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Treatment of Leber Congenital Amaurosis Due to *RPE65* Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results of a Phase I Trial

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Abstract

Leber congenital amaurosis (LCA) is a group of autosomal recessive blinding retinal diseases that are incurable. One molecular form is caused by mutations in the *RPE65* (retinal pigment epithelium-specific 65-kDa) gene. A recombinant adeno-associated virus serotype 2 (rAAV2) vector, altered to carry the human *RPE65* gene (rAAV2-CB^{SB}-h*RPE65*), restored vision in animal models with RPE65 deficiency. A clinical trial was designed to assess the safety of rAAV2-CB^{SB}-h*RPE65* in subjects with *RPE65*-LCA. Three young adults (ages 21–24 years) with *RPE65*-LCA received a uniocular subretinal injection of 5.96×10^{10} vector genomes in 150 μl and were studied with follow-up examinations for 90 days. Ocular safety, the primary outcome, was assessed by clinical eye examination. Visual function was measured by visual acuity and dark-adapted full-field sensitivity testing (FST); central retinal structure was monitored by optical coherence tomography (OCT). Neither vector-related serious adverse events nor systemic toxicities were detected. Visual acuity was not significantly different from baseline; one patient showed retinal thinning at the fovea by OCT. All patients self-reported increased visual sensitivity in the study eye compared with their control eye, especially noticeable under reduced ambient light conditions. The dark-adapted FST results were compared between baseline and 30–90 days after treatment. For study eyes, sensitivity increases from mean baseline were highly significant ($p < 0.001$); whereas, for control eyes, sensitivity changes were not significant ($p = 0.99$). Comparisons are drawn between the present work and two other studies of ocular gene therapy for *RPE65*-LCA that were carried out contemporaneously and reported.

Introduction

Incurable genetic retinal blindness became treatable for the first time when early results of ocular gene therapy were reported for two clinical trials, each treating three adult subjects with Leber congenital amaurosis (LCA) caused by mutations in the *RPE65* gene (Bainbridge *et al.*, 2008; Maguire *et al.*, 2008). RPE65 (retinal pigment epithelium-specific 65-kDa) is the isomerase enzyme in the RPE that catalyzes a critical step in the visual (retinoid) cycle that permits photoreceptor visual pigments to absorb photons and maintain sight (reviewed in Travis *et al.*, 2007). These clinical trial announcements followed years of fundamental and applied science in animal models of *RPE65*-LCA (e.g., Redmond *et al.*, 1998; Acland *et al.*, 2001, 2005; Dejneka *et al.*, 2004; Pang *et al.*, 2005; Jacobson *et al.*, 2005), dose-efficacy and safety studies (Jacobson *et al.*, 2006a,b), and retinal function–structure and visual pathway investigations of the human disease (Jacobson *et al.*, 2005, 2007, 2008; Aguirre *et al.*, 2007).

Despite similarities in disease diagnosis and age of the subjects in the two published clinical trials, there were important differences in the results and their interpretations

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(Miller, 2008). The present study reports an independent clinical trial of subretinal gene therapy in three young adults with *RPE65*-LCA that was carried out contemporaneously with the two published studies mentioned. Comparisons are drawn between the results of the three trials, adding data and perspective to these early initiatives to treat a rare autosomal recessive eye disease by somatic gene therapy.

Materials and Methods

Regulatory approvals and oversight

The clinical trial is being performed at Scheie Eye Institute of the University of Pennsylvania (UP, Philadelphia, PA) and at University of Florida (UF)/Shands Children's Hospital (Gainesville, FL). Trial conduct is in a manner consistent with the ICH-E6 Good Clinical Practice guideline document, and has been reviewed by the U.S. 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 (IRBs) and Institutional Biosafety Committees (IBCs) of UP and UF, the Vice Provost Research Review Committee of UP, the Western IRB, and the General Clinical Research Center of UF. A Data and Safety Monitoring Committee, appointed by the National Institutes of Health, monitors the trial. The tenets of the Declaration of Helsinki are being followed. Informed consent was obtained and subjects spoke with a Decision Monitor to ensure the voluntary nature of participation. Because of their severe visual abnormalities, all patients received paper and recorded versions of the informed consents at least 2 weeks before formal enrollment to enhance detailed understanding of the trial and to permit all questions to be formed and posed.

Study eligibility and protocol

The three young adult subjects had a clinical diagnosis of LCA. *RPE65* mutations were determined by the John and Marcia Carver Nonprofit Genetic Testing Laboratory at the University of Iowa (Iowa City, IA). Inclusion and exclusion criteria are listed in Supplementary Table 1 (see www.liebertonline.com/hum). A summary of the protocol study visits is to be found in Supplementary Table 2 (see www.liebertonline.com/hum).

cGMP vector production, purification, and titering

The recombinant adeno-associated viral (rAAV) vector, AAV2-CB^{SB}-h*RPE65*, has previously been described (Jacobson *et al*., 2006a). The vector was packaged and purified according to clinical Good Manufacturing Practices (cGMP). Briefly, HEK-293 cells (adenovirus serotype 2 [Ad2], E1A$^+$E1B$^+$) were cotransfected with the pDG packaging plasmid, which encodes three other adenoviral genes required for packaging (E2A, VA, and E4) and the AAV2 *rep* and *cap* genes, and with the AAV vector plasmid as previously published (Grimm *et al*., 1998). Downstream purification of vector was done by all-column purification methods (Snyder and Flotte, 2002; Snyder and Francis, 2005; Zolotukhin *et al*., 2002). The titer of the vector, expressed as vector genomes (VG), was determined by dot-blot assay (Zolotukhin *et al*., 2002).

