Two-Year Results After AAV2-Mediated Gene Therapy for Choroideremia: The Alberta Experience

IOANNIS S. DIMOPOULOS, STEPHANIE C. HOANG, ALINA RADZIWON, NATALIA M. BINCZYK, MIGUEL C. SEABRA, ROBERT E. MACLAREN, RIZWAN SOMANI, MATTHEW T.S. TENNANT, AND IAN M. MACDONALD

• PURPOSE: To assess the safety of a recombinant adeno-associated viral vector expressing REP1 (rAAV2.REP1) in choroideremia subjects.

• METHODS: Design: Phase I clinical trial. Participants: Six adult male subjects, 30-42 years of age, with genetically confirmed choroideremia (CHM) were enrolled. The eye with the worse vision, for all subjects, received a single subfoveal injection of 0.1 mL rAAV2.REP1 containing 10^{11} genome particles. Subjects were followed up for 2 years thereafter. Outcome Measures: The primary outcome measure was safety, determined by the number of ocular and systemic adverse events assessed by ophthalmic examination, spectral-domain optical coherence tomography (SD-OCT), and short-wavelength autofluorescence (FAF). Secondary outcome measures were the change from baseline in best-corrected visual acuity (BCVA) in the treated eye compared to the untreated eye, changes in visual function using microperimetry, and the area of retinal pigment epithelium (RPE) preservation by FAF.

• RESULTS: One subject had an 8-ETDRS-letter BCVA loss from baseline measured at 24 months, while 1 subject had a ≥15-letter BCVA gain. A similar improvement was noted in the untreated eye of another subject throughout the follow-up period. Microperimetry sensitivity showed no improvement or significant change up to 2 years after vector administration. The area of preserved RPE as measured by FAF was noted to decline at a similar rate between the treated and untreated eyes. One subject experienced a serious adverse event: a localized intraretinal immune response, resulting in marked decline in visual function and loss of SD-OCT outer retinal structures.

• CONCLUSIONS: One serious adverse event was experienced in 6 subjects treated with a subfoveal injection of AAV2.REP1. The area of remaining functional RPE in the treated eye and untreated eye declined at the same rate over a 2-year period. Fundus autofluorescence area is a remarkably predictive biomarker and objective outcome measure for future studies of ocular gene therapy in CHM subjects. (Am J Ophthalmol 2018;193:130–142. © 2018 Elsevier Inc. All rights reserved.)

FOR MANY YEARS, OUR CLINICAL RESEARCH LABORATORY has investigated the pathogenesis of choroideremia (CHM), an X-linked retinopathy caused by mutations in the *CHM* gene that generally results in the loss of Rab escort protein-1 (REP1). With this expertise, the Alberta Ocular Gene Therapy Team was established with the intent of building capacity for ocular gene therapy in Canada and testing the effect of gene replacement in human CHM subjects.

To maximize our early research success, our group liaised with the Oxford ocular gene therapy group to realize lessons learned when performing ocular gene therapy. In a phase I study of 6 CHM patients treated by MacLaren and associates[1] with the AAV2.REP1 vector, at 6 months, subjects had not lost "clinically significant" visual acuity while the retinal thickness remained unchanged despite surgically induced foveal detachment. Furthermore, 2 patients showed an improvement in retinal function. Longer follow-up of the 6 subjects demonstrated that 2 maintained improvements in visual acuity, providing encouraging signs of safety.[2]

This group had used an AAV2.REP1 vector that shares the same capsid, promoter, and poly-A signal as Luxturna (Spark Therapeutics, Philadelphia, Pennsylvania, USA), which in December 2017 became the first gene therapy treatment to be approved by the US Food and Drug Administration (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm589467.htm).

As questions remained as to what metrics would most accurately measure the safety and efficacy of subretinal delivery of an AAV2.REP1 vector, we tailored our first human study to include additional objective outcomes that

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From the Department of Ophthalmology and Visual Sciences, University of Alberta, 7-030 Katz. Bldg., Edmonton, Alberta, Canada (I.S.D., S.C.H., A.R., N.M.B., R.S., M.T.S.T., I.M.M.); Molecular Medicine Section, National Heart and Lung Institute, Imperial College London, London, United Kingdom (M.C.S.); and Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom (R.E.M.).

Inquiries to Ian MacDonald, Department of Ophthalmology and Visual Sciences, University of Alberta, 7-030 Katz Bldg, Edmonton, AB T6G 2E1 Canada; e-mail: macdonal@ualberta.ca

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might pick up subtler changes not identified in the original phase I study.

The design of clinical trials for CHM depends on subjective and objective measures of retinal structure and visual function. Cross-sectional data from large cohorts and prospective natural history studies of CHM patients help define expected rates of change and delineate how long patients should be followed to identify interventional effects. Visual field loss in CHM patients tends to be symmetric and progressive, with a loss rate of 8.3% (± 4.7%) per year.[3] This rate of change is matched closely with an estimated 7%-8% rate of loss of functional retinal pigment epithelium (RPE) per year, as measured by fundus autofluorescence (FAF).[4] A summary of structural and functional outcome measures (best-corrected visual acuity [BCVA], color vision, perimetry, microperimetry, spectral-domain optical coherence tomography [SD-OCT], and FAF imaging) that should be considered when designing a clinical trial of experimental therapies in choroideremia can be found in Dimopoulos and associates.[5]

Inclusion of various genotypes may also be important when performing a gene therapy study for choroideremia. Studies have shown that there is no specific genotype-phenotype correlation in CHM, thereby suggesting that all genotypes should be considered in clinical trials of gene therapy.[6]

This study was a 2-year prospective trial assessing the effects of subretinal delivery of AAV2.REP1 in 6 CHM patients.

METHODS

• **STUDY DESIGN:** This study was designed as a phase 1 clinical trial evaluating the safety and efficacy of a single subretinal injection of recombinant AAV vector expressing human REP1 (rAAV2.REP1). Only 1 eye received the intervention, with the fellow eye acting as an active comparator. Safety was monitored by evaluating adverse events, hematologic and clinical chemistry parameters, and vector dissemination in peripheral tissues. Efficacy was based on longitudinal change in anatomic and functional measures from baseline in the treated compared to the untreated eyes. Each patient was followed for a minimum of 24 months after treatment to assess the primary and secondary endpoints. This clinical trial was registered with Health Canada and ClinicalTrials.gov (NCT02077361). The University of Alberta Human Research Ethics Board provided ethics approval.

Eligibility Criteria. All study candidates required molecular or genetic confirmation of choroideremia, as previously described.[7] To be considered eligible they had to meet the following inclusion criteria: (1) BCVA equal to or worse than 20/30 but better than or equal to 20/100 in the study eye; (2) active degeneration of the retina with changes in OCT visible within the macula; and (3) the expectation of significant decline in visual function without any intervention over the subsequent 5 years. The latter was based on natural history studies of full-field sensitivity loss in CHM, which identified precipitous loss of sensitivity once the degeneration encroached upon the macula.[8] Exclusion criteria consisted of any of the following: (1) previous retinal surgery in the study eye (such as macular hole repair) or history of ocular inflammatory disease (uveitis); (2) grossly asymmetrical retinal disease or other ocular morbidity that might confound adopting the fellow eye as an active comparator; and (3) any other significant systemic disease or disorder that, in the opinion of the investigator, would either put the research subject at risk because of participation in the study or influence the result of the study, or the research subject's ability to participate in the study. This would include a contraindication to oral immunosuppression (prednisone), such as a history of peptic ulcer disease. Subjects were also excluded if they had participated in another research study involving an investigational product within the past year.

