Gene Therapy as a Treatment for Inherited Retinal Disorders

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Introduction

One of the leading causes of vision loss and blindness in individuals is inherited retinal disorders, commonly known as IRD's. For years doctors and researchers have sought to uncover a treatment that would halt the progression of these debilitating diseases. One of the most promising treatments that has been researched is gene therapy. Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes by making a beneficial protein by providing the necessary gene to restore the function of the protein. The gene is transferred using a carrier called a vector which is genetically engineered to deliver the gene. There have been gene therapy trials for a wide expanse of diseases such as cancer, hemophilia, Duchenne muscular dystrophy, autoimmune disorders, cardiovascular disease, and many more. Clearly gene therapy is the future of medicine, so understanding how gene therapy works is essential for healthcare workers. As a future optometrist, I was drawn towards understanding how gene therapy has had an effect on treating inherited retinal disorders especially since I hope to focus my studies on ocular disease. The eye has certain important features which makes it an optimal target for gene therapy, which is a reason why researchers have been successful in creating a gene therapy treatment called RPE65 gene therapy to treat Leber's Congenital Amaurosis. Since the accomplishment of creating the gene therapy drug for Leber's Congenital Amaurosis, researchers have sought to replicate this therapy for other inherited retinal disorders while also finetuning certain features of the treatment to create an even better drug. Additionally, in the past decade there has been advances made in genomic editing and a new technology called CRISPER-Cas9 has arose that can cause gene correction, gene disruption, or gene integration. Through understanding the background of gene therapy and how it specifically targets the eye, then delving into the effect it has had on several inherited retinal disorders, and what my role as a

future Optometrist will be in helping patients with IRD's, I hope to convey the important role gene therapy has in both the world of eyecare and medicine at large.

History of Gene Therapy

There were many scientific discoveries that helped pave the way towards the introduction of gene therapy. One important finding that microbiologist Joshua Lederberg and Norton Zinder found in 1952, is that bacteriophages may transfer genetic material into a cell through a process called transduction. This process was later applied to viruses by Howard Temin- he stated that genetic mutations could be inherited as a result of a viral infection. This led to the idea that viruses have properties that could make it useful in delivering genes into cells of interest and sparked the idea that genetic engineering could be used to treat genetic disease (Wirth 2013).

Edward Tatum wrote a paper in 1966 about the usage of viruses in gene therapy- and that one would need to remove the pathology causing genes from the virus and replace them with therapeutic genes. It wasn't until 1990 that the FDA approved the first gene therapy trial led by Michael R. Blaese. The trial focused on two children that suffered from adenosine deaminase (ADA) deficiency which leads to severe immunodeficiency, they made history by being the first patients to undergo an approved application of gene therapy. Retroviral vectors were packaged with functional ADA genes and were transferred into the T cells of their blood which was reinfused into the girls. The success of this treatment created the momentum that drove many other gene therapy trials. A setback in the gene therapy trials occurred in 1999 when Jesse Gelsinger, an 18 year old participant in a gene therapy trial, died from a high dose of the adenovirus administration- this led to the discourse over the safety and ethical ramifications of the treatments. Despite this setback, clinical trials continued to take place all over the world, with

60% of trials focused on battling cancer. The very first gene therapy product, called Gendicine, was approved for clinical use in 2003 in China for the treatment of head- and neck squamous cell carcinoma. Another noteworthy drug that was approved by the European Medicines Agency (EMA) in 2012 is Glybera. Even though the drug effectively helped combat lipoprotein lipase deficiency, the drug was too expensive to keep manufacturing, especially due to the limited amount of people who needed this treatment (Sagonwsky 2017). Although the progression of gene therapy research has had its opens and downs, the bumps in the road have all contributed to better understanding the challenges that gene therapy poses and have given scientists room to try and learn from their mistakes.

How Gene Therapy Works

The goal of gene therapy is to introduce genetic material into a patient using a viral or non-viral vector in order to regulate, repair, replace, add or delete a genetic sequence, in order to treat or prevent a disease (Lee 2018). Either cells are removed from the patients and after genetic modification they are reinjected, or genetic material can be directly introduced into the target organs or tissues. There are different types of gene therapy that are being researched, including: gene augmentation, gene- specific targeting, and genome editing. Gene augmentation replaces a disease-causing gene with a healthy copy of the gene to compensate for the faulty genes and aims to reach a sufficient level of expression of the needed protein. This type of gene therapy is usually used to target autosomal recessive and X-linked hereditary diseases. Gene- specific targeted therapy either introduces a new gene or deactivates a disease-causing gene by causing cell death, its designed to alter inappropriate gene activity that’s due to disease. It provides a long term therapeutic treatment for both nongenetic diseases and autosomal dominant disorders.

Genome editing, also called correction therapy, directly modifies and transforms mutated genes into normally functioning one. This therapy is used to treat autosomal recessive, autosomal dominant, and X-linked diseases that are caused by genes with specific spatial and stoichiometric expression.

The first steps in the gene therapy process is to create a working gene in the laboratory that can then be transferred to a patient, the scientist design genes that will code for the proteins that need to be expressed. A vector then needs to be prepared in order to transport the genes, while there are different types of vectors that can be used a viral vector is typically utilized. A virus is stripped of its viral DNA, and the genes are placed within the empty shell of the virus. Once the vector is administered it will enter the patients cells, and bring a copy of the functional gene into the nucleus. Once in the nucleus the functional gene allows for the production of the protein of interest and correct the underlying cause of the disease. Not all patients are necessarily eligible to receive gene therapy as a treatment, factors such as age, gender, organ health, if your blood contains antibodies for the vector (this would interfere with the gene therapy treatment) will determine whether or not one can receive the treatment. Once determined eligible for the treatment, the vector is either injected surgically or through IV therapy. The patient is regularly monitored by having blood drawn, in order to see how their body will react to the treatment. Researchers must understand the risks and the impact the drug has on individuals and how long its effects last on the patients, so patients are monitored for an extended period of time.