Vector administration

The eye with worse visual function was chosen for vector administration in each subject. After mild intravenous sedation, the surgical eye received retrobulbar anesthesia and was then prepped and draped in a standard sterile fashion. A standard three-port 23-gauge core and peripheral vitrectomy was performed. The conjunctiva over the right-sided sclerotomy was dissected with Westcott scissors and .3 forceps. Hemostasis was maintained by eraser-tipped cautery. The sclerotomy was enlarged with a 20-gauge MVR blade so that the subretinal cannula could easily be inserted into the eye. The vector was drawn into a 39-gauge injection cannula (Synergetics, O'Fallon, MO) and was introduced into the subretinal space. At the end of the procedure, the sclerotomy sites were secured with 7.0 Vicryl sutures and the conjunctiva was closed with interrupted sutures. Subconjunctival antibiotics and steroids were administered. Topical antibiotics and steroids were used for 20 days after surgery.

Safety parameters

Ocular safety was assessed with standard eye examinations at two baseline visits, daily for the first 10 days posttreatment, and on days 14, 20, 30, 60, and 90. To quantify the severity of inflammatory response, standard grading systems were used (Hogan *et al*., 1959; Nussenblatt *et al*., 1985; Ladas *et al*., 2005). 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 a physical examination at both baseline evaluations, daily after administration for 10 days, and on days 30 and 90 postadministration. Routine hematology, serum chemistry, prothrombin time (with international normalized ratio [INR]), partial thromboplastin time, and urinalysis were performed as part of baseline evaluations and 1, 3, 10, 30 and 90 days after vector administration.

Anti-AAV2 antibody titers

Serum samples from the patients were assayed for circulating antibodies to the AAV2 capsid proteins at baseline and on days 14 and 90. Briefly, 96-well plates were coated with 1.2×10^9 AAV2 particles per well overnight at 4°C. A wash with phosphate-buffered saline (PBS)–Tween was followed by blocking for 2 hr at 37°C with 10% fetal bovine serum (Cellgro; Mediatech, Herndon, VA). After a 1× wash with PBS–Tween, samples and a known positive human standard were diluted between 1:10 and 1:10,240 and allowed to bind overnight at 4°C. Each sample was run in duplicate. Washing was followed by addition of the detection antibody at a dilution of 1:50,000 (goat anti-human immunoglobulin, conjugated with horseradish peroxidase [HRP]; Biosource International/Invitrogen, Camarillo, CA) for 2 hr at 37°C. Finally, the plate was washed and exposed to tetramethylbenzidine (TMB) peroxidase detection substrate (KPL, Gaithersburg, MD) and read at 450 nm with an enzyme-linked immunosorbent assay (ELISA) plate reader (Molecular Devices, Sunnyvale, CA). Sample anti-AAV2 titers were then read relative to a human standard curve derived from the same plate. We did not run an assay for antibody against the vector transgene. Because no human antibody to human

RPE65 was available to use as a positive control with which to construct the relevant calibration curve in an ELISA for antibody against the vector transgene, negative results from patient samples could not be confidently interpreted.

Antigen-specific response

Anti-AAV2 antigen-specific lymphocyte proliferation responses were assessed as previously described (Hernandez *et al*., 1999). After isolation and purification from blood, lymphocytes were cultured at 1×10^5 cells per well in 200 μl of RPMI 1640 medium (10% human serum) in 96-well round-bottom plates. Lymphocytes were separated into four groups with three (controls) and six (unknowns) cultures per group: unstimulated (as negative control), stimulated with AAV2 (5000 particles/cell), stimulated with AAV2 (500 particles/cell), and stimulated with AAV2 (50 particles/cell). After 5 days of incubation the stimulation index (SI) was defined as: (mean counts per minute of [^{3}H]thymidine from stimulated cells)/(mean counts per minute of [^{3}H]thymidine from unstimulated cells). On the basis of antigen-specific lymphocyte proliferation response (ASR) results at baseline in another (nonocular) study, SI values greater than 2.0 (Brantly *et al*., 2006) or 3.0 (Hernandez *et al*., 1999) have been considered significant. The viability of each lymphocyte culture was confirmed by positive controls with mitogen-induced proliferation in response to phytohemagglutinin (PHA) (10, 1.0, and 0.1 μg/ml) and *Candida albicans*.