Selection Process. Twenty candidates were identified from the University of Alberta and Foundation Fighting Blindness patient registry. An expert panel of 4 vitreoretinal surgeons from 4 independent Canadian centers masked to the patient name or identity reviewed the following information: age, BCVA, FAF and color photographs, SD-OCT, and microperimetry. Symmetrical disease, degeneration edge not involving the fovea, and sufficient area of RPE to safely detach the underlying retina were favored. Those candidates meeting the above-mentioned criteria and selected by the expert panel as being excellent surgical candidates (inter-rater agreement, kappa statistic > 0.8) were invited and enrolled.

• **INTERVENTION:** *Viral Vector*. The viral vector used was a recombinant AAV vector expressing human REP1 (rAAV2.REP1) in a dose of 0.1 mL containing 10^{11} genome particles. Chicken beta actin (CBA) was used as a universal promoter, with inclusion of the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to enhance transgene expression.[9] All subjects received pretreatment with oral prednisone (1 mg/kg/day) and omeprazole (20 mg/day) for 2 days before surgery and 7 days thereafter. Prednisone was gradually tapered to 0.5 mg/kg/day in the second week and halved in each of the 2 days thereafter, before stopping.

Delivery Method. Subjects underwent a standard 3-port 23 gauge vitrectomy (Constellation; Alcon, Fort Worth, Texas, USA) under general anesthetic. In cases where the posterior hyaloid was still attached to the posterior pole, triamcinolone (0.1 mL of 40 mg/mL)-assisted vitreous detachment was performed, followed by complete vitrectomy without shaving of the vitreous base. Next,

TABLE 1. Baseline Demographic, Genetic, and Clinical Characteristics of 6 Included Male Subjects Affected With Choroideremia

Subject No.	Age (Years)	*CHM* Genotype	Protein Change	Treated Eye	Visual Acuity OD EDTRS	Visual Acuity OS EDTRS	MP Mean Threshold OD (dB)	MP Mean Threshold OS (dB)	FAF Area OD (mm^2)	FAF Area OS (mm^2)	NEI-VFQ Score
P1	42	c.315-?_1166+?del	Deletion of exons 5-8 (in frame)	OD	53	60	8.77	6.30	2.41	1.95	56
P2	35	c.1218C>A	p.Cys406*	OS	73	61	10.15	9.74	2.43	2.13	42
P3	29	c.1218C>A	p.Cys406*	OS	72	67	13.79	9.80	3.10	1.97	44
P4	38	c1245-521A>G	Splice defect between exon 9/10	OD	73	75	8.80	8.00	1.58	2.18	62
P5	32	c.224 G>A	p.Trp75*	OS	62	58	8.19	4.90	2.81	1.89	53
P6	30	c1245-521A>G	Splice defect exon 9/10	OS	75	75	12.10	11.80	14.29	10.58	76

the neurosensory retina was detached from the underlying RPE with balanced salt solution (BSS) injected through a 41 gauge cannula (Dutch Ophthalmic Research Center, Zuidland, The Netherlands) into the subretinal space within the clinical macula. The location of retinal penetration was chosen through consensus by the surgical team following a review of both the FAF and OCT images. The optimal site chosen was an area of pigmentation distant from the residual island of subfoveal RPE but close enough to allow elevation of the retina with subretinal BSS bleb into the foveal area. In all cases, the BSS-induced neurosensory retinal detachment was enlarged to include the subfoveal space. The viral vector solution was then infused into the subretinal space via the same retinotomy site with a separate 41 gauge cannula connected to a 1 cc syringe with 0.2 cc of viral vector (rAAV2-REP1). Controlled injection pressure was achieved using a viscous fluid injector customized connector attached to the vitrectomy machine. Further details of the surgical modification and the device are found in a manuscript by Xue and associates.[10] At least 0.1 mL of viral vector solution was injected in each case to achieve a minimum dose of 10^{11} genome particles. Upon completion of subretinal injection of viral vector, the peripheral retina was inspected for tears with scleral depression. The light pipe was then removed and all sclerotomy sites were closed and sutured with 8.0 Vicryl sutures (Ethicon Inc., a subsidiary of Johnson and Johnson). A subconjunctival injection of 0.2 mL of cefazolin (100 mg/mL) and 0.1 mL dexamethasone (10 mg/mL) was performed at the end of the surgery.

Eye Selection. The eye with the greatest reduction in visual acuity was selected as the treatment eye. This decision was based on lower BCVA measures at baseline or subjectively worse eye. Two patients, P4 and P6, had EDTRS visual acuities of 73 and 75 letters at baseline, putting them within 3 lines (15 letters) of 20/20 Snellen acuity (85 letters).

• **OUTCOME MEASURES:** *Primary Outcome Measure*. Safety was measured by the number and severity of ocular and systemic adverse events assessed by ophthalmic examination, SD-OCT, and short-wavelength FAF.

TABLE 2. List of Ocular and Systemic Adverse Events

Adverse Event	Frequency	Outcome
Ocular		
Subconjuctival hemorrhage	6/6 (100%)	Resolved 6/6 (100%)
Ocular pain	4/6 (66%)	Resolved 4/4 (100%)
Vitreous floaters	2/6 (33%)	Present 2/2 (100%)
IOP decrease (postoperative)	6/6 (100%)	Resolved 6/6 (100%)
Blurred vision	6/6 (100%)	Resolved 5/6 (83%)
Metamorphopsia	6/6 (100%)	Improved 5/6 (83%)
Anterior chamber reaction (3/4+ cells)	2/6 (33%)	Resolved 2/2 (100%)
Intraretinal hyperreflective material	1/6 (16%)	Resolved; Rx: prednisone PO × 7 days
Suture inflammation	1/6 (16%)	Resolved; Rx: Pred Forte QID × 7 days
Other		
Epigastric pain	1/6 (16%)	Resolved
Anemia	1/6 (16%)	Resolved
Elevated CRP (month 1)	1/6 (16%)	Resolved (month 6)

CRP = C-reactive protein; IOP = intraocular pressure; PO = per os; QID = 4 times a day; Rx = prescription.

Secondary Outcome Measures. Best-Corrected Visual Acuity

Visual acuity was recorded in each eye using the Early Treatment Diabetic Retinopathy Study (ETDRS) charts, following standardized refraction. BCVA was measured in accordance with the established protocols of the ETDRS.[11] Efficacy was estimated as change from baseline BCVA at 2 years in the treated eye compared to the untreated eye.

