



# Results at 2 Years after Gene Therapy for RPE65-Deficient Leber Congenital Amaurosis and Severe Early-Childhood–Onset Retinal Dystrophy

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**Purpose:** To provide an initial assessment of the safety of a recombinant adeno-associated virus vector expressing RPE65 (rAAV2-CB-hRPE65) in adults and children with retinal degeneration caused by RPE65 mutations.

**Design:** Nonrandomized, multicenter clinical trial.

**Participants:** Eight adults and 4 children, 6 to 39 years of age, with Leber congenital amaurosis (LCA) or severe early-childhood–onset retinal degeneration (SECORD).

**Methods:** Patients received a subretinal injection of rAAV2-CB-hRPE65 in the poorer-seeing eye, at either of 2 dose levels, and were followed up for 2 years after treatment.

**Main Outcome Measures:** The primary safety measures were ocular and nonocular adverse events. Exploratory efficacy measures included changes in best-corrected visual acuity (BCVA), static perimetry central 30° visual field hill of vision ( $V_{30}$ ) and total visual field hill of vision ( $V_{TOT}$ ), kinetic perimetry visual field area, and responses to a quality-of-life questionnaire.

**Results:** All patients tolerated subretinal injections and there were no treatment-related serious adverse events. Common adverse events were those associated with the surgical procedure and included subconjunctival hemorrhage in 8 patients and ocular hyperemia in 5 patients. In the treated eye, BCVA increased in 5 patients,  $V_{30}$  increased in 6 patients,  $V_{TOT}$  increased in 5 patients, and kinetic visual field area improved in 3 patients. One subject showed a decrease in BCVA and 2 patients showed a decrease in kinetic visual field area.

**Conclusions:** Treatment with rAAV2-CB-hRPE65 was not associated with serious adverse events, and improvement in 1 or more measures of visual function was observed in 9 of 12 patients. The greatest improvements in visual acuity were observed in younger patients with better baseline visual acuity. Evaluation of more patients and a longer duration of follow-up will be needed to determine the rate of uncommon or rare side effects or safety concerns. *Ophthalmology* 2016;123:1606-1620 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



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RPE65 is a 65-kDa isomerohydrolase produced by retinal pigment epithelium cells that catalyzes a key step in the conversion of all-trans-retinyl ester to 11-cis retinol, a step essential in regenerating the visual chromophore.<sup>1,2</sup> Mutations of RPE65 result in a profound deficiency of the active chromophore. Most patients with mutations of RPE65 have Leber congenital amaurosis (LCA), a heterogeneous disorder that presents in infancy with profound visual impairment, nystagmus, weakly reactive pupils, and a range of fundus appearances that can evolve from initially normal appearance to severe pigmentary changes.<sup>3</sup> The electroretinogram (ERG) results are profoundly abnormal in most forms of LCA and may be unrecordable over the

noise level.<sup>4</sup> A minority of patients with RPE65 mutations seek treatment after infancy, but still within the first decade of life, with similar but initially milder symptoms dominated by night blindness. This phenotype has been referred to by a number of names, including early-onset retinitis pigmentosa,<sup>5,6</sup> early-onset severe rod–cone retinal degeneration,<sup>7</sup> and severe early-childhood–onset retinal dystrophy (SECORD).<sup>8</sup> The ERG results are severely abnormal in SECORD,<sup>8,9</sup> but may still be recordable in childhood, including in patients who have mutations in RPE65.<sup>3,10,11</sup> Mutations in RPE65 account for 6% to 16% of cases of LCA<sup>12,13</sup> and 2% of cases of autosomal recessive retinitis pigmentosa or SECORD.<sup>14,15</sup>

Proof-of-principle studies demonstrating the safety and benefit of gene replacement have been conducted in murine<sup>16,17</sup> and canine<sup>17–21</sup> models of RPE65 deficiency and in human patients, in whom *RPE65* gene replacement has successfully restored cone and rod sensitivity, improved visual fields, and in some cases, improved visual acuity.<sup>22–28</sup> Improvement in visual function was reported to persist for at least 3 years after treatment,<sup>29</sup> despite continuing retinal degeneration documented by optical coherence tomography (OCT).<sup>30</sup> More recent studies have reported durable benefit, but a reduction in the magnitude of improvement, after a 5- to 6-year follow-up period<sup>31</sup> or during a 2-year follow-up period after administration of an adeno-associated virus (AAV) vector that used a weaker promoter.<sup>32</sup> Herein, we report the results of a clinical trial evaluating the safety and efficacy of gene replacement therapy in 12 patients with LCA or SECORD caused by *RPE65* mutations at 2 after subretinal injection of an AAV *RPE65* gene therapy vector.

## Methods

The study was supported by a grant from the United States Food and Drug Administration's Office of Orphan Products Development and was conducted according to the tenets of the Declaration of Helsinki. The protocol and Health Insurance Portability and Accountability Act-compliant informed consent forms were approved by institutional review boards, and each subject, or a parent or guardian of patients younger than 18 years, gave written informed consent. Pediatric subject assent was obtained as required by institutional review boards. Study oversight was provided by an independent data and safety monitoring committee. The study is listed on [ClinicalTrials.gov](https://clinicaltrials.gov) under identifier NCT 00749957.

## Study Design

This was an open-label, nonrandomized, multicenter, sequential, 2-arm, phase 1/2 clinical trial evaluating safety and efficacy parameters after subretinal injection of a recombinant AAV vector expressing human RPE65 (rAAV2-CB-hRPE65). Safety was monitored by evaluating adverse events, hematologic and clinical chemistry parameters, and the presence of the vector in blood. Efficacy was assessed by evaluation of best-corrected visual acuity (BCVA) and visual fields (primary efficacy parameters), ERG results, and quality-of-life questionnaire responses (secondary efficacy parameters). Additional outcome parameters included OCT, ERG, and fundus photography.

## Eligibility Criteria

Eligibility criteria included age of at least 6 years, retinal disease consistent with LCA or SECORD and documented mutations in both alleles of the *RPE65* gene, BCVA not better than 20/60 and not worse than hand movements in both the treated and fellow eye, and visible photoreceptor (outer nuclear) layer on an OCT scan in the region of the retina where the study agent administration was planned.

## Treatment

Vector design and production followed the methods of Jacobson et al.<sup>33</sup> A device compatibility study demonstrated no decrease in vector titer after exposure to the 39-gauge microinjection cannula (catalog no. 12.12; Synergetics, USA, O'Fallon, MO) used to

administer the vector. Patients were enrolled into 2 groups of 6 each. For each subject, the poorer-seeing eye underwent a vitrectomy and received a subretinal injection of 450  $\mu$ l containing  $1.8 \times 10^{11}$  (group 1) or  $6 \times 10^{11}$  (group 2) vector genomes of rAAV2-CB-hRPE65. For patients in group 1, the retinotomy used to administer the vector was made outside the retinal vascular arcade. For patients in group 2, the retinotomy was inside the vascular arcade. All patients received postoperative treatment with topical corticosteroids and antibiotics.

## Clinical Assessments

All patients underwent assessments of visual function at 14 specified visits during the 2-year clinical trial. These included a screening visit within 8 weeks of surgery, a 2-day baseline visit before treatment that occurred on day 0, and posttreatment visits 1, 7, and 14 days; 1, 3, 6, and 9 months; and 1, 1.5, and 2 years after treatment. At each visit, BCVA was measured using an electronic visual acuity system<sup>34</sup> based on the Early Treatment Diabetic Retinopathy Study (ETDRS) method,<sup>35,36</sup> and an ophthalmic examination (slit-lamp retinal biomicroscopy, tonometry, and indirect ophthalmoscopy) was performed.

Spectral-domain OCT imaging of both the treated and untreated eye was performed with the Heidelberg Spectralis (Heidelberg Engineering, Inc., Heidelberg, Germany). Horizontal and vertical line scans were obtained through the foveal depression of each eye at baseline and the year 1 visit. Automatic real-time eye tracking was used when possible. Scans were segmented manually by a grader (Peter Steinkamp) masked to the treatment eye with the manufacturer's provided software (HRA Spectralis viewing module version 6.0.9.0; Heidelberg Engineering, Inc.). Three retinal boundaries were delineated: the internal limiting membrane, the inner boundary of the outer plexiform layer, and Bruch's membrane. The location of minimum foveal thickness was marked. The coordinates of the linear segmentation were aligned at the central fovea location with a custom program written in MATLAB (version 8.2 (R2013b); MathWorks, Inc., Natick, MA). The data were standardized along the lateral scale and were processed in Excel (Excel 2013; Microsoft Corporation, Redmond, WA). A 6000- $\mu$ m width of each scan was analyzed. Three layer thicknesses were determined: inner retina (defined as the distance between the internal limiting membrane and the outer plexiform layer), outer retina (defined as the distance between the outer plexiform layer and Bruch's membrane), and total retina thickness.

