Objective: To determine the safety and efficacy of subretinal gene therapy in the RPE65 form of Leber congenital amaurosis using recombinant adeno-associated virus 2 (rAAV2) carrying the RPE65 gene.

Design: Open-label, dose-escalation phase I study of 15 patients (range, 11-30 years of age) evaluated after subretinal injection of the rAAV2-RPE65 vector into the worse-functioning eye. Five cohorts represented 4 dose levels and 2 different injection strategies.

Main Outcome Measures: Primary outcomes were systemic and ocular safety. Secondary outcomes assayed visual function with dark-adapted full-field sensitivity testing and visual acuity with Early Treatment Diabetic Retinopathy Study charts. Further assays included immune responses to the vector, static visual fields, pupillometry, mobility performance, and optical coherence tomography.

Results: No systemic toxicity was detected; ocular adverse events were related to surgery. Visual function improved in all patients to different degrees; improvements were localized to treated areas. Cone and rod sensitivities increased significantly in the study eyes but not in the control eyes. Minor acuity improvements were recorded in many study and control eyes. Major acuity improvements occurred in study eyes with the lowest entry acuities and parafoveal fixation loci treated with subretinal injections. Other patients with better foveal structure lost retinal thickness and acuity after subfoveal injections.

Conclusions: Gene therapy for Leber congenital amaurosis caused by RPE65 mutations is sufficiently safe and substantially efficacious in the extrafoveal retina. There is no benefit and some risk in treating the fovea. No evidence of age-dependent effects was found. Our results point to specific treatment strategies for subsequent phases.

Application to Clinical Practice: Gene therapy for inherited retinal disease has the potential to become a future part of clinical practice.

Trial Registration: clinicaltrials.gov Identifier: NCT00481546

response data, and toxicity testing.\textsuperscript{12,14} Human gene therapy in RPE65-related LCA seemed to be the next worthy step, and clinical trials began.

Early results of 3 contemporaneous human clinical trials were reported in 2008, and these preliminary results showed safety and modest efficacy after subretinal injections of adeno-associated virus 2 (AAV2) carrying RPE65.\textsuperscript{15-18} More recently, a fourth trial was initiated, and early results published.\textsuperscript{19} Questions about the longevity of the safety and efficacy in gene therapy for RPE65-related LCA have started to be addressed.\textsuperscript{20-22} Beyond the initial increases in visual sensitivity after treatment, we detected a slow and progressive movement of fixation over many months from the anatomical fovea to the treated retinal region. The region of therapy had become a preferred locus for use in this eye under certain conditions, suggesting cortical adaptations to the restored vision.\textsuperscript{24}

We have now performed gene vector administration in 5 cohorts of patients, representing children and adults, in a dose-escalation study using a single subretinal injection in the first 3 cohorts and 2 injections in the same eye at the time of surgery in the last 2 cohorts. Safety and efficacy results from our initially reported cohort\textsuperscript{17,18} now represent a 3-year interval since treatment. These results of relatively long-term follow-up, taken together with those from patients with shorter-term follow-up, lead to a perspective on how best to advance this trial and other retinal gene therapy clinical trials.

### METHODS

The clinical trial was performed at Scheie Eye Institute of the University of Pennsylvania in Philadelphia and at Shands at the University of Florida in Gainesville. The subjects had a clinical diagnosis of LCA. RPE65 mutations were determined at the John and Marcia Carver Nonprofit Genetic Testing Laboratory (at the University of Iowa in Iowa City). Study eligibility and protocol; regulatory approvals and oversight; Good Manufacturing Practice vector production, purification, and titration; and surgical procedure for vector administration have been reported in earlier studies of cohort 1.\textsuperscript{17,18,20} The conduct of the trial was in a manner consistent with the ICH E6 Good Clinical Practice guideline document and was reviewed by the US Food and Drug Administration (Investigational New Drug application BB-IND 12824) and the National Institutes of Health Recombinant DNA Advisory Committee (protocol 0410-677). Approvals were obtained from the institutional review boards and institutional biosafety committees of the University of Pennsylvania and the University of Florida, the Vice Provost Research Review Committee of the University of Pennsylvania, the Western Institutional Review Board, and the General Clinical Research Center of the University of Florida. A Data and Safety Monitoring Committee, appointed by the National Institutes of Health, monitored the trial. The tenets of the Declaration of Helsinki were followed. Informed consent or assent was obtained from all subjects. Brief summaries of the methods are given, and expanded methods can be found in the eAppendix (http://archophthalmol.com).

### VECTOR ADMINISTRATION

The eye with worse visual function was chosen for vector administration in all subjects except patient 10 (Table 1). Both eyes of patient 10 were severely affected, but the eye with worse function had keratoconus, and the contralateral eye was chosen for the procedure. Two methods of anesthesia were used. For the first 2 cohorts (6 patients aged 20-30 years), the procedure was performed with retrobulbar anesthesia. For the subsequent 3 cohorts (9 patients) that included 6 patients younger than 18 years of age, general anesthesia was used. A standard 3-port 23-gauge vitrectomy was performed in cohorts 1, 2, and 3 as previously described\textsuperscript{17}; in cohorts 4 and 5, a 25-gauge vitrectomy was performed. The vector was introduced into the subretinal space with a 39-gauge injection cannula (Synergetics Inc, O’Fallon, Missouri). A single injection was used in the first 3 cohorts, whereas 2 injection sites were used in the last 2 cohorts (Table 1).

### OCULAR AND SYSTEMIC SAFETY PARAMETERS

Ocular safety was assessed with standard eye examinations. To quantify severity of inflammatory response, standard grading systems were used.\textsuperscript{23-27} To document fundus appearance, fundus photographs (using an infrared camera to avoid excess visible light exposure) were taken at baseline and at posttreatment visits. Systemic safety was evaluated with physical examinations at baseline and postoperative visits. Routine hematology; testing of serum chemistry, prothrombin time (with international normalized ratio), and partial thromboplastin time; and urinalysis were performed at baseline and postoperatively. The schedule of study visits and list of measured parameters for each time point from baseline to 3 years after the operation have been published.\textsuperscript{17} In all safety and efficacy studies, the examiners were not masked to which eye was the study eye.

### IMMUNOLOGY PARAMETERS

Serum samples from the patients were assayed for circulating antibodies to AAV2 capsid proteins at baseline, at days 14 and 90, and at years 1, 2, and 3. Details of the testing process were previously described.\textsuperscript{17} Anti-AAV2 antigen-specific lymphocyte proliferation responses were assessed as previously described.\textsuperscript{17} Procedures for biodistribution of patient samples (AAV DNA in peripheral blood) in this trial were previously described.\textsuperscript{17} Details of blood collection and the interferon-γ (IFN-γ) enzyme-linked immunosorbent assay were described for cohort 1 patients.\textsuperscript{17} Further details are provided for all immunology parameters (eAppendix).