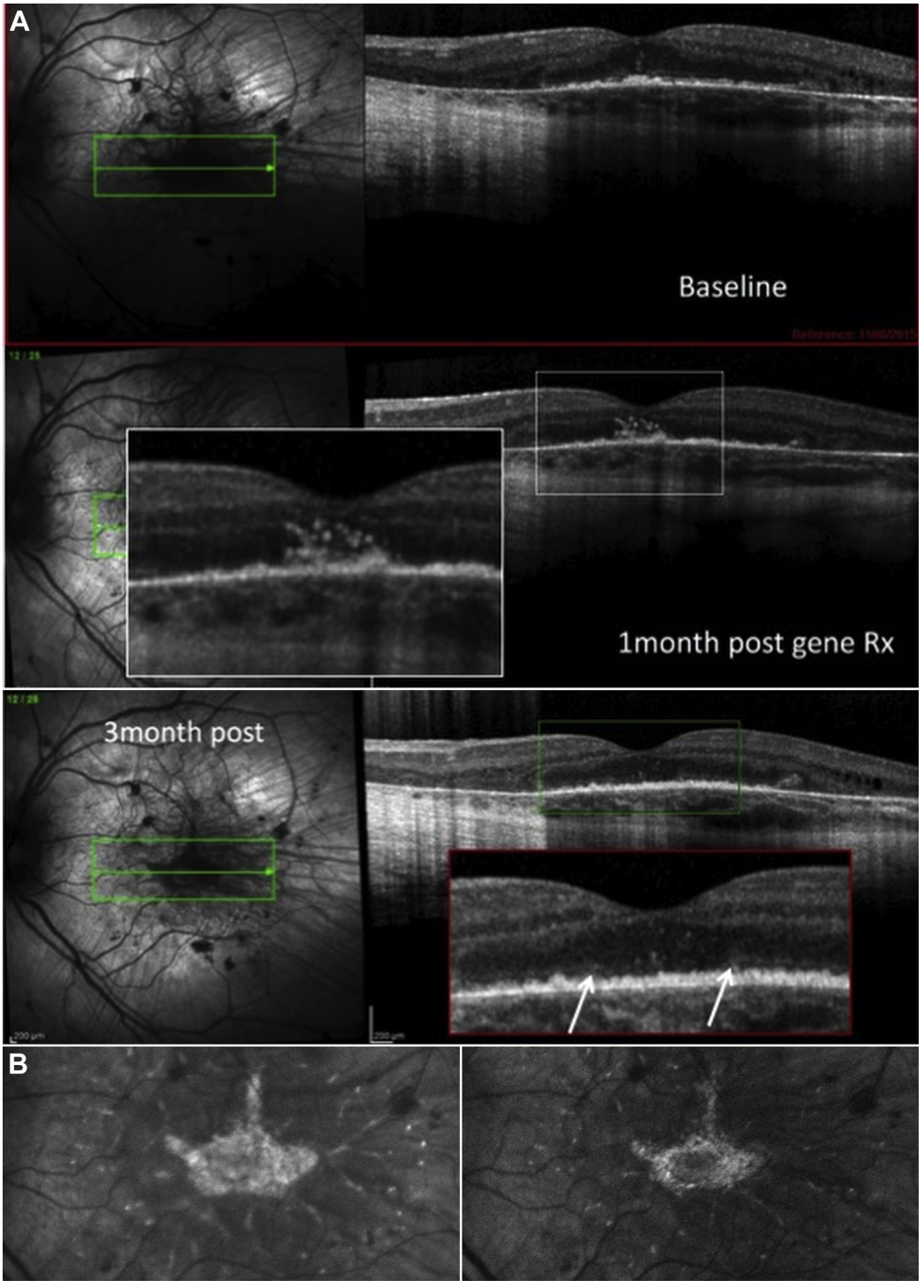

FIGURE 1. Overview of serious adverse event. (A) Spectral-domain optical coherence tomography evidence of hyperreflective material in the treated eye of Subject P3 at 1 month post-intervention (inset). Partial resolution 2 months after oral immunosuppression—a significant defect in the ellipsoid zone and external limiting membrane could be observed (arrows). (B) Baseline and 3 month short-wavelength fundus autofluorescence (FAF) images from the treated eye of Subject P3 demonstrating progressive loss of total and subfoveal FAF hyperfluorescence.

Fundus Autofluorescence

Short-wavelength (λ = 488 nm) fundus autofluorescence (SW-FAF) images were acquired for both treated and untreated eyes at baseline and at 1, 3, 6, 9, 12, 18, and 24 months using the Spectralis SD-OCT unit (Heidelberg Engineering, Heidelberg, Germany). A 30-degree lens

