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Improvement and Decline in Vision with Gene Therapy in Childhood Blindness

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Summary

Retinal gene therapy for Leber's congenital amaurosis, an autosomal recessive childhood blindness, has been widely considered to be safe and efficacious. Three years after therapy, improvement in vision was maintained, but the rate of loss of photoreceptors in the treated retina was the same as that in the untreated retina. Here we describe long-term follow-up data from three treated patients. Topographic maps of visual sensitivity in treated regions, nearly 6 years after therapy for two of the patients and 4.5 years after therapy for the third patient, indicate progressive diminution of the areas of improved vision.

Leber's congenital amaurosis, a childhood-onset autosomal recessive blindness that is known to be caused by mutations in at least 19 different genes, was considered to be untreatable and incurable until 2008, when gene therapy was successfully developed for patients with the disease caused by mutated *RPE65*. The gene encoding RPE65 (retinal pigment epithelium-specific protein 65 kDa), a key enzyme of the retinoid cycle of vision, was introduced into the retina of patients with *RPE65*-associated Leber's congenital amaurosis in three independent but contemporaneously conducted clinical trials. In all the trials, the safety and efficacy of the treatment was announced within a few months after the initiation of therapy, which consisted of a single administration of the vector containing *RPE65* to each patient.^{1–3} The studies were heralded as landmarks in the field of gene therapy.⁴

However, the therapeutic response, even in the short term, was complex: the restored enzymatic cycle had dramatically slowed kinetics, which complicated outcome measures and the usefulness of the increased night vision.⁵ Central visual acuity was not substantially altered by the therapy, but cone photoreceptors outside the central retina could show

dramatic improvement.^{5,6} In 2013, we determined the rate of photoreceptor-cell loss in the treated retinas versus the untreated retinas of 11 patients who had received vector containing *RPE65* at one or two injection sites. Although improved vision was maintained in the patients, the photoreceptors showed progressive degeneration.⁷ A case was made for a combinatorial approach in which neuroprotective agents would be used along with gene therapy to slow the degenerative disease that is present in patients of any age with this condition.^{8,9}

Here, we report analyses of vision over a period of 5 to 6 years after treatment in a subset of three treated patients from our earlier study.⁷ In these patients, the treated retina showed improved visual sensitivity, which slowly increased in area and then contracted.

Methods

Case Reports

Three patients with a clinical diagnosis of Leber's congenital amaurosis and mutations in *RPE65* (R44Q/R91W in Patient 1, Y368H/Y368H in Patient 2, and V287F/V287F in Patient 3) were enrolled in our clinical trial of retinal gene therapy, after providing written informed consent. The ages of the patients at enrollment were 23, 21, and 16 years, respectively. Their previously published patient numbers were P2, P3, and P8.^{3,5-7} The criteria for including these patients in the current analyses were as follows: 4.5 years or more had passed since their treatment, they had a single subretinal administration of vector gene (AAV2-*RPE65*) in the extrafoveal retina of one eye, they had no postoperative complications, they had no ocular media opacities (e.g., cataract) before or after treatment, they retained foveal fixation,⁶ and they had the ability to undergo psychophysical testing and retinal imaging throughout the post-treatment period. Of the 15 patients who were included in our earlier study,³ only the 3 patients we describe here met all the inclusion criteria.

Topography of Visual Sensitivity

After an extended period of dark adaptation (>4 hours), visual sensitivity was determined on a 12-degree grid with additional loci at 2-degree intervals along horizontal or vertical meridians. The stimuli used were achromatic (1.7-degree diameter, 200 msec duration) with a maximum luminance of 10,000 apostilbs, and sensitivities are reported in log₁₀ units of attenuation from the maximum luminance.⁵⁻⁷ There was good correspondence between the loci in which sensitivities were substantially increased after treatment and the region of retinal detachment from the subretinal injection.⁶ To allow for uniform analysis across visits, we used bivariate linear interpolation to derive a surface on a regularly spaced grid covering the full visual field. Contours at three sensitivity levels (chosen to be higher than the 99th percentile of test-retest variability above baseline⁵) were extracted for a rectangular, patient-specific subregion showing treatment effect. We evaluated the extent of retinal function at each sensitivity level as the planar area of the corresponding contour. The planar areas were plotted against time after treatment. Variability in sensitivity measures that is specific to *RPE65*-associated Leber's congenital amaurosis⁵ was used in Monte Carlo simulations to estimate the variability expected from the planar area calculations at each time point.

