thorough analysis of growing retinal Organoids with human retinas and the evaluation of major ocular cell types in disease settings. (Collin et al., 2019; Cowan et al., 2020; Hoshino et al., 2017; Kaya et al., 2019; Kim et al., 2019)

A retinal organoid template of CRX-LCA has been developed using clinician iPSCs, and it shows a shift in the molecular morphology of photoreceptors, including decreased expression of optical opsins, which is consistent with clinically observed depletion of different responses shown to light. The delivery of a right CRX transgene powered by a human CRX promoter via an AAV vector has been shown to partially restore both rod and cone gene expression in recent research. Recent research points to gene augmentation as a therapeutic approach for dominant CRX-LCA. (Kruczek, K., 2021).

Figure 1

(A graphical abstraction of the treatment of CRX-LCA by Gene Therapy)



Concluding the remarks, retinal organoids have been used by researchers to understand the mechanisms of LCA disorder. Scientists are able to form gene augmentation for the dominant form of CRX-LCA. They demonstrate that their experimental approach can be used to establish successful therapies for retinal and other neuropsychiatric disorders that are uncommon or even dominant. (Kruczek, K., 2021).

Corneal Disease

Corneal disease is still the leading cause of vision loss around the world, and advances in gene therapy are showing promise in reducing, controlling, and curing blindness. Gene therapy, in its ideal state, will involve a vector and gene delivery system that targets specific cells and tissues and results in a much safer response that is highly non-immunogenic. The cornea is a model tissue for quality treatment because of its simplicity of clinician access and safe special state. Enhancements in the previous 5–10 years have started to upset the way to deal with quality treatment in the cornea with attention on adeno-related infection and nanoparticle conveyance of single and mixed quality treatments. Furthermore, the gene editing tools involving TALENS, CRISPR-Cas9 and ZNFs have the potential to cure genetic diseases and are widely enhanced. (Mohan, R., 2020).

Despite the fact that there are far fewer experiments on the cornea than there are on the retina (Ginn et al., 2017), development in the field of corneal genome editing has been impressive despite the fact that it is still in the preclinical stage. According to the WHO, corneal blindness is the 4th leading cause of blindness worldwide, with over 39 million people suffering total blindness and 246 million having vision impairment. Corneal defects cause vision loss in approximately 4% of the United States population, and they are the second most common cause of blindness in the majority of developing countries. (Oliva et al., 2012). Much more shocking is the rising number of individuals who develop corneal disease and are forced to live with a disability for the rest of the time. According to the Eye Institute, ophthalmic care costs $70 billion per year and is expected to rise to $717 billion by 2050; as a result, clinicians and researchers must develop corneal blindness prevention and treatment options. Infections involving trachoma and onchocerciasis, as well as injuries that include trauma and combat wounds, are the most common causes of blindness, but iatrogenic etiologies are also a factor due to common medical procedures, including photorefractive keratectomy (PRK). (World Health Organization, 2020).

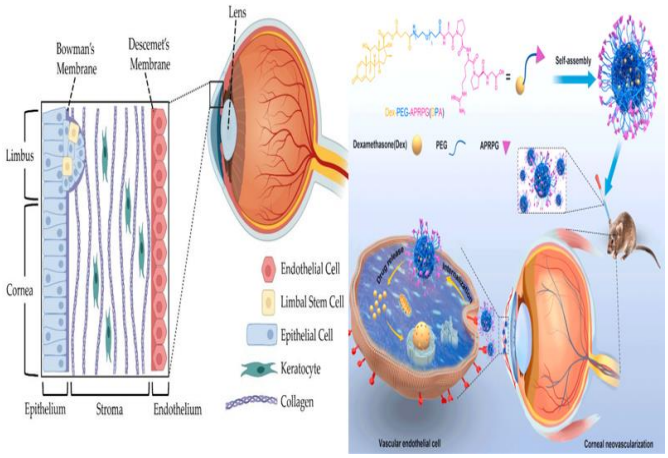
Because of its simple accessibility, immunologically favorable status, and position, the cornea is a perfect tissue for gene therapy. To deliver gene therapy vector throughout the cornea, all feasible methods, such as topical, mechanical, surgical, electrical, or chemical, may be used. In the rabbit and rodent corneas in vivo and in the human cornea ex vivo, easily accessible vector-delivery techniques resulted in tissue-targeted gene delivery into target cells. Furthermore, the cornea can be kept in an artificial physiological setting for several weeks for testing purposes as well as to apply treatments prior to corneal transplant surgery. This will minimize all the deleterious effects that occur due to graft rejection (Torrecilla et al., 2018).