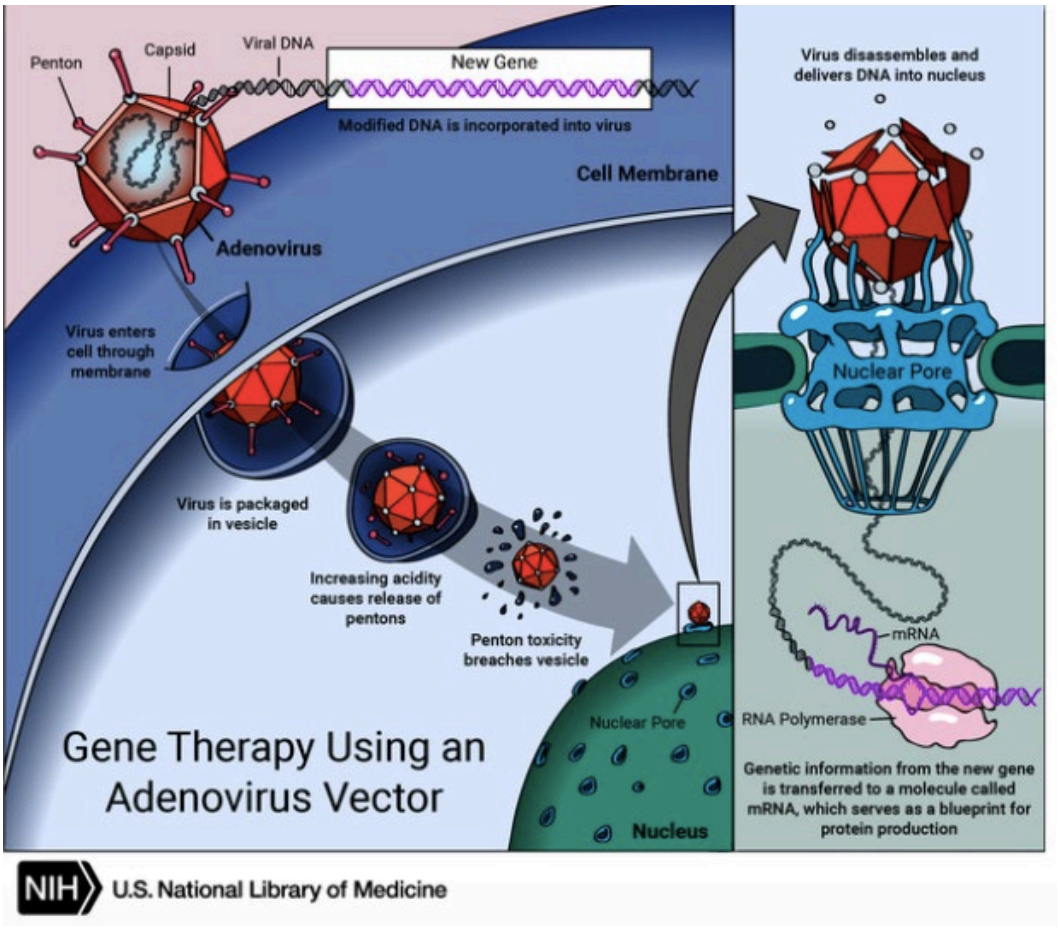

Figure 1: Gene therapy using an AAV vector: depicts how the gene of interest is packaged into the vector, and once inside the cell the vector enters the nucleus where the virus will dissemble and deliver the DNA. The DNA then serves as the instructions for the correct protein to be made in the cells. ("How Does Gene Therapy Work?" *Medline Plus.* https://medlineplus.gov/genetics/understanding/therapy/procedures/)

There are many benefits of using gene therapy as a treatment for certain diseases, but there are also various risk factors that have caused there to be both setbacks and reluctance for certain therapies to be FDA approved. One of the major benefits of using gene therapy is that there is a direct repair or compensation of the defective gene- since the disease is being targeted

at a genetic level, the therapeutic effects can last for longer periods of time without subsequent treatments (which if treated with pharmaceuticals would require). Despite this, there has been discrepancies in the longevity of the treatment among patients- especially those with retinal disorders, which will subsequently be elaborated on. Additionally, researchers have struggled with the cost effects of both the gene therapy clinical trials and even administrating the drug once approved, as seen with Glybera. Nevertheless, researchers have pushed through these obstacles and are still looking for ways to better gene therapy. By looking at the effects gene therapy has had on treating retinal disorders, one can gain a better understanding of both the complexity and variability that gene therapy encompasses, and how this research has impacted countless lives and will continue to do so for years to come.

Gene Therapy in the Eye

Before diving into the various genetic retinal diseases that have been studied under gene therapy trials, it's important to understand how gene therapy specifically works in the eye. First and foremost the anatomy of the eye gives it certain benefits that make it a more advantageous organ for gene therapy. The small size of the eye and the fact that it's an enclosed structure makes it an attractive target tissue because only small amount of the vectors are required to treat patients, which decrease the chances of an adverse response to the therapy and lowers the cost of the process (Trapani 2018). The easy accessibility of the eye allows for rapid examinations and one eye can remain the control while the other is the experimental target. The most beneficial quality of the eye is its immune privileged status. The immunological environment differs between the different compartments of the eye (anterior chamber, vitreous cavity, subretinal-

and suprachoroidal space), and while these tissues do have immunocompetent cells[1] and major innate immune receptors, the immune system in the eye has numerous idiosyncrasies that sets it apart. The eye is enclosed by blood-tissue barriers, which are formed by tight junctions at the retinal pigment epithelium cell layer (RPE, which are the endothelial cells of the inner-retina capillaries) and cornea, which lacks blood vessels. This blood-retinal barrier shields the non-regenerating ocular tissue from the systemic immune system thereby reducing the risk of blood-born infections and inflammation, which can cause damage of the ocular tissues and irreversible impairment of vision (Butcher 2020). This is extremely beneficial for gene therapy treatment because when the vector containing the gene of interest is injected by subretinal or intravitreal methods there is less of a chance of an immune response to amount to the foreign particle. Additionally, the physical barrier that the blood-tissue barrier creates prevents the dissemination of the vector and protects it from direct contact with the systemic immune system. Even with these attributes it's not a perfect system due to the fact that the blood-tissue barrier is not fully impermeable and cannot completely prevent the penetration of some cellular and humoral components of the immune system, therefore it's imperative to test the various vectors to ensure that it does not trigger a harmful immune response.

There are multiple methods in which the gene therapy drug can be administered to patients and have played in a role in the discussion of safety and efficacy of the treatment. The easiest and most non-invasive to deliver the drug is by topical instillation. This method is limited to only treating the anterior portion of the eye, since it's too hard for the size of the nucleic acids to pass through the cornea and conjunctival epithelia to the posterior segment. The drug can also

[1] Cells that can recognize antigens and amount an immune response

be delivered through subconjunctival injection, which is either injected under the eyeball conjunctiva or underneath the conjunctiva lining the eyelid (Stanley 2008). This method is slightly invasive but allows large volumes to be administered, and it can be repeated as necessary. The drugs can reach the anterior and posterior segment but size of the drugs can lead to complications- if the nucleic acid is too big then it won't penetrate to the ocular tissues (will remain in the subconjunctival space). Another method of administration is through intracameral injection, which injects the drug into the anterior chamber of the eye and induces transduction of anterior eye segment tissues but there is short contact time with ocular tissue which results in lower efficacy. Despite this there has been some stable protein expression using this method in corneal endothelial cells. Intravitreal injection delivers the vector directly into the space in the back of the eye called the vitreous cavity. This method allows for the high doses of the drug to be transported, but can lead to damage such as retinal detachment, high intra ocular pressure (IOP), and endophthalmitis[2]. Subretinal injection injects the drug into the subretinal space and has been the most successful from all the different methods. Allows for contact of the nucleic acids with photoreceptors, outer retinal layers, and RPE cells. (Solinis 2014) It is used for retinal degeneration caused by gene mutations, but is an invasive method and there's a risk of ocular damage, including lesions in RPE (retinal pigment epithelium), hemorrhages, retinal tears, pre-retinal fibrosis[3], and retinal detachment. There has been major success using this injection method for treating Leber Congenital Amaurosis which will later be discussed in further detail. Suprachoroidal injection is given below the sclera and above the choroid and is used to deliver

[2] Endophthalmitis is an infection of the tissues or fluid inside the eyeball, if not treated it can cause blindness.

