MYTH OR SCIENCE? KEEP YOUR EYE PEELED
GENETIC THERAPIES FOR INHERITED RETINAL DISORDERS

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Critical appraisal

"There is nothing we can do"; Creed Pettit and his family had heard this phrase over and over again [1]. Being born with an inherited form of retinal degeneration, Creed spent all his youth being fully dependent on flashlights and lamps to see properly. Unfortunately, his condition would only get worse, and Creed would eventually completely lose his sight as he got older. Yet, there was light at the end of the tunnel. At the age of nine, Creed was treated with Luxturna®, the first FDA-approved gene therapy drug for an inherited retinal disease, becoming the youngest person in the United States to receive this therapy [2, 3]. A few days after his treatment, Creed's vision had already improved. A month later, Creed was not in need of his flashlights anymore and could slowly start living a normal life. It was a miracle! Following Luxturna®, new versions of the technology have been developed over the years. Currently, the field is expanding rapidly, with hundreds of clinical trials investigating the efficacy of such gene therapies in hereditary retinal diseases. However, with scepticism around the feasibility, long-term efficacy, and costs of genetic therapies, their use in the clinic is still controversial. Can genetic therapies be used to cure inherited retinal disorders, and will they become a standard treatment option for all cases of such eye disorders?

Missing one of your senses might sound unbearable for most, but for blind people, it is reality. In 2017, it was estimated that, globally, about 36 million people suffer from blindness [4, 5]. Blindness can be the result of refractive errors, age, and trauma, or it can be inherited through genetic mutations [6, 7]. More than 190 genes are involved in inherited retinal disorders, and besides this high genetic variability, substantial clinical heterogeneity is also observed. For example, retinitis pigmentosa, which accounts for most cases of inherited retinal degeneration, can be caused by at least 50 genes [6, 7]. Examples of these are the *retinal pigment epithelium-specific 65* (*RPE65*) and *rhodopsin* (*RHO*), both expressed in the retina of the eye [6, 7]. Retinal degenerative diseases usually present as an impairment of night or colour vision and progress with loss of peripheral and central vision. Conventional strategies to manage vision impairment are limited to refractive correction (e.g. use of eyeglasses) or surgery [8, 9]. However, such therapies are not effective for inherited or complex forms of blindness [8]. Unfortunately, no pharmacological treatments are available either. Over the past years, however, considerable progress in molecular technologies has expanded the toolbox of scientists. With such increasing knowledge, will we be able to fully cure inherited retinal diseases?

How does it work?

Genetic mutations and consequent abnormalities in protein expression or function form the basis of hereditary disorders. Such genomic abnormalities can disrupt entire chromosomes or be of a smaller scale, affecting one or few genes within one chromosome [10]. Genetic therapies focus on the latter genomic alterations to treat a hereditary disease. Here, genetic material is introduced into specific cells of a patient to correct for the underlying genetic mutation (Figure 1) [6, 11]. The transferred genetic material can repress, enhance, or alter the expression of disease-causing genes. For instance, a functional copy of the gene can be inserted into the patient's cells to correct for the malfunctioning gene and restore the gene's normal protein activity (gene supplementation) [11]. Alternatively, genetic constructs can be used to repair the disease-causing mutation or inactivate the non-functional copy of a gene of interest. In the last two strategies, gene editing can be carried out in different ways, including CRISPR-Cas9 and exon skipping [12].

After their "manufacturing", these DNA constructs need to be delivered to the patient's cells. However, naked DNA cannot be introduced directly into your cells [13]. Hence, to do so, they are delivered in special carriers, known as vectors (Figure 1) [13]. For this purpose, viruses modified to carry the genetic material of interest can be used as vectors. Adeno(-associated)viruses, lentiviruses, and retroviruses are commonly used. Non-viral delivery methods exist as well; however, they have a lower gene transfer efficiency than viral vectors [11]. In the case of retinal diseases, the DNA/vector constructs can be directly injected into the eye (Figure 1). Common areas of injection include the vitreous cavity located behind the lens (intravitreal injection) and the subretinal space beneath the retina of the eye (subretinal injection) (Figure 1) [6].

Why the eye?