Approaches in Designing Gene Therapy for Corneal Diseases

The cornea is a special opaque tissue that provides the eye with almost two-thirds of refraction. The cornea's distinctive form and trilaminar layout, which consists of stratified epithelium, stroma, and metabolically active yet mitotically inactive endothelium monolayer, decide its refractive strength and transparency. The goals of the research, as scientists and physicians operating together, are to improve health care and sustain quality of life by reducing, preventing, or treating corneal disorders. When designing a new gene therapy, patient protection is the most important thing to remember. The preferred new gene could either suppress or substitute the mutated gene based on the corneal condition. Within, a simple approach to gene therapy production, as well as some considerations, will be adopted in the future. (Mohan, R. R., 2020).

Figure 2

Approaches in Designing Gene Therapy for Corneal Diseases



Identifying a Vector

Once a gene has been recognized as having a potential therapeutic effect for the corneal disease in question, a method of transporting the gene to particular cells within the cornea is required. The vector is the term for this carrier. Vectors are categorized as either viral or non-viral, as previously mentioned. It is necessary to know whether the corneal condition is congenital or acquired before choosing the best vector. Getting long-term or irreversible transgene expression would be preferable if the disorder was congenital. In this case, gene editing using a CRISPR/Cas9 method could be a viable option. AAV, LV, and RV viral vectors, on the other hand, normally have transgene expression for months to years. LV and RV vectors blend into host DNA and can induce mutagenesis. AAV has now become the most important and famous way of viral transport in laboratories due to all these reasons. In required to bring a bigger gene, NP vectors are more advantageous because they can quickly move via the cell membrane. Lengthy transgene expression, low price, and ease of processing are all advantages of the SB transposon. Following the selection of a vector, the delivery technique is calculated. (Mohan, R. R., 2020).

Mono Versus Dual Gene Therapy

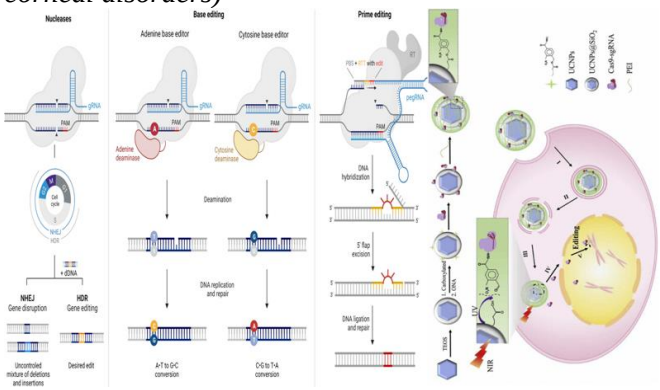
A corneal ulcer may develop as a result of a corneal injury, fracture, or inflammation and may be followed by corneal edema, neovascularization, and fibrosis. To encourage therapy implementation, clinicians need clinical resources that target several problems, are long-lasting, and secure for the patient with a lower intensity of treatment. Dual gene is an efficient therapeutic option for dealing with both of these issues. Combination gene therapy for disease control has only been tested in a few cornea trials. (Marlo et al., 2018) used a blend of pro-Smads (Smad2, Smad3, and Smad4) and anti-fibrotic (Smad7) Smads to silence and overexpress genes. Individual Smad2, 3, or 4 gene suppression or overexpression of Smad7 resulted in significant suppression of equine corneal fibroblasts to myofibroblasts and corneal fibrosis in vitro, but a combination gene therapy produced no important additional anti-fibrotic reaction. (Marlo et al., 2018). However, the integration of the two genes targeting two various factors or pathways showed radically different outcomes. For example, the combination of BMP7 and HGF gene therapy in rabbit in vivo and human in vitro cornea models demonstrated significant additive response associated with lower corneal fibrosis, inflammation, edema, and recovery of corneal clarity (Gupta et al., 2018). These are interesting results that will possibly spur further study in this field and lead to clinical studies relatively soon.