[3] Pre-retinal fibrosis is a condition in which an extremely thin membrane of scar-like tissue covers the surface of the macula which can cause visual distortion.

drugs to the posterior segment of the eye. This route does not interfere with the optical pathway and therefore shows great potential to circumvent many of the risks seen with subretinal injection (Barakat 2019). But there have been issues obtaining long-term sufficient protein expression using this method and if used would require the patient to undergo multiple treatments.

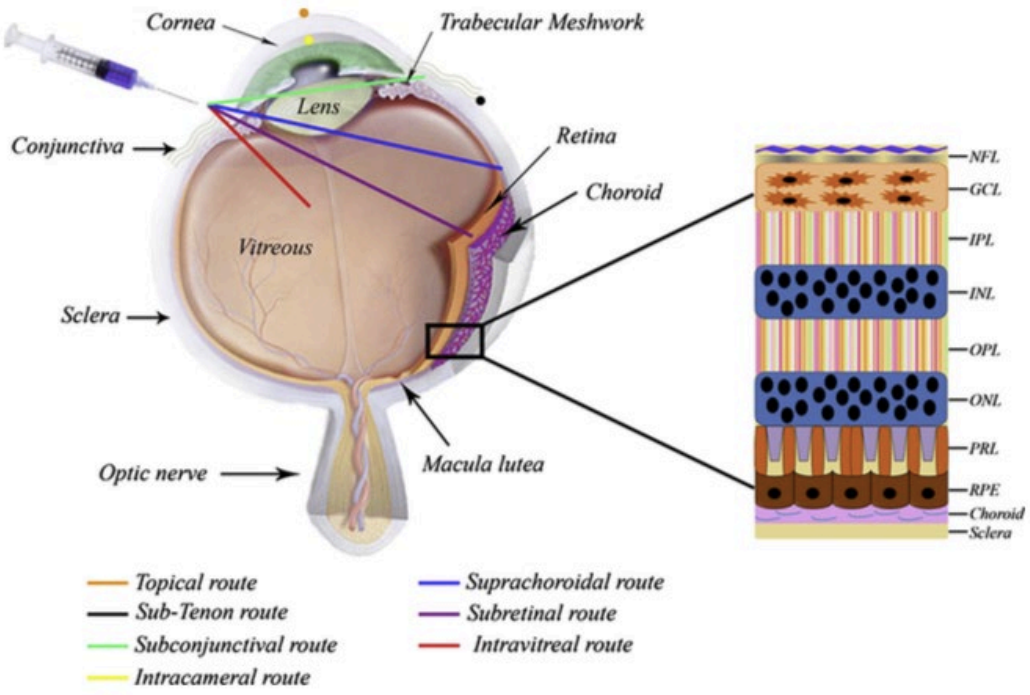

Figure 2: Depiction of the different methods of ocular administration of the gene therapy drug. The image on the right represents the various cell types in a retinal cross section. (M. Ángeles Solinís, Ana del Pozo-Rodríguez, Paola S. Apaolaza, Alicia Rodríguez-Gascón, *"Treatment of ocular disorders by gene therapy". European Journal of Pharmaceutics and Biopharmaceutics,* Volume 95, Part B, 2015, Page 332, ISSN 0939-6411, https://doi.org/10.1016/j.ejpb.2014.12.022.)

There are several different vectors that can be used to transport the gene therapy drug into the patients eye. There have been attempts to use a non-viral vector since compared to a viral

vector, there is a lower risk of causing an immune response and can deliver greater dosages and larger transgenes. The drawback with a non-viral vector is that there are low levels of transfection and that repeated administration would be required for long-term effects. There are three main viral vectors that have been tested for retinal gene therapy including, Lentiviruses (LV), Adenovirus (Ad), and Adeno-associated virus (AAV) vectors. Lentiviruses are single-stranded RNA viral vectors that can transduce both dividing and non-dividing cells. While lentiviruses successfully can transduce RPE cells, photoreceptors, and other retinal cells, there are concerns that it can cause insertional mutagenesis due to its ability to integrate into the hosts genome (because the DNA is able to translocate into the nucleus). An advantage of this vector is that is can carry up to 10 kilobases, whereas AAV vectors have a limit of 5 kilobases. While there are no FDA approved drugs using this vector, there have been clinical trials for Usher's Syndrome, Stargardt's disease, and age related Macular Degeneration using a Lentivirus (Lee 2019). Adenoviruses are double stranded DNA vectors and can package up to 37 kb of genetic material which it the largest capacity from all the vectors. The Ad vector is superior to the LV vector because the genetic material is non-integrating and episomal, which means that the DNA remains in the cytoplasm and does not translocate into the nucleus which lowers the risk of insertional mutagenesis (Nowakowski 2013). The most commonly used Ad vectors are Ad5 and Ad2 which can transduce retinal pigment epithelium and photoreceptors and have been used in age related macular degeneration clinical trials. The biggest issue with using an Ad vector is the immune response associated with its use.

The most widely used vector in retinal gene therapy clinical trials has been Adeno-associated virus (AAV) vector. AAV is a parvovirus, which are small, non-enveloped viruses that contain a linear, single-stranded DNA. AAV requires a helper virus (that is said to be the

natural human adenovirus) which is what allows it to be nonpathogenic. Like the Ad vector, the AAV vector is episomal which lowers the risk of mutagenesis. Little attention was paid to the AAV vectors until it was demonstrated that efficient transduction of mouse muscle tissue produced expression of the transgenes for up to a year with no decrease in expression. This spurred various clinical trials which then proved that the AAV virus can successfully transduce the eye, brain, and liver cells. The longest uninterrupted expression utilizing an AAV vector has been seen in the rpe65 gene in the eye of a deficient dog model for greater than 8 years. This has made AAV the vector of choice for achieving long term, persistent expression in animal models. Despite this incredible achievement there are limitations in the packaging capacity of the AAV vector. Only 5 kb can be packaged into the capsid, plus there are certain regulatory elements that are associated with AAV gene expression that reduces the amount of transgene that can be packaged to 3 kb. Never the less the AAV vector is widely used especially since as mentioned before, the AVV vectors are nonpathogenic. The vectors are nonpathogenic due to the fact that most humans have experienced prior exposure to AAV most likely along with an Ad infection, leading to the development of antibodies. The AAV virus also does not activate key components of the innate immunity which helps it have a low immunogenicity. The AAV vector has been tested for the treatment of a variety of retinal genetic disorders including Retinis Pigmentosa, Choroidermia, X-linked Retinoschisis, and Leber's Congenital Amaurosis. AAV particularly plays a large role in the RPE65 gene therapy which is used to treat Leber's Congenital Amaurosis and will further be explored.