Semiautomated kinetic perimetry (SKP) and full-field static perimetry were performed on 3 occasions before treatment and at posttreatment visits 14 days; 1, 3, and 6 months; and 1 and 2 years after treatment using the Octopus 101 perimeter (Haag-Streit, Koeniz, Switzerland) as described previously.<sup>8</sup> Static perimetry used the GATEi strategy (Haag-Streit AG, Koeniz, Switzerland)<sup>37,38</sup>, stimulus size V, and a radially designed, centrally condensed grid of 148 test locations that extended to 54° superiorly, 78° nasally and inferiorly, and 80° temporally. The set of  $x$ ,  $y$ , and  $z$  examination data, where  $x$  and  $y$  are the Cartesian coordinates of the test location and  $z$  is the threshold level, was exported from the perimeter and the threshold values converted to differential luminous sensitivity values using a published transformation formula.<sup>39</sup> The sensitivity values were imported for topographical display and volumetric analysis into a software application, Visual Field Modeling and Analysis, or VFMA (Office of Technology Transfer & Business Development [OHSU], Portland, OR). Once in the application, the sensitivity data were fit in non-Euclidian space with a thin-plate, radial-based spline, creating a 3-dimension model of the hill of vision.<sup>40</sup> The magnitude and extent of sensitivity was quantified in decibel-steradian (dB-sr) units by

interpolating the volume beneath the surface of the thin-plate spline representation of the hill of vision. The total volume of the entire hill of vision ( $V_{TOT}$ ) was determined using a selection process that conformed to the external boundary of the test grid. The central 30° portion of the hill of vision ( $V_{30}$ ) was measured by a circle of 30° radius as a separate metric of the central portion of the visual field. The details of this type of analysis have been published previously.<sup>39</sup>

### Normative Study of Static and Kinetic Perimetry

A normative visual field study, approved by the Institutional Review Board of Oregon Health & Sciences University, was conducted at the Casey Eye Institute using the Octopus 101 for both semiautomated kinetic perimetry and static perimetry. Informed consent was obtained for adults and both parental consent and assent of the individual were obtained for children. Normal subjects were tested once with SKP, with static perimetry using the 148-point test grid and stimulus size V, or with both. Static perimetry testing in 1 child (a 7-year-old girl) and test–retest variability of SKP in 2 children (an 8-year-old boy and a 9-year-old girl) also were evaluated. Results of testing in normal patients and a discussion are provided in the [Supplemental Material](#) (available at [www.aaojournal.org](http://www.aaojournal.org)).

### Electroretinography

Full-field ERGs were recorded before and 6 and 12 months after treatment as described previously<sup>41,42</sup> and according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards<sup>43,44</sup> with the following modifications. All patients were dark adapted for at least 30 minutes. For dim scotopic stimuli, a series of flashes were delivered measuring  $-3.2$ ,  $-2.6$ ,  $-2.0$ ,  $-1.6$ ,  $-0.6$ ,  $0.0$ , and  $0.6$  log cd-s/m<sup>2</sup>. One subject required sedation for ERG recordings, which were performed as previously described.<sup>45</sup> Vision-related quality of life was analyzed according to the National Eye Institute 25-Item Visual Function Questionnaire (NEI-VFQ-25).<sup>46</sup>

Samples for hematologic, clinical chemistry, and urinalysis testing were obtained at the screening visit, at the baseline visit, and at visits 1, 7, and 14 days and 3 months after treatment. Serum to measure neutralizing antibodies to wild-type AAV<sup>47</sup> was obtained at the screening visit and at visits 14 days and 1 and 3 months after treatment. Peripheral blood mononuclear cells to measure T-cell responses to AAV2, the serotype used to create the vector used for this clinical trial, and RPE65 peptides by enzyme-linked immunospot assay<sup>48</sup> were obtained at the screening visit, at the baseline visit, and at visits 1, 2, and 3 months after treatment. Whole blood for detection of vector DNA by polymerase chain reaction assay<sup>49</sup> was obtained at the screening visit and at visits 1, 7, and 14 days after treatment.

### Statistical Methods

Each of the 12 patients underwent 2 pretreatment visual acuity tests for each eye. The coefficient of repeatability was determined using the method of Bland and Altman<sup>50</sup> for all eyes and for 19 eyes after excluding 5 eyes that had an ETDRS letter score of 0 for both tests. Each of the 12 patients underwent 3 pretreatment (baseline) static and kinetic perimetry tests for each eye. For  $V_{30}$  and  $V_{TOT}$ , the limits of agreement among baseline tests for all patients were calculated pairwise between each of the baseline results, incorporating data for both eyes for all patients. The difference between the average baseline value for each subject and each posttreatment value for that subject then was determined and compared with the upper and lower limit of agreement as the criteria that would represent a statistically meaningful change for a subject.<sup>51</sup> Results for 6 of 72 baseline tests and 7 of 168

posttreatment tests that had a false-positive rate of more than 25% were excluded from this analysis. For kinetic visual fields, the mean  $\pm$  standard deviation of the total area with the V4e target was determined for the 3 pretreatment tests and was compared with the results at each subsequent visit.

## Results

Twelve patients, 6 to 39 years of age, with DNA sequence-confirmed mutations in *RPE65* were enrolled, 10 at the Casey Eye Institute and 2 at the University of Massachusetts, between July 2009 and September 2012. Baseline characteristics of these patients are shown in [Table 1](#). *RPE65* mutations were compound heterozygous in 9 patients and homozygous in 3 patients. The genotype was unique in all patients except 2 brothers (patients 202 and 203).

Pretreatment BCVA in the worse eye was between 40 and 62 ETDRS letters (0.90–0.46 logarithm of the minimum angle of resolution [logMAR]) in the 4 children, between 20 and 31 ETDRS letters (1.30–1.12 logMAR) in 3 adults, and counting fingers or hand movements in 5 other adults. The difference between the better eye and worse eye before treatment ranged from +2.5 to +13.5 letters in the 4 pediatric patients and 0 to +5 letters in the 8 adult patients.

The first 6 patients received a dose of  $1.8 \times 10^{11}$  vector genomes per eye and the second 6 patients received a dose of  $6 \times 10^{11}$  vector genomes per eye, administered by subretinal injection in a volume of 0.45 ml. The subretinal bleb included the fovea in 5 patients (patients 205, 206, 207, 208, and 210), and 1 subject (patient 204) received the 0.45-ml dose divided between 2 injection sites ([Fig 1](#)).

### Safety Evaluations

All patients tolerated the surgery and study agent administration with no treatment-related serious adverse events. The most common adverse events were those associated with the surgical procedure and included subconjunctival hemorrhage in 8 patients; ocular hyperemia in 5 patients; reduced visual acuity, eye pain, eye irritation, increased intraocular pressure, headache, or back pain in 2 patients each; and abnormal sensations in the eye or reduced visual acuity in 1 subject each. All of these were mild to moderate in intensity and generally resolved within 1 to 30 days. Adverse events considered possibly related to the rAAV2-CB-hRPE65 study agent were ocular hyperemia in 2 patients and photopsia in 1 subject. A list of all adverse events is provided in [Supplemental Table S1](#) (available at [www.aaojournal.org](http://www.aaojournal.org)).