### EFFICACY PARAMETERS

Best-corrected visual acuity (VA) was measured using Early Treatment Diabetic Retinopathy Study methodology\textsuperscript{28} at all baseline and postoperative visits.\textsuperscript{17,20} Best-corrected VA was scored as the number of letters correctly read after adjusting for distance and expressed as logMAR. The fixation locus of each eye at each visit was determined by video imaging the retina under invisible near-infrared light (MP1; Nidek Incorporated, Fremont, California) while the subject was gazing at a target.\textsuperscript{18,20} Further details are provided in the eAppendix.

Full-field stimulus testing (FST) was performed using a LED-based Ganzfeld stimulator (ColorDome; Diagnosys LLC, Littleton, Massachusetts) as previously described.\textsuperscript{34,35} In brief, blue and red stimuli were used for testing monocularly under 2 dark-adapted conditions: a standard dark adaptation of less than 2 hours and an extended dark adaptation of greater than 3 hours. The latter condition draws from our previous observations of prolonged kinetics of rod but not cone dark adaptation in RPE65-related LCA treated with gene therapy.\textsuperscript{10} Further details are provided in the eAppendix.
Table 1. Results of Clinical Trial for RPE65-Related Leber Congenital Amaurosis

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age at Baseline, y</th>
<th>RPE65 Mutations</th>
<th>Follow-up, mo</th>
<th>Anesthesia</th>
<th>Eye/Injection Sites, No.</th>
<th>Vector Dose, Log genomes</th>
<th>Total Volume, µL</th>
<th>Subfoveal Injection</th>
<th>Entry VA, logMAR a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/M/24</td>
<td>E417D/E417Q</td>
<td>24</td>
<td>L</td>
<td>Left/1</td>
<td>5.96 × 10^10</td>
<td>150</td>
<td>Yes</td>
<td>1.16, 1.00</td>
</tr>
<tr>
<td>P2/F/23</td>
<td>R440G/R91W</td>
<td>36</td>
<td>L</td>
<td>Right/1</td>
<td>5.96 × 10^10</td>
<td>150</td>
<td>No</td>
<td>1.04, 0.94</td>
</tr>
<tr>
<td>P3/M/21</td>
<td>Y368H/Y368H</td>
<td>36</td>
<td>L</td>
<td>Right/1</td>
<td>5.96 × 10^10</td>
<td>150</td>
<td>No</td>
<td>1.24, 1.06</td>
</tr>
<tr>
<td>Cohort 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4/M/30</td>
<td>G405H/I182Y</td>
<td>24</td>
<td>L</td>
<td>Left/1</td>
<td>11.92 × 10^11</td>
<td>300</td>
<td>Yes</td>
<td>1.72, 1.78</td>
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<tr>
<td>P5/F/20</td>
<td>97del20bp/97del20bp</td>
<td>24</td>
<td>L</td>
<td>Left/1</td>
<td>11.92 × 10^11</td>
<td>300</td>
<td>No</td>
<td>1.92, 2.00 b</td>
</tr>
<tr>
<td>P6/F/22</td>
<td>L341S/L341S</td>
<td>24</td>
<td>L</td>
<td>Left/1</td>
<td>11.92 × 10^11</td>
<td>300</td>
<td>Yes</td>
<td>1.48, 1.34</td>
</tr>
<tr>
<td>Cohort 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7/F/15</td>
<td>IVS1+1G/T/IVS1+1G&gt;T</td>
<td>18</td>
<td>G</td>
<td>Left/1</td>
<td>8.94 × 10^10</td>
<td>225</td>
<td>1.18, 1.04</td>
<td></td>
</tr>
<tr>
<td>P8/F/16</td>
<td>V287F/V287F</td>
<td>18</td>
<td>G</td>
<td>Left/1</td>
<td>8.94 × 10^10</td>
<td>225</td>
<td>0.90, 0.84</td>
<td></td>
</tr>
<tr>
<td>P9/M/11</td>
<td>IVS2-2A&gt;T/IVS31S</td>
<td>12</td>
<td>G</td>
<td>Left/1</td>
<td>8.94 × 10^10</td>
<td>225</td>
<td>1.02, 0.92</td>
<td></td>
</tr>
<tr>
<td>Cohort 4</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P10/M/24</td>
<td>R91W/R91W</td>
<td>6</td>
<td>G</td>
<td>Right/2</td>
<td>17.88 × 10^10</td>
<td>450</td>
<td>Yes</td>
<td>1.50, 1.48</td>
</tr>
<tr>
<td>P11/M/27</td>
<td>V353ins1bp/R91W</td>
<td>6</td>
<td>G</td>
<td>Right/2</td>
<td>17.88 × 10^10</td>
<td>450</td>
<td>No</td>
<td>0.32, 0.34</td>
</tr>
<tr>
<td>Cohort 5</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P12/M/15</td>
<td>IVS2-2A&gt;T/IVS31S</td>
<td>6</td>
<td>G</td>
<td>Right/2</td>
<td>17.88 × 10^10</td>
<td>450</td>
<td>No</td>
<td>0.64, 0.58</td>
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<tr>
<td>P13/F/11</td>
<td>R91W/R91W</td>
<td>3</td>
<td>G</td>
<td>Left/2</td>
<td>17.88 × 10^10</td>
<td>450</td>
<td>Yes</td>
<td>1.04, 0.94</td>
</tr>
<tr>
<td>P14/F/17</td>
<td>IVS1+5G&gt;AY239D</td>
<td>3</td>
<td>G</td>
<td>Right/2</td>
<td>17.88 × 10^10</td>
<td>450</td>
<td>No</td>
<td>1.06, 0.88</td>
</tr>
<tr>
<td>P15/F/18</td>
<td>Y368H/K354ins1bp</td>
<td>1</td>
<td>G</td>
<td>Right/2</td>
<td>7.95 × 10^10</td>
<td>200</td>
<td>No</td>
<td>0.72, 0.62</td>
</tr>
</tbody>
</table>

Abbreviations: G, general; L, local; VA, visual acuity.

a Best-corrected VA measured from back-lit Early Treatment Diabetic Retinopathy Study charts adjusted for distance (0.00 logMAR = 20/20 Snellen VA at 4 m).
b Baseline 1, baseline 2.
c No letters seen at 0.5 m.
d Intended dose was 17.88 × 10^10 vector genomes in 450 µL, but only 200 µL could be delivered.