Promising Approaches That Need Further Investigation

To use combination gene therapy in corneal treatment using gene therapy, there is still the need for extensive research. It is the aim shown by Marlo et al. (2018) that Smad2, which is a pathological gene, will be silenced in support of Smad7, which is a beneficial gene. As the Smad7 cannot be overexpressed alone while utilizing combination gene therapy, this will help the researchers to combine another gene with the overexpression of Smad7. That gene can effectively target the disorder or problem. In this way, combining Smad7 overexpression and another gene can inhibit corneal neovascularization. Histone acetylase is a protein that affects the gene expression which is Smad-mediated. This enzyme has an important function in gene transcription and epigenetic regulation. There is another way to search the development of both single and combination gene therapy which is by epigenetic modulation. In many research areas, the sleeping.

Figure 3

(CRISPR Cas9 Mechanism for gene editing to treat corneal disorders)



Due to the limited packing ability and capacity, however, all parts od prime and base editors are unable to deliver in a single AAV vector. The dual AAV method was used to deliver these editors, and in this regard, these new vector systems were eligible to deliver all necessary components required for gene editing with much better efficiency. For the safety and efficiency of the CRISPR method, a lot of research studies need to be focused on. CRISPR genetic engineering technologies will soon be widely used in the treatment of a wide range of ocular diseases thanks to improved efficacy, protection, an optimized vector and an administration path. (WenhanYu, 2021).

A Brighter Future Insight

There will be more success in the field of gene therapy to cure blindness and different eye disorders. Gene therapy will enter the market and will provide solutions for many disorders in the future. CRISPR Cas9 will revolutionize the whole world by making gene editing much more simples. The mutations causing blindness and many other eye disorders will be cured and treated in our retinal cells with the help of CRISPR. It is still in the early stages, but recently. Blindness caused by LCA will be cured by developing CRISPR therapy in the USA, with the collaborative work of Editas Medicine and Allegan. (Fernández, C. R. 2020). However, a huge improvement is still required in the success of gene therapy in treating specific diseases and rare mutations. It is limited to a very few patients. However, the days are not far away when gene therapy will cure all causes of blindness and will become the basic and important treatment. Advances in different techniques will help indicate problems that can be cured with gene therapy. Beauty transposons are very helpful, but they need to be examined before they can be used for corneal diseases. (Hudecek et al., 2017).

Table 2

Promising Approaches That Need Further Investigation

Promising Approach	Key Elements	Mechanism of Action	Potential Outcomes	Current Challenges	References
Combination Gene Therapy	Overexpression of Smad7 and silencing of Smad2	Smad7 promotes beneficial pathways, while Smad2 suppression mitigates pathological responses.	Inhibition of corneal neovascularization	Identifying compatible genes to pair with Smad7 for effective combined therapy	Zhang et al., 2020
Epigenetic Modulation	Histone acetylase regulation	Modifies chromatin structure to control Smad-mediated gene expression	Improved gene expression regulation and suppression of unwanted corneal pathologies	Understanding precise epigenetic changes and designing targeted interventions	van Grunsven et al., 2005
Utilization of Sleeping Beauty Transposons	Development of non-viral vector systems for gene delivery	Sleeping Beauty transposons integrate therapeutic genes into target DNA sequences	Stable and long-term gene expression with reduced risk of immune response	Safety concerns, integration efficiency, and evaluation in corneal-specific applications	Hudecek et al., 2018
Multi-target Gene Combinations	Pairing Smad7 with genes addressing specific corneal disorders (e.g., VEGF inhibitors or anti-inflammatory genes)	Synergistic effects on reducing pathological processes like neovascularization	Enhanced therapeutic efficacy by addressing multiple pathways simultaneously	Identifying optimal gene pairs and ensuring their compatibility within combination therapy frameworks	Cubillo et al., 2024
Gene Editing Tools	CRISPR/Cas9 for targeted editing of pathological genes (e.g., Smad2)	Directly edits pathological genes, preventing harmful protein production	Precision treatment of genetic causes of corneal disorders	Off-target effects, delivery challenges, and regulatory concerns in clinical applications	Ji et al., 2024
Viral Vector Enhancements	Improved AAV or lentiviral vectors for delivering combination therapies	Efficient and targeted delivery of therapeutic genes to corneal cells	Increased uptake and long-term expression in corneal tissues	Balancing efficiency with minimizing immune response or potential toxicity	Arsenijevic et al., 2022
Development of New Therapeutic Genes	Identification of genes that	Novel therapeutic pathways can be	Broader treatment possibilities for complex corneal pathologies	Need for extensive research to identify	Maslankova et al., 2022