AAV DNA in peripheral blood

Procedures for biodistribution studies have been described (Song *et al*., 2002). In brief, 1 μg of extracted genomic DNA was used in all quantitative polymerase chain reactions (PCRs); reaction conditions followed those recommended by Applied Biosystems (Foster City, CA) and included 50 cycles of 94°C for 40 sec, 37°C for 2 min, 55°C for 4 min, and 68°C for 30 sec. Primer pairs were designed to the cytomegalovirus (CMV) enhancer/chicken β-actin promoter as described (Donsante *et al*., 2001) and standard curves were established by spike-in concentrations of a plasmid DNA (CBAT) carrying the same promoter as mentioned previously and the α_1-antitrypsin cDNA (Song *et al*., 2002). DNA samples were assayed in triplicate. The third replicate was spiked with CBAT DNA at a ratio of 100 copies/μg of genomic DNA. If at least 40 copies of the spike-in DNA were detected, the DNA sample was considered acceptable for reporting vector DNA copies. When the copy number of the vector DNA found in that sample was >100 copies/μg, the sample was considered positive and the measured copy number per microgram was reported. The sample was considered negative if <100 copies/μg were present. When less than 1 μg of genomic DNA was analyzed to avoid PCR inhibitors copurifying with DNA in the extracted tissue, the spike-in copy number was reduced proportionally to maintain the 100 copies/μg DNA ratio. Biodistribution in blood was determined at the first baseline visit and on days 1, 3, and 14 postinjection.

Interferon-γ enzyme-linked immunospot assay

Blood samples were collected by venipuncture, using heparin as anticoagulant, and either shipped overnight (baseline and day 14 samples) or delivered immediately (day 90 samples) to the testing laboratory at UP. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque gradient centrifugation (GE Healthcare Life Sciences, Piscataway, NJ). All samples were processed on receipt and either evaluated in enzyme-linked immunospot (ELISpot) assays (see below) as fresh samples or cryopreserved in liquid nitrogen to be tested later. If cryopreserved, the PBMC samples were recovered and cultured in complete R10 medium (RPMI 1640 with 10% FBS) at 37°C overnight before being assayed.

Interferon (IFN)-γ ELISpot assays were performed according to previously described protocols (Fu *et al*., 2007). PBMCs were added at a density of 2×10^5 cells per well and stimulated for 18–20 hr at 37°C with peptide libraries derived from the AAV2 VP1 protein at a concentration of 2 μg/ml. Medium alone served as a negative control, and PHA as a positive control. In addition, responses to CEF (Mabtech, Stockholm, Sweden), which contains 23 MHC class I-restricted peptides (Mabtech) that represent T cell epitopes of viruses common in the human population (cytomegalovirus, Epstein–Barr virus, and influenza) and is able to bind to a broad range of HLA molecules, served as references. The peptide libraries were synthesized as 15-mers with a 10-amino acid overlap with the preceding peptide (Mimotopes, Clayton, Victoria, Australia). The AAV2 VP1 peptide library was divided into three pools such that pool 2A contained the first 50 peptides of AAV2 VP1, pool 2B contained peptides 51–100, and pool 2C contained peptides 101–145. Spots were counted with an ELISpot reader (AID Diagnostika, Strassberg, Germany). Peptide-specific cells were represented as spot-forming cells (SFCs) per 10^6 PBMCs. A positive response to any peptide pools was arbitrarily defined as three times over background (medium alone control) and the response was more than 55 SFCs per 10^6 PBMCs.

Visual function and retinal structure

Visual function was measured using ETDRS visual acuity (Ferris *et al*., 1982) and dark-adapted full-field sensitivity testing (FST; Roman *et al*., 2005, 2007b). Briefly, FST was performed with blue stimuli after subjects were dark-adapted for 1 hr. In each patient, baseline data were available for four pretreatment visits obtained on different days within a 6-month window before surgery. Posttreatment data were obtained up to 3 months after surgery. For each session, the average FST sensitivity was defined as the mean value of the four highest recorded sensitivities. FST sensitivity change was defined as the signed difference between each session and the mean baseline sensitivity for each eye; a positive change indicates improvement. Overall statistical analyses were performed separately in the study and control eyes by *t* test between pooled post- and pretreatment sessions. When the overall difference post- and pretreatment was statistically significant, further post hoc pair-wise comparisons were performed for each patient.

Retinal structure was assessed by cross-sectional imaging, using optical coherence tomography (OCT). Data were acquired using ultrahigh-speed and high-resolution OCT imaging with a Fourier domain (FD) OCT instrument (RTVue-100; Optovue, Fremont, CA). The high-definition line protocol (HD line) of the FD-OCT system was used to

obtain overlapping 4.5-mm-long horizontal scans composed of 4096 A-scans acquired at 26,000 A-scans per second. Lateral sampling density of the line scans were reduced by averaging groups of eight neighboring A-scans to increase the signal-to-noise ratio, aligned using a dynamic cross-correlation algorithm (Huang *et al*., 1998), and digitally stitched (Aleman *et al*., 2008). Foveal thickness measurements were performed as described (Sandberg *et al*., 2005).

Results

Study population

The three subjects ranged in age from 21 to 24 years and all had a clinical diagnosis of LCA. Molecular studies indicated that two subjects were homozygotes for point mutations in the *RPE65* gene and one subject was a compound heterozygote (Table 1). The mutations were previously reported to be associated with LCA (Morimura *et al*., 1998; Thompson *et al*., 2000; Lorenz *et al*., 2000; Simovich *et al*., 2001; Felius *et al*., 2002; Yzer *et al*., 2003; Booij *et al*., 2005) and showed little or no RPE65 isomerase activity by *in vitro* studies (Redmond *et al*., 2005; Takahashi *et al*., 2006).