Dark-adapted static visual field testing with computerized perimetry was able to be performed in 12 of 15 patients; 3 patients could not distinguish where in the visual field a light stimulus originated. A modified automated perimeter (Humphrey Field Analyzer; Zeiss Meditec Inc, Dublin, California) was used.18,20,32,33 To summarize the data, maps were constructed of loci showing significant sensitivity changes postoperatively. Further details are provided in the eAppendix.

The direct transient pupillary light reflex (TPLR) was elicited and recorded as previously described.3,18,34 Luminance-response functions were derived from the TPLR amplitude to increasing intensities (from −6.6 to 2.3 log units-candela m−2) of green stimuli with short duration (0.1 seconds) presented monocularly in the dark-adapted state. The TPLR luminance-response functions were recorded during the short-term postoperative time points at 1 month (patients 2, 3, 13, 14, and 15), at 3 months (patient 1, patients 4-10, and patient 12), or at 6 months (patient 11). Further details are provided in the eAppendix.

A mobility performance task was used to quantify the ability of the patients to move through an indoor obstacle course and determine whether there was any difference in this behavior before and after treatment (5 patients) or between treated and untreated eyes postoperatively (15 patients). The task is a version of published methods35-40 and further details can be found in the eAppendix.

**STATISTICAL ANALYSES**

Differences between baseline and postoperative values of efficacy parameters (FST blue, FST red, TPLR, and VA) were evaluated for control and study eyes with paired, 2-sided t tests. Results from multiple visits within baseline and postoperative time points were averaged before performing the t tests. A repeated-measures analysis of variance (ANOVA) was performed with a test of interaction comparing the magnitudes of differences between treated and control eyes. The repeated-measures factors included were eyes (study vs control) and visits (baseline vs postoperative). The between-subjects factors included in the ANOVA were age (11-20 years [younger] or 21-30 years [older]), for the analyses of all parameters, and fixation (foveal vs extrfoveal), for the analysis of VA. All group statistics are specified as mean (SE).

**OPTICAL COHERENCE TOMOGRAPHY**

Cross-sectional imaging with optical coherence tomography (OCT) was used to assess retinal structure before and after administration of the agent in the treated eye; comparable data were also acquired in the untreated eye. Ultra–high-speed and high-resolution OCT imaging with a spectral-domain OCT instrument (RTVue-100; Optovue Inc, Fremont, California) was used, as previously described.17,20 Measurements of foveal thickness were performed as previously described, and statistical comparisons were made between data from different visits.17,41

**RESULTS**

**STUDY POPULATION, ADVERSE EVENTS, AND COMPLICATIONS**

There were 15 patients (8 female and 7 male subjects) in the trial (Table 1). Other than a sibling pair (patients 9 and 12), the patients were unrelated. Patients 10 and 13 had the same mutant alleles (homozygous for R91W) but were not known to be related. Ninety-day and 1-year postinjection data from the 3 patients in cohort 1 have been published.17,18,20,24 The first 3 cohorts received single subretinal injections (Figure 1). Cohorts 1 and 2 were young adult patients (aged 20-30 years), and dose escalation occurred by doubling the initial injection volume of 150 µL of vector (cohort 1) to 300 µL (cohort 2).
hort 3 patients were younger than 18 years of age, and we were advised by a University of Pennsylvania regulatory body to reduce dosage in this first cohort of children, hence the 225-µL volume for these 3 patients. Cohorts 4 and 5 had 2 injections of 225 µL each (total, 450 µL; Figure 1), first in young adults (aged 24 and 27 years) and then in patients 18 years of age or younger. Patient 15 had 2 injections, but less total volume was injected.
Infrared views of all 15 study eyes are shown with superimposed locations of the injection site and estimated boundaries of retinal detachments caused by the subretinal injections. The treated eyes are all portrayed as left eyes for comparison (Figure 1); the actual eye treated is tabulated (Table 1). The postoperative course was similar in 13 of 15 patients with absorption of the subretinal fluid within 48 hours and no evidence of intraocular inflammation. Patients 7 and 11 were exceptions, with a second retinal detachment and choroidal effusions, respectively. By 30 to 60 days after surgery, all eyes were quiet and have remained so. The systemic safety parameter values, including those determined by physical examinations and blood (hematology, serum chemistry, and coagulation) and urine testing, showed no clinically significant abnormalities after gene transfer in all patients.

Among the postoperative adverse events were retinal detachment, choroidal effusions, ocular hypotension in the immediate postoperative period, and ocular hypertension associated with the administration of topical steroids. Patient 7, from cohort 3, had a retinal detachment in the region of the single subretinal injection, 1 day after it was deemed flat by use of ophthalmoscopy. This was surgically repaired, and there have not been any further complications. Patient 11, from cohort 4, was found to have choroidal effusions on the third day after surgery. The choroidals were treated with topical cycloplegics and additional topical steroids. The lack of resolution of the choroidals by day 30 led to a 3-week course of systemic steroids. It was determined after the patient underwent a clinical examination and ultrasonography that the choroidals had resolved on postoperative day 149. There was no measured ocular hypotension from the first measurement (postoperative day 3) through day 238. This patient, however, developed increased intraocular pressure, which was detected on day 149 and presumed to be secondary to the extended use of topical steroids. Cessation of steroids followed, and topical β-blockers were added. After a period of quiescence, the choroidals reappeared on day 190 and were treated with further cycloplegics and a short course of topical steroids. There was resolution by day 238. Ocular hypotension was documented in 4 patients (patients 4, 9, 12, and 15) during the early postoperative period (days 2–5; intraocular pressures were ≤6 mm Hg with an interocular difference of 5-10 mm Hg). By postoperative day 7, pressure in the study eyes increased, and interocular asymmetry decreased in all patients. Ocular hypertension was also documented after topical steroid use in the postoperative period in patients 8, 12, and 15. Cessation of steroids and treatment with topical β-blockers led to the eyes becoming normotensive.