	complement Smad7 activity	targeted alongside Smad7 overexpression		and validate new gene targets	
Epigenetic Regulation in Therapy	Use of inhibitors or activators of histone acetylases and other epigenetic factors	Influences Smad7 and Smad2 expression by modifying transcriptional accessibility	Controlled gene expression and improved therapeutic outcomes	Lack of precise control and understanding of long-term effects	Ding et al., 2024
Nanotechnology in Gene Therapy	Nanoparticles for delivering combination gene therapy (e.g., Smad7 paired with VEGF inhibitors)	Non-invasive delivery systems for corneal disorders	Enhanced delivery efficiency and reduced systemic exposure	Optimization of nanoparticle composition and safety evaluations	Amador et al., 2022

Potential Challenges and Limitations

For the purpose of getting into clinical trials from preclinical settings, there is a lot of research required to explain the different challenges faced by advanced molecular biology. There are various beneficiary improvements have been seen in the treatment of corneal disorders using gene therapy. Firstly, the safety of the patient should be prioritized and there should be the development of vector-based delivery methods and non-immunogenic vectors in order to transport genes into the targeted cells. This will create fewer adverse effects in the patients, and the pre-existing viral antibody challenges will also be minimized in human beings. Secondly, there is the need for new vectors to be used in combination therapy having AAV so that the vectors can have bigger gene packages and possess multiple genes. Thirdly, the therapeutic gene delivery's efficiency will be enhanced if highly specific vectors for tissues are developed that will minimize the unwanted problems or destruction to normal cells and tissues. Hence, these gene therapies and editing techniques should be strictly regulated as these generate various serious risks to the patients as well as can be exploited for bioterrorism. (Mohan, R. R., 2020)

Future Directions

If we have a look overall, corneal gene therapy is far behind in success and progress than the other ocular gene therapies as well as retinal disorders. However, there are various improvements in the research that are going to take corneal gene therapy to clinical applications and trials in human beings. In the cornea, the approach of gene therapy focuses on using nanoparticle and AAV delivery methods for single as well as combination therapies, and a lot of improvement has been made in this decade. Furthermore, the gene editing tools involving CRISPR, ZNFs and TALENS are improving their potential day by day. If more clinical trials are performed, gene therapy is going to be the permanent cure in some cases and an effective, useful and long-term option in many cases. Gene therapy will soon treat patients having eye disorders, including corneal graft rejection, corneal neovascularization, corneal dystrophies and corneal fibrosis. (Mohan, R. R., 2020).

