Gene therapy for Leber congenital amaurosis

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Abstract

Introduction: Leber congenital amaurosis (LCA) is a group of recessively inherited, early infantile-onset, severe rod-cone dystrophies that can result from defects in at least 25 genes, including *RPE65*, *CEP290*, *RDH12*, *AIPL1* and *GUCY2D*. The possibility of benefit is offered by therapeutic intervention to provide the functional gene that is otherwise lacking. Areas Covered: We searched PubMed for publications using the relevant keywords.

Expert Commentary: Clinical trials of gene therapy for LCA owing to defects in *RPE65* have demonstrated benefit with improved function of rod photoreceptor cells. A gene therapy for this condition has been approved by the FDA. Ongoing clinical trials aim to determine whether cone photoreceptor cell function can be protected by appropriate gene delivery at an early stage of the disease. Clinical trials of gene therapy for LCA owing to defects in 5 other genes are planned.

Keywords

Leber congenital amaurosis, LCA, LCA2, *RPE65* associated LCA, gene therapy, clinical trials

Introduction

Leber Congenital Amaurosis (LCA) was first described in 1869 by Theodore Leber. LCA is now used to define a group of recessively inherited, severe, early infantile-onset, rod-cone dystrophies[1]. LCA accounts for greater than 5% of inherited retinal disease[2], with a prevalence of between 1 in 33,000[2] and 1 in 81,000[3]. LCA classically presents at birth or in early infancy with severe sight impairment, nystagmus, poor pupillary responses, and undetectable responses to full-field electroretinography (ERG). However, the condition demonstrates significant phenotypic variability and may present later in infancy or early childhood as an 'early onset severe retinal dystrophy' (EOSRD) with impaired but residual visual function and highly attenuated but detectable ERG responses.[1] There is significant overlap between the molecular causes of classical LCA and EOSRD, with some genes implicated in both clinical phenotypes. To date, defects in 25 identified genes account for approximately 70-80% of affected individuals.[1] Since the condition is typically the consequence of lack-of-function mutations, it has the potential to benefit from therapeutic delivery of the functional gene. In its simplest form, gene 'supplementation' therapy compensates for loss-of-function mutations by provision of the normal gene to the cells in which it is required.[4] The therapeutic gene, typically delivered using a viral vector, is utilised by the transcriptional machinery of the target cell to generate the normal gene product that is otherwise lacking. Vector-mediated gene delivery can also be used to establish sustained local expression of proteins that may be neuroprotective.[5] Alternative gene therapy techniques can be used to suppress the undesirable expression of a harmful protein product resulting from gain-of-function mutations, with or without simultaneous provision of the normal gene.[6,7] More recently, gene editing strategies to correct harmful

mutations in endogenous genes, and anti-sense oligonucleotide mediated exon skipping to mitigate their impact, are also being investigated.[8,9]

The retina has specific advantages as a target organ for gene therapy. The transparency of the ocular media provides accessibility for microsurgical delivery of vector suspension to the retina under direct visualisation and for high-resolution optical imaging to assess its impact. Vector suspension can be targeted to the retina with minimal systemic dissemination owing to the contained nature and compartmentalisation of the intraocular tissues. The intraocular environment provides the retina with a degree of immune privilege, which helps protect against immune responses that could adversely affect retinal function and limit expression of the therapeutic gene. Since inherited retinal diseases such as LCA typically cause bilateral disease with a significant degree of symmetry, the untreated contralateral eye offers a valuable control for natural history, intra-individual variability in performance and learning effects.

For gene transfer to retinal cells in LCA, most clinical applications currently use recombinant adeno-associated virus (AAV) or lentivirus vectors. AAV is a small, non-pathogenic single stranded DNA virus widely used for gene delivery in inherited retinal diseases. AAV vectors can mediate efficient and sustained transduction of photoreceptor cells, retinal pigment epithelium (RPE), and ganglion cells. First-generation AAV2 vectors are limited by relatively slow onset of expression and small capacity (4.7 kB).[10] However, the isolation of alternative serotypes and the development of self-complementary vectors and novel variants by rational design and/or directed evolution, have provided a broad range of

alternatives with more rapid expression and wider cell tropisms.[11,12] Measures to address the limited capacity include dual AAV vector strategies in which a large gene delivered in component parts by multiple AAV vectors is reconstituted by splicing.[13] Lentiviral vectors have substantially greater capacity (approximately 8 kB) than AAV and can naturally accommodate larger genes. Lentiviral vectors mediate efficient transduction that is typically limited to RPE cells.[14]

Since defects in genes involved in phototransduction or the visual cycle account for many forms of LCA, photoreceptors and RPE cells are important target populations for gene therapy. Viral vectors deliver genes to these cells most efficiently when the vector is placed in direct contact by injecting the vector suspension into the potential space between the RPE and the overlying photoreceptor cell layer. Injection into this subretinal space is typically performed using a fine cannula that is advanced through the sclera anteriorly, across the vitreous cavity and through the neuroretina. Injection into this site generates a bleb of vector suspension that temporarily separates the neurosensory retina from the underlying RPE, before it is absorbed over a period of hours or days.