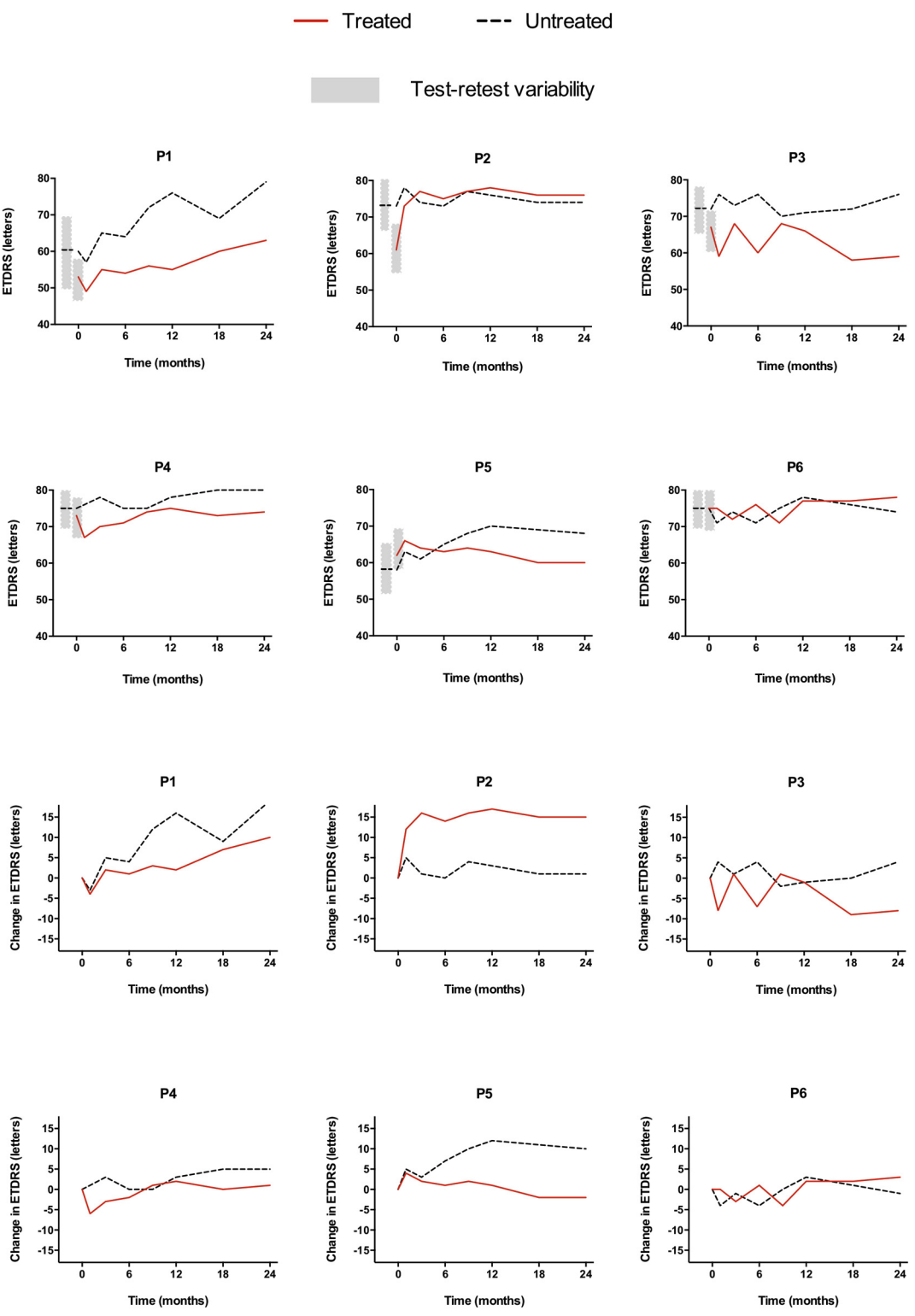

FIGURE 2. Longitudinal changes in best-corrected visual acuity over 2 years. (Top graphs) For each subject, serial visual acuity measurements for both treated and untreated eyes are presented. Gray boxes represent individual test-retest variability limits from multiple measures at baseline (month 0). (Bottom graphs) Letter change from baseline for each subject. Red continuous line: treated eye; black dotted line: untreated eye.

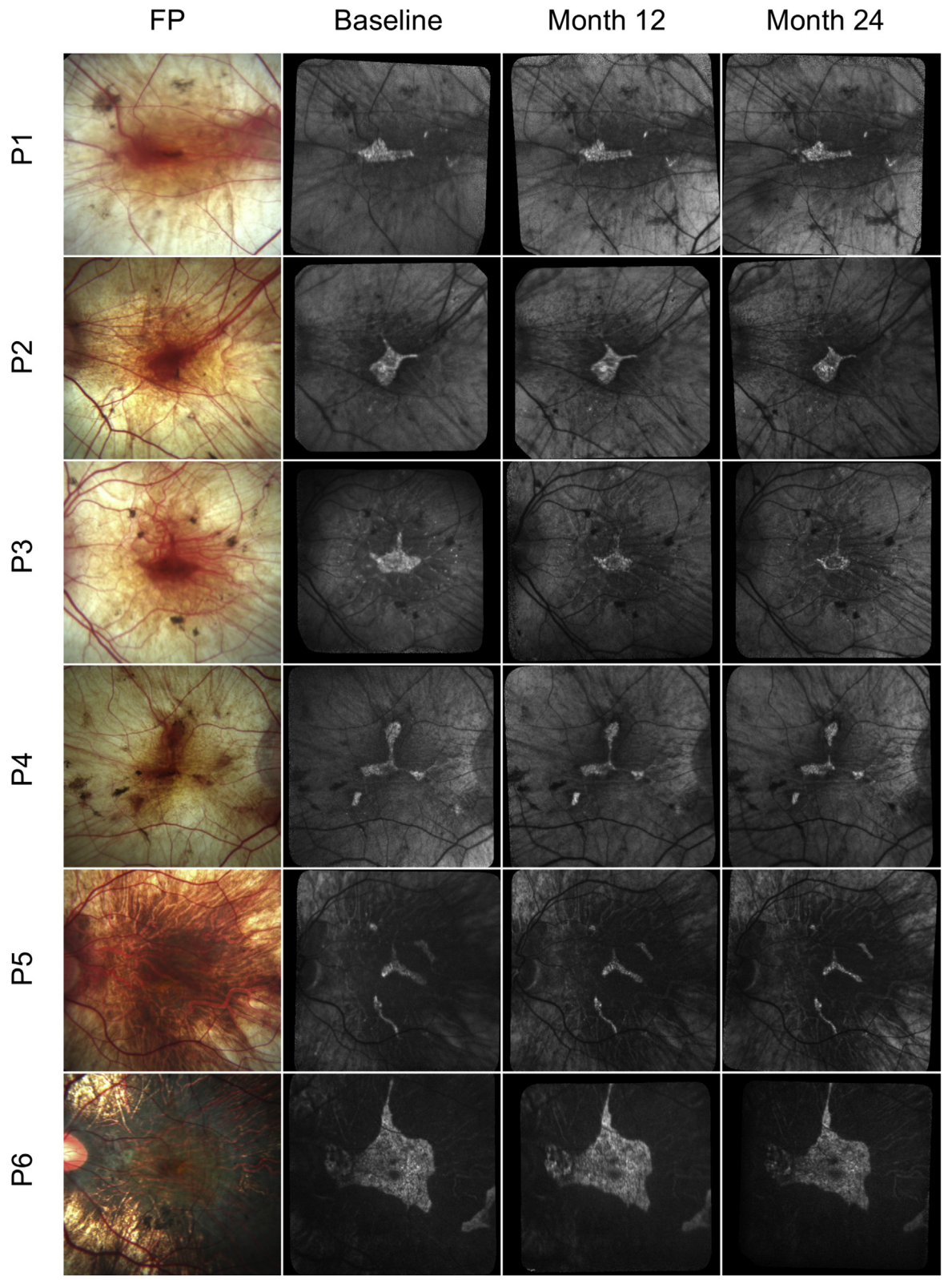

FIGURE 3. (Left) Serial short-wavelength fundus autofluorescence (FAF) images from rAAV2.REP-treated eyes. Baseline, 12-month, and 24-month images have been co-registered and aligned. Corresponding color fundus photographs (FP) are provided in the first column. (Right) FAF area change over 2 years. For each subject, linear plots of serial FAF area measures across time are presented for both treated and untreated eyes (left column). Corresponding percentage area changes from baseline (month 0) are shown in the right column. Red continuous line: treated eye; black dotted line: untreated eye.