Vector administration and safety

An infrared view of a normal fundus is shown and major retinal landmarks consisting of the optic nerve head, fovea, and macula, defined as the central 5.5–6 mm (diameter) of retina (Hogan *et al*., 1971), are indicated (Fig. 1). The locations of the retinal detachments created by subretinal injection in each subject are also shown. The treated eyes were the left eye in P1, and the right eye in P2 and P3; to permit comparisons in Fig. 1, all images are shown as left eyes. P1 had two attempted injections: the first, a retinotomy near the superior macular region, did not cause a retinal detachment; the second, a retinotomy inferior and temporal in the macula, achieved the goal of delivering the 150 μl of vector. The retinal detachment involved the inferior macular region (including the fovea) and extended beyond it inferiorly. P2 had a single subretinal injection attempt at a site superior and temporal to the macula. The retinal detachment extended away from the fovea and into the superotemporal retina, not including the fovea. P3 had three attempted injections: two attempts were at the same retinotomy site in the superotemporal retina outside the macula and both failed to cause a retinal detachment; the third injection site was inferior to the original sites and almost directly temporal. The retinal detachment extended away from the fovea and into the temporal extramacular retina. P2 had laser treatment applied to a vitreoretinal tag in the far peripheral superior retina. P3 had very light laser treatment applied to the retinotomy site that did not produce retinal detachment.

For all three patients, there was absorption of most subretinal fluid by 5–6 hr postsurgery. There were no retinal tears or other complications from the surgery. Retinotomy sites continued to be visible by ophthalmoscopic examination for at least 1 month. There was no clinical evidence of intraocular inflammation in any patient; inflammation of the

Table 1. Comparison of Results of *RPE65*-LCA Clinical Trials

	Age at baseline (years)/gender	*RPE65 mutation*	*Follow-up (months)*	*Anesthesia*	*Vector dose*	*Volume (ml) delivered*	*Injection location*	*Systemic immunosuppression*	*Ocular complications reported*	*Entry visual acuity (study eye)*	*Posttreatment visual acuity*	*Considered significant visual acuity change*	*Patient vision in dim lighting (self-report)*
Current study													
P1	24/M	E417Q/E417Q	3	L	5.96×10^{10}	0.15	M	No	FT	20/240[a]	20/317	No	+
P2	23/F	R44Q/R91W	3	L	5.96×10^{10}	0.15	ST	No	No	20/195[a]	20/138	No	+
P3	21/M	Y368H/Y368H	3	L	5.96×10^{10}	0.15	T	No	No	20/283[a]	20/191	No	+
Bainbridge *et al.* (2008)													
Patient 1	23/M	Y368H/Y368H	12	G	1.0×10^{11}	1.0	M	Yes	No	20/286	20/145	No	NC
Patient 2	17/F	IVS1+5G>A/G40S	12	G	1.0×10^{11}	1.0	M	Yes	No	20/662	20/662	No	NC
Patient 3	18/M	E6X/D167Y	6	G	1.0×10^{11}	1.0	M	Yes	No	20/115	20/115	No	+
Maguire *et al.* (2008)													
Patient 1[b]	26/F	E102K/E102K	4.75	G	1.5×10^{10}	0.15	SN	Yes	No	<20/2000	20/1050	Yes	+
Patient 2[b]	26/M	E102K/E102K	2.75	G	1.5×10^{10}	0.15	M	Yes	MH	<20/2000	20/710	Yes	+
Patient 3	19/F	R234X/R234X	1.25	G	1.5×10^{10}	0.15	M	Yes	No	20/640	20/290	Yes	+

Abbreviations: LCA, Leber congenital amaurosis; L, local; G, general anesthesia; M, macula including fovea; ST, superotemporal retina; SN, superonasal retina; T, temporal retina; MH, macular hole; FT, foveal thinning; NC, no change; NM, not measured; +, patient-reported improvement.

[a]Average of two baseline measures. Refractive errors: P1 = $-0.25 + 1.50 \times 090$; P2 = $-1.50 + 0.50 \times 075$; P3 = $-2.70 + 1.75 \times 100$.

[b]Fraternal twins.