### IMMUNE RESPONSE ASSAYS

Humoral immune responses were monitored by measuring levels of circulating antibody to AAV2 capsid at baseline and at postoperative days 14, 90, and 270 and years 1, 2, and 3, depending on the treatment cohort (Table 2). All patients exhibited titers at baseline and at posttreatment time points well below the normal population mean of 2 148 715 mU/mL (99 random samples), except for patient 12, who exhibited a titer of 2 789 606 mU/mL at day 90, which is approximately 60% above this patient’s baseline value. Ten of the 14 patients with at least day-14 data showed no increase in antibody titer greater than 2-fold from baseline, and most experienced a decline. Of the remain-

### Table 2. Anti–Adeno-Associated Virus 2 Serum Antibody Titers at Baseline and Posttreatment Time Points for 15 Patients With RPE65-Related Leber Congenital Amaurosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
<th>Cohort 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 14</td>
<td>Day 90</td>
<td>Year 1</td>
<td>Year 2</td>
</tr>
<tr>
<td>P1</td>
<td>11 884</td>
<td>3576</td>
<td>14 221</td>
<td>4861</td>
<td>Not tested</td>
</tr>
<tr>
<td>P2</td>
<td>38 086</td>
<td>22 497</td>
<td>169 700</td>
<td>12 583</td>
<td>Not tested</td>
</tr>
<tr>
<td>P3</td>
<td>118 861</td>
<td>38 125</td>
<td>38 251</td>
<td>59 141</td>
<td>Not tested</td>
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<td>P4</td>
<td>23 062</td>
<td>11 745</td>
<td>12 704</td>
<td>22 105</td>
<td>4241</td>
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<tr>
<td>P5</td>
<td>22 333</td>
<td>68 065</td>
<td>15 704</td>
<td>24 554</td>
<td>174 245</td>
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<td>605 249</td>
<td>392 069</td>
<td>31 808</td>
<td>12 815</td>
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<tr>
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<td>136 837</td>
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<td>495 714</td>
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<tr>
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<td>1 164 327</td>
<td>950 041</td>
<td>527 275</td>
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<td>181 096</td>
<td>71 863</td>
<td>21 862</td>
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<tr>
<td>P12</td>
<td>1 746 927</td>
<td>1 668 567</td>
<td>2 789 606</td>
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</tr>
<tr>
<td>P13</td>
<td>90 444</td>
<td>30 072</td>
<td>25 962</td>
<td>...</td>
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</tr>
<tr>
<td>P14</td>
<td>477 156</td>
<td>568 951</td>
<td>546 801</td>
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<td>...</td>
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<td>P15</td>
<td>2285</td>
<td>3271</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviation: Ellipses indicates that testing was not yet performed.

- aTesting at year 2 was added to the protocol after the patients in cohort 1 had already completed this time point.
- bPatient 2 (P2) was additionally tested on day 270 (antibody titer, 21 059 mU/mL).
- cP6 was additionally tested on day 60 (antibody titer, 707 306 mU/mL).
ing 4 patients, patient 2 exhibited a 4-fold increase at day 14 that returned to below baseline at day 90 and years 1 and 3. Patient 5 experienced a 3-fold increase at day 14, which returned to baseline at day 90 and year 1, but then spiked again at year 2. This pattern of episodic antibody spikes over multiple years with a return to baseline is not consistent with a humoral immune response to a one-time vector administration but is more likely a result of periodic reexposure to wild-type AAV2 through natural viral infections. Patient 6 experienced a 7-fold titer increase at day 14 that peaked at 11-fold at day 60 and subsequently declined to just over 2-fold at year 2. Patient 8 also showed an increase in titer at day 14 (3-fold) that peaked at day 90 (4-fold) and was decreasing at year 1 (2-fold over baseline). Patients 6 and 8 are circumstantially the only patients with serum antibody titers potentially consistent with a response to the vector, but coincidental exposure to wild-type AAV2 either directly or by reactivation of latent AAV2 through natural adenoviral or herpes viral infection cannot be ruled out. Overall, the patterns of patient serum antibody titers to AAV2 over time posttreatment suggest limited or no systemic immune response to subretinal AAV2 vector delivery. AAV2 capsid antigen-specific reactivity of peripheral lymphocytes was monitored at baseline and at posttreatment days 14 and 90 and years 1, 2, and 3 (eTable 1). Only patients 1 and 2 exhibited a significant increase in the stimulation index at any time point (the minimal level of significance for the stimulation index ranges from 2 to 3). For both patients, the change in stimulation index was only marginally significant and only a small increase from baseline: Patient 1 at year 1 had a stimulation index of 2.02, a small increase from the baseline value of 1.62, and patient 2 at day 90 showed a stimulation index of 2.10, also a small change from 1.89 at baseline. We conclude that the AAV2 capsid antigen-specific lymphocyte proliferation response to a single subretinal AAV2 treatment elicits neither a consistent nor pronounced AAV2 antigen-specific immune response.

The T-cell immune response to the AAV2 capsid was monitored by IFN-γ enzyme-linked immunospot (ELISpot) assays. Peripheral blood mononuclear cells from subjects at baseline and at days 14 and 90 and years 1, 2, and 3 after treatment, depending on the patient, were stimulated with AAV2 peptide library pools and assayed for IFN-γ secretion. With one exception, there were no positive responses to AAV2 peptide pools at any of the time points tested in the subjects thus far (eTable 2). Patient 9 exhibited a response to peptide pool 2C at day 90, but not before or after. This is inconsistent with the T-cell response to the AAV2 vector, and a clear reason for this modest and transient increase at day 90 is not apparent. The study of AAV2-specific memory T cells measured by the cultured ELISpot showed that some patients had preexisting AAV2-specific T cells at baseline (patients 2, 6, and 9 and patients 11-14) (eTable 3). Most of the patients that tested positive at baseline remained positive as measured by the cultured ELISpot, and most of the patients that were negative remained negative. Only patients 5 and 7, who were negative at baseline, became positive after treatment as measured by cultured ELISpot but not by ex vivo ELISpot. Although preexisting AAV2-specific memory T cells were found in some patients, AAV2 administration to the eye did not expand these resting cells as demonstrated by the negative data obtained by the ex vivo ELISpot. The data suggest that, even in the presence of peripheral AAV2-specific memory T cells, AAV2 administration to the eye is not sufficient to activate them.

The biodistribution of the vector in the peripheral blood was monitored at baseline and at days 1, 3, and 14 after treatment by use of quantitative polymerase chain reaction with spike-in assays to control for polymerase chain reaction inhibition in specific samples. For all patients at all time points, there were no vector genome copies detectable, thus confirming the lack of escape of a subretinally administered AAV2 vector into the circulation.

**FULL-FIELD AND FOCAL PSYCHOPHYSICS, PUPILMETRY, AND MOBILITY**

Visual function was assessed before and after treatment using FST, TPLR, dark-adapted static visual field testing, mobility performance, and VA as measured using Early Treatment Diabetic Retinopathy Study methodology. We did not assume that the vector used in each patient was bioactive but tested it. At the end of each surgery, the unused residual vector was injected subretinally into rd12 (Rpe65-deficient) mice and an electoretinographic bioassay performed to quantify vector activity.18,42,65 In all patients, the residual vector was proven to be active by this method (eFigure 1).