One serious adverse event occurred in this study. Noncardiac chest pain and hypertension developed in patient 201 at the year 1 follow-up visit that resulted in overnight hospitalization, was considered not related to study agent, and resolved within 1 day. Patient 205 had dispersion of subretinal pigment within the subretinal bleb during study agent administration that was associated with persistent reduction in BCVA and mild reduction of visual field measures in the treated eye, but by the end of the study, he reported that the treated eye was the better-seeing eye in dim illumination. This subject also had a persistent elevation of intraocular pressure (41 mmHg on day 8) that responded to conventional management with topical dorzolamide and timolol for 7 weeks. In 3 patients, a small amount of subretinal fluid was detected in the treated eye on OCT examination 1 day after surgery that resolved by the next visit (patients 204 and 206) or by the month 2 visit (patient 201). Anterior chamber cells of grade 0.5 (1–5 cells per field) were observed at

Table 1. Baseline Demographic, Genetic, and Clinical Characteristics of 12 Patients with RPE65 Leber Congenital Amaurosis or Severe Early-Childhood–Onset Retinal Dystrophy

Patient No.	Age (yrs)	Gender	Race	Ethnicity	RPE65 Genotype		Visual Acuity*			Visual Field		National Eye Institute 25-Item Visual Function Questionnaire Score <sup>§</sup>
							Better Eye	Worse Eye	Kinetic (deg <sup>2</sup> ) <sup>†</sup>	Static (Decibel-Steradian) <sup>‡</sup>		
										Total Visual Field of Vision	Central 30° Visual Field Hill of Vision	
301	39	Male	White	Non-Hispanic	Gly40Ser	His182Tyr	5	0 (HM)	558	3.5	1.4	54
201	33	Female	Black	Non-Hispanic	Arg124Stop	Tyr318Asn	23.5	20.5	14 727	44.4	5.2	31
303	35	Male	Black	Non-Hispanic	Arg124X	Trp402X	6.5	2.5 (CF)	1038	3.6	0.7	54
202	6	Male	White	Hispanic	Arg118Ser	Val443Ala	53.5	40	14 408	29.4	9.0	75
203	11	Male	White	Hispanic	Arg118Ser	Val443Ala	63.5	57.5	13 738	33.0	8.9	69
204	6	Female	White	Non-Hispanic	Tyr368His	Tyr431His	57.5	48	11009	27.9	8.7	55
205	37	Male	White	Hispanic	Arg124Stop	Arg91Gln	33	31	181	6.8	28	57
206	30	Female	Asian	Non-Hispanic	c289dupA	c289dupA	0 (CF)	0 (CF)	51	1.0	0.8	45
207	32	Female	White	Hispanic	c.1336dupA	c.1336dupA	4.5	1 (CF)	1144	7.0	2.9	48
208	28	Female	White	Hispanic	Tyr144 stop	Asn451Tyr	30	25.5	14 050	21.1	6.8	36
209	35	Female	Asian	Non-Hispanic	H182R	H182R	0 (HM)	0 (HM)	1086	6.5	1.9	50
210	6	Male	White	Non-Hispanic	Lys294insIgcA	Ala415Val	64.5	62	9448	18.4	5.7	76

CF = counting fingers; HM = hand movements.

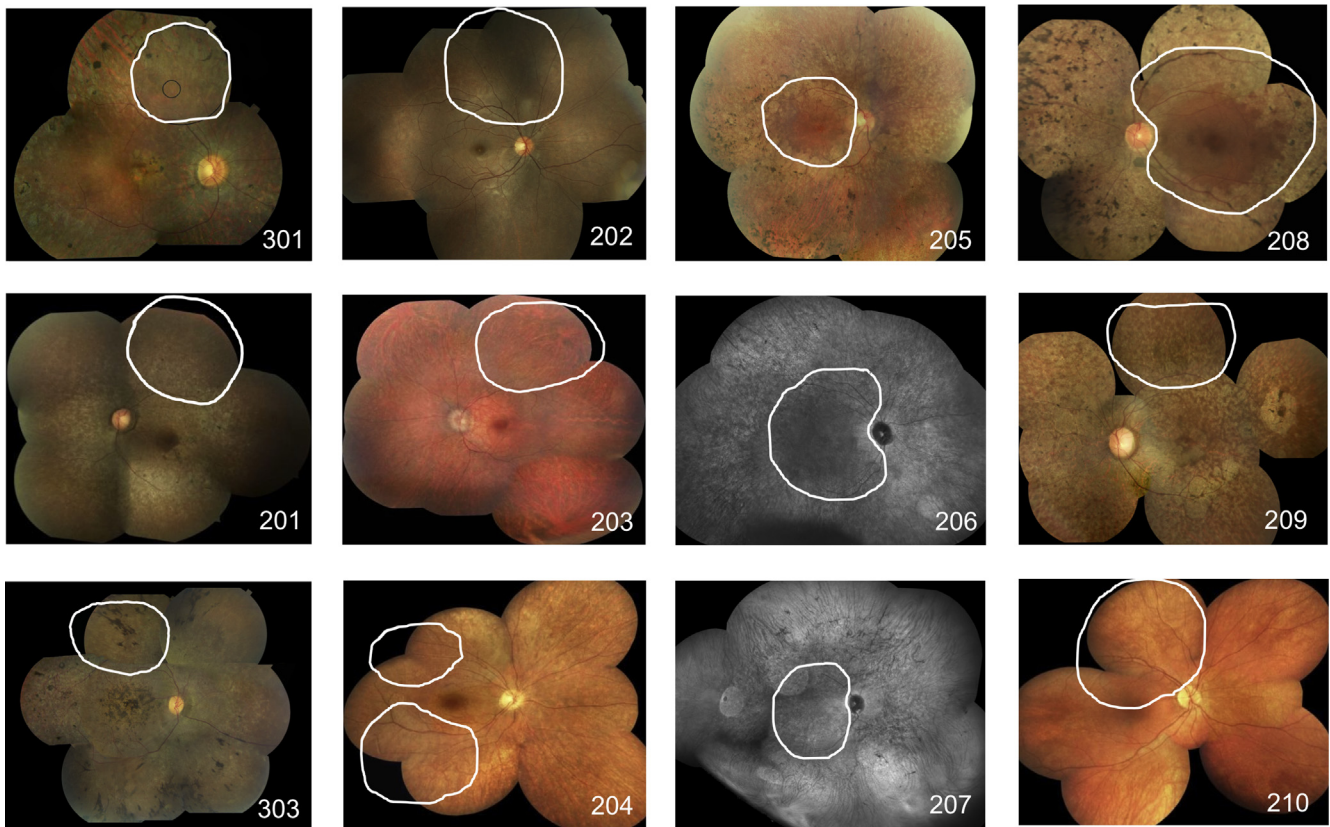
Patients are listed in the order in which they entered the trial.

\*Mean of 2 pretreatment Early Treatment Diabetic Retinopathy Study letter scores.

<sup>†</sup>Average area in square degrees for 3 pretreatment tests in the treated eye with a V4e target.

<sup>‡</sup>Average retinal sensitivity in decibel-steradians for 3 pretreatment tests in the treated eye for the total or central 30° field.

<sup>§</sup>Composite score, for which the maximum score is 100.



**Figure 1.** Images showing the locations of subretinal blebs after administration of recombinant adeno-associated virus vector expressing RPE65. The bleb included the subfoveal area for patients 205, 206, 207, 208, and 210.

days 1 and 7 in patient 210 and at day 14 in patients 208 and 209. No other patients showed signs of ocular inflammation, and no patients demonstrated prolonged retinal detachment, vitreous hemorrhage, or development of a cataract.

There were no clinically important changes in hematologic or serum chemistry parameters after study agent administration. Titers of neutralizing antibodies to AAV increased in 5 of 12 patients, including 1 of 4 children and 4 of 8 adults (see [Supplemental Table S2](#), available at [www.aaojournal.org](http://www.aaojournal.org)). No patient demonstrated an enzyme-linked immunospot response to RPE65 or AAV2 capsid peptides and no patient had vector DNA detected in blood at any time point tested.

