3. A hope for vision : Gene-replacement therapy for RPE65 associated Leber's Congenital Amaurosis

Review Article

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Abstract

Leber's Congenital Amaurosis (LCA) is a group of inherited blinding diseases which leads to congenital retinal dystrophies and is difficult to treat. However, gene replacement therapy holds great potential in treating LCA type 2 caused to Rpe65 gene mutations. This restores the isomerohydrolase activity of the expressed RPE65 protein by correcting the defect at the molecular level. Adeno-Associated Virus mediated subretinal delivery of RPE65 cDNA regulated by constitutive or tissue specific promoter shows dramatic improvement in vision in terms of subjective and objective aspects which indicates efficacy of the therapy. Absence of immune responses, adverse effects and vector dissemination underlines the safety of the therapy. Successful findings observed in pre-clinical and clinical trials pave a way to its practical application. Response to the treatment however varies with the extent of retinal degeneration and age of the patient. Moreover, currently developing non-viral gene delivery and nanoparticle mediated approaches gives an insight to its future prospects too.

Keywords: Leber's congenital amaurosis (LCA) type 2, Rpe65 gene, gene replacement therapy, Adeno-associated virus, subretinal delivery, efficacy, safety.

1. Introduction

1.1. Leber's Congenital Amaurosis

Leber's Congenital Amaurosis (LCA) is a group of inherited retinal dystrophies characterized by severe loss of retinal and visual functions from birth due to progressive degeneration of the cellular structure of the retina.[1, 3, 5]LCA which was first reported by Theodor Leber,affects almost 1/81,000 individuals and accounts for

at least 5% of all retinal dystrophies.[2,3]This degenerativedisorder is identified by nystagmus,highly attenuated electroretinographicresponses and pupillary light reflexes, oculodigital sign (habitual rubbing or poking the eyes), keratoconus, cataracts and fundus abnormalities. Since childhood, vision starts getting deteriorated and ultimately total blindness is observed by the third or fourth decade of life.[4, 5]No effective treatment for LCA has been reported yet. [1]

1.2. RPE65 gene, its function and pathogenesis of LCA-Type 2

LCA is reported to be caused due to mutations in 15 different genes and is inherited in an autosomal recessive manner although autosomal dominant forms are also reported. LCA2 resulting due to mutations in the Rpe65 gene accountfor 10% of LCA. [5] Rpe65 gene is expressed predominantly in the retinal pigment epithelium (RPE) cells producinga 65kD protein which isessential for the functioning of a visual retinoid cycle. The RPE65 protein is a carotenoid oxygenase whose isomerohydrolase activityconverts all-trans-retinylesters to 11-cis-retinal.This facilitates constant supply of a light-sensitive 11-cis-retinal to the photoreceptor outer segments for re-association with opsin to form a visual pigment, Rhodopsin. Moreover, RPE also contributes to the survival of the photoreceptors through the phagocytosis of rod and cone outersegment discs. Hence, an optimum functioning of the photoreceptors ultimately depends on the RPE.[2, 4, 6,7]

