

Leber's congenital amaurosis and the role of gene therapy in congenital retinal disorders

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Abstract

• **Leber's congenital amaurosis (LCA) and recent gene therapy advancement for treating inherited retinopathies were extensive literature reviewed using MEDLINE, PubMed and EMBASE. Adeno-associated viral vectors were the most utilised vectors for ocular gene therapy. Cone photoreceptor cells might use an alternate pathway which was not reliant of the retinal pigment epithelium (RPE) derived retinoid isomerohydrolase (RPE65) to access the 11-cis retinal dehydrochromophore. Research efforts dedicated on the progression of a gene-based therapy for the treatment of LCA2. Such gene therapy approaches were extremely successful in canine, porcine and rodent LCA2 models. The recombinant AAV2.hRPE65v2 adeno-associated vector contained the RPE65 cDNA and was replication deficient. Its *in vitro* injection in target cells induced RPE65 protein production. The gene therapy trials that were so far conducted for inherited retinopathies have generated promising results. Phase I clinical trials to cure LCA and choroideremia demonstrated that adeno-associated viral vectors containing RPE genes and photoreceptors respectively, could be successfully administered to inherited retinopathy patients. A phase III trial is presently ongoing and if successful, it will lead the way to additional gene therapy attempts to cure monogenic, inherited retinopathies.**

• **KEYWORDS:** retina; Leber's congenital amaurosis; Leber's congenital amaurosis type 2; choroideremia; achromatopsia; cyclic nucleotide gated channel alpha 3; retinoid isomerohydrolase; gene therapy

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INTRODUCTION

One of our most appreciated senses, vision, relies on the mechanisms of phototransduction, in which extremely specialized photoreceptor cells located in the human retina, the rods and cones act^[1]. Rod photoreceptor cells are located in the retina of the eye and function in combination with cone photoreceptor cells. There are approximately 125 million rod cells located in the human retina^[2]. Rod photoreceptor cells are found on the outermost edges of the retina and are predominantly responsible for peripheral vision. These cells are more sensitive to light compared to cone photoreceptor cells. Night vision relies on rod photoreceptor cells which have the ability to react to very few photons. Cone photoreceptor cells on the other hand become activated under bright light, and are essential for colour vision.

Impaired vision can affect numerous individuals throughout their lifetime; individuals can be born with impaired vision or suffer from age associated vision defects, such as macular degeneration as they get older.

Genetically inherited eye disease affects approximately 1 in 2300 people worldwide. They have been found to be genetically and phenotypically heterogeneous and are unfortunately quite difficult to treat. More than 150 genetic mutations have so far been identified, caused by loss of protein function in single genes that have been shown to be responsible for correct visual function^[3]. These genes manifest expression in photoreceptors of the retina and the retinal pigment epithelium (RPE). Dysfunction or absence of these genes can give rise to a number of genetically inherited eye conditions, including retinitis pigmentosa, Stargardt disease, choroideremia and Leber's congenital amaurosis (LCA).

LCA involves a spectrum of hereditary, autosomal recessive conditions such as retinal degeneration, which results in loss of vision, firstly observed during infancy. They are the most prevalent genetic cause of congenital visual defects of retinal origin in children and infants. These disorders are associated with various eye defects, the most obvious of which are abnormal roving-eye movements, also known as nystagmus. Other symptoms include slow pupillary reactions and poor or absent electroretinographic reactions in early life. In order to provide an accurate diagnosis of the disease, electroretinographic responses and pupillary light reflexes are recorded. In the case of LCA individuals, they

are both abnormal^[4-5]. LCA patients suffer from severe visual impairment during childhood, with their vision continuously deteriorating, the final outcome of which usually is complete loss of vision by their thirties or forties^[6]. So far, no successful treatment for LCA exists but the condition is thought to be a result of mutations in three different genes; cone-rodhomeobox (CRX), guanylatecyclase and retinoid isomerohydrolase (RPE65)^[7].