### Visual Acuity

Results of visual acuity testing over time are presented in [Figure 2](#). The repeatability coefficient was 0.145 logMAR, equivalent to 7 ETDRS letters, for all eyes, and 0.164 logMAR, equivalent to 8 ETDRS letters, for the 19 eyes that had an ETDRS letter score of more than 0 on both pretreatment tests. (One subject had count fingers at both pretreatment tests, with an ETDRS letter score of 0 on one test and 1 on the other.). In the treated eye, BCVA did not change in the 5 patients with counting fingers or hand movements vision, but decreased immediately after treatment as a result of surgical elevation of the retina in the other 7 patients. In 6 patients, BCVA improved to pretreatment levels or more by day 14 (patients 202 and 204), month 1 (patients 201, 203, and 210), or month 2 (patient 208). In 5 of these patients, improvement in

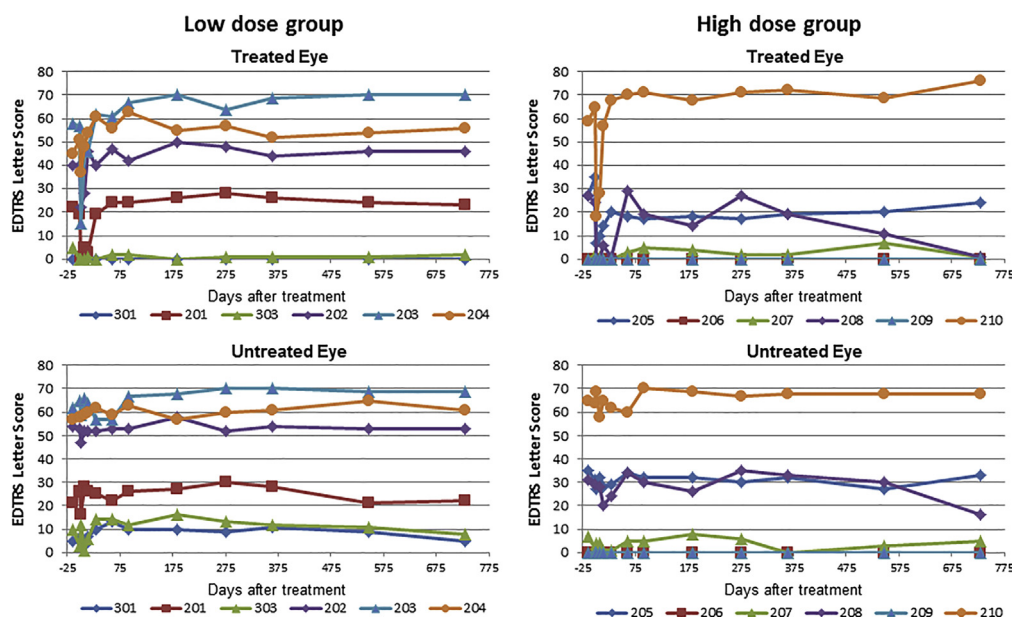
BCVA compared with pretreatment BCVA persisted during the 2-year study and was greater in the treated eye than in the untreated eye ([Fig 3](#)). In patient 205, BCVA in the treated eye remained at less than pretreatment levels throughout the 2-year study. In patient 208, there was worsening of BCVA during the second year of the study in both the treated and untreated eye, with a loss of 24.5 letters in the treated eye and 14 letters in the untreated eye at the year 2 visit compared with baseline.

### Optical Coherence Tomography Analysis

Because of advanced degeneration in some patients, OCT segmentation was difficult and could be completed in both eyes of only 6 patients (patients 202, 203, 204, 205, 208, and 210). After treatment, the outer retinal thickness was increased variably or decreased at different eccentricities from the fovea. Both treated and untreated eyes demonstrated changes in thickness; however, the patterns were quite variable and there was neither evidence suggestive of a treatment effect, nor for a toxic effect. Interpretation of the data was limited by the overall poor quality of the data primarily because of poor fixation in these patients.

### Static Perimetry

Results of analyses of static perimetry for all patients in the trial are presented in [Figures 4 and 5](#) and in [Supplemental Table S3](#) (available at [www.aaojournal.org](http://www.aaojournal.org)). A graphic example of the visual field modeling and analysis results for patient 202 is shown in [Supplemental Figure S1](#) (available at [www.aaojournal.org](http://www.aaojournal.org)).



**Figure 2.** Graphs showing serial visual acuity measurements in patients treated with recombinant adeno-associated virus vector expressing RPE65 by subretinal injection on day 0. Early Treatment Diabetic Retinopathy Study (ETDRS) letter score of 85 = 0.00 logarithm of the minimum angle of resolution (logMAR) = Snellen 20/20. An ETDRS letter score of 35 = 1.0 logMAR = Snellen 20/200.

There was more than a 10-fold variation among patients in the average baseline retinal sensitivity determined by static perimetry, which in the treated eye ranged from 0.67 to 9.03 dB-sr (mean, 4.56 dB-sr; median, 1.42 dB-sr) for  $V_{30}$  and from 1.04 to 44.37 dB-sr (mean, 16.87 dB-sr; median, 3.58 dB-sr) for  $V_{TOT}$  (Table 1). Comparison of pretreatment values for  $V_{30}$  and  $V_{TOT}$  found no significant between-test differences. The average limit of agreement for all baseline retinal sensitivity results was  $-3.50$  to  $+3.12$  dB-sr for  $V_{30}$  and  $-9.73$  to  $+8.17$  dB-sr for  $V_{TOT}$ .

Six patients (patients 202, 203, 204, 207, 208, and 210) showed improvement in  $V_{30}$  in the treated eye at 1 or more posttreatment visits, and 4 of these patients (patients 202, 207, 208, and 210) also showed improvement in  $V_{30}$  in the untreated eye at 1 or more posttreatment visits (Fig 4; Supplemental Table S3, available at [www.aaajournal.org](http://www.aaajournal.org)). Five patients (patients 202, 204, 207, 208, and 210) showed improvement in  $V_{TOT}$  in the treated eye at 1 or more posttreatment visits, and 3 of these patients (patients 202, 207, and 210) also showed improvement in  $V_{TOT}$  in the untreated eye at 1 or more posttreatment visits (Fig 5; Supplemental Table S3, available at [www.aaajournal.org](http://www.aaajournal.org)).

One patient (patient 204) showed a decrease in  $V_{30}$  in the treated eye at a single posttreatment visit and 2 patients (patients 202 and 208) showed a decrease in  $V_{30}$  in the untreated eye at 1 or 2 posttreatment visits (Fig 4; Supplemental Table S3, available at [www.aaajournal.org](http://www.aaajournal.org)). Four patients (patients 201, 203, 204, and 208) showed a decrease in  $V_{TOT}$  in the treated eye at 1 or more posttreatment visits, and 3 patients (patients 202, 203, and 208) showed a decrease in  $V_{TOT}$  in the untreated eye at 1 or more posttreatment visits (Fig 5; Supplemental Table S3, available at [www.aaajournal.org](http://www.aaajournal.org)).