Future Perspectives of Gene Therapy for Eye Disorders

Gene and Cell-Based Therapies Are Promising

There have been great improvements in the fields of gene therapy in this decade to treat eye disorders. Cell-based and gene therapies, as well as CRISPR gene editing techniques, are going to be promising. Ophthalmic and eye disorders are at the front side of all these therapeutic developments. In comparison to all other pathological diseases, ocular diseases are more accessible for the strategies used to treat them. In order to analyze many treatment problems, control studies that are randomized and continual translational studies are required. These therapies are promising in order to benefit patients who are currently going through many kinds of irreversible vision loss and blindness. (Yu, W., 2020)

The success of CRISPR Cas9 in Treating Eye Disorders

A large number of ocular disorders will be treated in the future by using CRISPR Cas9 gene editing tools, specifically IRDs that are inherited retinal disorders. CRISPR Cas9 bases gene editing has been started in clinical trials for a retinal disease LCA10 that occurs due to some intronic mutations happening in the gene CEP290. The diseases that occur due to dominant mutations can be treated by utilizing CRISPR-dependent allele specific editing of the genes as well as its high efficiency. One of the most important diseases caused by dominant mutations is retinitis pigmentosa, which occurs due to a mutation in the rhodopsin gene. Some point mutations that cause IRDs will be cured by utilizing the HDR mechanism of CRISPR, but there are efficiency and safety concerns about this method. There are various base editing repair mechanisms as well as prime editing methods that have been recently developed. These methods will correct the point mutations. The point mutations will be corrected by creating the least DSBs as well as minimizing all kinds of safety problems and concerns.

REFERENCES

Amador, C., Shah, R., Ghiam, S., Kramerov, A. A., & Ljubimov, A. V. (2022). Gene Therapy in the Anterior Eye Segment. *Current Gene Therapy*, *22*(2), 104–131. https://doi.org/10.2174/1566523221666210423084233

Apte, R. S. (2018). Gene therapy for retinal degeneration. *Cell*, *173*(1), 5. https://www.cell.com/action/showCitFormats?doi=10.1016%2Fj.cell.2018.03.021&pii=S0092-8674%2818%2930301-5

Arsenijevic, Y., Berger, A., Udry, F., & Kostic, C. (2022). Lentiviral Vectors for Ocular Gene Therapy. *Pharmaceutics*, *14*(8), 1605. https://doi.org/10.3390/pharmaceutics14081605

Collin, J., Queen, R., Zerti, D., Dorgau, B., Hussain, R., Coxhead, J., ... & Lako, M. (2019). Deconstructing retinal organoids: single cell RNA-Seq reveals the cellular components of human pluripotent stem cell-derived retina. *Stem Cells*, *37*(5), 593-598. https://doi.org/10.1002/stem.2963

Cubillo, L. T., Gurdal, M., & Zeugolis, D. I. (2024). Corneal fibrosis: From in vitro models to current and upcoming drug and gene medicines. *Advanced Drug Delivery Reviews*, *209*, 115317–115317. https://doi.org/10.1016/j.addr.2024.115317

den Hollander, A. I., Roepman, R., Koenekoop, R. K., & Cremers, F. P. M. (2008). Leber congenital amaurosis: Genes, proteins and disease mechanisms. *Progress in Retinal and Eye Research*, *27*(4), 391–419. https://doi.org/10.1016/j.preteyeres.2008.05.003

Ding, Y., Zhou, G., & Hu, W. (2024). Epigenetic regulation of TGF-β pathway and its role in radiation response. *International Journal of Radiation Biology*, *100*(6), 834–848. https://doi.org/10.1080/09553002.2024.2327395

Fernández, C. R. (2020). Can Gene Therapy Become? The Cure For Blindness? Labiotech. Eu, Labiotech UG, 13.

Ginn, S. L., Amaya, A. K., Alexander, I. E., Edelstein, M., & Abedi, M. R. (2018). Gene therapy clinical trials worldwide to 2017: An update. *The Journal of Gene Medicine*, *20*(5), e3015. https://doi.org/10.1002/jgm.3015

Glenisson, W., Castronovo, V., & Waltregny, D. (2007). Histone deacetylase 4 is required for TGFβ1-induced myofibroblastic differentiation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1773*(10), 1572–1582. https://doi.org/10.1016/j.bbamcr.2007.05.016