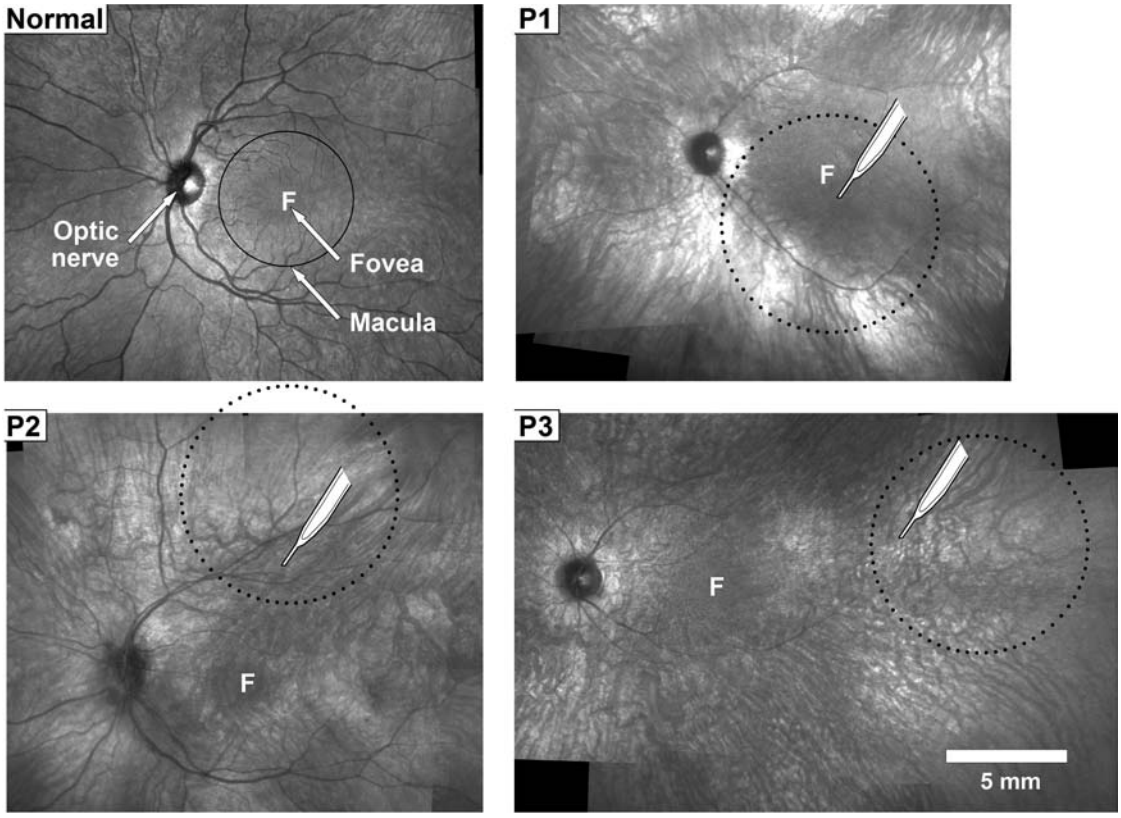

FIG. 1. Fundus images with near-infrared illumination of a normal subject and of P1, P2, and P3 with *RPE65*-LCA. The macula is defined by a circle on the normal image; the fovea (F) and the optic nerve head are indicated. Dotted circles on the images of individual patients represent the estimated areas of retinal detachment from drawings at time of surgery. White "syringe" indicates the site of the retinotomy that produced the detachment. All images depicted as left eyes to enable comparisons.

conjunctiva diminished slowly over the first month and the study eyes were quiet on evaluation at 60 days and thereafter.

Physical examinations were unchanged from baseline and there were no clinically significant abnormalities in hematology, serum chemistry, coagulation parameters, and urinalysis after retinal gene transfer in all subjects.

Immune response assays and vector biodistribution to the peripheral blood

Humoral immune responses were monitored by measuring levels of circulating antibody to AAV serotype 2 capsid at baseline and on days 14 and 90 posttreatment (Fig. 2A). For P1 and P3 at all time points there were no significant changes in AAV2 antibody titers. P2 experienced a 4.5- to 7.5-fold increase in titer on day 90 compared with baseline or day 14, but her day 90 titer (168,700 mU/ml) was similar to the baseline value for P3 (118,861 mU/ml) and ~16% of the mean titer (1,042,089 mU/ml) determined contemporaneously from 79 independent samples.

The AAV2 capsid-specific reactivity of peripheral lymphocytes (ASR) was also monitored at baseline and on posttreatment days 14 and 90 (Fig. 2B). Again P1 and P3 showed no significant rise in stimulation index (SI) at any time point. P2 on day 90 showed a marginally increased SI of 2.10 from a baseline SI of 1.89 (minimal level of significance for SI ranges from 2 to 3).

The T cell immune response to AAV2 capsid was monitored by IFN-γ ELISpot assays. PBMCs from subjects at baseline and on days 14 and 90 after treatment were stimulated with AAV2 peptide library pools and assayed for IFN-γ secretion. There were no positive responses to AAV2 peptide pools at any time points tested in the three subjects (Fig. 2C).

Biodistribution of vector in the peripheral blood was monitored by quantitative PCR (Poirier *et al.*, 2004) at baseline and on days 1, 3, and 14 posttreatment. For all patients at all time points, there were no vector genome copies detectable.

Visual function and retinal structure

All three patients noted increased light sensitivity in the study eye but to different degrees. These observations were most evident to the patients under low ambient light conditions and when using their control eyes as comparators. P1 described small regions of higher light sensitivity around the center of vision in the study eye, and also reported decreased clarity in this eye. P2 noted a region of increased light sensitivity below the point of fixation in the study eye. P3 described considerable improvement in light sensitivity across a wide region in the nasal visual field of the study eye.