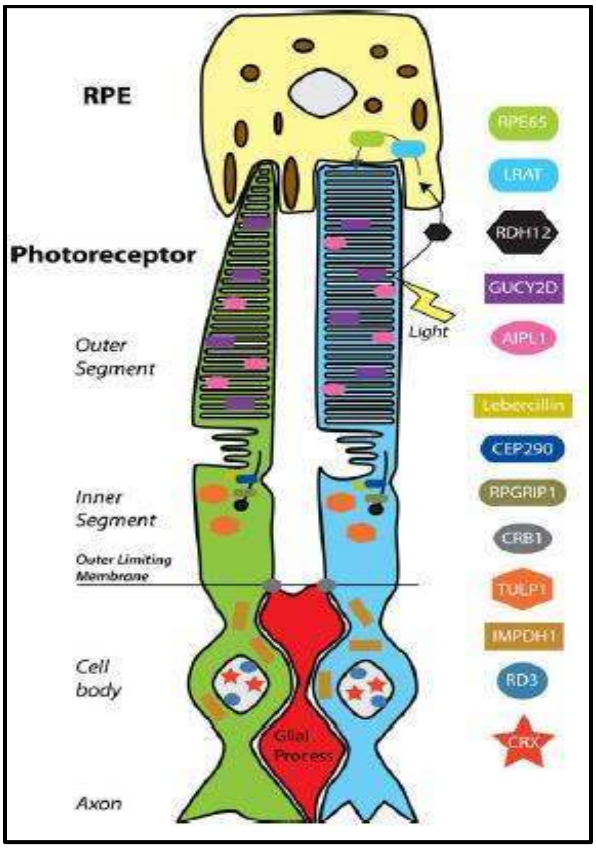

Figure 1: Location of the LCA-associated proteins within the photoreceptor-RPE complex.[2]

Mutations in Rpe65 gene resulting in RPE65 deficiency cause biochemical blockade of the visual cycle. This leads to the accumulation of all-trans-retinyl esters in RPE cells and the diminished levels of 11-cis-retinal is observed in LCA2 patients. As

a result,rod photoreceptor cells are unable to respond to light. Cone photoreceptor cellscan sustain their function through an alternative pathway that does not depend on RPE65, thus, allowing cone-mediated vision initially in children with LCA. However, cone photoreceptor cells progressively degenerateleading to loss of cone-mediated vision. Overall,a profound impairment in visual function along with delayed histological degeneration of retinal cells is the ultimate outcome. [1,5]

1.3. Potential of Gene Therapy

Gene-replacement therapy may serve as a potential therapeutic strategy to correct the defects in Rpe65 gene owing to the features of the disorder. Vector mediated delivery of a wild typeRPE65cDNA shows improvement in the visual function. [1,5, 8]

2. Gene therapy-Process

2.1 RPE as a target for gene therapy

The eye seems to be a promising target for gene therapy as it's immune privileged, has a small compartment size, easily visualized and examined, and readily accessible with minimal risk to patients undergoing surgery. The prime targets for gene therapy include the photoreceptors and the RPE (the phagocytotic nature of the RPE further facilitates gene delivery). Retina is exploited for the accurate delivery of therapeutic agent to the photoreceptors and RPE by injecting a fluid suspension containing the therapeutic particles into the subretinal space. Temporary detachment of the retina is seen following to the injection; however serious damage to the other parts is not reported. [6]

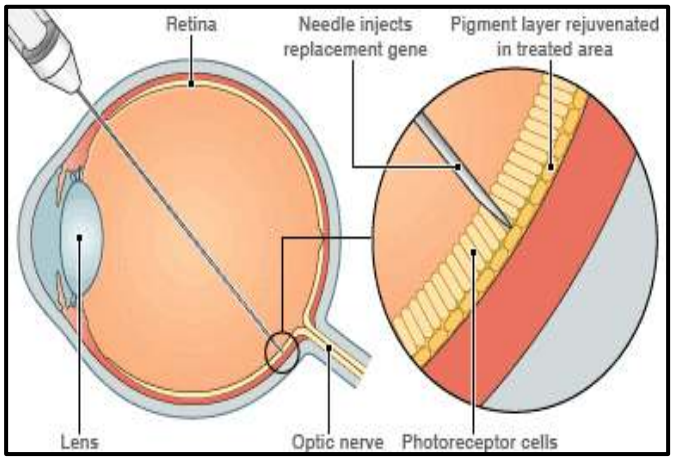

Figure 2: Gene-replacement therapy through Rpe65 genesubretinal delivery. [17]

2.2. Adeno-associated virus mediated gene therapy

The efficiency with which the transgene is delivered to the appropriate cell type determines the success of gene therapy technique. Of the two major approaches for the gene delivery - viral and non-viral types, varied tropisms are exhibited by different viral vectors for the specific eye tissues. Of all the viral vectors, adeno-associated virus

proves to be a major vector in the gene therapy for LCA2. [6]