One of the types of LCA disease is the LCA2 form that is instigated by transformations in the RPE65 gene. The RPE65 gene codes a 65 kDa protein that is crucial for a biochemical pathway involved in the regeneration of visual pigment as a result of exposure to external light, called the visual cycle. The RPE65 protein is accountable for the activity of isomerhydrolase in the retinal pigment epithelium, leading to the production of 11-cis-retinal from all-trans-retinylesters^[8]. The 11-cis-retinal is a naturally occurring "ligand and chromophore of the opsins of rod and cone photoreceptors"^[9]. The absence of 11-cis-retinal, leads to the inability of opsins to capture and transduce light into electrical responses which consequently initiate the phenomenon of vision^[10]. It is thus expected that such absence of a crucial protein will cause the immediate and severe impairment of visual function. Nevertheless, in LCA2 patients as well as the LCA2 animal models, there is a delay in the histological degeneration of retinal cells, even though examinations indicate that the patients' visual ability and electrophysiological responses are severely compromised^[10-11]. It has been suggested that cone photoreceptor cells may make use an alternate pathway which is not reliant of the RPE derived RPE65 to access the 11-cis retinal dehydechromophore^[11]. This would explain why LCA children maintain their cone-mediated vision. Nevertheless, the continuous deterioration of cone cells will eventually lead to the loss of cone cell facilitated vision.

While the symptoms of LCA are extremely severe, no effective treatment for LCA caused by the RPE65 genetic defects exists so far. The fact that children retain their visual function and that retinal imaging studies show that photoreceptor cell death is a relatively late event in the progression of the disease, suggest that gene replacement therapy might be a valid option.

Gene Therapy for Inherited Retinopathies Gene therapy is the process by which new DNA is inserted into cells. The aim of this method is either to offer the cell a gene that it is lacking or not functioning, or to provide the cell with a gene that could serve a therapeutic purpose. Gene therapy has been used for a number of tissues and defective cells, with variable success. In the case of providing treatment for retinopathies, the retina is the best target for gene therapy for numerous reasons as will be discussed below.

Initially, the retina is formed of ordered epithelial layers that make the administration of a DNA vector, in which the

therapeutic gene is contained, easy to be injected and inserted in all the eye cell populations. In addition, the physiology of the eye itself makes it feasible to insert the DNA into it, by employing a relatively non invasive approach. The intraocular environment can be easily accessed through the pars plana. This is very different to most internal organs that cannot be accessed easily and hence this makes gene therapy approaches for them tricky. It should also be noted that the amount of retinal tissue is small compared to other visceral organs, again permitting an efficient, targeted approach towards the insertion of the DNA vector into the cells. Furthermore, the blood-retinal barrier allows the isolation of the intraocular environment from the immune system and this is important, as it is likely to control for extreme immune responses initiated by the administration of foreign antigens. Finally, it is relatively easy to monitor the efficacy of gene therapy treatment by assessing the patient's visual acuity or by employing electrophysiology and optical coherence tomography approaches.

Various vector systems have been adopted for retinal gene delivery. These include lentiviral vectors, adenoviral vectors and recombinant adeno-associated viral vectors^[12]. The latter are the most utilised vectors for ocular gene therapy as instead of viral genes, they have been engineered to contain distinct DNA sequences that have been shown to have therapeutic applications. Significantly, rAAVs can "transduce both dividing and non dividing cells"^[9] and hence they are able to target all cells at different phases of the cell cycle^[12]. When containing specific modifications, they have been capable of targeting individual types of cells, including, photoreceptors, retinal pigmented epithelia and ganglion cells^[13].

Gene Therapy Approaches for Leber's Congenital Amaurosis

As previously mentioned, LCA involves a spectrum of hereditary, autosomal recessive conditions such as retinal degeneration, which results in loss of vision, firstly observed during infancy. They are the most prevalent genetic cause of congenital visual defects of retinal origin in infants and children. These disorders are associated with various eye defects, the most obvious of which are abnormal roving-eye movements, also known as nystagmus. Other symptoms include slow pupillary reactions and poor or absent electroretinographic reactions in early life. It thus becomes obvious that gene transfer could be a promising approach for improving visual function or for preserving existing vision in affected individuals. Stemming from these findings in a number of human conditions, research efforts have dedicated on the progression of a gene-based therapy for the treatment of LCA2. Such gene therapy approaches have already been extremely successful in a number of animal models. Recombinant adeno-associated viral vectors have already been used in canine, porcine and rodent LCA2 models. The recombinant AAV2. hRPE65v2 adeno-associated vector contains the RPE65 cDNA