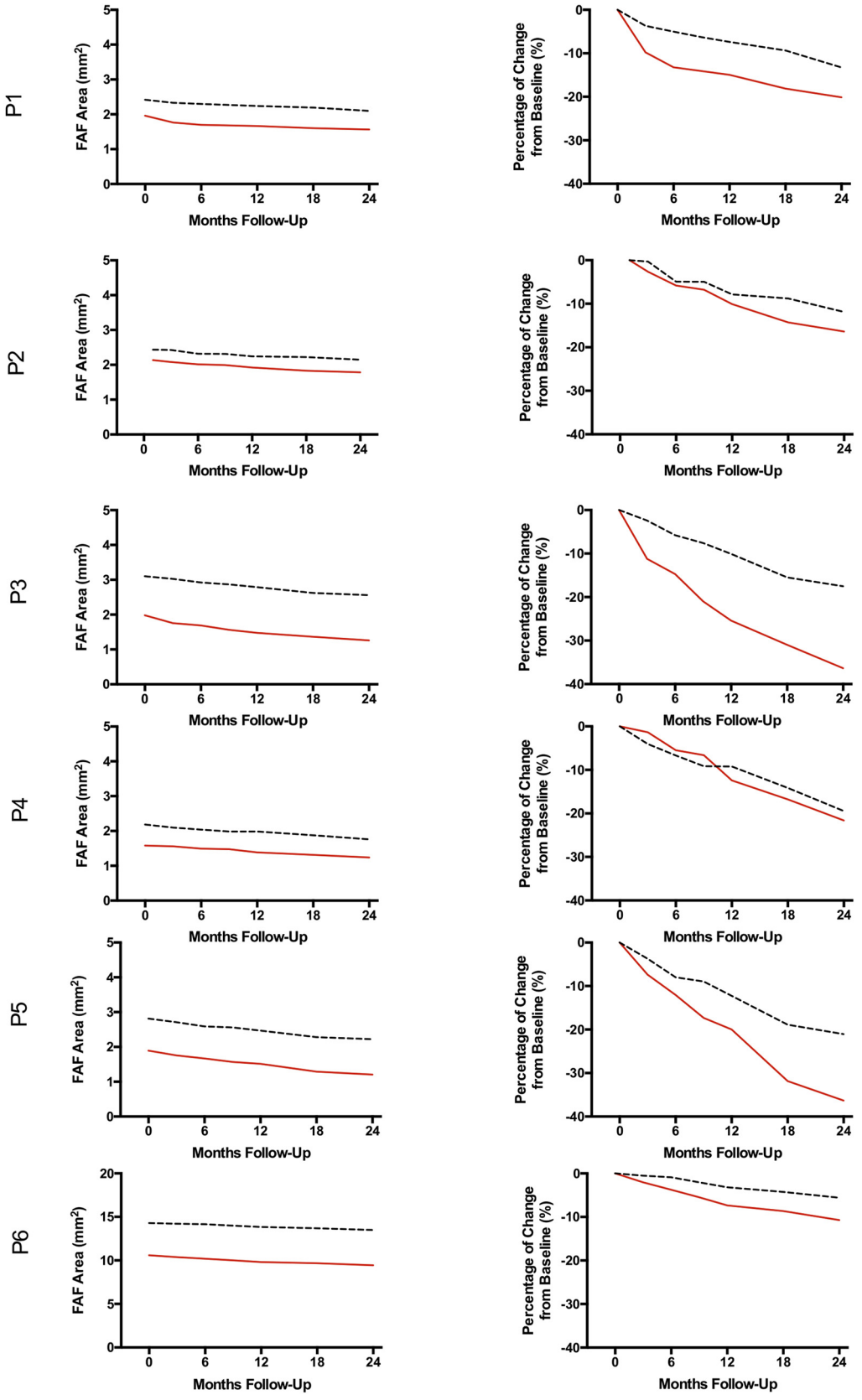

FIGURE 3. (Continued)

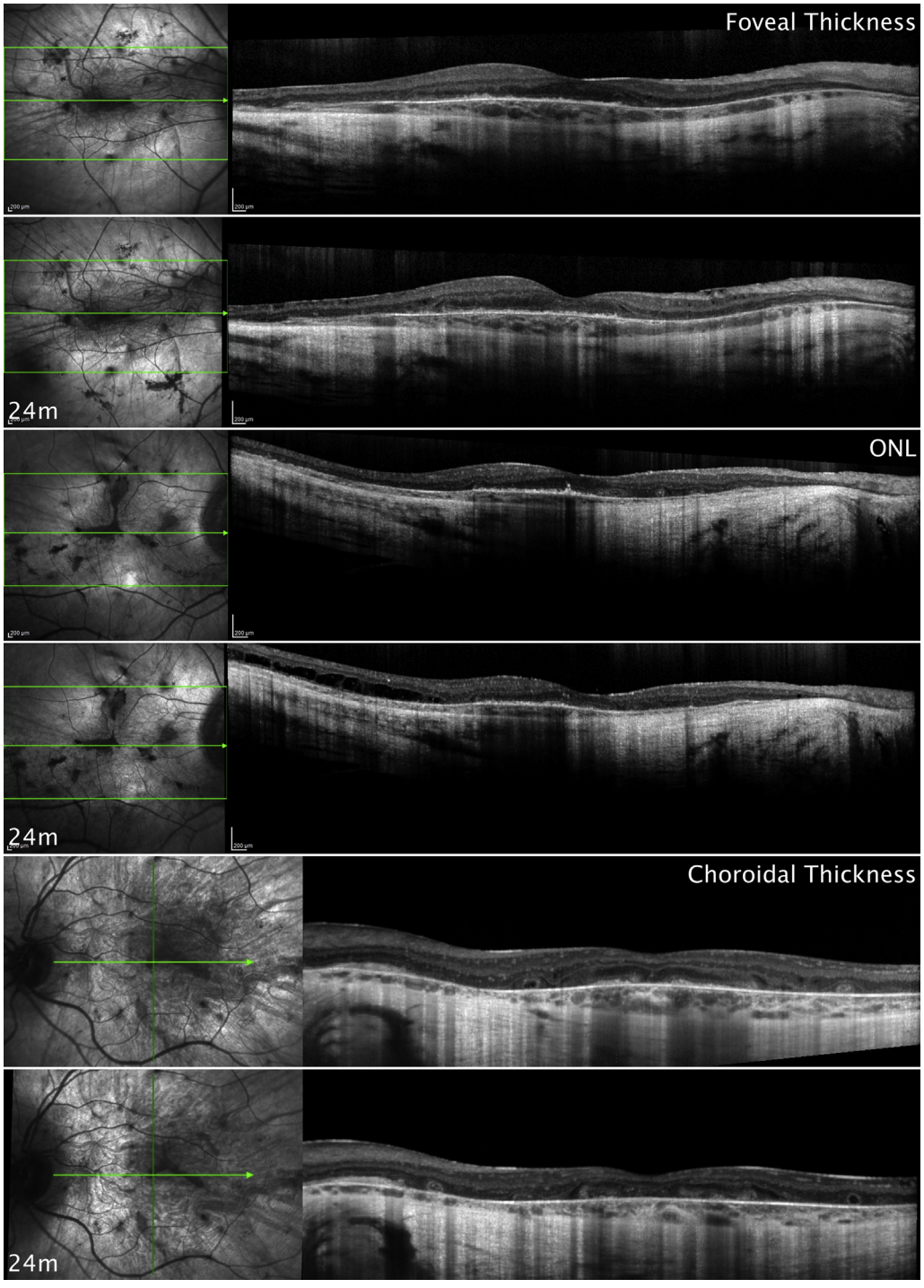

FIGURE 4. Representative examples of spectral-domain optical coherence tomography changes observed in rAAV2.REP1-treated eyes after 24 months. (Top) Foveal thickness thinning in Subject P1. (Middle) Outer nuclear layer (ONL) changes in Subject P4. (Bottom) Subfoveal choroidal thinning in Subject P5.

captured residual RPE at the macular region with a sampling rate of 1536 × 1536 pixels (high resolution mode). Automatic Real Time (ART) function was set to at least 30 frames. Subjects P1 and P2 underwent FAF imaging using a modified fundus camera at baseline and week 1, before switching to the Spectralis SD-OCT unit for the rest of the study.