Breakdown of the Retina

Retinal disorders are widely studied within gene therapy clinical trials due to the ability of easily being able to visualize the effects of the disease and after the gene therapy treatment by both photographical and by psychophysical measures (Including visual acuity, field, and color contrast). Retinal disorders tend to be slowly progressive so people with the disease may not become severely visually handicapped until later in life, which gives leeway for genetic studies to be conducted (Francis 2006). Loss of vision in retinal degenerative disorders is usually caused by the inability of the retina to transmit light signals to the brain. In a normal eye the light is transmitted by first entering through the cornea, it then passes through the pupil and the iris (which will control how much light passes through). The light then hits the lens, which will focus the light rays onto the retina, and once it passes through the vitreous humor (the clear, jelly-like substance that fills the center of the eye) it will hit the retina- the light sensitive nerve layer that lines the back of the eye that will invert the image. The retina is also responsible for capturing all the light rays and then processing them into light impulses through millions of tiny nerve endings and then send them to the optic nerve. The retina processes the light through a layer of photoreceptor cells. Photoreceptors are light sensitive cells consisting of rods and cones. Cone photoreceptors are responsible for central, high-resolution, color vision. Unlike rod photoreceptors which support vision in dim, night-time conditions, cones are needed in order to perform daily tasks in bright lighting. Diseases which compromise the integrity of this cell type are debilitating because they can induce daytime blindness, preventing the patient from performing ‘normal’ tasks such as driving. The neural output originating from the visual signal in rods and cones is sent through secondary order neurons (horizontal and bipolar cells) to the retinal ganglion cells (RGCs). The process of vision culminates in the retina with transmission of

these signals from the RGCs to the visual cortex. According to Dr. Sherry Bass who runs the ocular disease clinic at SUNY College of Optometry and actively does research in the genetics of hereditary retinal disorder, the two most common retinal disorders that she encounters in patients are Retinis Pigmentosa and Stargardt disease which both involve rod and cone death.

History of Treating Retinal Disorders

Prior to the 20th century there wasn't really any research done on both understanding and treating retinal disorders, largely because people didn't live long enough to worry about the long term effects of the diseases. By the 20th century, medical advances expanded the life expectancy which brought along the concerns of the debilitating effects of retinal disorders, especially since the number of elderly people who fell blind due to retinal disorder increased. Since the retina posed the greatest issue in the eye for doctors to treat the Retina Research Foundation was created in 1969 in order to spearhead the research in tackling IRD's. A key figure in inherited retinal disorders research was Dr. Eliot Berson; he found groundbreaking discoveries in understanding retinis pigmentosa and developing treatment and therapies for various IRD's. In the 1960's he discovered that Electroretinography (ERG) could detect photoreceptor dysfunction up to a decade prior to the onset of visual deterioration in retinis pigmentosa and can measure the retina's sensitivity to light. The ERG is still an instrumental tool used to diagnose this condition and estimate visual prognoses. Dr. Berson and his co-workers at Harvard Medical School, Massachusetts Ear and Eye Infirmary discovered that if patients between the ages of 18-60 with retinis pigmentosa or Usher's syndrome would take vitamin A palmate, oily fish, and lutein it could slow the progression of the disease. It was reported that these patients had a 20% annual slower loss of retinal function, which was measured by an ERG, as compared to patients who

didn't take these supplements. The reason Lutein slows the progression of the IRD's is because Lutein serves as a free radical scavenger in the retina that cause photoreceptor apoptosis (Koushan 2013). Additionally, Lutein absorbs blue light which is also useful because blue light can cause photoreceptor damage. Vitamin A is necessary for the conversion of *cis*-retinal to all-*trans*-retinal when light is absorbed by the visual pigments rhodopsin or cone opsin, which leads to phototransduction. Vitamin A is crucial in visual cycle and for normal vision, so the supplementation of vitamin A in patients with RP or Stargardts will help to delay progressive visual loss. While taking these supplements have helped many patients, it's not evidence based medicine and is not an approved method of treatment. The very first true form of treatment that has been discovered to aid with IRD's has been gene therapy. While there is still a vast amount of research that needs to be done in order to find a definitive cure for different IRD's, there have been incredible milestones in the achievements that gene therapy has brought to treating IRD's, most specifically when a gene therapy treatment was found for Leber's Congenital Amaurosis.

Leber's Congenital Amaurosis

Leber's Congenital Amaurosis (LCA) is the most severe and earliest type of inherited retinal disease responsible for childhood blindness. While LCA only effects 2-3 per 100,000 births and makes up approximately 5% of retinal dystrophies researchers sought to find some form of treatment to push off its debilitating effects. LCA was first discovered in 1869 by German Ophthalmologist, Theodor Leber in a child that displayed nystagmus (rapid involuntary movements of the eye), amaurotic pupil (eyes that are unreactive to light), retinis pigmentosa (RP), and vision loss. It's predominately a recessive disorder and there are 28 genes that have been linked to the pathogenesis of LCA. Mutations in these genes are the stepping stone to

debilitating effects of LCA due to the fact that they cause disruption of functions such as photoreceptor morphogenesis, retinal differentiation, phototransduction, ciliary transport process, retinoid cycle, guanine synthesis, and signal transduction. Researchers have found that 75% of LCA cases are due to mutations in said genes (Kondkar 2019). The three main genes that LCA is said to be traced to are cone-rodhomeobox (CRX), guanylatecyclase and retinoid isomerohydrolase (RPE65) (Sharif 2017).