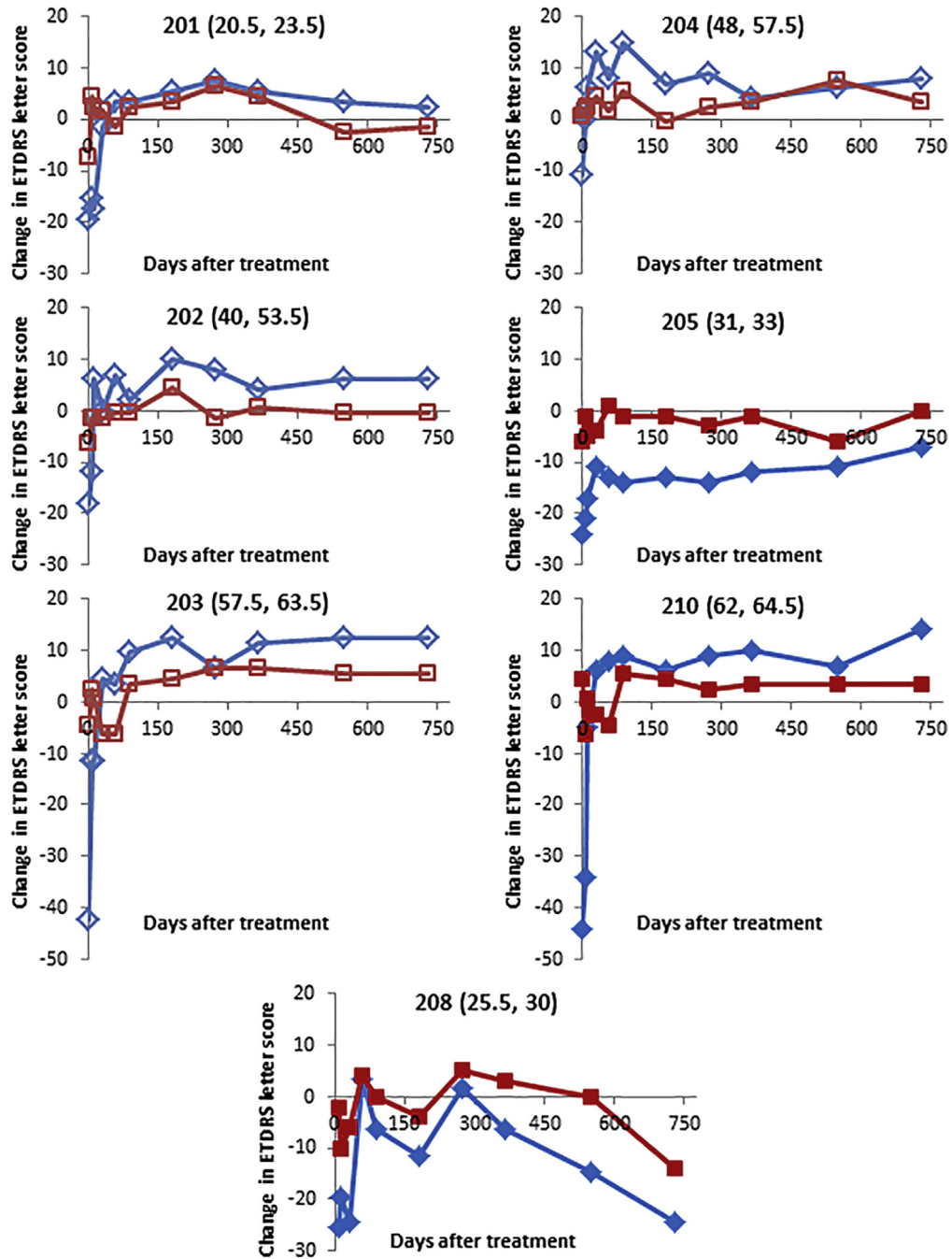
### Kinetic Perimetry

There was more than a 100-fold variation among patients in the average baseline kinetic visual field area, which for the V4e target

ranged from 51 to 14 727 deg<sup>2</sup> (mean, 7308 deg<sup>2</sup>; median, 5296 deg<sup>2</sup>) in the treated eye (Table 1). Four patients showed improvement in kinetic visual fields during the study (Fig 6). Patients 301, 303, and 207 showed an increase in kinetic visual field area in the treated eye compared with baseline at the month 6 and year 1 and 2 visits, with a decrease in visual field area in the untreated eye at the month 6 and year 1 and 2 visits in patient 303 and at the year 2 visit in patient 301 (Table 2). Patient 201 showed resolution of a central scotoma on kinetic testing in the treated eye (Fig 6). Although this was not more than the limit of agreement of pretreatment values, she also showed a modest increase in  $V_{30}$  decibel-steradian units on static perimetry and reported improvement in her ability to navigate independently when traveling through airports. Two patients showed worsening of kinetic visual fields during the study (Fig 7). Patient 209 showed a loss of kinetic visual field area in the treated eye compared with baseline at the month 6 and year 1 and 2 visits, with an increase in visual field area in the untreated eye at these visits, and patient 208 showed a substantial decrease in the kinetic visual field area in both eyes during the study that was larger in the treated eye than in the untreated eye (Table 2). The other 6 patients had no apparent changes in kinetic visual field area with the V4e target. Changes in kinetic visual field area with the V4e target for all patients are displayed in Supplemental Figure S2 (available at [www.aaajournal.org](http://www.aaajournal.org)). Patient 205 showed a small increase in kinetic visual field area with the II4e target, from a mean  $\pm$  standard deviation of  $4 \pm 2$  deg<sup>2</sup> before treatment to 25.4 deg<sup>2</sup>, 39.8 deg<sup>2</sup>, and 40.8 deg<sup>2</sup> at the month 6, year 1, and year 2 visits, respectively. None of the other patients had any apparent changes in kinetic visual field area with the III4e or II4e targets.

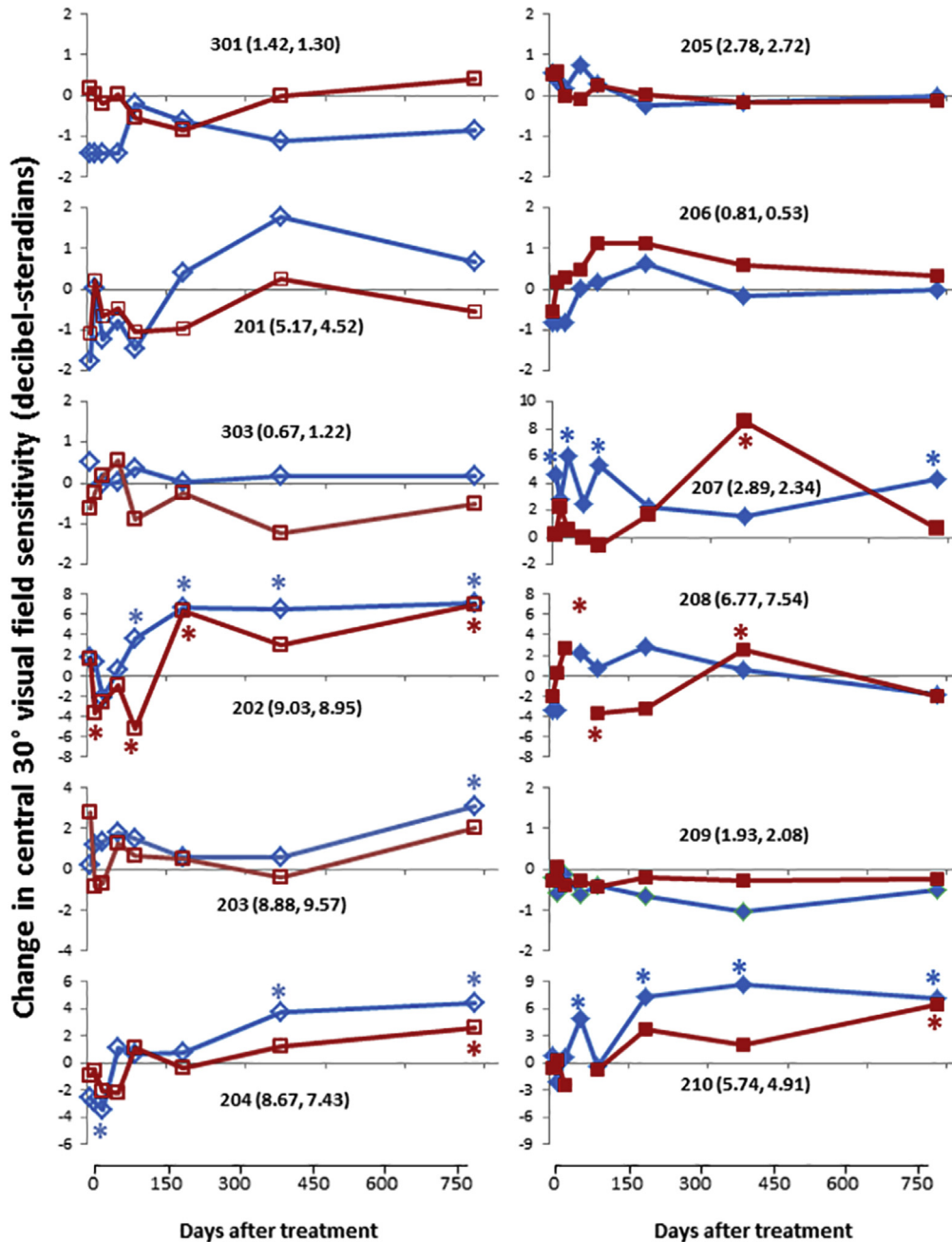
### Normative Data for Static and Kinetic Perimetry

Fifteen normal controls (6 males and 9 females; mean age, 34.7 $\pm$ 17.7 years) were tested using SKP and 13 normal controls



Subject	201	202	203	204	205	208	210
Treated eye	+2.5	+6	+12.5	+8	-7	-24.5	+14
Untreated eye	-1.5	-0.5	+5.5	+3.5	0	-14	+3.5

Figure 3. Graphs showing visual acuity changes from baseline after subretinal injection of recombinant adeno-associated virus vector expressing RPE65 in 7 patients with pretreatment visual acuity better than counting fingers. Each patient's pretreatment visual acuity in the treated and untreated eye is in parentheses after the patient's study number. Diamond symbols are for treated eyes, square symbols are for untreated eyes, open symbols are for the lower-dose group, and solid symbols are for the higher-dose group. Changes from baseline in the treated and untreated eye at the year 2 visit are shown in the table at the bottom of the figure.