2.2.1. Adeno-associated Virus (AAV) - Vector construction and gene packaging

AAV is a non-pathogenic, non-enveloped virusapproximately 25 nm in diameter containing a single stranded DNA genome with a maximum length of 5.1 kb. [6, 9]For constructing the recombinant AAV (rAAV) vector, the coding sequence of AAV (rep and cap genes) is replaced with the transgene cassette comprising of a promoter (either constitutive or tissue-specific), gene of interest (here Rpe65 gene) in the form of cDNA (human RPE65 cDNA is 1.6 kb long)[11] and a poly-Adenylation tail. [9] The Inverted terminal Repeats (ITRs) sequences of the AAV are retained as they are required for cis-packaging of a recombinant vector genome. Due to the necessary inclusion of ITRs, the total coding capacity of AAV vectors is reduced to approximately 4.7 kb, which limits the size of the therapeutic gene that can be incorporated. [6]rAAV-mediated Rpe65 gene delivery resulted in a stable transgene expression in RPE cells, by maintaining the transgene mostlyin an episomal fashion.[12]

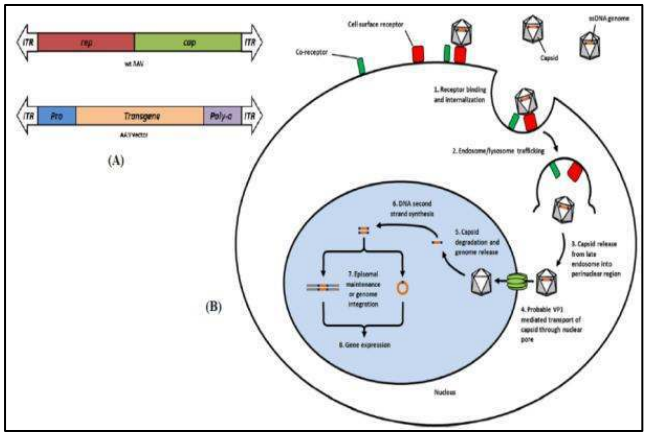

Figure 3: (A) rAAV vector genome, (B) Ways of transgene expression on viral transduction. [6]

2.2.2. Pseudotyping of rAAV Serotypes

By the 'pseudotyping' process, rAAV vectors are produced. For example, an AAV2 (AAV serotype 2) based transgene cassette is packaged into a capsid from a virus originating from a different serotype. By using this technique, gene delivery to RPE occurs effectively with several pseudotypes, particularly rAAV2/1 (AAV2-based genome in AAV1 capsid), rAAV2/2 and rAAV2/4. [6]

2.2.3. Regulation of Transgene Expression

Expression is primarily regulated by inclusion of appropriate promoters such as constitutive ones like Chicken β actin (CBA) promoter or RPE-tissue specific promoter. [1, 4, 6, 10] Temporal regulation of

transgene expression is achieved by using tetracycline, doxycycline, etc. [6]

2.3. Methodology

Following two processes are carried out each based upon the different promoter types used. Methods A and B utilize CBA and tissue specific promoters respectively.

Method A: The transgene cassette in the AAV2.hRPE65v2 vector carrying a CBA promoter drives the expression of the human RPE65 (hRPE65) cDNA. Co-administration with a surfactant prevents the loss of the vector to surfaces which are in contact with the product. During surgery, an injection of vector genome of AAV2.hRPE65v2 in a phosphate-buffered saline supplemented with Pluronic F-68 NF Prill Poloxamer 188is administered into the subretinal space which creates a localized retinal detachment which ultimately resolves later. [4, 11]

Method B: The rAAV2 (specifically tgAAG76) vector containing the human Rpe65 coding sequence driven by the hRPE65promoter and terminated by the bovine growth hormone polyA site is filled in a buffered saline solution and allowed to freeze in 1-ml aliquots at −70°C. rAAV2 efficiently transduce RPE cells following subretinal delivery. The possibility of the occurrence of intraocular inflammation was minimized by using immunosuppressive agents. [1]