and is replication deficient^[14]. Its *in vitro* injection in target cells induced RPE65 protein production. Hence, rAAV vectors have been used to deliver functional retinoid isomerohydrolase, restoring retinal function with dramatic visual improvement^[15]. A single sub-retinal injection of the AAV2. RPE65 vector has resulted in long term improvement of visual capacity in specifically selected breeds of dog. One of the first dog breed chosen was the Swedish briard dog which is a naturally occurring LCA2 canine model^[16]. Finally, sub-retinal delivery of the RPE65 cDNA-containing recombinant adeno-associated viral vector enhances retinal capacity and subsequent vision as has been demonstrated through visual mobility studies under low light levels^[17-18]. Hence all these research findings postulated the basis for Phase I clinical trials in humans for the restoration of RPE65 function by gene therapy approaches in LCA patients.

A study conducted in 2008 by Bainbridge *et al*^[19], assessed the impact of RPE65 gene therapy on LCA patients^[19]. The study was based on results taken from young adults aged between seventeen and 23 years old who were experiencing the early onset of severe retinal dystrophy which was as a result of RPE65 mutations^[20]. The type of gene therapy adopted involved recombinant adeno-associated viral vector of serotype-2, which is referred to as tgAAg76. The sequence coding of RPE65 is made by fragment of 1400 base pairs in this vector^[19]. The RPE65 transcription and promoter was concluded following the presence of the “bovine growth hormone polyadenylation site”^[21]. After three-port vitrectomy, a predetermine amount of recombinant adeno-associated viral vector was dispensed through a “subretinal cannula” directly into the subretinal space in a single eye^[20].

In order to be able to confidently assess the impact of the RPE65 gene therapy on the LCA patients, their retinal structure and function was checked prior to the dispensing of the recombinant adeno-associated viral vector. The evaluation of the implantation was carried out through the use of retinal imaging, electrodiagnostic methods and psychophysical techniques. Retinal imaging techniques are particularly useful for determining retinal thickness and integrity, in addition to colour fundus photography^[19]. Subsequent to the adeno-associated viral vector administration, the patients’ sensitivity to contrast, visual acuity, cone flicker sensitivity and colour vision were measured. Furthermore, the visual field of each patient was analysed through the use of techniques including Goldmann dynamic perimetry, microperimetry, and automated static perimetry. In addition, the patients’ visual mobility was recorded at various lightning conditions by examining their performance to mobilise through simulated road environment. ERG studies were also used. “The assessments of visual function and immune status were repeated at 2, 4, 6, and 12mo subsequent to the administration of the vector”^[19].

The findings of this gene therapy study were indeed striking. The use of transvitreal, transretinal therapy after pars planavitrectomy in patients who are experiencing the late stages of retinal degeneration, has been found to have a positive impact on the symptoms of LCA patients. Consistent improvement of microperimetry as well as dark-adapted perimetry was observed in one of the patients. In particular, an improvement in reference visual acuity in both the experimental group and control group was observed in this patient. Furthermore, the patient’s visual mobility in response to low levels of light significantly improved. The enhancement in the patient’s visual mobility under low levels of light was significantly higher than that owed to the modest learning effect and the result is comparable with an enhancement of visual function which is founded by perimetry^[19]. Although these findings highlight the fact that gene therapy approaches could be beneficial for LCA patients, it still remains unclear if enhancement in visual reactions observed in the outer macula of the LCA patient was rod or cone effected. However, the results reported by Bainbridge *et al*^[19] (2008) suggest that subretinal administration of recombinant adeno-associated viral vectors isn’t related with instantaneous unfavourable incidents “in patients with severe retinal dystrophy and that adeno-associated virus-mediated RPE65 gene therapy”^[19] could result in humble advances in visual performance^[22]. These findings critically demonstrated the important impact that gene therapy approaches can play on inherited retinopathies and indicated that further clinical trials should be conducted to allow for a more thorough evaluation of gene therapy approaches in LCA. In order to further these findings, additional phase 1 human clinical trials have been performed. A phase 1 study of 15 patients aged 11 to 30y who were observed over a period of three years and who were treated with sub-retinal injection of a rAAV vector expressing *RPE65*, showed that there was improved, striking visual function in all patients^[11]. It also confirmed that the administration of the adeno-associated viral vector resulted in no systemic toxicity and in good ocular tolerance. A 24-patient phase 3 trial is currently recruiting patients in Iowa and Pennsylvania^[23] (clinical trial ID: NCT01461213) and the scientific community is keenly awaiting the findings of this more extensive study.