Although subretinal administration appears generally well-tolerated, a risk of harm from consequent thinning of the outer neurosensory retina is recognised.[15,16] The safety of subretinal administration may be improved by appropriate control of the pressure and flow rate of injection, and the height and duration of neurosensory separation. Injection of vector suspension into the vitreous cavity is a less invasive

alternative to subretinal administration. Using current vector systems, the efficiency of gene delivery to the outer retina from intravitreal administration is low because vector penetration across the inner retina is limited, but novel capsid variants such as AAV7M8 offer the potential for greater vector penetration and more efficient gene delivery.[17]

Whilst vectors injected subretinally appear to be relatively protected from systemic immune responses, vectors injected into the vitreous cavity can generate deleterious immune responses[18] possibly owing to flow of vector particles within the intraocular fluid compartments and via the outflow pathways to the systemic circulation. Measures to protect against intraocular inflammation, which presents a risk of harm and could limit the potential for benefit, include appropriate selection of vector and the use of immunomodulatory medication around the period of vector administration.

***RPE65*-associated LCA**

Mutations in the gene *RPE65* account for approximately 5-10% of LCA.[19] *RPE65* encodes a 65kD retinoid isomerase that is expressed in the RPE and is essential for the production of 11-*cis* retinal, a critical component of the retinoid (visual) cycle.[20,21] A lack of functional *RPE65* results in deficiency of 11-*cis* retinal such that rod photoreceptor cells are unable to respond to light, causing profound night blindness from

birth.[1] Cone photoreceptor cell function can be relatively preserved initially because cones have access to 11-*cis*–retinaldehyde chromophore through an alternative retinoid pathway.[22] However, cone-mediated vision deteriorates during childhood and early adulthood owing to progressive degeneration of the retina that involves both rod and cone photoreceptor cells.

Subretinal injection of AAV-vectors encoding the cDNA for *RPE65* can improve visual function in rodent models of *RPE65*-LCA, and in the Swedish Briard dog, which has a naturally occurring mutation in *RPE65*. [23] In the *Rpe65* knock-out mouse, gene therapy not only improves rod photoreceptor function but also preserves cone function and protects against degeneration.[24,25] In the naturally occurring Rpe65-deficient *Rd12* mouse gene therapy can improve rhodopsin levels, improve ERG responses dose-dependently to near normal levels[26] and improve dim-light vision.[27] In affected dogs, [28] AAV-mediated expression of *RPE65* can result in improved responses on ERG, pupillometry and flash-evoked cortical potentials in the dark-adapted state, with functional improvements sustained for as long as 10 years [23] and preservation of outer nuclear layer thickness evident on OCT scanning.[29]

In early-phase clinical trials in humans with *RPE65*-LCA, gene therapy has resulted in improved aspects of sight for up to 5 years.[15,16,30,31]. However, improvements in photoreceptor function in affected individuals have been relatively modest compared to those in animal models, even when retinal degeneration is less advanced, and the durability of benefit can be limited by progressive retinal degeneration.[31-34] In

one trial of subretinal administration of an AAV2/2 vector, improvements in retinal sensitivity, dark-adapted perimetry and vision-guided mobility were evident in 6 of 12 participants but were not sustained, with only 2 participants benefitting for up to 3 years.[32] Similar outcomes were noted in the other clinical trials. In a separate trial variable improvements in visual function localised to the treated area of retina were evident in all 15 participants but the benefit declined over time.[34,35] A phase III trial of subretinal administration of an AAV2/2 vector has also reported benefit at 1 year, reaching its primary endpoint for efficacy with improved performance on a novel test of multi-luminance mobility.[36] This product, voretingene neparvovec (Luxturna, Spark Therapeutics Inc) has recently been approved by the FDA for the treatment of RPE65-associated retinopathy. Findings of relatively modest efficacy and limited durability of benefit in other studies suggest that current vectors may not fully meet the demand for RPE65 in humans.[32] An optimized AAV2/5 vector carrying an optimized hRPE65 promoter and a codon-optimised hRPE65 gene is at least 300-fold more potent in mouse models.[15,37] and is the subject of a phase I/II clinical trial (clinicaltrials.gov: NCT02781480).