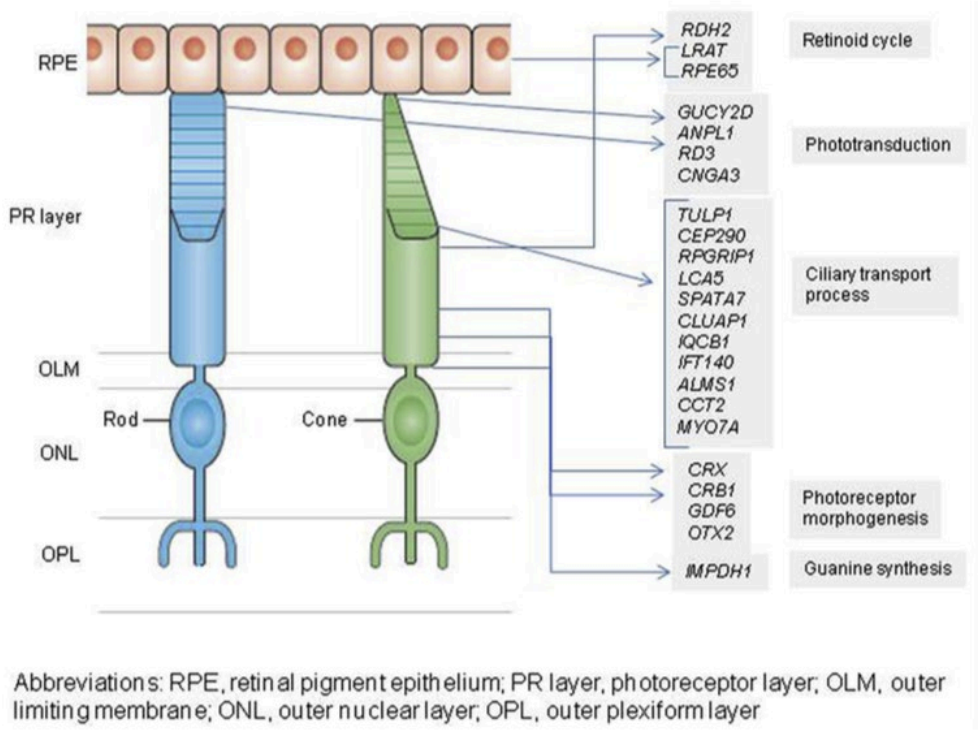

Figure 3: Representation of rod and cone cells and the various genes implicated in LCA that may undergo mutation and their resulting downstream effects. (Altaf A. Kondkar, Khaled K. Abu-Amero,"Leber congenital amaurosis: Current genetic basis, scope for genetic testing and personalized medicine", *Experimental Eye Research,* Volume 189, 2019, 107834, ISSN 0014-4835, https://doi.org/10.1016/j.exer.2019.107834)

LCA not only has genetic variability but phenotypically expresses itself differently in every case. LCA can cause early visual impairment, sluggish or near absent pupillary response, photophobia (light sensitivity or intolerance to light), nyctalopia (night blindness), Franceschetti's oculo digital sign (eye poking), keratoconus (corneas thins and bulges outward), and abnormal electroretinogram[4] results. While some patients progress to complete blindness by adolescence others are slower to reach that point and become fully blind by their 30 or 40's. It's clear that this IRD greatly effects a person's quality of life which is why it's been a target for gene therapy treatment and has been the very first successfully treated IRD by gene therapy.

The groundbreaking gene therapy termed RPE65 gene therapy has been key in treating LCA and has been a vital step in uncovering other gene therapy treatments for other IRD's. In order to properly understand how RPE65 gene therapy works, it's important to understand how LCA targets the eye and causes such damaging effects. The Retinal Pigment Epithelium (RPE) is the pigmented cell monolayer positioned between the neurosensory retina and the choroidal blood supply. The RPE sustains the metabolic needs of the underlying neural retina by controlling the transfer of small molecules between the blood stream and the retina. It plays a crucial role in the Vitamin A cycle by isomerizing all trans-retinol to 11-cis retinal. RPE65 (retinal pigment epithelium-specific 65 kDa protein), expressed predominantly in RPE cells, is an isomerase enzyme which converts all-*trans*-retinyl ester to 11-*cis*-retinol. Improper functioning of RPE65 results in a lack of 11-cis retinal production, which causes an accumulation of all-*trans*-retinyl ester in the RPE, which in turn creates and an inability to form visual pigments (rhodopsin and cone opsin). Without these light-sensitive opsins, photoreceptor

[4] An electroretinogram (ERG) measures the electrical activity of the retina in response to a light stimuli.

cells of the retina lack the ability to usefully absorb photons and initiate the conversion of light into a visual signal. These mutations in RPE65 are associated with 16% of LCA cases in humans and are a major contributor to the degenerative effects of the disease.

In order to evade the underlying defect in the retinoid cycle (the lack of 11-cis retinal and failure to regenerate visual pigment), three therapies have been assessed; RPE cell transplantation, retinoid delivery and gene based intervention. RPE cell transplantation is mechanically difficult and invasive. Oral administration of retinoid is not optimal because it requires repeated administration of drug. Gene-based intervention has had the most success from all three therapies, especially since LCA caused by mutations in RPE65 is monogenic.

RPE65 therapy was tested using various viral vectors to transfer the genes and different methods of administration. The lentivirus was used to transfer mouse cDNA to another mouse that was lacking the *Rpe65* gene which led to long-lasting transgene expression in the RPE cells and normal electroretinogram results. While there was some success using a lentivirus, typically an AAV vector it utilized due to its ability to induce efficient long-term transgene expression while also stimulating minimal immune response. When testing various methods of administering the vector researchers found that it the way the drug was adminisitered impacted gene expression. REP65 gene expression in the RPE was present when given by a subretinal injection but not if administrated by a intravitreal injection; therefore the AAV virus containing the gene of interest is administered by subretinal injection. Animal models, such as dogs and rats, that are lacking the RPE65 gene have had positive results when treated with the gene therapy drug created in the lab. In 2001, one of the first dog breeds to have successful outcomes with the RPE65 gene therapy was the Swedish briard dog, which is especially positive due to the fact that it has a very similar phenotype to humans that suffer from LCA. There was significant

improvement in the dogs visual function, retinoid content, and visual behavior which lasted for more than seven years.

There has been variable results in RPE65 gene therapy, especially depending on whether it's administered early in life versus if it's given later on in the progression of the disorder. When tested on dogs, researchers saw that when the dog merely showed photoreceptor dysfunction and severe visual impairment but had yet to reach retinal degeneration, there was rapid improvement in retinal, subcortical, and cortical visual function, which remained stable long term. Data also demonstrated the distinct preservation of photoreceptor layers and structure in the area treated around 5-10 years after treatment. However, when the dogs were treated at mid-life and already exhibited retinal degeneration, there was mixed results; some showed initial improvement in retinal function, while in others degeneration was not halted.