Gupta, S., Rodier, J. T., Sharma, A., Giuliano, E. A., Sinha, P. R., Hesemann, N. P., Ghosh, A., & Mohan, R. R. (2017). Targeted AAV5-Smad7 gene therapy inhibits corneal scarring in vivo. *PLOS ONE*, *12*(3), e0172928. https://doi.org/10.1371/journal.pone.0172928

Huang, L., Xiao, X., Li, S., Jia, X., Wang, P., Guo, X., & Zhang, Q. (2012). CRX variants in cone–rod dystrophy and mutation overview. *Biochemical and Biophysical Research Communications*, *426*(4), 498–503. https://doi.org/10.1016/j.bbrc.2012.08.110

Hudecek, M., & Ivics, Z. (2018). Non-viral therapeutic cell engineering with the Sleeping Beauty transposon system. *Current Opinion in Genetics & Development*, *52*, 100–108. https://doi.org/10.1016/j.gde.2018.06.003

Hudecek, M., Zsuzsanna Izsvák, Johnen, S., Renner, M., Thumann, G., & Zoltán Ivics. (2017). Going non-viral: the*Sleeping Beauty*transposon system breaks on through to the clinical side. *Critical Reviews in Biochemistry and Molecular Biology*, *52*(4), 355–380. https://doi.org/10.1080/10409238.2017.1304354

Ibrahim, M. T., Alarcon-Martinez, T., Lopez, I., Fajardo, N., Chiang, J., & Koenekoop, R. K. (2018). A complete, homozygous CRX deletion causing nullizygosity is a new genetic mechanism for Leber congenital amaurosis. *Scientific Reports*, *8*(1), 1-6. https://doi.org/10.1038/s41598-018-22704-z

Ji, P., Li, Y., Wang, Z., Jia, S., Jiang, X., Chen, H., & Wang, Q. (2024). Advances in precision gene editing for liver fibrosis: From technology to therapeutic applications. *Biomedicine & Pharmacotherapy*, *177*, 117003. https://doi.org/10.1016/j.biopha.2024.117003

Kruczek, K., & Swaroop, A. (2020). Pluripotent stem cell-derived retinal organoids for disease modeling and development of therapies. *Stem Cells*, *38*(10), 1206-1215. https://doi.org/10.1002/stem.3239

Kruczek, K., Qu, Z., Gentry, J., Fadl, B. R., Gieser, L., Hiriyanna, S., Batz, Z., Samant, M., Samanta, A., Chu, C. J., Campello, L., Brooks, B. P., Wu, Z., & Swaroop, A. (2021). Gene therapy of dominant CRX-leber congenital amaurosis using patient stem cell-derived retinal Organoids. *Stem Cell Reports*, *16*(2), 252-263. https://doi.org/10.1016/j.stemcr.2020.12.018

Liu, M. M., Tuo, J., & Chan, C. (2010). Gene therapy for ocular diseases. *British Journal of*

Ophthalmology, *95*(5), 604-612. https://doi.org/10.1136/bjo.2009.174912

Marlo, T. L., Giuliano, E. A., Tripathi, R., Sharma, A., & Mohan, R. R. (2017). Altering equine corneal fibroblast differentiation through Smad gene transfer. *Veterinary Ophthalmology*, *21*(2), 132-139. https://doi.org/10.1111/vop.12485

Maslankova, J., Vecurkovska, I., Rabajdova, M., Katuchova, J., Kicka, M., Gayova, M., & Katuch, V. (2022). Regulation of transforming growth factor-β signaling as a therapeutic approach to treating colorectal cancer. *World Journal of Gastroenterology*, *28*(33), 4744-4761. https://doi.org/10.3748/wjg.v28.i33.4744

Mitton, K. P., Swain, P. K., Chen, S., Xu, S., Zack, D. J., & Swaroop, A. (2000). The leucine zipper of NRL interacts with the CRX Homeodomain. *Journal of Biological Chemistry*, *275*(38), 29794-29799. https://doi.org/10.1074/jbc.m003658200

Mohan, R. R., Martin, L. M., & Sinha, N. R. (2021). Novel insights into gene therapy in the cornea. *Experimental Eye Research*, *202*, 108361. https://doi.org/10.1016/j.exer.2020.108361