***CEP290*-associated LCA**

CEP290 encodes a centrosomal protein involved in trafficking through the connecting cilia of photoreceptor cells. Mutations in *CEP290* account for 15- 20% of LCA and represent one of the most common causes. At 7.5kb, the full-length gene exceeds the capacity of AAV vectors but can

be accommodated by lentiviral vectors. Given the lack of suitable animal models, gene therapy based intervention for *CEP290* associated LCA has been explored *in vitro*; transduction of patient-specific induced pluripotent stem cell-derived photoreceptor precursor cells rescues the cellular phenotype.[38] Alternative molecular therapeutic strategies focus on the common deep intronic *CEP290* sequence variant, which creates a strong splice donor site that leads to the insertion of a cryptic exon encoding a premature stop codon. Techniques include anti-sense oligonucleotide mediated exon skipping to abrogate the disease-causing variant, and correction of the splice defect using CRISPR/Cas9-mediated gene editing.[8,9] A clinical trial investigating the safety and tolerability of intravitreal RNA antisense oligonucleotide is ongoing (clinicaltrials.gov: NCT03140969).

AIPL1-associated LCA

Aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) is a molecular chaperone of phosphodiesterase 6, which mediates phototransduction in both rod- and cone- photoreceptors. Mutations in *AIPL1* cause a particularly severe, rapidly progressive disorder, which accounts for less than 5% of LCA.[19] Affected children have severe sight impairment in early infancy, and rapid retinal degeneration. Although the outcome is very poor, some preservation of retinal structure during infancy indicates a window of opportunity for intervention by gene replacement therapy,[39] with proof of principle demonstrated using an AAV2/8 vector in a rodent model of the disorder.[40]

***RDH12*-associated LCA**

RDH12 encodes a broad specificity aldehyde reductase localised in photoreceptor inner segments. The protein is not essential in the visual cycle, but is believed to protect against toxic accumulation of all-*trans*-retinal under persistent illumination.[41] Disease-causing sequence variants in *RDH12* account for approximately 10% of LCA/EOSRD.[19,42] AAV2/8-vector-mediated *RDH12* gene replacement therapy in *Rdh12* knockout mice indicates the potential for benefit in affected humans.[43]

***GUCY2D*-associated LCA**

Retinal guanylate cyclase-1 (GUCY2D) is essential in photoreceptor cells for timely recovery from photoexcitation. Mutations in the *GUCY2D* gene account for 10-20% of LCA. Photoreceptor architecture in *GUCY2D*-LCA is relatively well preserved[44] and preclinical studies of gene augmentation therapy in animal models have demonstrated benefit. HIV1-based lentiviral vector *in ovo* improves optokinetic reflexes and volitional visual behaviour in a chicken model.[45] In the *GC1* knock-out mouse, both AAV serotype 5 (AAV5) and AAV8 vectors can protect retinal function and preserve of cone photoreceptor cells.[46-48] In the GC1/GC2 double knock out mouse, the tyrosine capsid mutant

AAV8(Y733F) restores both cone and rod- mediated vision. [49] Proof of principle in experimental models and relative preservation of photoreceptor cells in affected humans suggest that affected individuals stand to benefit from gene augmentation therapy.

***RPGRIP1*-associated LCA**

Retinitis pigmentosa GTPase regulator (RPGR) is anchored in the connecting cila of photoreceptor cells by RPGR-interacting protein (RPGRIP). Mutations in *RPGRIP1* account for about 5 % of LCA.[50,51] In contrast to other forms of LCA, *RPGRIP1*-associated LCA appears to be relatively non-progressive, following an initial rapid decline in visual function.[52] Furthermore, photoreceptor structure is preserved despite deterioration in visual function.[53] In the *RPGRIP1* knockout mouse and in a mouse model carrying a recessive *RPGRIP1* mutation (designated Rpgrip1^{nmf247}), AAV-mediated expression of RPGRIP1 can protect photoreceptor cells against degeneration and preserve retinal function.[54,55] In a canine model carrying a spontaneous homozygous *RPGRIP1* ins44 mutation, subretinal injection of AAV vector expressing canine cDNA an under the control of a human rhodopsin kinase promoter improves photoreceptor function for as long as 24 months.[56] These findings suggest the potential for individuals affected by *RPGRIP1*-LCA to benefit from gene therapy.