The eye is a particularly suitable organ for genetic therapies for multiple reasons. Firstly, eyes are immune-privileged sites, meaning that immune responses are suppressed here [14]. This property of the eyes is favourable for genetic therapy, as no strong immune responses against the viral delivery vectors can be mounted [15]. Besides, since the eyes are positioned in tight cavities separated from the rest of the body, the chance of systemic viral contamination is low [15]. Furthermore, retinal cells do not regenerate or spontaneously mutate, which is suitable for the long-term expression and functioning of the DNA constructs delivered through genetic therapies [16]. Finally, due to their location, eyes are easily accessible by injections, which makes them highly suitable for such therapies [15].

The promise of genetic therapies

Following the success and overall safety of gene therapies in rodent and larger animal models, such therapies have been tested in multiple clinical trials for hereditary retinal diseases [17, 18]. The first gene therapy for inherited blindness approved in the clinic, Luxturna®, contains the functional (wild-type) copy of *RPE65*, which encodes an enzyme involved in vision [2]. Mutations in *RPE65* are reported in multiple retinal degenerative disorders, such as Leber congenital amaurosis type 2 (LCA2), where dysfunctional *RPE65* disrupts the conversion of light into electrical signals that stimulate vision [2, 3]. Subretinal injections of Luxturna® in patients with *RPE65*-related retinal dystrophy improved their visual and navigational abilities. Besides, patients treated with the *RPE65*-based gene therapy

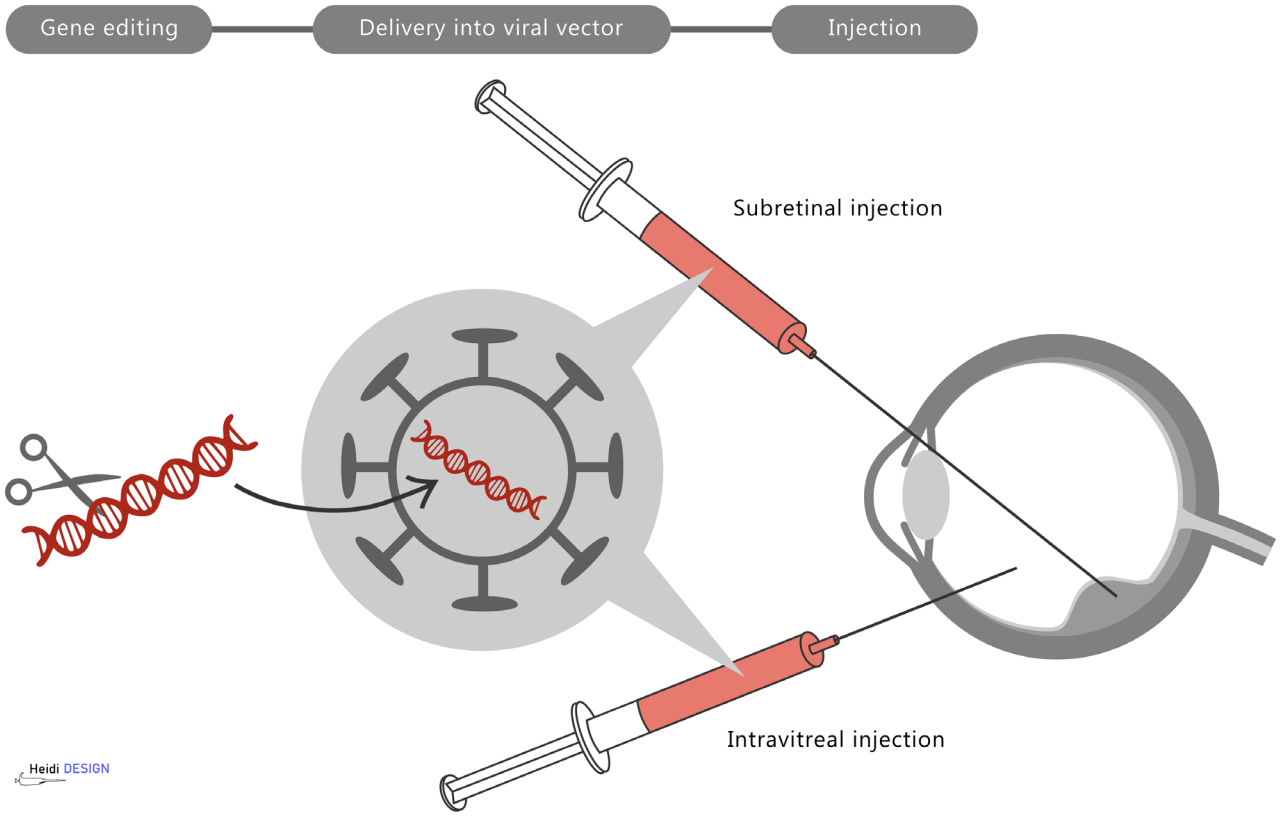

Figure 1: *The general approach of genetic therapies. After manufacturing the DNA constructs of interest (gene editing), they are packaged into viral vectors and administered to the eye of the patient by subretinal or intravital injections.*

also showed changes in the visual cortex, suggesting that the brain is responsive to the gene therapy as well [19].