Recombinant adeno-associated viral vectors have already been used in dog like, pig like and rodent LCA2 models. The recombinant AAV2.hRPE65v2 adeno-associated vector contains the RPE65 cDNA and is replication deficient. Its *in vitro* injection in target cells induced RPE65 protein production^[22]. Hence, rAAV vectors have been used to deliver functional retinoid isomerohydrolase, restoring retinal function with dramatic visual improvement^[14-15]. A single sub-retinal injection of the AAV2. RPE65 vector has resulted in long term improvement of visual capacity in specifically selected breeds

of dog. The dog breed chosen was the Swedish briard dog which is a naturally occurring LCA2 canine model^[16].

Besides LCA, gene therapy treatments have been employed for other hereditary eye disorders. Choroideremia is considered to be an X-linked recessive degenerative retinal condition which effects approximately 1 in 50 000 people. It manifests in the first decade of one's life by loss of night vision. These symptoms are gradually followed by peripheral visual loss eventually advancing to total blindness when the patient reaches their fifties. Retinal degeneration is a result of mutations in the choroideremia Rab-escort 1 (CHM) gene. Mutations in CHM lead to loss of the Rab escort protein 1 which leads to a prenylation deficiency^[22]. A gene therapy study involving sub-retinal injections of a rAAV vector expressing Rab-escort protein-1 has already been conducted. This study was a phase I study and it involved 6 men aged 35-63y^[14,24]. The findings of this study indicated an improvement in average visual acuity. In particular, an improvement in visual acuity by 3.8 letters was observed in all patients, with two of these patients exhibiting a dramatic improvement in visual acuity by 11 and 21 letters.

Further to replacing dysfunctional proteins, gene therapy has also been used as a means of drug delivery for eye disorders. For example, conditions that may affect the vasculature of the eye have benefited from gene therapy approaches that block the vascular endothelial growth factor (VEGF). These approaches are used in neovascular age related vascular degeneration and have very effectively suppressed VEGF expression^[25-26]. Given however the short half lives of these administered proteins, frequent, almost monthly administration of the proteins is required to achieve maximum clinical effect and therapy, resulting in increased patient risks and inconvenience.

Gene therapy can also be used for drug delivery, as many retinal diseases can benefit from the local production of a specific RNA or protein. As such, adenoviral vectors that express the pigment epithelium-derived factor in order to inhibit angiogenesis were successfully administered in a phase I clinical study^[27]. Another phase I clinical study is now evaluating whether the intravitreal administration of a recombinant adeno-associated vector that expresses soluble VEGF receptor can be used to block VEGF^[28].

CONCLUSION

It hence becomes apparent that the gene therapy trials that have so far been conducted for inherited retinopathies have generated promising results. Phase I clinical trials to cure LCA and choroideremia have demonstrated that adeno-associated viral vectors containing RPE genes and photoreceptors respectively, can be successfully administered to inherited retinopathy patients. Follow up reports from UK and Florida studies showed significant improvement in dark adapted visual function and subjective mobility performance^[29-30]. However,

it was reported that ideal timeframe for human intervention requires further investigation. Concerning recent choroideremia gene therapy follow up study involving 6 patients, Barnard *et al*^[31] reported "mean visual acuity improved to 3.8 letters phase III trial". Recent approval for achromatopsia cyclic nucleotide gated channel alpha 3 (CNGA3) gene therapy and retinitis pigmentosa MerTK AAV gene therapy trials are currently ongoing^[31]. Therefore if current trials are successful, they will further lead the way to additional gene therapy attempts to cure monogenic, inherited retinopathies.

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