In 2008, there were multiple clinical trials that tested the RPE65 gene therapy on human patients between the ages of 17 to 23 who as a results of the RPE65 mutation were undergoing the early onset of severe retinal dystrophy. The trial utilized a recombinant AAV vector, termed tgAAg76 and it was delivered directly into the patients subretinal space in a single eye. To assess the efficacy of the therapy, retinal imaging, ERG, electrodiagnostic methods, and psychophysical methods were used. The patients sensitivity to contrast (tests a person's ability to detect low contrast images, such as an image containing fog or with glares), visual acuity, visual field, and color vision were measured. Participants of the study experienced an improvement in visual acuity and visual mobility in response to low levels of light. The positive results from this clinical trial was great proof that gene therapy can truly make a difference in patients suffering with retinal disorders and paved the way for the first gene therapy drug for retinal disorders to be approved. After multiple years of testing the safety of the gene therapy treatment, the US FDA

approved the first AAV gene therapy drug called Voretigene neparvovec, or commonly known as Luxturna that was produced by Spark Therapeutics which is based in Philadelphia Pennsylvania. Luxturna is administered by a subretinal injection in an AAV vector one eye at a time. The injection in the second eye takes place 6-18 days after the first eye. Patients are given a corticosteroid before and after the injections in order to decrease the potential immune response to either the AAV2 vector or the RPE65. A new test called the Multi-Luminance Mobility Test was created in order to measure functional vision, which is a person's ability to use vision to carry out activities of daily living under different levels of light. An obstacle course was built in the lab, which consists of a pathway of arrows that the patients must follow, along the pathway are objects of varying height and contrast in order to simulate everyday life. For example, along the way a pool noodle was placed to represent an ankle height obstacle that a person could potentially encounter in real life, such as tree branch laying on the sidewalk. There were a total of 15 obstacles throughout the course and there were 7 different light levels that were tested on the patients that reflect varying light levels (called a lux level) that can be seen throughout ones day, such as an office elevator, a train car and station at night, a city one hour after sunset, a parking lot at night, and the darkest level representing a moonless summer night. Before undergoing the test, patients had to go through 40 minutes of dark adaptation and went through the test three times: once with one eye patch, another with the other eye patched, and lastly without any eye patch (each time the test was given the layout of the obstacle was changed). The scoring of the test was based on if they remained on course and were able to detect the various obstacles in their path and the time it took to complete the course. A majority of patients were able to complete the course at the darkest light level after treatment, which indicates an improvement in their functional vision.

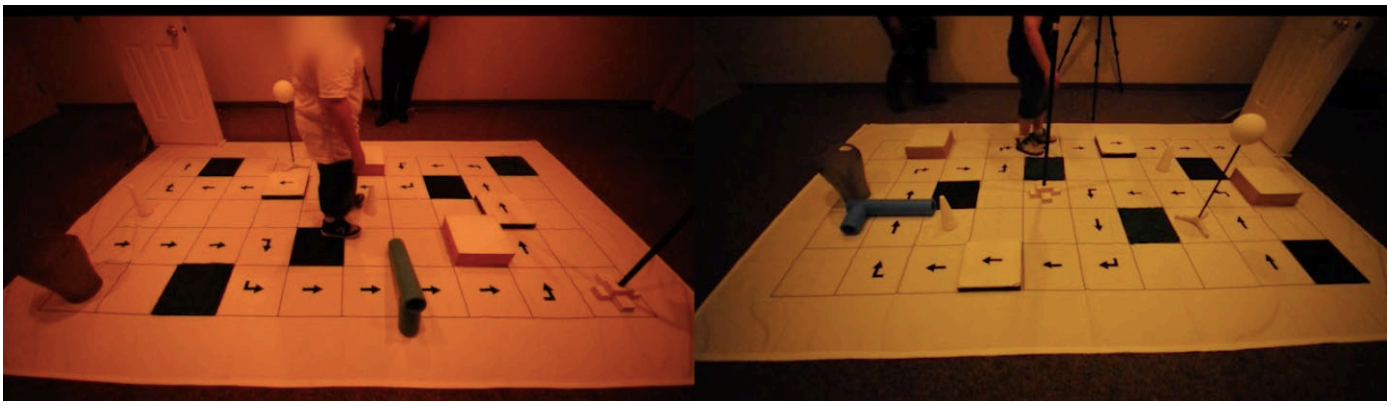

Figure 4: The image on the left illustrates a man going through the Multi-Luminance Mobility Test before treatment at the darkest light level (camera distorts the actual level of light in the room), and the image on the right is the man going through the pathway one year after treatment. One can see that before treatment the mean veered off course and was unable to follow the arrows, whereas after the treatment he successfully was able to pass the test and was able to follow the arrows and obstacles along the way. (This image was taken from a video found on https://luxturnahcp.com/efficacy/MLMT-clinical-trial/)

While there have been great results seen from Luxturna, there are some side effects that can occur such as redness of the eye, cataract, increased pressure inside the eye, just to name a few. Even though there are some risks involved with the surgery, these risks can outweigh the effects of LCA is left untreated. Overall the creation of Luxturna has been a great indication to researchers that gene therapy truly can be successful and has been the model for researching gene therapy treatments for other IRD's.

Future of Gene Therapy for IRD's

While many obstacles were overcome in the clinical trials that led to Luxturna becoming an FDA approved gene therapy drug, there is still much research to be done since every IRD is associated with different genes and offers its own challenges to overcome. An IRD that has been

under various clinical trials to try and create a gene therapy drug is choroideremia. This IRD is an X-linked disorder that primarily occurs in males. It causes a severe degeneration of the RPE, choroid (blood vessel layers between the retina and sclera), and photoreceptors. It causes night blindness in childhood and later on there is loss of peripheral or "tunnel vision" and eventually loss of central vision. The fact that choroideremia has a slower disease progression allows for potential therapeutic treatments to be administered in the first few decades of life before its progresses too far. There are multiple clinical trials in the works to test the AAV vector in patients with this disease. Trials have included both administering the drug by either subretinal injection or intravitreal administration. There have also been attempts to use intravitreal administration of the vector to treat X-linked retinoschisis. This IRD is caused by a mutation in the RS1 gene, which codes for an extracellular cell adhesion protein that binds to cell membranes of rods and cone inner segments, photoreceptor outer nuclear layer, and bipolar cells. The abnormal cell-cell adhesions that arises with the mutated gene leads to the schisis (the splitting) of the retinal layers. This can cause macular holes to form or retinal detachment which can lead to blindness. An intravitreal administration is favorable for treating both X-linked retinoschisis and choroideremia since it prevents the patient from having to undergo surgery which comes with its own group of risks. While there was efficient transduction of RS1 in a mouse model that was lacking the gene when administered, there was limited efficacy seen in the reduction of schisis.