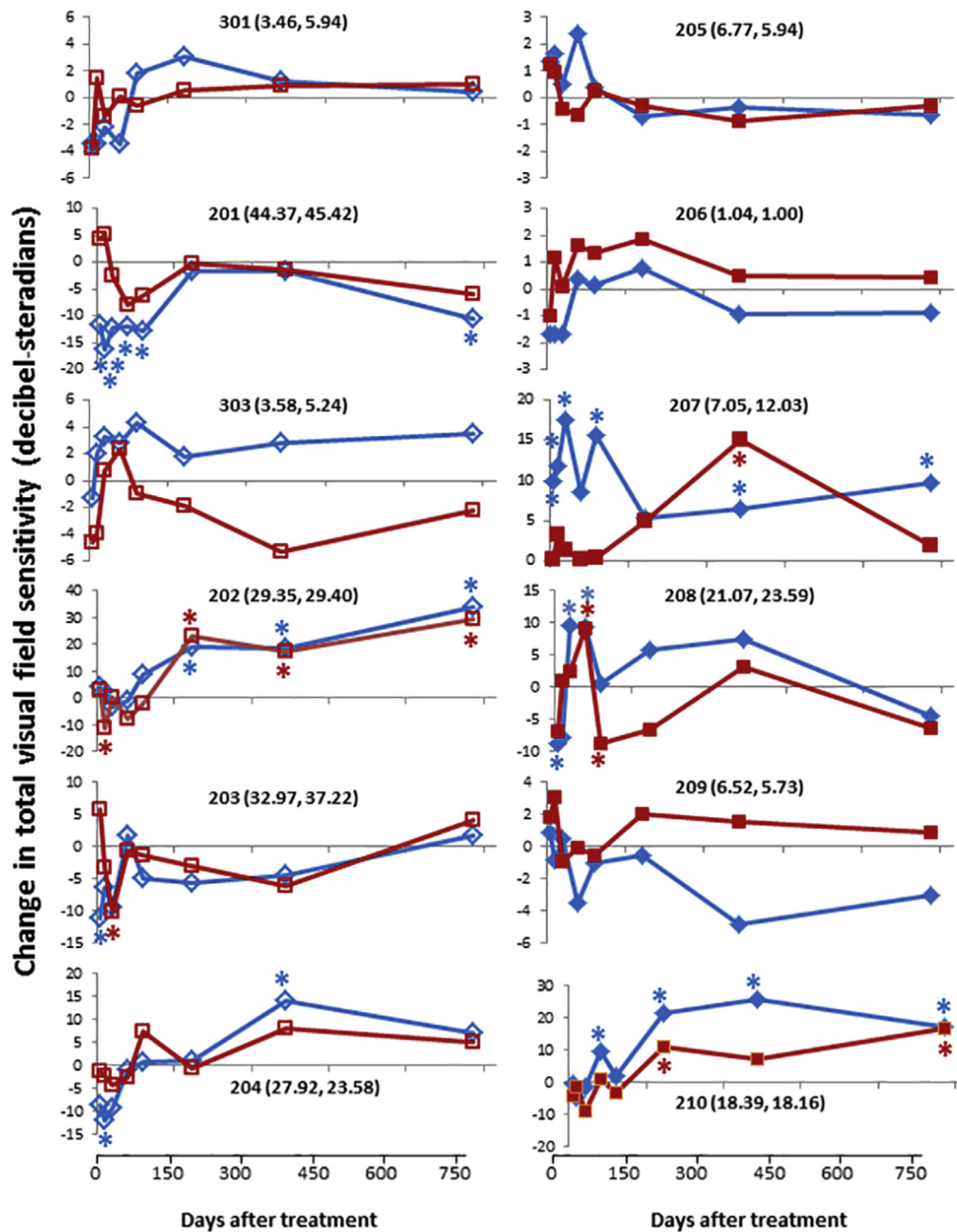


**Figure 4.** Graphs showing central 30° visual field hill of vision ( $V_{30}$ ) changes from baseline. The mean baseline  $V_{30}$  value in the treated and untreated eye is listed after each patient number. Diamond symbols are for treated eyes, square symbols are for untreated eyes, open symbols are for the lower-dose group, and solid symbols are for the higher-dose group. Asterisks indicate changes larger than the limit of agreement of pretreatment values for all patients.

(4 males and 9 females; mean age,  $38.9 \pm 19.6$  years) were tested using static perimetry. To evaluate whether the results obtained in children younger than 18 years, and in particular those in the first decade of life, were comparable with those of adults, the SKP values for the isopter areas (in square degrees) for target sizes V4e, III4e, II4e, and I4e and the indices  $V_{TOT}$  and  $V_{30}$  from static perimetry were graphed by age of testing (see Supplemental Figs S4 and S5, available at [www.aaojournal.org](http://www.aaojournal.org)). Further discussion of the normative findings is presented in the Supplemental Materials (available at [www.aaojournal.org](http://www.aaojournal.org)). The graphs of SKP isopter areas fit best with a linear equation with age and were reasonably

flat except for possibly mild decline in later years. The values in the children 10 years of age and younger appeared to be consistent with the graph obtained over the entire range of adult ages. The volumetric measures of  $V_{TOT}$  and  $V_{30}$  derived from hill of vision modeling appeared to fit notably better using a quadratic equation with smaller root mean square error (RMSE) values and larger  $R^2$  values. The maximum peak of the equation that best fit the data was in the second to third decade of life. Again, the volumetric measures of sensitivity for children in the first and second decades of life seemed comparable with those for young adults.