**Full-Field Sensitivity**

Visual function was measured psychophysically using FST in dark-adapted eyes with blue and red flashes (Figure 2A, eFigure 2, and Table 3). At baseline, the mean (SE) FST sensitivity with blue flashes in all RPE65-related LCA eyes (study and control) was 1.17 (0.09) log10, a value that was substantially reduced compared with 6.61 (0.10) log10 in normal eyes. At baseline, there were no significant differences (P=.16) between control and study eyes of trial patients. Intervisit test-retest variability of the FST measure at baseline in trial patients was similar to that published previously.30,31 The mean (SE) FST sensitivity to red flashes in all RPE65-related LCA eyes at baseline was 0.72 (0.11) log10, which is greatly reduced compared with the normal mean (SE) value of 4.37 (0.08) log10 measured under dark-adapted conditions, or the normal mean (SE) value of 2.48 (0.09) log10 measured at the cone plateau (eFigure 2 and Table 3). Chromatic differences were used to define the photoreceptor types that were mediating the FST responses. In all patients, red FST flashes were detected by cones, whereas blue FST flashes could be detected by rods (patients 1, 3, 5, 7, 8, 9, and 10) or cones (patients 11, 12, and 15), or by both rods and cones (patients 2, 4, 6, 13, and 14), at baseline (eFigure 2).

During the postoperative period, FST sensitivities to blue flashes showed highly significant differences compared with baseline in study eyes but not in control eyes (P < .001, by repeated-measures ANOVA test of interaction) (Figure 2A and Table 3). The postoperative improvement in study eyes was 1.59 (0.23) log10. Chromatic differences supported mediation of blue FST flashes by rods postoperatively in all study eyes except for patient 6 (eFigure 2).
The FST sensitivities to red flashes showed highly significant differences compared with baseline in study eyes but not in control eyes (P = .008, by repeated-measures ANOVA test of interaction). The postoperative improvement in study eyes was 0.45 (0.10) log10 (Table 3). Chromatic differences supported mediation of red FST flashes by cones postoperatively in all study eyes (eFigure 2). The ages of the clinical trial participants did not have a significant effect on blue FST (P = .25, overall; P = .53, magnitude) or on red FST (P = .10, overall; P = .63, magnitude).

Pupillometry

The transmission of information from retina to the brainstem was quantified objectively with the TPLR (Figure 2C and D). Measurements were made under fully dark-
adapted conditions, and luminance-response functions were available in 27 of 30 eyes. For patient 10 at baseline, the control eye showed a subcriterion contraction, whereas the study eye showed no contraction to maximal stimulation; his sensitivities were assigned to the reciprocal of the maximum stimulus luminance for statistical purposes. For the control eye of patient 13, the TPLR was not recorded owing to time constraints. At baseline, RPE65-related LCA eyes required on average 5.6 \text{log}_{10}-unit higher luminance of a full-field green flash in order to produce a criterion pupillary contraction compared with normal eyes (mean [SE], −0.88 [0.15] \text{log}_{10} for RPE65-related LCA eyes vs 4.74 [0.06] \text{log}_{10} for normal eyes). The magnitude of this defect was similar to the 5.5 \text{log}_{10}-difference observed with blue FST between RPE65-related LCA and normal eyes. At baseline, there were no significant differences (P = .81) between the control and study eyes of trial patients (Figure 2C).

During the postoperative period (1-6 months), TPLR sensitivities showed highly significant differences compared with baseline in study eyes but not in control eyes (P < .001, by repeated-measures ANOVA test of interaction) (Figure 2C and Table 3). The magnitude of the postoperative TPLR sensitivity improvement in study eyes was 1.17 (0.20) \text{log}_{10} (Figure 2D and Table 3), which corresponded to an intermediate value between the blue and red FST improvements observed. The ages of the clinical trial participants did not have a significant effect on the TPLR (P = .67, overall; P = .56, magnitude of the treatment effect).

**Static Visual Fields**

The significantly increased light sensitivity after surgery in treated eyes using FST and TPLR prompted us to ask whether we could localize the increases in the visual field and how any localization of function was related to the sites of subretinal injection (Figure 3). Visual field maps of sensitivity change from baseline in the study eyes showed good correspondence between the loci, where there were significantly increased responses to stimuli, and the estimated region, where the retinal detachment with subretinal injection of agent occurred. Patients in cohorts 4 and 5 who had a second subretinal injection site in the nasal retina had loci in the temporal visual field that showed a significant response. In summary, 11 of the 12 patients with visual field maps showed correspondence between most detected loci and the area of injection; these include patients 1, 2, 3, and 4, patients 7, 8, and 9, and patients 11, 12, 14, and 15. It is of interest to note, however, that some of the detected loci in the patients were outside the estimated injection area. The basis for this effect remains uncertain. It is to be noted that most of these unexpectedly responsive loci were in the far periphery. The results of patient 6 indicated a response at a single peripheral locus but no evidence of a response in the subretinal injection area. This temporal inferior peripheral field locus was consistently detected, and it is likely to be the source of the FST and TPLR responses in this patient (Figure 2), who described this location of perception and its appearance postoperatively.

**Mobility Testing**

We also asked whether these localized changes from baseline had any effect on the ability of the subjects to negotiate an obstacle course. In 5 patients, representing cohorts 4 and 5, we performed the study both before and after treatment (Figure 4A). A comparison of mobility performance for study eyes relative to postoperative values indicates overall a better performance after treatment for ambient illuminations between 0.2 and 4 lux. For the 100-lux illumination, however, patients were able to navigate the course practically without errors, both at baseline and after surgery and regardless of which eye was used. For the control eyes, there were less pronounced differences in performance for the lower illumination levels, which suggests a learning effect. We also determined whether the difference in performance between eyes (interocular difference) changed after treatment. The results in patients 11 and 13 indicate a greater interocular difference after treatment, with better performance of the treated eye relative to the control eye, at the lower illumination levels. Patient 12 performed better with the treated eye only at the lowest illumination level; for patients 10, 14, and 15, there were no notable effects.