Longitudinal SW-FAF images were co-registered and aligned using the i2K Align Retina platform (Dual-Align LCC, Clifton Park, New York, USA) to compensate for

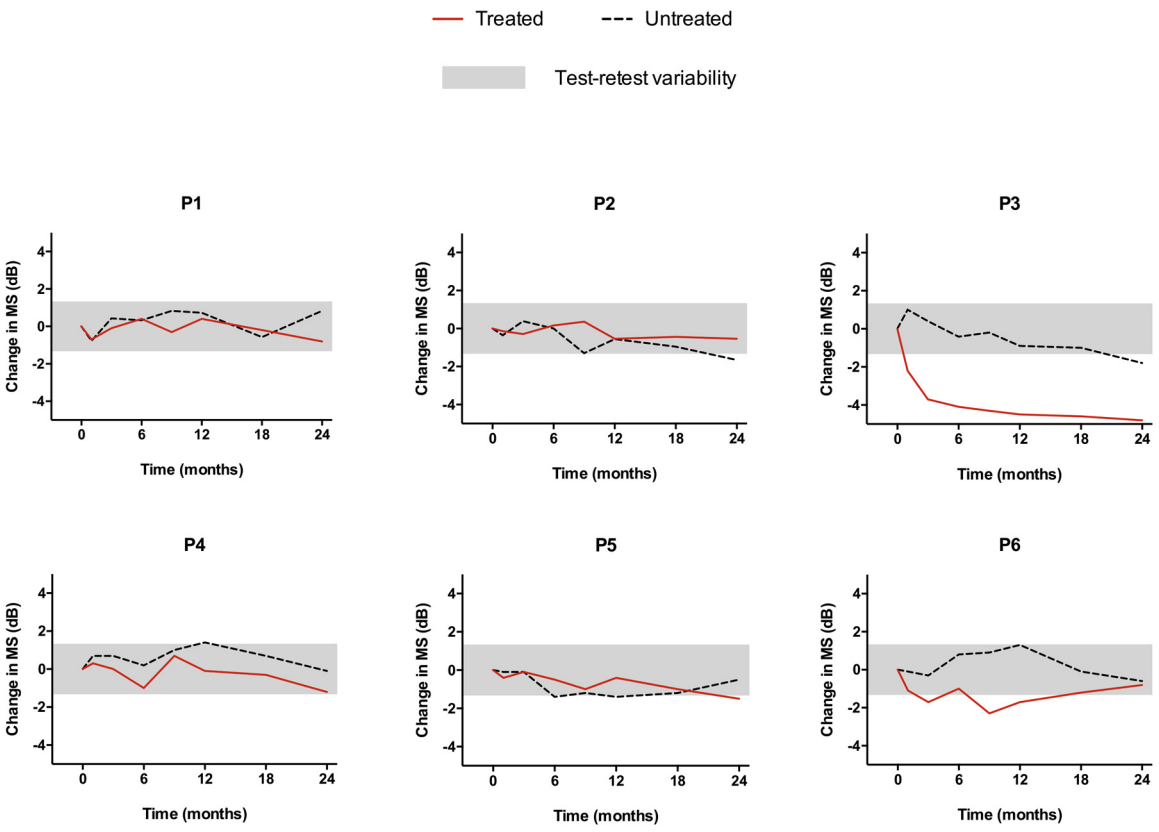

FIGURE 5. Longitudinal microperimetry changes over 2 years. Change from baseline is provided for each subject and for both treated and untreated eyes. Gray boxes represent individual test-retest variability limits from multiple measures at baseline (month 0). Red continuous line: treated eye; black dotted line: untreated eye. P1-P6 = patients 1 to 6.

image distortion, changes in resolution, pixel density, and focus.[12] A MATLAB-based image-processing algorithm was developed to allow semi-automated segmentation of residual RPE islands and measurement of AREA change (in mm^2) across time points. An overview of the segmentation algorithm used is provided as Supplementary Material (available at AJO.com).

Spectral-Domain Optical Coherence Tomography

At each visit, subjects underwent SD-OCT imaging using the Spectralis SD-OCT unit (Heidelberg Engineering, Heidelberg, Germany). Two separate scans were acquired for both eyes: (1) 20 × 20 macular volume consisting of 37 B-scans with at least 12 frames averaged per B-scan; and (2) enhanced depth imaging (EDI) horizontal and vertical B-scans entered at the fovea with at least 50 frames averaged.

Resulting scans were analyzed in a masked fashion using in-built Heidelberg manually guided segmentation and measuring tools. The following structures were analyzed: (1) central foveal thickness; (2) outer nuclear layer (ONL) thickness; (3) subfoveal choroidal thickness; and (4) ellipsoid zone (EZ) width. Central foveal thickness was determined using the automated retinal thickness measurements between the inner limiting membrane (ILM) and Bruch membrane (BM). The ONL was defined as the thickness between the outer plexiform layer (OPL) and the external limiting membrane (ELM). For longitudinal assessments, the thickest ONL point was selected. Choroidal thickness was defined as the thickness between the Bruch membrane and the choroidal-scleral interface. The EZ width was measured temporally and nasally from the foveal center, as previously described.[13,14] The central foveal thickness and ONL were analyzed on volume scans, while the EZ and subfoveal choroidal thickness were analyzed on horizontal and vertical EDI images. OCT analysis was performed at 3-month intervals during the first 12 months, and then every 6 months thereafter.

Microperimetry

Macular functional changes were longitudinally assessed with the MAIA microperimeter (Macular Integrity Assessment; CenterVue, Padova, Italy). Real-time fundus imaging was achieved using a near-infrared line scanning laser ophthalmoscope (850 nm, 1024 × 1024 pixels resolution, 36 degrees field of vision). Eye-tracking technology was employed at a frequency of 25 Hz to correct for brief saccadic eye movements. Perimetry testing followed a 4-2

staircase strategy using white stimuli (size: Goldmann III, duration: 200 ms) against a background of 1.27 cd/m^2 luminance. The dynamic range of stimulus intensity achieved was 36 dB, with a minimum of 0 and maximum of 318 cd/m^2, respectively. Testing was performed according to the manufacturer's instructions in a dimly lit room, after 20 minutes of dark adaptation and with nondilated pupils. A small red circle of 1 degree diameter was used as a fixation target. The stimulus grid consisted of a standard 37-stimuli grid for all subjects except for Subject P6, who was tested with a 10-2 grid (61 stimuli). The "follow-up protocol" was used for longitudinal testing to ensure automatic alignment and registration of follow-up examinations with the baseline infrared fundus image.