Expert Commentary

The aim of gene therapy for LCA is to protect affected children from disabling impairment of sight by correcting the genetic defect responsible. In the last 20 years, progress in the field has led from proof of concept of retinal gene transfer to licensing of the first approved treatment. The ideal gene therapy will promote normal visual development and provide durable benefit in the long term. This will depend on timely intervention by targeted local delivery of vectors that can deliver genes to surviving cells retinal cells at appropriate doses, while protecting against harm from the surgical procedure and immune responses. The development of better validated outcome measures of retinal function in children is required to provide relevant endpoints for clinical trials and to estimate the value of novel therapies. Reliable comprehensive assessment of retinal structure by wide-field high-resolution optimal imaging is required to enable optimal targeting of vector, and to provide a potentially valuable surrogate outcome indicating the potential for protection of sight. Gene therapies developed for children with LCA, like those for other rare diseases, will need to be made available at a cost that is both justified by the benefit to quality of life and affordable to payers.

Five-year review

Positive outcomes of clinical trials of gene therapy for LCA- *RPE65* have led to the recent licensing of a gene therapy product for this indication. The results of ongoing trials will help define the potential window of opportunity for effective intervention. Early intervention, while retinal

structure and cortical plasticity are relatively preserved, is likely to offer the best outcomes. Proof of principle for gene therapy in experimental models will support clinical trials of gene therapy for other forms of LCA. Further developments in vector design and delivery will provide greater efficiency and safety of gene transfer. Rapid reliable assessment of outcomes will be accelerated by optimisation of clinical trial design.

Key Issues

- Leber congenital amaurosis (LCA) is a group of severe recessively-inherited infantile-onset rod-cone dystrophies that result from mutations in at least 25 genes.
- In rodent and canine experimental models, gene augmentation therapy for several causative gene defects can improve retinal function and protect against retinal degeneration.
- Clinical trials of gene therapy for LCA-RPE65 demonstrate benefit with improvement in aspects of sight. The magnitude and durability of benefit in humans may be limited by established degeneration and the potency of current vectors.
- A clinical trial of anti-sense oligonucleotide mediated exon skipping for *CEP290*-LCA is ongoing.
- Efficacy of gene therapy in experimental models of LCA owing to mutations in *AIPL1*, *RDH12*, *GUCY2D and RPGRIP* support its application in affected humans.

- Further developments in vector design and delivery will provide greater efficiency and safety of gene transfer.

Declaration of interests

Alexander Smith, Michel Michaelides, Robin Ali and James Bainbridge declare financial interests in MeiraGTx.

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Tables

LCA subtype	Gene	Vector	Mode	Phase	Current Status	clinicaltrials.gov	Sponsor	Location
LCA-2	*RPE65*	AAV2	Subretinal	III	Ongoing	NCT00999609 NCT01208389	Spark Therapeutics	Iowa and Philadelphia (USA)
LCA-2	*RPE65*	AAV2	Subretinal	I	Ongoing	NCT00516477	Spark Therapeutics	Pennsylvania (USA)
LCA-2	*RPE65*	AAV2/5	Subretinal	I/II	Recruiting	NCT02781480 NCT02946879	MeiraGTx UK II Ltd	London (UK)
LCA-2	*RPE65*	AAV2	Subretinal	I	Unknown	NCT00821340	Hadassah Medical Organization	Jerusalem (Israel)

LCA-2	*RPE65*	AAV2	Subretinal	I	Ongoing	NCT00481546	Unversity of Pennsylvania and NEI	Florida and Pennsylvania (USA)
LCA-2	*RPE65*	AAV2	Subretinal	I/II	Ongoing	NCT00749957	Applied Genetic Technologies Corp	Massachusetts and Oregon (USA)
LCA-2	*RPE65*	AAV2/2	Subretinal	I/II	Completed	NCT00643747	University College London	London (UK)
LCA-2	*RPE65*	AAV2/4	Subretinal	I/II	Completed	NCT01496040	Nantes University Hospital	Nantes (France)
LCA-10	*CEP290*	N/A*	Intravitreal	I/II	Recruiting	NCT03140969	ProQR Therapeutics	Iowa, Pennsylvania (USA) and Ghent (Belgium)

Table 1: Summary of clinical trials for LCA. *RNA antisense oligonucleotides are administered without a vector.