### Table 3. Measures of Ocular Function at Baseline and After Surgery

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control Eyes, Mean (SE)</th>
<th>Study Eyes, Mean (SE)</th>
<th>P Value</th>
<th>Control Eyes, Mean (SE)</th>
<th>Study Eyes, Mean (SE)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FST sensitivity, blue</td>
<td>1.22 (0.10)</td>
<td>1.17 (0.11)</td>
<td>.05 (.06)</td>
<td>1.12 (0.099)</td>
<td>2.72 (0.21)</td>
<td>.59 (.25)</td>
</tr>
<tr>
<td>FST sensitivity, red</td>
<td>0.75 (0.10)</td>
<td>0.74 (0.12)</td>
<td>.02 (.08)</td>
<td>0.70 (0.12)</td>
<td>1.14 (0.12)</td>
<td>.45 (.10)</td>
</tr>
<tr>
<td>TPLR sensitivity</td>
<td>−0.90 (0.20)</td>
<td>−0.91 (0.16)</td>
<td>.01 (.05)</td>
<td>.87 (.09)</td>
<td>.28 (.27)</td>
<td>.17 (.20)</td>
</tr>
<tr>
<td>VA, logMAR</td>
<td>0.96 (0.13)</td>
<td>0.91 (0.13)</td>
<td>.05 (.02)</td>
<td>.02 (.01)</td>
<td>.97 (0.11)</td>
<td>.12 (.05)</td>
</tr>
</tbody>
</table>

Abbreviations: FST, full-field stimulus testing; TPLR, transient pupillary light reflex; VA, visual acuity.

a Postoperative value − baseline value.
b Determined by use of the 2-sided paired t test.
c Normal mean (SE) value, 6.61 (0.10) \text{log}_{10}.
d Normal mean (SE) values, 4.37 (0.08) \text{log}_{10} when rod-mediated at dark-adapted conditions and 2.48 (0.09) \text{log}_{10} when cone-mediated during the cone-plateau period.
e Normal mean (SE) value, 4.74 (0.06) \text{log}_{10}.
f Normal value, 0.00 \text{logMAR} corresponding to 20/20 Snellen VA at 4-m distance.

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All study participants were assessed by the differences in performance between eyes, averaged across all posttreatment visits (Figure 4B). Results indicate a consistently lower number of incidents while navigating with the treated eye, with varying degrees of performance gain across participants. The mobility performance results (the interocular differences) for all participants are summarized in Figure 4C. Mean differences between study and control eyes were significantly different from 0 for the 4 lower illumination levels, indicating that patients as a group navigated more efficiently when using their treated eyes under such conditions.

**VA, FIXATION, AND FOVEAL OPTICAL COHERENCE TOMOGRAPHY**

At baseline, the mean (SE) VA was 0.96 (0.13) logMAR (corresponding to a mean Snellen acuity of 20/182; range, 20/39 to worse than 20/2000) in control eyes and 1.09 (0.11) logMAR (corresponding to a mean Snellen acuity of 20/246; range, 20/43 to 20/1824) in study eyes (Tables 1 and 3). Postoperatively, the mean (SE) VA increased to 0.91 (0.13) logMAR in control eyes and increased to 0.97 (0.11) logMAR in study eyes, and both changes were significant (Table 3). In contrast to other psychophysical and pupillometric measurements, repeated-measures ANOVA showed no indication ($P = .16$) of a difference in the magnitude of the postoperative VA change between study and control eyes of $−0.12$ (0.05) and $−0.05$ (0.02) logMAR, respectively. Furthermore, in the great majority of the eyes (28 of 30 eyes), the mean postoperative VA change did not reach or surpass the 0.30-logMAR (3-line) halving of visual angle limit, which is a commonly accepted criterion for clinical significance. It was noted that the second baseline VA was better than the first baseline measurement in 10 study eyes (range, $−0.18$ logMAR better to 0.08 logMAR worse) and in 9 control eyes (range, $−0.10$ logMAR better to 0.10 logMAR worse). Regression to the mean and learning curve effects could have potentially contributed to this tendency. Thus, an alternative analysis was performed using only the second baseline VA. This led to a decrease in the logMAR improvement postoperatively in both the study eyes (mean [SE], $−0.09$ [0.05]; $P = .10$) and the control eyes (mean [SE], $−0.04$ [0.02]; $P = .08$).
To understand better any possible VA changes resulting from gene therapy, fixation properties were analyzed in each eye at each visit. At baseline, in 12 of 15 study eyes, the mean fixation location corresponded to the anatomical foveal depression (Figure 5A), and, not unexpectedly, these eyes had the highest VA (Table 1). The mean (SE) instability of fixation (including high-frequency nystagmus and lower-frequency wandering eye movements) around the mean was 1.97° (0.21°) and correlated \( r^2 = 0.42 \) inversely with VA as previously published in this patient population.29 Control eyes showed less fixation instability with a mean (SE), -0.30 (0.14)° from eccentricities of 2.1°, 5.1°, and 3.4°, respectively. The control eyes of these 3 patients also fixated eccentrically; the mean locus of fixation was 2.0° and 2.3° eccentric for patients 4 and 5, respectively, whereas it could not be quantified in patient 10. Postoperatively, fixation remained extrafoveal for all 3 patients (Figure 5D).

Next, VA changes were considered in the context of the type of fixation. There was a larger VA improvement in patients with extrafoveal fixation (mean [SE], -0.30 [0.14] logMAR) (Figure 5E) than in those with foveal fixation (mean [SE], -0.09 [0.04] logMAR) (Figure 5C).
A repeated-measures ANOVA demonstrated a statistically significant difference ($P = .04$, test of interaction) between the magnitude of postoperative VA improvement in the patients with the 2 types of fixation. In subgroup analyses, VA change in foveal-fixating study eyes or in extrafoveal-fixating study and control eyes did not achieve statistical significance ($P = .07$, .17, and .56, respectively); counterintuitively, foveal-fixating control eyes showed a significant ($P = .02$) improvement in VA. Of note were the large VA improvements (~0.29 logMAR for patient 4 and ~0.55 logMAR for patient 10) in 2 of the extrafoveal-fixating eyes where the central retina, including the fixation, was involved in the subretinal injection, as compared with the lack of any such large improvement in the extrafoveal-fixating eye without a central detachment (~0.06 logMAR for patient 5) or in 2 foveal-fixating eyes with a foveal detachment (0.20 logMAR for patient 1 and ~0.11 logMAR for patient 13). Until there are physiologically based hypotheses for improvements in untreated control eyes or in eyes without a foveal detachment, the law of parsimony would suggest that small VA improvements in extrafoveal-fixating eyes may have been influenced by the high expectations and motivation of the patients taking part in an open-label study as well as by a possible learning effect. Clinically significant unilateral VA improvements in extrafoveal-fixating eyes, on the other hand, appear consistent with independent data showing improvements in extrafoveal cone sensitivity following gene therapy.