2.4. Efficacy and safety of the therapy

Administered AAV2-hRPE65v2 is well tolerated and improvement in subjective and objective measurements of vision (i. e, dark adaptometry, pupillometry, electroretinography, nystagmus, and ambulatory behaviour) is noticed in pre-clinical and clinical trials as described elsewhere. The response to subretinal Rpe65 gene therapy depends onthe extent of retinal degeneration andthe ageof the patient. The greatest improvement is seen in children as seen in a study. [8] Absence of the clinically significant adverse effect of subretinal vector delivery underlines the safety of the therapy at all administered doses whereas absence of the systemic dissemination of the vector is indicative of the minimal extraocular leakage of vector from the subretinal space. [1, 8, 9] However, a macular hole is reported during a trial conducted in a patient but it occurred due to contraction of a preexisting membrane stimulated by the surgical procedure,and does not affect the vision. [4] No significant humoral immune responses and neutralizing antibodies areobserved against viral capsid or expressed RPE65. [11] There's a

possibility that rAAV particles can spread to adjacent cells and transduce ganglion cells. This can result in expression in the visual pathways present in the brain. However, evidence which can support the occurrence of CNS toxicity in preclinical or clinical studies is not reported. [12]The safety, extent, and stability of improvement in vision as noted in trials described elsewhere support the use of AAV-mediated gene therapy for the treatment of LCA2.[8]

3. From Research to Practice

3.1. Preclinical Trials

3.1.1. Description

In this trial, a naturally occurring murine model of LCA with a recessive nonsense Rpe65 mutation, the rd12 mouse, was employed to test the potential of recombinant adeno-associated virus (rAAV) -mediated gene therapy in rescuing this defect and the associated loss of retinal structure and function. rd12 mouse is a natural model of RPE65. Due to mutation in Rpe65 gene, small lipid-like droplets in RPE cells were first detected at 3 weeks of age, followed by progressive retinal degeneration. Fundoscopic examination also revealed evenly dispersed yellowish- white spots throughout the retina from 5 months onward.

The recombinant adeno-associated virus constructs with AAV2 inverted terminal repeats and pseudotyped AAV5 capsids were based on the pTR-UF2 vector. Serotype 5 rAAV vectors carry a normal hRPE65 cDNA (AAV5-CBA-hRPE65). The viral preparations were made, with average physical particle titers of 10^{13} viral particles/ml and biological titers of approximately $2x10^{11}$ infectious particles/ml. In each mouse, 1 microlitre of vector suspension was administered subretinally. The other eye remained uninjected. Animals with indications of ocular damage, and other complications were removed from further study. For this study, C57BL/6 mice were used as controls for various later tests. The control mice received no injections whatsoever.

3.1.2. Results

a) rAAV-mediated Rpe65 gene therapy was employed into rd12 mice with the aims of rescuing the RPE-origin functionaldefect and ultimately restoring vision in these animals. 3 weeks after single injection, stable RPE65 expression was observed in the injected eye. No RPE65 expression was noted in untreated rd12 retinas of the same animals.

b) Changes in fundus were also noted. Retinas treated with AAV5-CBA-hRPE65 appeared nearly normal, in contrast to yellow-white spots devoid of pigment observed in untreated eyes.

c) Rhodopsin levels in retinas injected with AAV5-CBA-hRPE65 were restored to 70% of normal levels and had a molecular weight identical to those noted in the control mice.

d) Using light and electron microscopy, the retinal morphology after vector treatment was noted. Retinas of normal control C57BL/6 mice and the age-matched rd12 eyes treated with AAV5-CBA-hRPE65 were comparable, with 10-12 layers of outer nuclear layer (OLN) cells. In contrast, untreated rd12 eyes displayed striking differences. The OLN contained only 6–8 layers of cells.

3.1.3. Discussion

Through this trial, it was concluded that gene therapy could reverse the congenital defect, improve retinal structure and vision. Retinas treated with AAV5-CBA-hRPE65 showed results comparable to those in normal eyes. Several other preclinical studies have concluded that gene replacement therapy using a recombinant adeno-associated virus (rAAV) vector carrying a wild-type canine Rpe65 cDNA restored partial retinal function. Success has also been noted in similar rescue using gene therapy in a strain of Swedish briard dogs carrying a functionally null allele of canine $Rpe65^{-/-}$ that also exhibit a retinal dystrophy. Hence, there was a further need to validate existing data through human clinical trials. [7]

3.2 Clinical trials

3.2.1 Description

Three consecutive patients who had LCA2 and were between the ages of 19 and 26 years were selected. The eye with worse function was selected for delivery of AAV2.hRPE65v2. Method utilized for the therapy was the same as described in the Method A under the section 2.3 (Methodology) of this article.