The most common subtype of IRD's that causes retinal degeneration is retinis pigmentosa (RP), which is responsible for vision loss in 1 in 4000 people. It can be inherited as either an autosomal dominant, autosomal recessive, or X-linked disorder and can occur alone or in conjunction with other systemic disorders. The severity of the disorder varies among patients and

can include night blindness, loss of mid-periphery vision, and later can progress to loss of central vision leading to blindness. These effects occur due to apoptosis of the rod photoreceptors which causes the outer nuclear layer of the retina to become thinner. The goal of gene therapy for patients with retinis pigmentosa would be to slow down or stop retinal degeneration from occurring. While RP can be caused from a mutation in one of 60 genes, a phase 1 clinical trial was done specifically looking at X-linked RP caused by mutations in the RPGR gene, which is the most common form of recessive X-linked RP (M. Ángeles Solinís 2015). Similarly to RPE65 gene therapy, this study utilized an AAV vector which was administered by subretinal injection into only one of the patients eyes. Even though the study aimed at discovering the safety of the procedure so the patients part of the study had advanced retinal degeneration, they still saw positive outcomes from the therapy. The patients visual acuity reached baseline level 3 months post-surgery, there was also a gain in retinal sensitivity, reversal of some of the visual field loss, and improvement in visual clarity and field of vision. These improvements were only seen in the eye that was treated. While patients who received a higher dosage experienced mild retinal inflammation, this was reduced using oral prednisolone. Lastly they hypothesized that the functional gains were attributed to an anatomical improvement that could be caused by regeneration of photoreceptor cells in response to the gene therapy (Cehajic-Kapetanovic, Jasmina 2020). Even though this is only a phase 1 clinical trial there is immense potential with using gene therapy as a treatment for retinis pigmentosa.

A factor of RPE65 gene therapy that limits its usage towards other IRD's is the small packaging size of the AAV vector (only can carry about 5 kb), this proves to be a problem since some of the IRD's have larger genes that need to be packaged. Two IRD's which have a larger transgene load are Stargardt disease and Usher's Syndrome type 2; the former effecting the

ABCA4 gene which therein effects both the retina and macula, and the latter effects the gene MYO7A causing vision loss beginning in adolescence. Since both these genes are unable to fit in the AAV vector, an alternative viral vector was created to provide a larger packaging capacity. The vector is an equine infectious anemia virus (EIAV), which is a non-HIV non-primate lentivirus that is able to package up to 8 kb. The successful transduction in animals models in the various clinical trials being run using this vector has been a step in the right direction towards finding a vector that could package larger genes. There have also been attempts to try and utilize the AAV vector even if the gene of interest is too large to package. Methods such as an "overstuffed" AAV vector or a dual AAV vector have been researched. Overstuffed vectors package the vector beyond the usual capacity, and in some cases this has shown to cause decreased transduction efficiency. But there has been success in packaging up to 8.9 kb in an AAV5 vector with effective expression of the MYO7A and CEP290 (which is another gene that causes LCA) in the mouse retina. Using a dual vector involves dividing the transgene of interest into 5' and 3' halves. There are limitations with transferring the gene in this manner because it would require co-transfection of two vectors in a single cell and results have reported only a 5% transduction efficiency rate which is much lower than using a AAV single vector.

CRISPR-Cas 9

While there has been many advances made with gene therapy, gene editing has been on the rise- specifically the genome editing technique clustered regularly interspaced short palindromic repeats, which more commonly is referred to as CRISPR/Cas9. What makes gene editing more advantageous over gene therapy is that it allows for the precise and targeted repair of faulty genes. The faulty DNA sequence is removed and the desired DNA sequence is inserted

at the specific location on the genome. Gene therapy on the other hand focuses on the addition of a functional gene rather than actually changing the DNA sequences- this limits the diseases that gene therapy can be used for because it will only aid the patient if the faulty gene is repressed by the addition of the functional one. Due to the advantages of gene editing there has been strides to use this technique for treating IRD's.

The gene editing technique CRISPR/Cas9 has been abuzz within the research and science community for potentially treating ocular diseases, cancer, blood disorders, AIDS, and even Covid-19. CRISPR/Cas9 is a natural immune system that is used in bacteria and archaea to defend against invading viruses and phages. When a virus infects the bacteria, the bacteria will cut some of the viral DNA and incorporate it into the bacterial chromosome. This sequence of viral DNA that is incorporated into the bacteria is called CRISPR. The bacteria does this to keep a memory of virus, so if invaded again down the line the bacteria will have the artillery to attack it. When the bacteria recognizes the virus again it uses a guide RNA (gRNA) to locate the newly invading viral DNA and then a Cas protein binds to it and cuts out the viral DNA. The goal is to utilize this system to either inactivate or add new genes in humans to alter genetic mutations that lead to diseases.

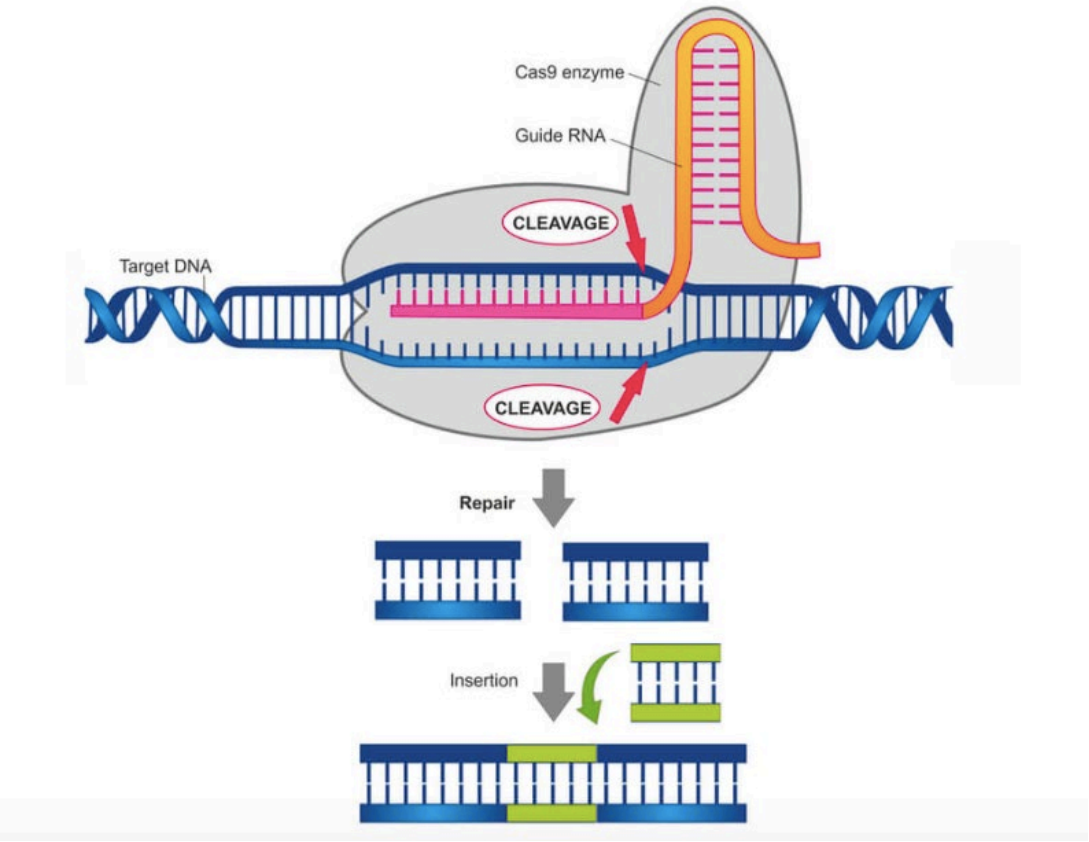

Figure 5: Illustration of the CRISPR/Cas9 system: depicts the guide RNA bringing the Cas9 enzyme to the target DNA so that it can cleave the DNA of interest. Subsequently correct DNA is inserted. (Fernandez, Rodriguez Clara. "CRISPR-Cas9: The Gene Editing Tool Changing the World" *Labiotech.eu*. https://www.labiotech.eu/in-depth/crispr-cas9-review-gene-editing-tool/)

