

EDITORIAL



Preliminary Results of Gene Therapy for Retinal Degeneration

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In this issue of the *Journal*, two groups of investigators — Bainbridge et al.¹ and Maguire et al.² — describe the first results of separate clinical trials investigating the short-term safety and preliminary efficacy of gene therapy for Leber's congenital amaurosis. Both groups present short-term data (12 months and 5 months, respectively) on three patients with Leber's congenital amaurosis in each study; the patients were enrolled in trials of recombinant adeno-associated viral delivery of the human retinal pigment epithelium-specific 65 kDa protein gene (*RPE65*), which was administered as a subretinal injection during vitrectomy.

Originally described by Leber in 1869, Leber's congenital amaurosis has the earliest onset of all inherited retinal dystrophies causing congenital blindness; it is also the most severe form.³⁻⁵ Infants with Leber's congenital amaurosis have profound visual impairment or blindness at birth. However, the visual impairment is usually recognized only later when parents note the infant's inability to track objects or light. Severe visual impairment persists throughout childhood, resulting in an inability to read or ambulate independently, and finally in total blindness by the third or fourth decade of life. Other findings include nystagmus (roaming eye movements), abnormal pupillary responses, and flat or nearly undetectable signals on electroretinography (ERG). The appearance of the retina is normal early on but progresses to a pigmentary retinopathy over time. There is no treatment for Leber's congenital amaurosis.

Most cases of Leber's congenital amaurosis are inherited in an autosomal recessive fashion, and disease-associated mutations have been found in seven genes expressed preferentially in the pho-

toceptor or retinal pigment epithelial (RPE) cell.⁴ Mutations in *RPE65* account for approximately 6% of cases.^{4,6} *RPE65* encodes an enzyme in the RPE that catalyzes the conversion of all-*trans*-retinyl esters to 11-*cis*-retinal. The latter is the precursor of rhodopsin, which is required for phototransduction and vision. The biochemical defect that is associated with the *RPE65* mutation leads to immediate, severe vision impairment with subsequent degeneration of the rods and cones.

Animal models of Leber's congenital amaurosis exist, including a strain of Briard dogs with a spontaneous defect in *RPE65*. Proof-of-principle experiments restored visual function in dogs treated with subretinal adeno-associated virus (AAV) carrying *RPE65* complementary DNA.⁷ Visual function has remained stable for almost 8 years after a single treatment.

Both Bainbridge et al. and Maguire et al. used recombinant AAV in their clinical trials. AAV was selected because it is noninfectious. However, it can elicit an immune response, since most people have been exposed to AAV and may carry antibodies. Both groups therefore administered perioperative local and systemic corticosteroids and observed no significant inflammation in treated patients. Although both groups delivered DNA coding for human *RPE65*, Bainbridge et al. used a tissue-specific promoter to limit expression to RPE and excluded patients with null mutations to minimize the possibility of an immune response to the transgene product. Maguire et al. did not use a tissue-specific promoter, having demonstrated effective transgene expression in RPE after subretinal injection in animal studies, as well as some expression in optic nerve, chiasm, and brain.^{7,8} Maguire et al. did not exclude patients with null mutations, although the re-

ported patients all had missense mutations. Maguire et al. used surfactant to reduce adsorption of viral particles to the contact surfaces of delivery devices and processed the vector to remove empty capsid (which reduced empty capsid from >80% to <2%) in order to increase the potency and minimize immunogenicity to capsid proteins. They injected a vector volume of 0.15 ml, and Bainbridge et al. injected approximately 1 ml; the difference in volume is probably related to the more rapid resolution of the localized retinal detachment observed by Maguire et al. (14 hours vs. 2 to 3 days).

In both these studies, patients had severe vision loss secondary to Leber's congenital amaurosis that was documented by visual acuity and ERG, as well as by microperimetry, which measures retinal sensitivity at precise locations (carried out by Bainbridge et al.), and pupillary light reflex, measured by pupillometry (carried out by Maguire et al.). Bainbridge et al. showed no change in patients' visual acuity, whereas all three patients in the study by Maguire et al. found a gain in visual acuity. Visual acuity is a subjective measure, and since the patients could not be masked in either study, there is certainly a possibility of placebo effect for this outcome measure. Furthermore, measurement of visual acuity at such low levels of acuity is not reliable. The fact that three patients in the study by Maguire et al. showed improvement and the three patients in the study by Bainbridge et al. did not show improvement is of uncertain significance. With additional subjects and longer follow-up, this outcome may prove more informative, particularly if continued safety justifies the inclusion of patients with better baseline visual function.

Bainbridge et al. observed improvement in both daylight and dark-adapted microperimetry (consistent with improved visual function) in one of three patients. Maguire et al. observed visual-field improvement using Goldmann perimetry and decreased nystagmus after treatment in all three patients. ERG responses were extremely low or undetectable in patients in both studies at baseline and remained unchanged after treatment. Both studies showed some improvement in navigational testing in at least one patient. This outcome measure has yet to be proven as an accepted measure of vision function.

Maguire et al. tested the pupillary light reflex using pupillometry as an objective measure of reti-

nal function and found improvement in each of the treated eyes. The pupillary light reflex is a consensual response in that a stimulus to either eye will cause both pupils to contract similarly. Fundamentally, it is a measure of the amount of signal input from photoreceptors and light-sensitive ganglion cells conveyed through the afferent arc to the brain, with the output driving bilateral pupillary constriction. The pupillary light reflex measures photoreceptor input if the test is limited to studying the early-phase response and uses low-intensity light.⁹ Such responses in patients with Leber's congenital amaurosis are diminished, consistent with decreased photoreceptor input into the afferent arc of the reflex. Maguire et al. found that the pupillary light reflex in treated patients became asymmetric, consistent with a marked increase in signal input from the retina of the treated eye. These objective data are very useful in monitoring the outcome of intervention in this group of patients with retinal degenerations and severely limited vision function, in which changes in more standard clinical outcomes (visual acuity and visual fields) are difficult to quantify.

Maguire et al. observed the development of a macular hole in one patient, which they believed was caused by contraction of a preexisting epiretinal membrane after surgery. Alternatively, as the authors note, subretinal injections in atrophic retina may cause complications, and this observation warrants further study. Given the limited central vision in the enrolled patients, the hole was of no clinical significance; however, it would be clinically significant in patients with better baseline retinal function. Both groups of investigators followed patients with optical coherence tomography, which provides some retinal anatomic detail. High-resolution spectral-domain optical coherence tomography provides greater resolution of the retinal-cell layers before and after treatment; this approach should be considered as these studies move forward and in future studies.¹⁰

The preliminary results from these investigations suggest that in the short term, the procedure is safe. Moreover, the data are suggestive of efficacy. Both groups recognize that longer follow-up and additional subjects are necessary to provide satisfying safety data. Certainly, efficacy data will be available only in larger trials. Some of the remaining issues include the repro-

ducibility and persistence of the improved retinal function and whether further retinal degeneration is delayed or averted. In addition, systemic or ocular complications may yet be encountered with the treatment of additional patients, with higher doses of vector, and with longer follow-up. One might also speculate that treatment of younger patients with less advanced retinal degeneration might allow greater improvement of visual function. Finally, larger studies may show advantages of a particular vector preparation, promoter selection, or technique.

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