Visual acuities in study and control eyes were plotted in all patients (Fig. 3A and C). P1, the only subject with an in-

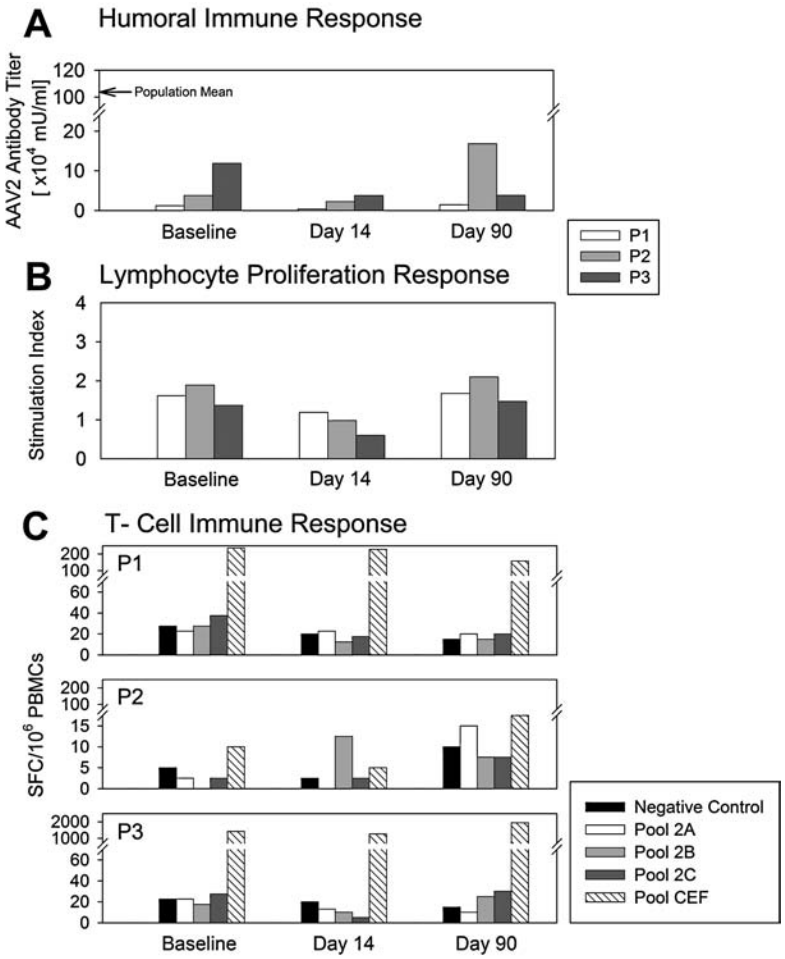

FIG. 2. Immune assays before surgery (baseline) and at 14 and 90 days after surgery. (**A**) Humoral immune response to AAV serotype 2 (AAV2) assayed by determining circulating serum antibody titers against AAV2 capsid in each study subject before and after surgery. Arrow along vertical axis indicates mean levels from a normal reference population ($n = 79$). (**B**) Antigen-specific lymphocyte proliferation response (ASR) assayed in peripheral blood lymphocytes incubated in the presence versus in the absence of AAV2 capsid antigen. The stimulation index (the ratio of $[^3H]$thymidine uptake in the presence of antigen to the uptake in its absence) in each study subject after surgery is compared with baseline values. (**C**) T cell immune response to AAV2 capsid in peripheral blood mononuclear cells (PBMCs). PBMCs isolated from each study subject before and after surgery were stimulated with three AAV2 capsid peptide pools (2A, 2B, and 2C) and assayed for IFN-γ secretion by ELISpot. The number of spot-forming cells (SFC) per 10^6 PBMCs before and after surgery is compared with a negative control (medium alone); responses to a CEF pool served as reference.

jection that included the fovea, showed the longest recovery time of visual acuity after surgery. At 90 days, the results remained three letters below the lowest of the baseline measurements. P1 has returned to baseline, using a conventional statistical limit of 3 lines (corresponding to 15 letters) of visual acuity loss or gain as significant (Sieving *et al.*, 2006). P2 showed a more rapid time course of recovery of visual acuity and returned to baseline. P3 had almost no visual acuity loss postoperatively and remains at baseline (Fig. 3A). All control eyes were unchanged over the 90 days after surgery (Fig. 3C).

The retinal anatomic basis of the visual acuity measurements in all subjects was studied by OCT (Fig. 3B and D). Differences in foveal thickness between pretreatment and 90 days posttreatment in control eyes of all subjects varied from 1.1 to 6.2 μm, which are all within published 98% confidence limits of intervisit variability in a retinal degeneration population ($\pm$16.7 μm; Sandberg *et al.*, 2005). In the study eyes of P2 and P3, there was also no significant difference between pretreatment and 90 days posttreatment (P2, 4.1 μm; P3, 8.2 μm). P1, however, showed a significant difference in the study eye of 80.3 μm between pre- and posttreatment foveal thickness. Inspection of the scans shows that the foveal architecture in the study eye of P1 before surgery did not show the typical normal depression. This abnormality may have been due to an epiretinal membrane or, as observed in other retinal degenerations, possible swelling of central Muller glial cells (Jacobson *et al.*, 2006c). There was evidence, however, of epiretinal membranes in all eyes of all patients pre- and postoperatively (arrows, Fig. 3B and D). At 90 days after surgery, the fovea of P1 had a more typical depression but thickness was reduced overall.