Topography of Photoreceptor Layer Thickness

Retinal cross sections were obtained with a spectral-domain optical coherence tomography (OCT) system (RTVue-100, Optovue). Postacquisition data analysis was performed with custom programs (MatLab, version 7.5; MathWorks).⁷ Photoreceptor outer-nuclear-layer thickness was quantified manually with the use of both signal intensity and slope information⁷ and with consideration of the complicating hyperscatter originating from the Henle fiber layer.¹⁰ For topographic analysis of the outer nuclear layer, first the precise location and orientation of each OCT scan relative to anatomical landmarks (blood vessels and optic disc) were determined by registering images of integrated backscatter intensity calculated from each raster OCT scan within larger en face images of near-infrared reflectance. Next, longitudinal reflectivity profiles making up the OCT scans were allotted to regularly spaced bins in a rectangular coordinate system centered at the fovea; the waveforms in each bin were aligned, averaged, and segmented. Lastly, the measured outer-nuclear-layer thicknesses were mapped to a pseudocolor scale, missing data were interpolated bilinearly, and landmarks were overlaid for reference. The thickness of the outer nuclear layer in patients was divided locus-by-locus by the mean normal value (representing data from three persons without Leber's congenital amaurosis; age range, 23 to 45 years) to calculate the outer-nuclear-layer fraction. Rates of thinning of the outer nuclear layer over time were estimated on the basis of an exponential progression of degeneration,⁷ which has previously been found to explain the time course of loss of photoreceptors or retinal function.

Results

Changes in Visual Sensitivity over Time

Serial visual maps from baseline (before treatment) to nearly 6 years after treatment (for Patients 1 and 2) and from baseline until 4.5 years after treatment (for Patient 3) are shown in Figure 1A. Baseline maps are shown as full visual fields for the treated eye. The region that was injected with the vector gene was magnified to enable visualization of the contours at the various time points sampled after treatment. Patient 1 had a visual response at 6 months; the area of enhanced visual sensitivity increased until 3 years after treatment, when the contour representing 5 log units of increased sensitivity reached its maximal peak area. The area of this contour diminished at later time points. Patient 2 also had a visual response at 6 months; visual sensitivity peaked between 1 and 3 years after treatment and then diminished. Patient 3 had an increase of 3 log units in visual sensitivity, which peaked 1 year after treatment and then declined, although patches of increased visual sensitivity (as compared with baseline) remained at 4.5 years after treatment, the latest time point assayed.

Areas of improved visual sensitivity, although present at 6 months in all three patients (and detectable before that time⁵), expanded slowly — over a period of 3 years in Patient 1, over a period of 1 to 3 years in Patient 2, and over the course of a year in Patient 3 (Fig. 1B). We observed a subsequent decline in the area of improved sensitivity in all patients; the decline in Patient 3 occurred earlier than in the other patients.

Relationship between Visual Sensitivity and the Outer Nuclear Layer

We determined the topographic relationship between visual function and photoreceptor structure in part of the treatment zone in Patients 1 and 3 (Fig. 2A). (Data for Patient 2 were available at 1 month and later time points and only unidimensionally along the horizontal meridian.) Topographic maps of the thickness of the outer nuclear layer in Patients 1 and 3, across a wide extent of central retina that included part of the superior treated zone, are shown in Figure 2A. At baseline, Patient 1 had a relatively thicker outer nuclear layer than did Patient 3. During the extended follow-up period, the thickness of the outer nuclear layer in both patients showed generalized reductions in treated and untreated regions.⁷ As shown in Figure 2B (upper graphs), there were early post-treatment increases in light sensitivity of 2.6, 1.6, and 0.6 log units in Patients 1, 2, and 3, respectively, followed by slower increases of approximately 1 log unit, peaking between 1 and 3 years after treatment. Thereafter, there was a slow diminution of sensitivities by approximately 0.8 log unit. The thickness of the outer nuclear layer continued to decrease steadily throughout the long post-treatment period while the complex changes in sensitivity were occurring. The yearly rate of protracted loss of sensitivity after the peak response was substantially greater than that predicted on the basis of photoreceptor degeneration in these patients or from the natural history of retinal degeneration in a large cohort of patients with *RPE65*-associated Leber's congenital amaurosis (Fig. 2B, lower graph).

Discussion

There is consensus among the clinical trials of gene therapy for *RPE65*-associated Leber's congenital amaurosis that visual gain is detectable within the first month after treatment,^{1-3,5,11} and there is persistence of efficacy at 1 year¹² and 3 years.^{6,7,13} Two-dimensional mapping of sensitivity across the injected retina has not been used previously to assay efficacy. Unexpectedly, the early improvement, which has been reported as stable,^{6,7,12,13} actually shows a slow expansion of extent during a period of 1 to 3 years and then undergoes a contraction.

It is likely that the abrupt early improvement within days to weeks after treatment is due to partial reconstitution of the canonical retinoid cycle within the retinal pigment epithelium. Slower expansion of the extent of improvement over months to years may be due to lower gene expression at the edges of the localized retinal detachment created by the subretinal injection (bleb), resulting from a combination of the lateral spread of the vector beyond the initial detachment¹⁴ and less opportunity for transfection at shallower parts of the detachment that reattach faster. Alternatively (or in addition), recovery of the photoreceptor-retinal pigment epithelium interface¹⁵ after the detachment may be delayed near the edges of the initial bleb. Previously, qualitative evidence of spatial variation in *RPE65* expression within the bleb has been presented.¹⁶ It is not known whether the expansion of the area of visual sensitivity in patients is driven by rod-based vision, cone-based vision, or both. In theory, it is possible that cones have two phases of improvement, which is consistent with the regeneration of pigment from two distinct retinoid cycles.¹⁷