Quality-of-Life Assessment

All subjects completed the Visual Function Questionnaire (VFQ-25) of the National Eye Institute (NEI)[15] prior to surgery and at the completion of the trial.

• **STATISTICAL ANALYSIS:** Summary statistics are presented for both eyes (treated vs untreated eye groups). For categorical/binary data, the number and proportion of patients pertaining to each category are presented with its 95% confidence interval (CI). For continuous data, mean (and 95% CI) and standard deviation (SD) are presented. At each time point, the change from baseline in ETDRS letters, area of autofluorescence, and microperimetry mean sensitivity are computed for each eye. The average change from baseline at 12 and 24 months is presented for both treated and the untreated eyes. Wilcoxon matched-pairs signed rank test is used to compare the 2 groups at these time points. Slopes of linear regression lines are compared using analysis of covariance (ANCOVA).

RESULTS

• **BASELINE CHARACTERISTICS:** Six adult male subjects with genetically confirmed choroideremia were enrolled in this study. The subject's ages ranged from 30 to 42 years. Baseline demographics are provided in Table 1. Pretreatment BCVA ranged from 53 to 75 ETDRS letters in the treated and 58 to 75 ETDRS letters in the untreated eyes. The baseline interocular BVCA difference was between 0 and 12 ETDRS letters (mean: 3.6). The interocular difference in FAF area ranged from 0.31 to 3.71 mm^2.

• **PRIMARY OUTCOME:** *Adverse Events*. All subjects tolerated the surgery and study agent administration without known intraoperative delivery-related adverse events (AEs). The most common postoperative AEs occurring in all subjects (6/6) included subconjunctival hemorrhage, reduced visual acuity, blurred vision, metamorphopsia, and decreased intraocular pressure. Five of the 6 subjects had resolution of these AEs within 1-14 days. For 1 subject (P3) blurred vision and metamorphopsia in the treated eye did not resolve and continued at all study time points. Anterior chamber reaction (3/4+ cells) was noted transiently in 2 of 6 subjects after weaning off peritreatment systemic steroids. Elevated C-reactive protein was detected at the 1-month time point for 1 subject, with resolution after 2 months. A list of all adverse events is provided in Table 2.

A significant adverse event of presumed intraretinal inflammation was noted for Subject P3. At 1 month after surgery, SD-OCT revealed hyperreflective foci located in the outer retinal layers of the subfoveal region without apparent involvement of the choroid (Figure 1A, Top three rows). This event occurred after the patient had discontinued oral steroid use. The subject was re-started on oral steroid (1 mg/kg/day) for 1 week. After 2 months, the inflammation and hyperreflective material had subsided, with residual defect in the EZ and ELM persisting (Figure 1A, white arrows). Progressive loss of the RPE by FAF signal was also observed, especially in the subfoveal region (Figure 1B, Bottom row). Interestingly, despite these changes, there was preservation of the ONL thickness in the fovea.

Subsequent review of the surgical video for Subject P3 revealed that during the initial retinal penetration by the 41 gauge cannula and subsequent injection of viral vector, a small amount of subretinal, intraretinal, and vitreous hemorrhage was released. In addition, several bubbles of air were injected into the subretinal space during injection of the viral vector, the largest of which was approximately 1000 μm in diameter.

• **SECONDARY OUTCOME MEASURES:** *Best-Corrected Visual Acuity*. Results of visual acuity testing over time are presented in Figure 2. Repeatability coefficients—ranging from 5 to 10 ETDRS letters—were determined individually for each subject based on multiple pretreatment measures. In all cases but 1, BCVA decreased immediately post-intervention but returned to baseline levels by day 14. As previously discussed, Subject P3 had a serious AE (SAE) that resulted in persistent loss of BCVA. At 24 months, an 8-ETDRS-letter loss from baseline was measured. Five of 6 treated eyes did not experience any significant gain in BCVA compared to baseline (Figure 2, Bottom graphs). One eye, of Subject P2, demonstrated a significant and sustained improvement of >15 ETDRS letters over the 2 years of the study. Interestingly, similar visual acuity gains were observed in 1 untreated eye throughout the 2-year study period (Subject P1: +19 ETDRS letters); no significant loss in BCVA was noted in the remaining untreated eyes.

Area of Fundus Autofluorescence Analysis. For both treated and untreated eyes, the FAF area showed a linear decline with time (Figure 3, Right). The rate of decline

was very similar for all the subjects, ranging from -0.012 to -0.046 mm^2/month (mean [SD]: -0.021 [0.008]). There was no difference in the rate of decline between treated and untreated eyes for 5 of 6 subjects ($P > .05$ DFd = 10; ANCOVA). The only exception was Subject P3, who encountered an early SAE that resulted in pronounced loss of FAF area (>30% loss from baseline). Excluding Subject P3, the average change from baseline FAF area at 12 months was -14.65% (SD: 6.40) for the treated and -9.09% (SD: 3.64) for the untreated eyes. At 24 months, average decline of FAF from baseline was -19.63% (SD: 6.85) and -14.80% (SD: 5.72), respectively. Differences between the 2 groups of eyes did not reach statistical significance at any of these 2 time points (Wilcoxon signed rank test; $P = .062$). The average change in FAF area between months 12 and 24 was -8.32% (SD: 4.18) and -7.13% (SD: 3.36) for the treated and untreated eyes, respectively (ns; $P = .31$).

Spectral-Domain Optical Coherence Tomography Analysis. Both treated and untreated eyes demonstrated changes in retinal thickness; however, the patterns were variable. After 24 months, the majority of treated eyes (4/6) showed more pronounced loss of central foveal thickness when compared to untreated eyes. ONL and subfoveal choroidal thickness were better preserved in only 1 of 6 and 2 of 6 treated eyes, respectively. Subject P2 showed EZ width preservation in the treated eye, with the remaining subjects revealing less attenuated EZ in their untreated eyes or no interocular difference. Representative examples of SD-OCT changes observed in rAAV2.REP1-treated eyes are illustrated in Figure 4. Longitudinal changes of analyzed OCT measures are provided as Supplementary Material (available at AJO.com).

Microperimetry. Results of longitudinal microperimetry testing are presented in Figure 5. The repeatability coefficient for mean sensitivity measures in choroideremia eyes has been previously estimated by our group (±1.33 dB).[16] Individual test-retest repeatability limits were also calculated based on at least 3 pretreatment measures. None of the treated eyes exhibited any significant improvement in mean sensitivity throughout the 2-year study period. Subject P3 experienced a profound loss of his baseline mean sensitivity in the treated eye (-4.8 dB) owing to the encountered SAE. Visual acuity improvement for Subject P2 could not be corroborated with microperimetry testing. However, at study exit a trend toward preservation of mean sensitivity was observed for his treated eye. Nevertheless, the 2-year follow-up period was not sufficient to observe any sustained decline in mean sensitivity that exceeded test-retest limits.