The retinal laminar architecture across the fovea was quantified in all patients. The foveal thicknesses in both eyes of the 15 patients before and after surgery are summarized in Figure 6A. For control eyes, changes from baseline are within published intervisit variability for a retinal degeneration population. For study eyes, there are 2 notable examples of foveal thinning in the short term: patients 1 and 13. Long-term follow-up in patient 1 showed that foveal thinning was still present. In both patients 1 and 13, the fovea was detached in the subretinal injection (Figure 1). Patient 4, another patient with a foveal detachment, showed less pronounced but still significant thinning in the long term but not in the short term. Patient 4's control eye also showed long-term thinning but not to the degree as in the study eye. As a counterexample, patient 5, who did not have a foveal detachment, showed similar results as patient 4. The other patients with a foveal detachment, patients 6 and 10, showed no such effects. All other study eyes without foveal detachment did not have thinning within the time period studied.

Representative horizontal OCT cross sections and longitudinal reflectivity profiles through the fovea (high-
Figure 6. Foveal structure and quantitation of thickness using optical coherence tomography (OCT) scans in control and study eyes. A, Foveal thickness measurements in control and study eyes at baseline, and at short-term and long-term postoperative time points, are shown. Changes from baseline are displayed adjacent to the foveal thickness measurements. B, OCT scans along the horizontal meridian are shown for 4 representative patients: 1 without foveal detachment (patient 7 [P7]) and 3 with foveas detached at the time of the procedure (P1, P13, and P6). The ellipses denote the central retinal region of interest that shows changes in the inner segment/outer segment (IS/OS) laminations in 3 of the 4 study eyes at early time points but with some resolution at later times. C, Longitudinal reflectivity profiles (LRPs) through the fovea in the patients compared with a normal LRP (N). The LRPs are color coded and labeled for the outer nuclear layer (ONL), the entire region encompassing inner and outer segments (IS + OS), and the retinal pigment epithelium (RPE) to illustrate the postoperative changes. The ONL and IS + OS measurements are shown to the right of the LRPs. F, indicates fovea; FD, foveal detachment as part of the subretinal injection procedure.

lighted and labeled for outer retinal laminae and with histograms of layer thickness) are shown in Figure 6B and C for 4 study eyes at baseline and at early and later postoperative times. Patient 7, a patient without a foveal detachment, showed no remarkable changes at 30 days and 18 months after surgery. Patients 1, 13, and 6 had foveal
detachments, and all showed a disturbance in the laminar architecture of inner and outer segments 30 days after surgery; however, there was recovery at later visits. Unlike patient 6, however, patients 1 and 13 showed loss of the outer nuclear layer at 30 days and at later time points. How do these structural findings relate to VA? The only patient with clinically significant loss of VA was patient 1 at last visit (Figure 5), and this patient had the most prominent foveal abnormalities determined by OCT.

COMMENT

This 5-cohort 15-patient RPE65-related LCA retinal gene therapy clinical trial can be summarized as follows. There were no detectable systemic safety concerns; certain ocular adverse events occurred, and these were attributable to the surgical procedure and improved visual function (determined by FST) was present in all patients, albeit to different degrees. Like the other concurrent early-phase RPE65-related LCA clinical trials,15,16,21,22 our trial of a relatively small number of patients has limitations. The study design did not involve the randomization of eyes, and there was an inherent imbalance between eyes with regard to variables related to outcome because the study eyes, by definition, had worse vision. The statistical approach was thus confounded by such issues. Examiners were not masked to the study eye vs the control eye (study eyes showed residual conjunctival injection for weeks after surgery). Later phase trials for this disease and for other rare genetic retinal degenerations may be able to confront these issues. Despite the limitations of the ongoing RPE65-related LCA clinical trials, the fact remains that the longstanding concept that genetic retinal degenerations are incurable and that vision cannot be improved has been revised. The advantages of beginning the era of treating rare inherited retinal diseases with RPE65-related LCA are discussed in the eAppendix.

Some key questions to consider in planning future phases of gene therapy clinical trials for RPE65-related LCA are as follows: (1) Where in the retina should we treat or not treat? (2) Is there sufficient evidence for “age-dependent effects” of this gene therapy to recruit younger and younger patients with RPE65-related LCA in future cohorts? (3) Is cone-based vision improved by the currently used vectors?

RETNAL REGIONS FOR TREATMENT

Where in the retina should treatment be directed and where do we avoid injecting? All 15 patients in this trial and an additional 15 patients in 2 other trials received subretinal injections in 1 eye. Where have the injections been delivered? Sixteen of 30 procedures (53%) detached the macula and fovea. All but 2 of these 16 patients were reported to have some measure of efficacy in the macula after surgery; however, there was recovery at later visits. Unlike patient 6, however, patients 1 and 13 showed loss of the outer nuclear layer at 30 days and at later time points. How do these structural findings relate to VA? The only patient with clinically significant loss of VA was patient 1 at last visit (Figure 5), and this patient had the most prominent foveal abnormalities determined by OCT.
(patients 4 and 10) showed an increase in VA of greater than 0.3 logMAR after surgery. These 2 patients had the 2 lowest VAs at baseline in our study and had foveal and macular detachments, and their conditions improved by maintaining extrafoveal fixation loci in the treated areas. One of the other RPE65-related LCA trials reported no improvement in VA, whereas group statistics in a third trial showed significant changes in study eyes (see Supplemental Table 5 from this third trial); individual results suggest improvements in many control eyes, but group statistics were not provided. Using the 0.3-logMAR criterion as a clinically significant change in VA, this third trial found improvement in 4 of 8 patients who received macular injections that included the fovea, and 2 of 12 untreated control eyes also showed large improvements in VA. Fixation was not reported. Complicating the risk-benefit issue for this trial is the report of a decrease in VA in a 10-year-old subject’s treated eye.

Taking a conservative, patient safety—first approach, we conclude that the lack of consensus in VA results within and between the contemporaneous trials and our evidence of foveal thickness loss lead to the recommendation that the fovea should not be included in macular subretinal injections except when there is foveal atrophy (determined by use of OCT) or very reduced VA and fixation is parafoveal (readily detectable on OCT scans). This approach also opens the door to the treatment of patients at later disease stages when there is foveal atrophy but a preserved extrafoveal outer nuclear layer, best exemplified by patients 4 and 10 in the present study.