3.2.2. Results

a) Pupillometry: Baseline testing showed that the pupillary light reflexes of the three patients prior to injection were much less sensitive to light than those of control subjects, even when the strength of the stimulus was increased. After injection, the responsiveness of the patients' injected eyes was reliably greater than that of their uninjected eyes. In summary, after injection of AAV2.hRPE65v2, each of the three eyes that received injection became more effective in driving the pupillary response,

more sensitive to light and surpassed the sensitivity of the other eye.

b) In the weeks following injection, patients reported having improved vision in dimly lit environments. In tests of ability to navigate an obstacle course before the injection of AAV2.hRPE65v2, patients collided with most of the obstacles and remained off-course throughout. However after, administration of injection, patients were able to navigate through the obstacle course.

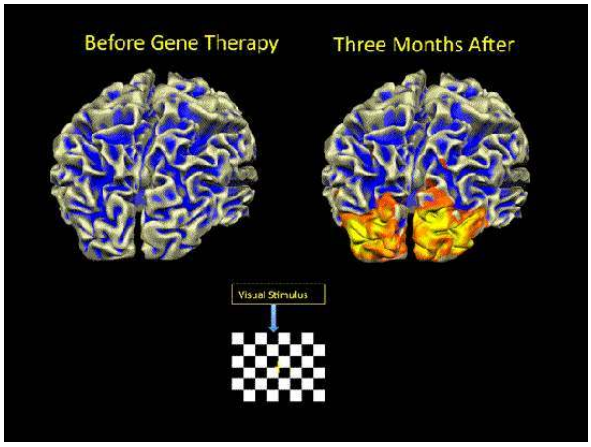

Figure 4: Comparative analysis of the responses to visual stimuli before and after the gene therapy.[18]

3.2.3 Discussion

All three patients with LCA2 who received AAV2.hRPE65v2 by subretinal injection showed evidence of improvement in retinal function. There was improvement in the pupillary light reflexes and increase in vision. There were no apparent local or systemic adverse events observed throughout. Thus, this trial provides the foundation for gene-therapy approaches in humans for treatment of LCA and other forms of retinal degeneration.[4]

4. Advantages and Limitations of the Therapy

AAV mediated subretinal Rpe65 gene delivery show promising results in the trials. Vision improvement both in objective and subjective aspects along with the safety of the therapy underlines the clinical significance. Even the re-administration in the previously treated eye shows dramatic improvements in the vision. However, the delivery of larger genes using AAV is still a problem. Efforts to expand AAV packaging capacity artificially are in progress. [2]

5. Conclusion

Practical application of gene therapy for Leber's Congenital Amaurosis has not yet started. . However, various preclinical and clinical trials have yielded optimistic results. The many benefits reaped by the patients after gene therapy, suggests that practical use of gene therapy for Leber's Congenital Amaurosis should be carried out at the earliest. These findings along with earlier

successes are very promising in terms of the potential of gene therapy for patients with certain forms of congenital and degenerative eye disorders and open the possibility of rescue of a wider variety of recessive mutations originating in RPE and rod photoreceptors.

6. Future Prospects

Alternative functions of RPE65 in photoreceptors, if discovered, may play a role in the disease process and treatment effects. Apart from AAV, other gene delivery vectors encompassing various viral (example- lentivirus) or non-viral vectors may modify the efficacy of the therapy. Non-viral gene delivery techniques like protein-based nanoparticle carriers, electroporation, lipofection, etc., if exploited, may prove as more effective approaches. Nanoparticle-mediated therapy prove to be an effective technique since nanoparticles are easily manufactured, may have less immunological responses, and can readily pass through membranes. Nanoparticles (NPs) can be modified to produce sustained-release compounds.[2, 13]CK30PEG10k NPs have shown high levels of expression by distributing throughout the RPE cell layer. A major clinical phenomenon is observed by transduction of CK30PEG10k NPs in RPE cells away from the site of injection. Moreover, targeted expression in RPE cells is also reported in the case of VMD2-eGFP NPs. [13, 14] Targeted gene delivery using Zinc Finger Nucleases also have potential to prove as a promising strategy in the coming years. In addition to this, oligochitosan polyplexes and low-molecular weight oligochitosan (non-viral technique) mediated gene delivery may serve as an active research area, too. [15, 16]

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