

EDITORIAL



Long-Term Effects of Retinal Gene Therapy in Childhood Blindness

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Current ocular imaging techniques provide extraordinarily high-resolution visualization of the retinal layers, amounting to what has been called *in vivo* microscopy. Combine this with a battery of sophisticated retinal-function tests, and the retina becomes unusually accessible in the evaluation of trials of retinal gene therapy for the treatment of conditions such as Leber's congenital amaurosis.

Leber's congenital amaurosis is a group of inherited retinal dystrophies associated with blindness early in life and can be caused by mutations in at least 19 different genes. Loss-of-function mutations in one of these genes, *RPE65*, account for 5 to 10% of recessive Leber's congenital amaurosis.¹ Its translation product is highly abundant in retinal pigment epithelium, where it catalyzes the key isomerase step in the visual cycle, by which 11-*cis* retinal chromophore is regenerated after its light-induced isomerization to all-*trans* retinal.² Unlike persons with most other forms of inherited retinal dystrophy, those with Leber's congenital amaurosis caused by mutated *RPE65* have a disproportionately normal outer retinal structure given the degree of visual loss, and this provides a "window of opportunity" for restoring vision with the use of gene-replacement therapy.³⁻⁵

Two reports in the *Journal* now describe the long-term follow-up of persons with *RPE65*-associated Leber's congenital amaurosis treated by means of subretinal delivery of recombinant adeno-associated virus (rAAV2) vectors; these vectors carry the human *RPE65* complementary DNA driven either by a ubiquitous promoter (which is active in a wide variety of cell types)⁴ or by its native promoter.⁵ Both studies report on phase 1-2 open-label clinical trials (ClinicalTrials

.gov numbers, NCT00481546 and NCT00643747). The first study (conducted in the United States) describes the 5-to-6-year posttreatment follow-up of three patients with *RPE65*-associated Leber's congenital amaurosis (16 to 23 years of age at enrollment) who were given a single extrafoveal subretinal injection into one eye, with the contralateral eye serving as the control.⁴ All three patients had improvement in visual sensitivity in the treated region, which peaked at 1 to 3 years after treatment but, disappointingly, declined thereafter for up to 5 to 6 years after treatment, alongside a continuing loss of photoreceptors. The degeneration continued at much the same rate as in the untreated retina, despite the initial improvement.^{3,4}

The second study (conducted in the United Kingdom) involved 12 patients with *RPE65*-associated Leber's congenital amaurosis (6 to 23 years of age at enrollment) who were treated with a similar rAAV2-*RPE65* vector and single subretinal injection, although in this study the investigators sought to include the fovea, to try to improve central as well as extrafoveal vision.⁵ Six of the 12 treated patients had modest improvements in visual sensitivities, which peaked at 6 to 12 months after injection, but the effect again declined or was lost by 3 years after injection. Neither group reported improvements in visual acuity resulting from foveal function,^{4,5} and no improvement was detected by means of electroretinography (ERG),⁵ a panretinal measure of function, but the results of dark-adapted vision and ambulatory tests were initially improved. The need for an unusually prolonged period of dark adaptation (≥ 4 hours) was previously noted as necessary to achieve maximum rod sensitivity,⁶ which probably indicated an in-

adequate supply of RPE65 and only partial restoration of the visual cycle.

Vector dosage was therefore explored further.⁵ Five of the eight patients given a higher vector dose had improved visual sensitivities, as compared with one of four who received the lower dose. A systematic dose-escalation study using the same vector was then performed in Briard dogs that carried a naturally occurring RPE65 mutation. This showed a strong dose-response relationship: the highest vector dose achieved the most substantial improvements in RPE65 expression, 11-*cis* retinal production, ERG b-wave amplitude, and vision-guided behavior.⁵

Other lessons were learned from these studies, including the dangers of subfoveal injections in humans, which often reduced visual acuity and caused macular thinning, a finding consistent with photoreceptor loss due to the detachment.⁵ Evidence of an immune response to the vector was also found in five of the eight patients who were given a higher vector dose, which suggested that dose-dependent toxicity may limit further dosage increases.

Where does this leave retinal gene therapy for RPE65-associated Leber's congenital amaurosis and almost 200 other potentially treatable inherited retinal disorders (<https://sph.uth.edu/retnet>)? Extensive preliminary work with the use of mouse and dog models of the disease showed that, as long as gene replacement occurs at an early enough stage in the disease, stable and substantial restoration of rod and cone vision can be achieved.⁷ The failure to sustain visual improvement in humans may be due to a greater requirement for RPE65, a more aggressive disease, or the later stage when treatment can be applied. A fall-off in vector expression due to epigenetic or immune factors may also be a contributing factor.^{4,5} The photoreceptor dysfunction in RPE65-associated Leber's congenital amaurosis is clearly caused by the visual-cycle defect, but it is less clear what drives the photoreceptor degeneration. In the *Rpe65*^{-/-} null mouse, the absence of 11-*cis* retinal in rods leads to constitutive activation of the opsin apoprotein, which promotes degeneration, whereas in cones, mislocalization of the opsin apoprotein appears to be a major factor.⁸ There are also profound non-cell autonomous changes in the energetic and metabolic environment of the outer retina after degeneration sets in⁹: when enough photoreceptors are lost, neurotrophic support may be attenuated, and a more

proapoptotic environment could make it effectively impossible to prevent continuing degeneration.

Rescue may be possible, provided there are enough surviving photoreceptors: dogs with RPE65-associated Leber's congenital amaurosis treated at an early stage in the disease process maintain good rescue at more than 7 years after treatment. However, the human disease may necessitate more efficient RPE65 delivery, perhaps with the use of a lentiviral vector or one of the newer rAAV vectors. In principle, the clinically routine intravitreal delivery route would be ideal, if only the outer retina could be transduced efficiently enough, but this has yet to be achieved.¹⁰ In the meantime, both *Journal* reports suggest that without a highly efficient vector delivery system and sufficient surviving retinal pigment epithelium and photoreceptors, treatment success will be transient.

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