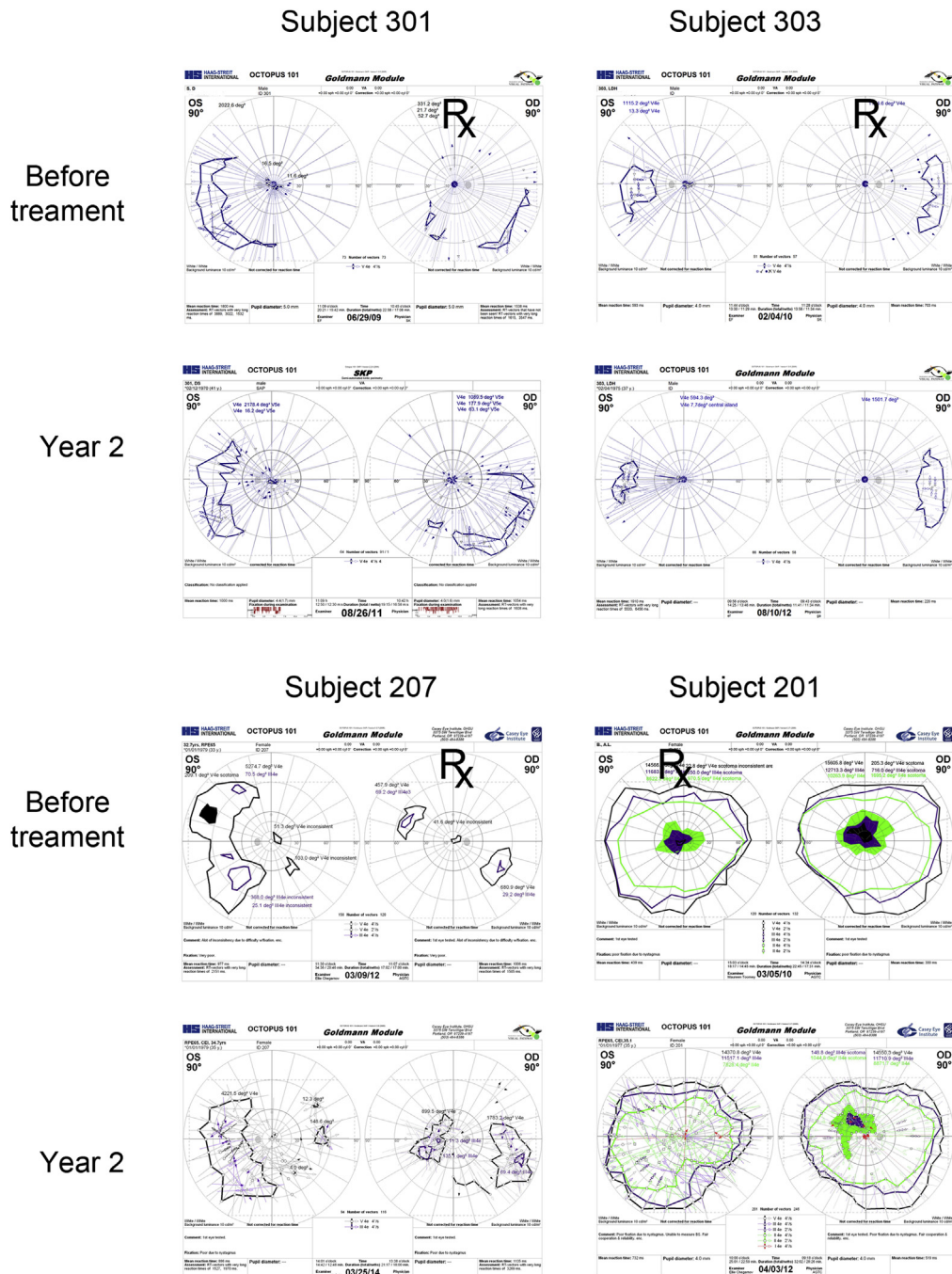


**Figure 5.** Graphs showing total visual field hill of vision ( $V_{TOT}$ ) changes from baseline. The mean baseline  $V_{TOT}$  value in the treated and untreated eye is listed after each subject number. Diamond symbols are for treated eyes, square symbols are for untreated eyes, open symbols are for the lower-dose group, and solid symbols are for the higher-dose group. Asterisks indicate changes greater than the limit of agreement of pretreatment values for all patients.

### Electroretinography

Scotopic and photopic ERG responses were reduced severely in all patients. In 8 of 12 patients, dim scotopic, bright scotopic, and single flash photopic responses were not recordable. A 30-Hz flicker response of amplitude 0.2 to 1.1  $\mu\text{V}$  was discernible in 6 of these 8, but only after filtering techniques to isolate the fundamental frequency. The meaningfulness of these submicrovolt responses is questionable. None of these 8 patients demonstrated a convincing increase in the ERG responses in either eye after treatment.

Four patients (patients 201, 202, 203, and 204) had small but recordable ERG responses during the study (Supplemental Table S4, available at [www.aojournal.org](http://www.aojournal.org)). For the 30-Hz flicker response, patient 203 showed no changes in either eye after treatment, patient 201 showed a decrease in the treated eye with no change in the untreated eye, and patients 202 and 204 showed an increase in the treated eye that was more than that in the untreated eye. Patient 204 also demonstrated small but recordable a- and b-waves to the brightest stimuli under scotopic and photopic conditions (Fig 8). At the month 12 visit,



**Figure 6.** Kinetic visual fields in patients 301, 303, 207, and 201 before and 2 years after subretinal injection of recombinant adeno-associated virus vector expressing RPE65. The right eye was treated in patients 301, 303 and 207 and the left eye was treated in subject 201, indicated by the R symbol.

there was an increase in the scotopic b-wave amplitude (from 9 to 17  $\mu$ V) in the treated eye compared with a decrease (from 15 to 11  $\mu$ V) in the untreated eye, and the increase in the photopic single flash b-wave amplitude was slightly more in the treated eye (from 5 to 13  $\mu$ V) than in the untreated eye (from 4 to 8  $\mu$ V). The changes in photopic responses may reflect intervisit variability, but the improvement in scotopic recordings in the treated eye of patient 204 could reflect a functional improvement.

### Quality-of-Life Assessment

The NEI-VFQ-25 composite scores at baseline ranged from 55 to 76 for the 4 pediatric patients with better visual acuity and from 31 to 57 for the 8 adult patients with worse visual acuity (Table 1). The NEI-VFQ-25 composite score was improved at all or most time points after treatment in 11 of 12 patients and worse at all time points after treatment in patient 205 (Supplemental Fig S3, available at [www.aaojournal.org](http://www.aaojournal.org)).

Table 2. Changes from Baseline Kinetic Visual Field Area after Treatment

	Patient No.				
	301	303	207	208	209
Treated eye					
Baseline	558 ± 145	1038 ± 119	1144 ± 132	14 050 ± 2219	1086 ± 454
Month 6	1199	1380	3462	14 406	209
Year 1	1028	1386	2752	1201	9
Year 2	1330	1502	2683	2866	0
Untreated eye					
Baseline	2047 ± 277	1213 ± 100	2837 ± 2065	13 297 ± 4346	773 ± 133
Month 6	2708	466	4093	16 112	1451
Year 1	2011	484	2966	14 892	2074
Year 2	234	602	4386	6523	1241

Values are the kinetic visual field area in square degrees tested with a V4e target for the 5 patients who showed a change from baseline. The baseline value for each patient is the mean ± standard deviation of 3 pretreatment test results. The other 7 patients showed no consistent changes in total kinetic visual field area after treatment.

### Discussion

Results of this study confirm that rAAV2-CB-hRPE65 delivered by subretinal injection had an acceptable safety

profile and was associated with improvement in 1 or more measures of visual function in 9 of 12 patients. However, the visual function parameters that improved varied among patients, and 2 patients showed a decrease in BCVA or

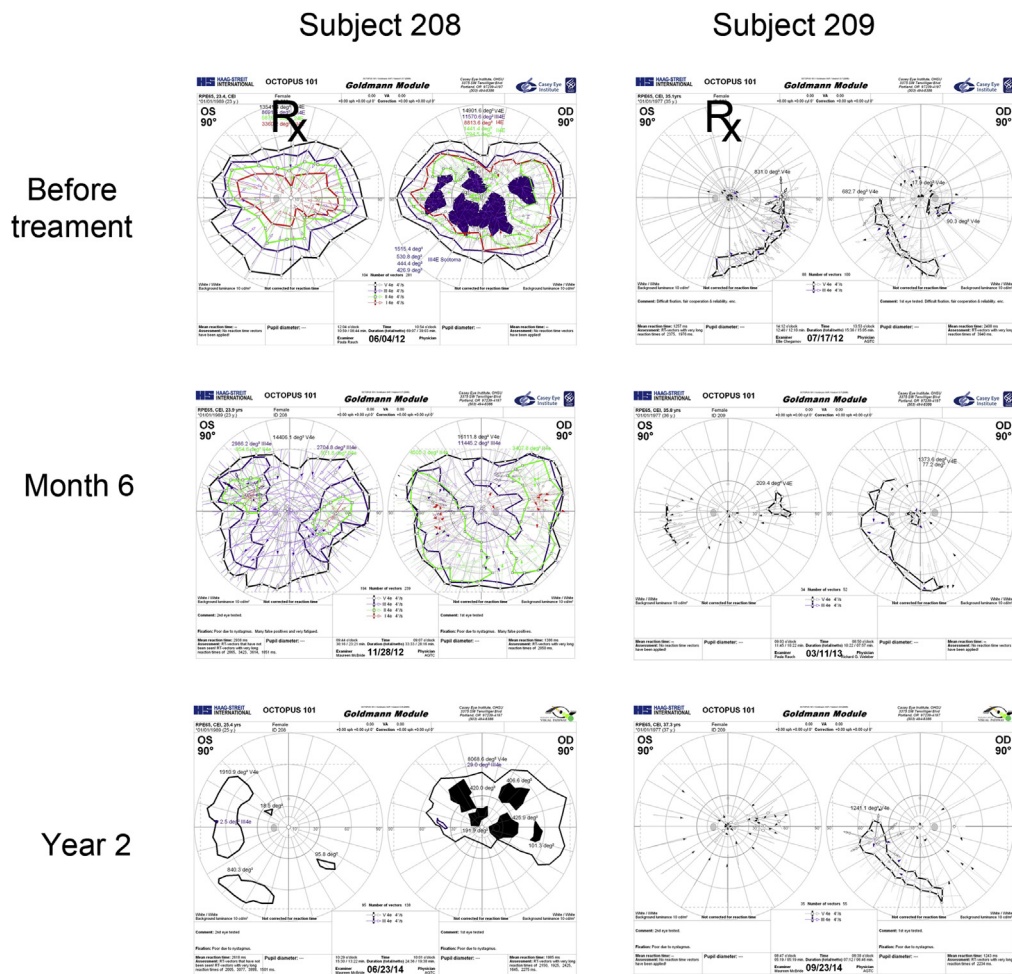
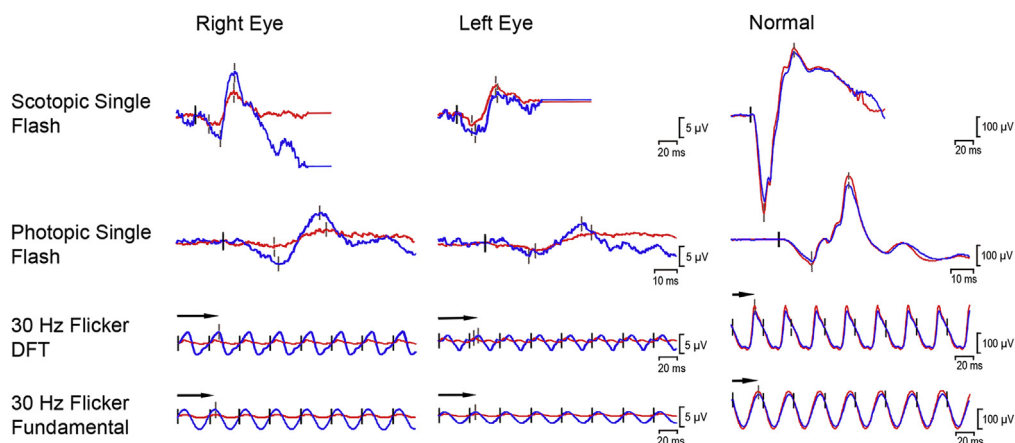