While there have been other gene editing tools used such as TALEN and zinc-fingers, these were slower and less efficient since a new gene editing protein had to be made from scratch for each specific DNA modification made. CRISPR on the other hand can use the same Cas9 molecule to cleave the DNA by providing it with a guide RNA, which is much easier to produce in the lab. CRISPR can either be conducted ex vivo or in vivo, the former being similar to gene therapy where the cells are extracted from the patient, engineered in the lab and then reinjected. The latter method directly delivers the CRISPR-Cas9 into the patients cells to edit their DNA

and once completed will be cleared out of the body- human testing using this method had begun in 2020. While there is much more research that needs to be done on CRISPR and navigating the ongoing debate about the ethics of having such a tool, the potential for the good it can do to treat IRD's holds immense promise.

Optometry and Gene Therapy

While there is a vast amount of literature on gene therapy for inherited retinal disorders, most of that literature is through an ophthalmological perspective and as a future optometrist I was inclined to uncover what the role of an optometrist is within the realm of gene therapy. Looking comparatively at the scope of practice of an optometrist and ophthalmologist, one can see that an optometrist wouldn't be able to deliver the gene therapy drug since they are not trained to perform surgery.[5] Even though optometrists can't administer gene therapy to their patients, it's still vital for them to be educated on gene therapy for IRD's since many IRD's are first diagnosed by optometrists. Optometrists are responsible for two thirds of vision and eyecare in the United States, and according to the American Optometric Association thirty-nine percent of counties in the United States have access to an optometrist but not an ophthalmologist. This really illustrates how optometrists are the primary eyecare providers for many Americans so it's essential that they are able to guide their patients to the right care once patients are diagnosed with different IRD's. I had the opportunity to correspond with Dr. Sherry Bass, who is the head

[5] An optometrist differs from an ophthalmologist in that they don't attend medical school but specifically go to optometry school. Beyond merely prescribing glasses, optometrists examine, diagnose, treat, and manage diseases, injuries, and disorders of the visual system. An ophthalmologist can also perform surgery in addition to being able to being able to do what an optometrist does.

of the retina clinic at the University Eye Center (which is associated with SUNY College of Optometry), and she related how at SUNY Optometry students are taught about gene therapy in relation to IRD's and treatment of LCA. She also noted that while optometrists can't perform the injection themselves she states: "*However, it is in our scope of practice to perform genetic testing and refer patients for either approved gene therapy or on-going gene therapy trials depending on the disease and the type of mutation. Genetic testing currently consists of providing a buccal or saliva sample and that is why optometrists can do this."* The optometrist plays a central role in helping patients uncover what IRD's they may be genetically inclined to get in order to take preventative action.

While some optometrists do ensure that their patients get genetic testing, some don't necessarily offer these tests in their offices or are even so knowledgeable about the subject. According to a study done in 2019 led by Eyes on Eyecare, Dr. Matt Geller, Dr. Melody Huang, Dr. Sathi Maiti, and Dr. Collin Robillard surveyed 427 optometrists, who represent various optometric settings[6] and who graduated from optometry school in different years (ranging from 1973 to 2019), in order to learn about their knowledge and of their opinions of the future of genetic testing in eyecare. They found that merely 6%, which is only 27 out of the 427, of the optometrists surveyed actually offered genetic testing in their practices now. Additionally when asked to rate their level of understanding of diagnostic genetic testing in the eyecare space from 1-10, the average rate of their knowledge was only at a 4.79. For those 6% of optometrists who do perform genetic testing for their patients 81.5% only do genetic testing specifically for retinal conditions (such as Stargardts disease, retinitis pigmentosa, Leber congenital amaurosis, etc.). A

[6] The optometrists range from working in private practices, corporate practices, ophthalmology practices, hospitals, etc.

large barrier that prevents many optometrists from providing patients with genetic testing is high cost of the test, which in most cases patients would have to pay out of pocket (the average cost for an out of pocket genetic retinal testing is $164.67 per patient). Through a genetic testing study run by the Foundation Fighting Blindness, a person who is clinically diagnosed with an IRD can receive free genetic testing and genetic counseling. The test could only be used to identify the causative gene for a confirmed clinical diagnosis but not to screen for eye diseases. More opportunities such as this should be accessible to patients and there should even be opportunities for people to screen for ocular diseases if not for free than at a lower price in order to make genetic testing more wide-spread for patients and for its administration by optometrists. Lastly, while only a small percentage of optometrists currently offer genetic testing to patients, over half of the respondents from the survey run by Eyes on Eyecare felt that genetic testing and genetic testing for treatment (such as gene editing) has a strong future within eyecare. In order for that future to be actualized it's imperative that optometry schools increase the exposure and knowledge its students[7] have of genetic testing so that once they move on in their professional lives they have the tools to understand and utilize this essential test.

Conclusion

There have been remarkable steps taken towards using gene therapy as treatment for IRD's. The success seen with treating LCA and the positive implications of trials done with treating retinis pigmentosa have been milestones in the long history of treating IRD's and in the

[7] Especially since as noted before from the survey done, many optometrists rated their knowledge of genetic testing for IRD's pretty low (the average being only a 4.79/10), which indicates that there needs to be more of a focus on this topic within optometry schools.

evolution of gene therapy. The progress that has been made from telling patients to merely take supplements such as vitamin A and Lutein (which was not evidence based research) to having a drugs such as Luxturna which has caused improvements in patients vision and has been measured by using tests such as the Multi-Luminance Mobility Test, is an incredible feat. Understanding how gene therapy works by delivering the gene of interest in a vector, and how various methods of administration can affect the outcome of the treatment has shed light on the arduous path researchers have taken to create such a therapy and how they constantly need to make small changes to accommodate each IRD. By looking into the future of what gene therapy can hold and understanding the gene editing system CRISPR/Cas9 really highlights what's to come in both eyecare and medicine at large. By learning about this fascinating subject not only have I gained a better appreciation for the eye and ocular disease but as a future optometrist have learned what my role will be in preventing the progression of IRD's by stressing genetic testing to patients. Hopefully with more research done of both gene therapy and gene editing there will be more effective treatments available for IRD's and improve the quality of life of so many who suffer from IRD's across the globe.

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