



New gene therapy approach shows promise against Leber's congenital amaurosis

New research has shown that gene therapy may be effective in treating Leber's congenital amaurosis. The findings, by researchers on both sides of the Atlantic, provide further data in support of gene therapy intervention as a viable treatment for patients with certain retinal degenerative diseases over the coming decades.

The recent success resulted from collaboration between researchers at the Berman-Gund Laboratory, Harvard Medical School, and Massachusetts Eye and Ear Infirmary in the United States and the Division of Molecular Therapy, Institute of Ophthalmology, at University College London in the United Kingdom.

Their joint publication appeared in the journal *Investigative Ophthalmology and Visual Science* 2005; 46:3039-3045.

Leber's congenital amaurosis (LCA) is one of the most clinically severe retinal degenerations causing near total blindness in infancy. The disorder results from mutations in any one of eight different genes. Three of the genes are expressed in the retinal pigment epithelium cells; the remainder are expressed in photoreceptor cells.

LCA made the news in 2001 when a team of American scientists working at Cornell University, the University of Pennsylvania and the University of Florida corrected a particular form of the disease caused by mutations in the RPE-65 gene in dogs.

In healthy humans, the RPE-65 gene makes a critically important protein involved in the biochemical visual transduction cascade. When RPE65 is missing, a key component of visual transduction is lost, causing photoreceptor cells in the retina to progressively degenerate. The initial report of successful RPE-65 gene replacement, published in *Nature Genetics*, was greeted with much excitement.

Given the various genes and mutations known to cause LCA, scientists soon began to test if such gene therapy approaches could similarly work for the other genetic mutations involved in LCA.

The current research, led by Dr. Tiansen Li at Harvard and by Dr. Robin Ali at University College London, has focused in on the "RPGRIP" gene. RPGRIP refers to "retinitis pigmentosa GTPase regulator" which is a key component of both the rod and cone photoreceptor cells.

RPGRIP resides in the connecting cilia of these cells which connects the nuclear and outer parts of photoreceptor cells. RPGRIP has been shown to function in

regulating protein traffic across the connecting cilia and as such is critical to the health and correct functioning of the entire retina. Animal models without RPGRIP in their retinas show disorganised and degenerating retinal tissues within 15 days of birth.

In humans, mutations in the RPGRIP gene – located on chromosome 14 – cause severe retinal degeneration and there is currently no therapy available to treat the disorder. The pathology observed in animals, according to the researchers, is similar to that found in humans.

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Viruses as vectors for gene therapy

To try and correct such pathology, the researchers attempted to deliver a functioning copy of the RPGRIP gene to mice without any functional RPGRIP using a modified human virus as a vector. That virus, the adeno-associated virus, or AAV, carries the hopes of an entire technology – gene therapy – aimed at providing therapeutic benefit by introducing copies of functioning genes to diseased tissue or organs.

Viruses occupy a unique branch on the tree of life. Incapable of independent existence, viruses are the ultimate parasites requiring a living cell in order to reproduce and consequently they have become masters at burglary – gaining entry into every cell type on earth and hijacking the biological apparatus within for their own purposes.

It is these stealth qualities that have resulted in viruses becoming the number one tool of choice for gene delivery. Modified in the laboratory, these viruses can be adapted into potent tools for drug delivery.

To date there are at least 8 different serotypes of AAV which exhibit tropisms for different cell types. For example the first serotype of AAV, known as AAV1, demonstrates efficiency at targeting the retinal pigment epithelial cells of the retina whereas serotype 2 or 5 have been shown to be effective in targeting both retinal pigment epithelial cells and photoreceptor cells such as rods and cones. These properties are significant in a clinical context when a specific cell type needs to be accessed if therapeutic benefit is to be achieved. For the current studies aimed at re-introducing RPGRIP the research team used the second serotype of the adeno-associated virus, known as AAV2.

Using a carefully developed procedure, the researchers made a 0.5 mm incision in the cornea and then inserted a 33-gauge blunt needle to target the sub-retinal space in the superior retina. The researchers then injected a volume of functioning copies of the RPGRIP gene with AAV2 into the right eye of each animal and an equivalent volume of saline into the left eye. The animals were then assayed two, three, four, and five months after the injection to record the effects of the treatment.

Essentially the important questions to answer were:

- was RPGRIP expressed in the correct cell type?
- was RPGRIP showing up in the correct part of target photoreceptor cell?
- was the RPGRIP having a beneficial effect on the structure of the retina?
- was there any evidence of physiological benefit; in other words, could the animals actually see any better?

Preservation of retinal structure and function

On all counts, the results proved encouraging. Retinas from treated animals five months after injection of RPGRIP showed positive staining with an RPGRIP antibody that was designed to detect RPGRIP in the right eye but not the left eye. Detailed microscopy additionally indicated that the injected RPGRIP was localised to the connecting cilia of photoreceptor cells.

In terms of retinal structure and architecture, the results indicated that thickness of the outer nuclear layer of photoreceptors increased from as little as 5µm to as much as 35µm, showing an increase from two rows of nuclei in untreated eyes to nearly five rows in

treated eyes, and on some occasion as high as seven rows.

The key test, however, was functional benefit as recorded with standard electroretinography. Here the results were promising but certainly not as dramatic as the histological data. Results indicated that treated eyes had a rate of decline 72% slower in electroretinography amplitude than untreated eyes.

The researchers came up with a number of explanations for this finding. The first explanation was that only a single injection to the superior retina was performed and so probably less than half the retina was exposed to the injected RPGRIP. Secondly, timing was an issue: injections were performed at around days 18 to 20, at which point degeneration had already begun; also, the type of viral vector used generally did not permit RPGRIP expression until three to four weeks after the injection.

Consequently, results might be improved by performing earlier injections with a vector that permitted earlier gene expression. Nevertheless, the results provide considerable cause for optimism in that tweaking of the system should hopefully bring about improved functionality.

Before such gene therapy for LCA can become a reality, however, researchers may need to conduct more tests on the eyes of larger animals because of their valuable anatomic similarities to the human eye. If those results can build on the successes of this current study, researchers may one day face the challenge of efficiently transferring such advances to the clinic.

Glossary

Gene expression: the synthesis of a protein from amino acid subunits as directed by a messenger RNA generated directly from the DNA sequence of a gene.

Parvovirus: the classification name for a family of single stranded DNA viruses that infect vertebrates.

Recombinant AAV: an AAV virus genetically engineered containing genetic material from at least two different sources.

Retinal pigment epithelium (RPE): a layer of cells forming a part of the retinal anatomy which function in the maintenance of the photoreceptor outer segment discs.

Serotype: a variant with distinct antigenic properties.

Transgene: a gene that has been introduced into the genome by genetic manipulation.

Virus vectors: genetically engineered viruses altered to remove pathogenic viral genes and replace them with therapeutic genes for delivery to disease tissues and organs.