Oliva, M., Schottman, T., & Gulati, M. (2012). Turning the tide of corneal blindness. *Indian Journal of Ophthalmology*, *60*(5), 423. https://doi.org/10.4103/0301-4738.100540

Prado, D. A., Acosta-Acero, M., & Maldonado, R. S. (2020). Gene therapy beyond luxturna: A new horizon of the treatment for inherited retinal disease. *Current Opinion in Ophthalmology*, *31*(3), 147-154. https://doi.org/10.1097/icu.0000000000000660

Rivolta, C., Berson, E. L., & Dryja, T. P. (2001). Dominant Leber congenital amaurosis, cone-rod degeneration, and retinitis pigmentosa caused by mutant versions of the transcription factor CRX. *Human Mutation*, *18*(6), 488-498. https://doi.org/10.1002/humu.1226

Roger, J. E., Hiriyanna, A., Gotoh, N., Hao, H., Cheng, D. F., Ratnapriya, R., Kautzmann, M. I., Chang, B., & Swaroop, A. (2014). OTX2 loss causes rod differentiation defect in CRX-associated congenital blindness. *Journal of Clinical Investigation*, *124*(2), 631-643. https://doi.org/10.1172/jci72722

Samiy, N. (2014). Gene therapy for retinal diseases. *Journal of Ophthalmic and Vision Research*, *9*(4), 506. https://doi.org/10.4103/2008-322x.150831

Sengillo, J. D., Justus, S., Tsai, Y., Cabral, T., & Tsang, S. H. (2016). Gene and cell-based therapies for inherited retinal disorders: An update. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *172*(4), 349-366. https://doi.org/10.1002/ajmg.c.31534

Swaroop, A. (1999). Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of the retinal transcription factor CRX: direct evidence for the involvement of CRX in the development of photoreceptor function. *Human Molecular Genetics*, *8*(2), 299–305. https://doi.org/10.1093/hmg/8.2.299

Swaroop, A. (1999). Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of the retinal transcription factor CRX: Direct evidence for the involvement of CRX in the development of photoreceptor function. *Human Molecular Genetics*, *8*(2), 299-305. https://doi.org/10.1093/hmg/8.2.299

Tran, N. M., Zhang, A., Zhang, X., Huecker, J. B., Hennig, A. K., & Chen, S. (2014). Mechanistically distinct mouse models for CRX-associated retinopathy. *PLoS Genetics*, *10*(2), e1004111. https://doi.org/10.1371/journal.pgen.1004111

Trinity College Dublin. (2020, November 26). *Scientists develop new gene therapy for eye disease*. ScienceDaily. www.sciencedaily.com/releases/2020/11/201126085921.htm

Van Grunsven, L. A., Verstappen, G., Huylebroeck, D., & Verschueren, K. (2005). Smads and chromatin modulation. *Cytokine & Growth Factor Reviews*, *16*(4-5), 495-512. https://doi.org/10.1016/j.cytogfr.2005.05.006

Veleri, S., Lazar, C. H., Chang, B., Sieving, P. A., Banin, E., & Swaroop, A. (2015). Biology and therapy of inherited retinal degenerative disease: Insights from mouse models. *Disease Models & Mechanisms*, *8*(2), 109-129. https://doi.org/10.1242/dmm.017913

Yu, W., & Wu, Z. (2021). Ocular delivery of CRISPR/Cas genome editing components for treatment of eye diseases. *Advanced Drug Delivery Reviews*, *168*, 181-195. https://doi.org/10.1016/j.addr.2020.06.011

Zhang, T., Wang, X., Wang, Z., Lou, D., Fang, Q., Hu, Y., Zhao, W., Zhang, L., Wu, L., & Tan, W. (2020). Current potential therapeutic strategies targeting the TGF-β/Smad signaling pathway to attenuate keloid and hypertrophic scar formation. *Biomedicine & Pharmacotherapy*, *129*, 110287. https://doi.org/10.1016/j.biopha.2020.110287