Figure 7. Kinetic visual fields in patients 208 and 209 before and 6 months and 2 years after subretinal injection of recombinant adeno-associated virus vector expressing RPE65. The left eye was treated in both patients, indicated by the Rx symbol.



**Figure 8.** Electroretinogram responses for each eye of subject 204 before (red) and after (blue) subretinal injection of recombinant adeno-associated virus vector expressing RPE65 (rAAV2-CB-hRPE65) in the right eye. The scotopic single flash b-wave amplitude at 12 months following treatment was notably increased for the right eye and was essentially unchanged for the left eye. The photopic single flash b-wave amplitude and 30 Hz flicker responses were also increased over baseline for the treated right eye and, to a lesser degree, increased in amplitude over baseline for the untreated left eye. The black vertical bar for the single flash responses and 30 Hz flicker responses indicate the stimulus occurrence. The length of the black arrow above each train of flicker responses depicts the implicit time from the initial stimulus to the positive b-wave peak.

visual field area that was more in the treated eye than in the untreated eye.

The 4 pediatric patients (patients 202, 203, 204, and 210) with baseline BCVA of 40 to 63 ETDRS letters showed improvement in BCVA, ranging from a 6- to 14-letter increase in the treated eye at the 2-year visit (Fig 3), which was more than the upper bound for intervisit variability of 7 or 8 ETDRS letters in 3 of the 4 patients. Visual acuity improvement was evident as early as 2 weeks after injection and often demonstrated progressive improvement for 3 months after treatment before stabilizing. This may correspond to transcriptional activation of the transgene in the AAV vector and is consistent with temporal observations after treatment in prior studies.<sup>22–24,26</sup> These 4 patients also showed improvement in  $V_{30}$  and 3 showed improvement in  $V_{TOT}$  at 1 or more posttreatment visits, but none showed improvement in kinetic perimetry area. Clinical examination also revealed reduced nystagmus in these patients after treatment.

Among the 3 adults with baseline BCVA of 20 to 30 ETDRS, changes in the treated eye included a 2.5-letter increase in BCVA and loss of a central scotoma in patient 201; a 7-letter decrease in BCVA, a small increase in visual field area with the II4e target, and subjective improvement in vision under dim light conditions in patient 205; and a 24-letter decrease in BCVA and kinetic perimetry area, but with an increase in  $V_{30}$  and  $V_{TOT}$  at some visits, in patient 208. Among the 5 adults with baseline visual acuity of counting fingers or hand movements (patients 301, 303, 206, 207, and 209), none showed a change in BCVA. Three of these patients showed an increase, 1 showed a decrease, and 1 showed no change in kinetic visual field area in the treated eye. Patient 207 showed a small increase in  $V_{30}$  and  $V_{TOT}$  at some visits.

In 3 of the 4 pediatric patients with an increase in visual acuity after treatment, the subretinal bleb visualized at surgery did not include the fovea, and the subretinal bleb in

patient 201 did not extend to the area of the central scotoma that resolved after treatment. It is possible that the size of the bleb increased in the first few days after subretinal injection, as has been described after subretinal injection of AAV vectors in dogs,<sup>52</sup> and expanded toward the fovea. The edges of the subretinal bleb are visible for extended periods in dogs and can be monitored over time, but in humans, the edges of the bleb are not apparent after the subretinal fluid is absorbed.

The increase in the visual field sensitivity in the untreated eye of several patients is consistent with observations in at least some of our patients that the visual acuity also improved to some degree in the untreated eye (Fig 3), and other investigators also have reported improvements in visual function for the untreated eye in their clinical trials of gene replacement therapy for RPE65-deficient LCA.<sup>27</sup> Nystagmus was a common finding in our patients, and in several instances, the parents or examining physicians noted a decrease in nystagmus after treatment. Previous studies have reported that use of an opaque occluder for the nontested eye during visual field testing will bring out a latent nystagmus component, leading to an increase in the apparent nystagmus, whereas use of a semitranslucent occluder can promote more stable fixation, can eliminate the retinal rivalry-based Ganzfeld blankout phenomenon, can increase retinal visual field sensitivity, and can improve global perimetric indexes.<sup>53,54</sup> These reports are consistent with our experience that worsening of an existing nystagmus is less likely when using the semitranslucent occluder provided by Haag-Streit. A substantial portion of the central nervous system is involved in eye tracking, saccades, and fixation stability, functions for which the eyes and brain work as a single unit. Because a dampening in nystagmus would be expected to produce an increase in foveal fixation time and a concomitant increase in viewing stability at the locations of visual field testing in each eye, the improvement in visual acuity and visual field sensitivity

in the treated eye in this way might have contributed to the improvement in acuity in the untreated eye over time.

Because of the large variation in baseline visual function, the small number of patients per group, and the differences in the location of the site of vector administration, it is not possible to draw meaningful conclusions about any potential dose-related differences in safety or efficacy or the effect of subfoveal vector administration, which was reported to be associated with a negative outcome in 1 previous study of gene replacement therapy in patients with *RPE65* mutations,<sup>29</sup> but not in patients in other studies.<sup>27,32</sup> The improvement in visual acuity in the 1 pediatric patient in the higher-dose group who received a subfoveal injection (patient 210) was similar to the improvement in the 3 pediatric patients in the lower-dose group who did not receive subfoveal injections (patients 202, 203, and 204), and 3 of 6 patients with improvement in  $V_{30}$ ,  $V_{TOT}$ , or both (patients 207, 208, and 210) were in the higher-dose group and received a subfoveal injection. The NEI-VFQ-25 composite scores improved in 11 of 12 patients, but interpretation of these results is hindered by the lack of an untreated control group.

In summary, analysis of results from this clinical trial shows that AAV2-mediated *RPE65* gene replacement therapy generally was safe and was associated with improvement in at least 1 measure of visual function in most individuals with LCA or SECORD caused by *RPE65* mutations. There was a trend for this improvement to be clustered in the younger patients, suggesting that treatment at an early age may prevent progression of photoreceptor degeneration.<sup>55–59</sup> Evaluation of more patients and a longer follow-up will be needed to determine the rate of uncommon or rare side effects or safety concerns.

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### Abbreviations and Acronyms:

**AAV** = adeno-associated virus; **BCVA** = best-corrected visual acuity; **dB-sr** = decibel-steradian; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **ERG** = electroretinogram; **LCA** = Leber congenital amaurosis; **logMAR** = logarithm of the minimum angle of resolution; **OCT** = optical coherence tomography; **rAAV2-CB-hRPE65** = recombinant adeno-associated virus vector expressing RPE65; **SECORD** = severe early childhood-onset retinal degeneration; **NEI-VFQ-25** = National Eye Institute 25-Item Visual Function Questionnaire; **SKP** = semiautomated kinetic perimetry; **V<sub>TOT</sub>** = total visual field hill of vision; **V<sub>30</sub>** = central 30° visual field hill of vision.

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