To date, our 2-site injection protocol in cohorts 4 and 5 was safe in adults and children. This is a positive step in the direction of a subretinal injection protocol with the goal of increasing even further the visual area affected by the vector. Most of the single injections in our study were directed at the superior retina in order to preserve inferior visual field function. When 2 injections were used, a nasal retinal injection was added to enhance temporal peripheral visual function, which can be detected in untreated patients with RPE65-related LCA even at relatively late stages. We propose that the next step for previously untreated patients would be to administer the agent to 3 sites of injection: (1) the superior retina, involving the macula but not the fovea, unless there is foveal atrophy and extrafoveal fixation; (2) the nasal−superior retina; and (3) the temporal retina. Ideally, a preoperative map of the remaining outer nuclear layer would help guide the injections, or at least a preoperative visual field (using sufficiently bright stimuli) would help to determine where residual vision is detectable.

To date, we have elected to use the eye with worse vision as the study eye. In some cases, this nonpreferred eye has been strabismic and possibly amblyopic. This strategy was motivated by safety, but it has also been convenient for patients who can continue to be visually active during the postoperative period despite a protective occluder worn on the operated eye. For the initial 5 cohorts in this safety trial, that decision was appropriate. Some patients, however, have continued to use their preferred (control) eye after treatment and are only aware of visual gain in the treated eye when asked to occlude the control eye for our studies. The 3-injection protocol thus proposed would ideally be used in the preferred eye. Factoring in the remarkable visual improvements in our cohort 1 patients who received 150 µL at a single site and the lack of toxicity to date of 2-site injections totaling 450 µL, we recommend 3 injections of 150 µL each. A single eye treated with such a protocol will test the safety and efficacy of this approach and leave the contralateral eye for further advances in the field.

AGE AND EFFICACY OF GENE THERAPY

Is there evidence of an age dependence on the effects of gene therapy? The RPE65-related LCA phenotype includes not only a severe visual dysfunction but also a progressive retinal degeneration, and the relationship between severity of retinal degeneration and age can be complex. On an individual patient basis, there is certainly age dependence of severity of disease; visual field extent decreases with age when followed longitudinally over more than a decade. But this longitudinal progression in individual patients does not simply translate to cross-sectional studies across different patients at different ages, and this is especially true in the first 3 decades of life. Our studies of visual function and photoreceptor topography have indicated that severity of dysfunction and degeneration can be as profound in some young patients in the first decade of life as in some patients in the third decade of life. Given comparable numbers of photoreceptors remaining in the retina and placement of the vector injection(s) in the region(s) of these photoreceptors, there should be an equal chance of efficacy of therapy, and this would be independent of age (assuming that the health of the RPE is similar). Did we find an age-dependent effect of gene therapy in our study, as reported in another trial? Therefore, what is the basis of the previously reported conclusion of age dependence of gene therapy outcomes? It seems to be a matter of emphasis. For example, the improved light sensitivity of an 8-year-old patient (ie, patient CH08; see Figure 2C from the third trial) is emphasized, but there is equal improvement in light sensitivity in a 35-year-old patient in the same trial (ie, patient CH13; see Figure 2C from the third trial). The mobility performance of younger patients is also emphasized, but these patients are studied uniconically and with lower room illuminations, whereas most older patients are only studied binocularly at 250-lux light levels (see Supplementary Table 6 from the third trial), a condition that we found does not reveal any differences in performance before and after treatment or between eyes. Considerable heterogeneity of disease severity in RPE65-related LCA is a fact, and when determining candidacy for this therapy, there should be evaluation on an individual basis, independent of age. The extent of retinal disease in human RPE65-related LCA at different ages has not been as predictable.
as, for example, a murine model.31 The temptation should be resisted to assume there is a common natural history among humans without measuring it. With greater understanding of the human disease, a better evidence-based formula may emerge for candidacy, in which age is one of several parameters.

TREATMENT EFFECT ON CONES

Are cones affected by the vectors? There is consensus from all current trials that some measures of visual function improve in response to gene therapy in patients with RPE65-related LCA.15,24 Evidence of rods and extrafoveal cones responding to the vector gene has been presented in our previous work,18,20 and this is confirmed and extended in the present work to larger numbers of patients. Foveal cones have not been proven conclusively to show either increased vision from the current vector-gene treatments or protection from further cell loss. It is thus possible that there is a difference in targeting efficiency between foveal RPE and extrafoveal RPE in RPE65-related LCA with the current vectors, or it is possible that there is localized toxicity (directly or indirectly) to foveal cones. Foveal and extrafoveal cones, for example, do not have the same relationship with RPE apical processes.32 Alternatively or additionally, contributions of the chromophore required from retinal and RPE visual cycle pathways may also differ between foveal and extrafoveal cones.5,53 There is also evidence of RPE65 localization in cones,34,35 suggesting a pathway that may need to be subserved by vectors capable of expressing RPE65 not only in the RPE but also in cones. In this context, using a promoter in the vector that does not limit expression to just the RPE may turn out to be beneficial.

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Author Affiliations: Scheie Eye Institute, Perelman School of Medicine at the University of Pennsylvania (Drs Jacobson, Cideciyan, Aleman, Sumaroka, and Swider, Ms Schwartz, Olivares, and Mullins, and Mr Roman), and Gene Therapy Program, Department of Pathology, Laboratory of Medicine, University of Pennsylvania School of Medicine (Dr Calcedo), Philadelphia; Department of Ophthalmology (Drs Ratnakararama, Peden, Conlon, Pang, Kaushal, and Hauswirth and Mr Boye), Powell Gene Therapy Center (Ms Erger and Drs Byrne and Hauswirth), University of Florida, Gainesville; and Bascom Palmer Eye Institute, Miller School of Medicine, University of Miami (Mr Feuer), Florida; Department of Ophthalmology and Vision Sciences, Hospital for Sick Children, University of Toronto, Ontario, Canada (Dr Heon); Hamilton Eye Institute, Department of Ophthalmology, University of Tennessee Health Science Center, Memphis (Dr Iannaccone); The Pancrege Center for Inherited Retinal Diseases, The Chicago Lighthouse, Illinois (Dr Fishman); and Department of Ophthalmology, University of Iowa Carver College of Medicine, Iowa City (Dr Stone).

Correspondence: Samuel G. Jacobson MD, PhD, Scheie Eye Institute, University of Pennsylvania, 51 N 39th St, Philadelphia, PA 19104 (jacobss@mail.med.upenn.edu).

Author Contributions: Dr Jacobson had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Jacobson and Hauswirth contributed equally to the planning and conduct of this clinical trial.

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REFERENCES