To quantify the patients' observations of increased light sensitivity in their study eyes (under low light conditions), we measured dark-adapted visual sensitivity with FST (Fig. 4), a psychophysical test designed for patients with severe visual loss (Roman *et al.*, 2005, 2007b). In an earlier study, untreated *RPE65*-LCA subjects showed visual sensitivities in response to full-field light stimuli that were 3–4 log units decreased compared with normal (Aguirre *et al.*, 2007); the three patients in the current study showed similar pretreatment results (Fig. 4A). After gene therapy, control eyes did not show consistent sensitivity changes; study eyes showed reductions in sensitivity immediately after the surgery which recovered generally within the first week (data not shown). Sensitivities obtained on days 30, 60, and 90 were pooled and compared with pooled sensitivities obtained pretreatment. Study eyes showed consistent increases in sensitivity compared with baseline; changes in control eyes were not consistent (Fig. 4A). To normalize for pretreatment differences between eyes, sensitivity changes were calculated from mean pretreatment baselines. Sensitivity changes were highly significant ($p < 0.001$) for all study eyes as a group (Fig. 4B, right); there were no obvious trends for sensitivity change

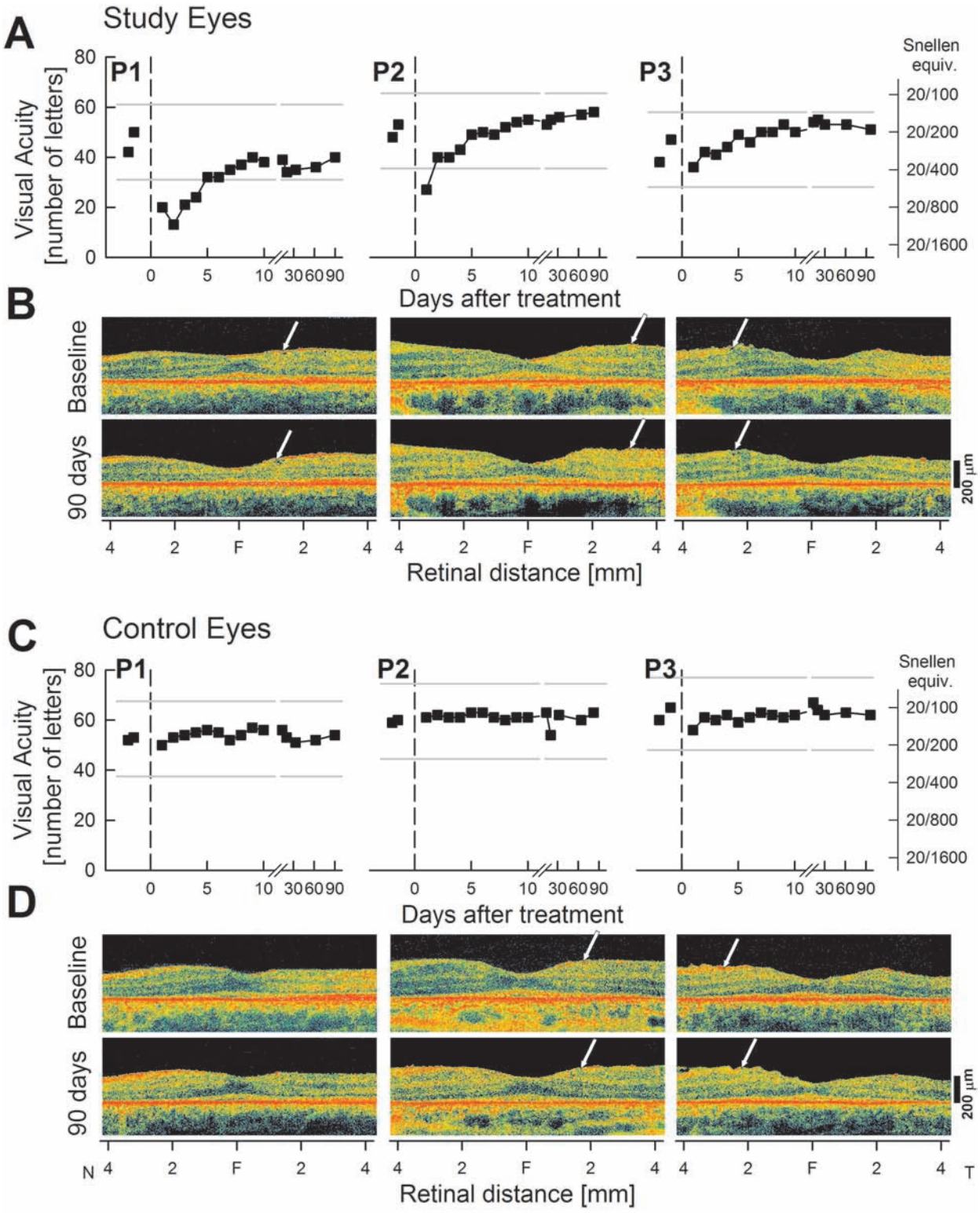

FIG. 3. Central vision and retinal structure in the *RPE65*-LCA patients. (**A**) Visual acuity as a function of time before (baselines) and after the day of surgery (0) in the study eyes of P1, P2, and P3. (**B**) OCT scans along the horizontal meridian before surgery and at 90 days after surgery in study eyes. (**C**) Visual acuity and (**D**) OCT scans in control eyes for comparison. Arrows, epiretinal membranes (detectable in all scans).

between days 30 and 90. For the control eyes (Fig. 4B, left), sensitivity changes were not significant ($p = 0.99$). Post hoc analysis in individual patients showed no significant changes in the control eyes of three patients; study eyes of P1 and P2, however, showed significant ($p = 0.04$ and $p = 0.002$, respectively) positive changes from mean baseline sensitivity after treatment; P3 changes were not significant ($p = 0.07$).