National Eye Institute Visual Function Questionnaire-25. The NEI-VFQ-25 composite scores at baseline ranged from 42 to 76 (Table 1). The NEI-VFQ-25 composite score remained relatively unchanged 24 months after treatment in 4 of 6 subjects. Subject P3 reported the greatest loss in function (-8) and Subject P1 the greatest improvement (+31).

DISCUSSION

IN A PHASE I TRIAL OF A NEW INTERVENTION, WE HAD intended to demonstrate the safety of ocular gene therapy in a small number of CHM subjects. All subjects tolerated the surgery and study agent administration with no intraoperative delivery-related adverse events. However, a significant adverse event associated with presumed intraretinal inflammation was encountered in 1 subject during the postoperative period that resulted in permanent structural and functional impairment of the retina in the treated eye. Although a similar effect has not been reported with rAAV2.REP1, strong clinical evidence of an inflammatory response to ocular gene therapy has emerged from the Leber congenital amaurosis (LCA) gene therapy trials. In particular, Bainbridge and associates[17] reported that 5 out of 8 subjects treated with a high dose (10^{12} genome particles) of recombinant AAV2 vector encoding RPE65 developed various degrees of intraocular inflammation. However, the dose ranges used in trials with rAAV2.REP1 remain at the low end of ranges previously used in LCA trials (10^{10} to 10^{11} genome particles) that were without any adverse events. Furthermore, preclinical studies of rAAV2.REP1 in CHM mice failed to demonstrate any detrimental structural or functional effects in the retina of treated animals.[18] The underlying causes of the SD-OCT retinal changes observed in our study thus remain unclear. To our knowledge, discrete hyperreflective spots have only been described in a single participant of a retinal gene therapy trial for *CNGA3*-based achromatopsia using an AAV8 recombinant vector.[19] In this case, the spots resolved following a course of systemic steroid treatment but led to no functional sequelae. Furthermore, activation of both innate and adaptive immunity was demonstrated in preclinical experiments with nonhuman primates undergoing subretinal injections of clinical-grade AAV8.[19] Taken together, an immune response to AAV viral capsids seems to be the most plausible explanation. Alternative causes include hemorrhagic damage or damage from the subretinal air that was injected at the time of surgery.

Visual function measures including microperimetry and visual acuity did not demonstrate improvement in our trial, with the exception of 1 subject whose visual acuity improved significantly in the treated eye. Our results are comparable to the only published trial on rAAV2.REP1 to date,[1] which reported improvement in visual acuity greater than 15 ETDRS letters in only 1 treated subject. In this study, improvements were also measured with

microperimetry for 2 out of the 6 treated individuals. However, microperimetry changes should be interpreted with caution, given the high test-retest variation associated with this particular type of testing.[16] Our study found that visual acuity gains might not necessarily be corroborated by microperimetry testing and that no significant loss of function should be expected within a 2-year period, suggesting that preservation rather than improvement of visual function should be a more realistic long-term goal of gene therapy for choroideremia.

Outcome measures used in our trial were intended to test both structural and functional changes that would be anticipated to occur over a chosen timeframe of the study. Some tests, however, proved to be less than reliable and may not be considered a priority for inclusion in future studies. For example, multifocal ERG was ineffective to measure outcome, as baseline testing showed that test responses were either absent or too variable in this cohort for it to be included. In a similar manner, test-retest variation in microperimetry[16] and visual acuity testing in CHM precludes accurate determination of disease progression and an intervention's efficacy.[3,20] Information collected on other measures such as contrast sensitivity, color vision, and full-field stimulus threshold test over the 2-year follow-up did not reveal clinical significance when subjected to critical analysis.

Of the various outcome measures tested in this study, FAF area proved to be the most informative. Our trial is the first to report on 2-year RPE structural outcomes following gene therapy for choroideremia. Based on cross-sectional data, the area of residual RPE in the posterior pole was expected to decline logarithmically, with an estimated rate of 7%-8% per year.[4] Our longitudinal data suggest that the rate of decline over a 2-year period is linear, with the progression in the treated and untreated eye plotting a parallel course. Growing evidence[21–23] supports our opinion that the health of the RPE is key to maintaining photoreceptor function in CHM. For future studies, we would recommend that FAF area measurements of intact RPE be used as a biomarker to test the potential benefit of any treatment.

The current trial delivered rAAV2.REP1 with injection into a bleb that had detached the central fovea. Concerns about the safety of gene therapy delivery to the subfoveal space have been raised previously. In the original trial by MacLaren and associates,[1] 1 patient who received a lower dose of viral vector demonstrated severe visual acuity loss owing to possible damage to the papillomacular bundle from retinal stretch.[1] In addition, measures of visual function (microperimetry sensitivity and color vision) have been shown to decline without recovery to pretreatment levels after subretinal detachment in another cohort of 5 rAAV2.REP1-treated CHM individuals.[24] In other retinal disorders, a clinical study of patients treated for macular detachments by Sasoh and associates[25] showed that function can be impaired, as measured by reduced amplitudes of the multifocal ERG in affected eyes compared to the fellow eyes. In our current trial, 1 subject experienced significant loss in central macular function post-treatment. We cannot determine the extent to which this loss of function was related to the physical detachment of the retina or the effect of an immune reaction to the viral capsids—as previously described. While perioperative immunosuppression was provided with oral steroid, it was insufficient to prevent the occurrence of intraretinal inflammation in this subject. Strategies to minimize potential surgical complications, such as avoidance of excessive retinal stretch, air bubbles in the injection system, and reflux of viral vector, have been described by Xue and associates.[10] The use of intraoperative OCT may also enhance surgical precision of subretinal delivery and reduce risk of complications. The results of additional trials of rAA2.REP1 gene replacement will likely provide additional evidence for the safety and efficacy of this vector and its safe delivery in a subretinal injection.

Many questions regarding gene therapy and choroideremia remain to be answered. For example, how much REP1 is required to normalize function when performing gene therapy? Preclinical experiments to rescue and restore wild-type REP1 in cell models or organoids may provide important insights as to the levels of REP1 that are necessary and sufficient to maintain normal function of cellular pathways. In addition, at which disease stage should gene therapy be delivered to maximize efficacy? As previously suggested by LCA trials,[26,27] the lack of long-term photoreceptor/RPE rescue with gene therapy in CHM could signify the presence of a "point of no return" at advanced disease stages, beyond which cell death is unpreventable. In the future, larger studies enrolling younger patients may provide insight into the optimal therapeutic window for gene therapy in CHM.

In summary, our phase I choroideremia gene therapy trial was characterized by 1 SAE with reduction in vision and thinning of retina. Throughout the 2-year study period, there was no slowing of RPE loss in any of the treated eyes when compared to untreated eyes. Only 1 of 6 treated eyes showed improvement in vision greater than 15 ETDRS letters. Interestingly, 1 of 6 untreated eyes also had an improvement of greater that 15 ETDRS letters, suggesting that improvement in visual acuity should likely not be used as a primary outcome for future choroideremia gene therapy trials.

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