Why would the area of the retina that shows improved sensitivity slowly contract? We previously speculated that healthier photoreceptors survived in the treated retina, whereas

others, perhaps in a preapoptotic cellular stress state, degenerated.⁷ The loss of visual function at later times after treatment is in keeping with progression of the degenerative process. Our observation that the late decrease in visual sensitivity was greater than would be expected, given the thickness of the outer nuclear layer, suggests that the visual cycle may no longer be functioning at the same level as at earlier time points. The basis for this is unknown, but there may be declining transgene expression leading to reduced metabolic capacity of the supporting retinal pigment epithelial cells that could result in their loss, followed by photoreceptor loss. In addition, the continued reduction in the number of photoreceptors in spite of the therapy may eventually lead to a loss of trophic support for the photoreceptors that initially had a response to the therapy.

RPE65-associated Leber's congenital amaurosis now joins the many medical diseases for which the consideration of treatment algorithms may be helpful in initiating a dialogue about the emerging complexity and optimization of management.^{18,19} Given the relatively small number of patients with this form of the disease,²⁰ we suggest that independent investigators form a consortium to share data and develop phenotyping scales and treatment protocols. This is especially important not only because of the findings of the current study but also because another study, published in this issue of the *Journal*, describes improvement and then decline in vision within 3 years after treatment,²¹ whereas a different trial has reported stable efficacy at 3 years.¹³

A decision tree for *RPE65*-associated Leber's congenital amaurosis could begin with disease identification at clinical and molecular levels, leading to the prediction of prognosis on the basis of the stage of severity of retinal degeneration at diagnosis. It is important to note that severity is not simply related to the patient's age.^{6,9} Disease staging can be performed noninvasively with OCT. From OCT maps, such as those presented in Figure 2A, the mean thickness of the photoreceptor layer can be calculated, and the patient's condition can be classified as mild, moderate, or severe. Such staging is analogous to a tissue biopsy, and the "pathology report" is the segmented OCT from a wide area of retina with topographic measurement of the photoreceptor layers. The life expectancy of photoreceptors can be estimated from the delayed exponential model of the disease.⁷

In conclusion, we report the longer-term effects of retinal gene therapy for *RPE65*-associated Leber's congenital amaurosis. There were fast and slow phases of improvement in vision, as well as a subsequent decline, all overlaid on a progressive, degenerative loss of photoreceptor cells.

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Figure 1. Time Course of the Changes in Vision after Gene Therapy

Panel A shows maps of visual sensitivity of the treated eye in three patients at baseline (full visual field) and at different times after treatment (in enlarged views of the treated region); time labels for the last two columns have been rounded. All log values are base 10. After an extended period of dark adaptation (>4 hours), visual sensitivity was determined on a 12-degree grid with additional loci at 2-degree intervals along horizontal or vertical meridians; in this figure, the increments shown along the axes at baseline denote 24 degrees. Contours delineate regions of equal sensitivity. Note that the color-scale ranges and contour-sensitivity levels have been expanded for the enlarged views of Patient 3. Ocular fundus

landmarks (optic nerve and central retinal vessels) are drawn schematically on the full visual field maps for reference. F denotes fovea. Panel B shows plots of the area of visual sensitivity (the area of contours shown in Panel A) during the post-treatment period. The area was quantified at 44.5, and 5 log sensitivities for Patients 1 and 2 and at 22.5, and 3 log sensitivities for Patient 3. Error bars (denoting 1 SD) were calculated on the basis of variability in sensitivity measures that is specific to *RPE65*-associated Leber's congenital amaurosis.⁵

Figure 2. (facing page). Relationship of Photoreceptor Structure to Vision

Panel A shows the outer-nuclear-layer thickness topography of Patient 1 and Patient 3 at baseline and at 5.7 or 4.5 years after treatment. Treated regions, delimited by the availability of data regarding colocalized sensitivity and photoreceptor-layer thickness, are outlined. For a comparison with patient data, a map representing the mean outer nuclear layer (ONL) from three normal controls is shown (upper right). Panel B (upper graphs) shows the time course of change in sensitivity (log unit change from baseline) and ONL thickness (fraction of normal mean) within the treated regions. The lower graph compares the observed rates of

slow change in sensitivity (occurring after 1 month) with that expected from the change in the ONL over two distinct periods. In the earlier period, there is an increase in sensitivity despite a decrease expected from the loss of photoreceptors. In the later period, there is a decrease in sensitivity as expected from continued loss of photoreceptors, but the rate of the loss of sensitivity exceeds that predicted from the change in the outer nuclear layer. The rates of change in the outer nuclear layer in the patients are similar to the mean rate reported previously for a cohort of patients with *RPE65*-associated Leber's congenital amaurosis (*RPE65*-LCA cohort).⁷ The significance of the differences between the observed and expected rates were assessed with the use of Student's t-test.