Following Luxturna®, more genetic therapy technologies have been tested for multiple forms of hereditary vision loss [20]. Supplementation of *MERTK (MER Proto-Oncogene, Tyrosine Kinase)* and *CHM (Choroideremia)* in patients suffering from inherited retinal diseases has shown encouraging results in early phase clinical trials [20]. In 2020, 43 clinical trials using genetic therapy against inherited retinal disorders had been reported, with promising candidates among them [20]. Interestingly, following gene therapy, improvement of vision in the non-treated eye has been reported as well. In a phase III trial, intravitreal injection of the functional copy of the disease-causing gene in one eye of the patient improved vision in both eyes [21]. Even though more research is needed, such improvement is thought to be the result of the movement of the injected genetic construct from one eye to the other [21]. Moreover, whether this is an event specific to this subset of inherited retinal disorder is also not known.

Besides gene supplementation, other genetic therapy methods have been tested in patients [22]. A promising example is the use of antisense oligonucleotides (AONs) [22]. AONs are short synthetic DNA or RNA sequences that are complementary to and can bind to RNA to alter protein expression [22]. Through AONs, errors during pre-mRNA splicing can be repaired. In a recent clinical trial, Sepofarsen®, an AON targeting the *CEP290* mRNA, has been tested for the treatment of LCA [23]. Intravitreal injections of Sepofarsen® showed a safe profile and improved the ability of LCA patients to discern visual details [23].

More advanced versions of the technology are being developed and tested in preclinical models with the promise to be introduced in the clinics [24]. Among them is the use of a modified CRISPR-Cas9 version, dead Cas9 (dCas9) [24]. With dCas9, scientists can activate the expression of proteins functionally equivalent with the disease-causing protein [24]. A recent study used this technology in a mouse model of retinitis pigmentosa to stimulate the expression of a gene that can functionally replace *RHO*. Subretinal injections of viral vectors containing this dCas9 construct resulted in improved retinal activity, which was stable for at least one year after the injection [24].

Is genetic therapy suitable for all patients?

Even though genetic therapies are promising, they might not be the solution for all patients [12]. Inherited retinal degeneration can be the result of a large variety of mutations in multiple genes [12]. To date, many disease-causing genes have been identified but, due to limitations in the technologies used to identify causal genes, many also remain to be discovered. As a consequence, patients with an unknown cause of genetic blindness cannot benefit from genetic treatment. Furthermore, with genetic therapies, only one gene can be targeted at a time [12]. When considering the genetic heterogeneity of retinal disorders, the number of patients that can benefit from a single genetic therapy correction is low as only a few patients would be eligible for each one. Besides, patients suffering from retinal degenerative disorders with more complex genetics will be more difficult to treat with genetic treatment. The disease stage should be considered as well; in advanced stages, extensive retinal degeneration limits the efficacy of viral vector delivery. Thus, in such cases, intervening with a genetic therapy would be ineffective. Finally, the high costs associated with the development and distribution of

genetic therapies can be limiting for some patients and the public healthcare system as a whole.

Conclusion

A decade ago, no cure for severe forms of visual impairment existed. Genetic therapies not only revolutionised medicine but also promised what no other therapy could, a possible cure for previously incurable diseases such as genetic blindness. Currently, new and improved versions of genetic therapies are being developed and tested for their efficacy in treating different types of hereditary retinal diseases. Even though such therapies hold great promise, several steps need to be taken before they become the standard of care for all patients with genetic visual impairment. Still, the incredible story of Creed Pettit gives hope to many patients around the world and inspires to perform more research into genetic therapies**.**

Acknowledgments

RAMS would like to thank Prof. Rob Collin, PhD, Radboudumc, Nijmegen, the Netherlands, for providing feedback to the author of this article, as well as Aimée de Croon, BSc, for reviewing the article.

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