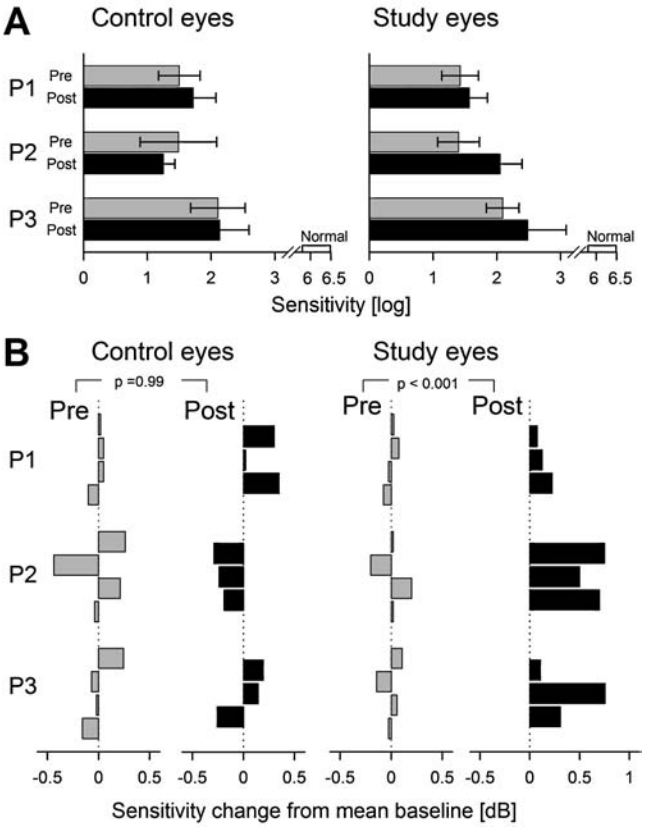

FIG. 4. Dark-adapted full-field sensitivity tests (FSTs) before (Pre) and 30 to 90 days after (Post) surgery in study and control eyes of the three patients. (**A**) Mean FST sensitivity and its variability (error bars, SD) at four visits presurgery compared with three visits postsurgery for the study and control eyes. Normal range for the FST is shown along the horizontal axis (Roman *et al*., 2007b; Aguirre *et al*., 2007). (**B**) Changes in sensitivity from mean baseline postsurgery show all positive numbers in study eyes compared with positive and negative changes in control eyes or pretreatment. Groups of three bars shown for each patient postsurgery refer to days 30 (*top*), 60 (*middle*), and 90 (*bottom*). *p* values refer to *t* test statistics between the pooled pre- and postsurgery results shown.

Discussion

What has been learned after nine *RPE65*-LCA young adult patients, representing three different studies, underwent uniocular subretinal gene therapy and were monitored for at least 1.25 months (Maguire *et al*., 2008) and at most 1 year (Bainbridge *et al*., 2008) (Table 1)? There has been no evidence to date of systemic toxicity of the retinal injections of vector in any of the patients. The concern over immune responses in gene therapy trials is high (reviewed in Zaiss and Muruve, 2008). All three *RPE65*-LCA trials performed studies to identify humoral immune reactions to AAV2 and assayed by ELISpot for a T cell-mediated response. In each case no positive result was evident. In the ASR test for AAV2 capsid-stimulated lymphocyte proliferation, which only this study included, one patient at the 90-day time point exhibited a stimulation index that just exceeds one of the previously established levels of significance (Brantly *et al*., 2006) but not another (Hernandez *et al*., 1999). This value was only slightly higher than at baseline and its relevance is unclear at present. This patient's ASR stimulation index will be reassessed at follow-up (see Supplementary Table 2) to understand better these results and their variation with time. Overall, however, the fact that little if any immune response was measurable in any patient may not be surprising given the small doses delivered subretinally in each of these studies relative to rAAV2 vector doses commonly administered in nonocular gene therapy trials; these are typically 2–4 orders of magnitude higher. In addition, the relative immune privilege of posterior ocular spaces is well appreciated (Streilein, 2003) and may serve to further limit any potential immune response to subretinal rAAV2 vector.

A key question for future *RPE65* LCA clinical trials concerns how the outcomes of the three current trials relate to the respective titers of vector delivered in each case. Unfortunately, there are at least four levels of uncertainty directly related to the vector in such an analysis that largely preclude any clear conclusions. First, each study made, purified, and titered their vector in a different facility using different protocols without reference to a common standard or to each other's vector. Thus, each stated titer cannot be related with confidence to the other two. Second, the area of RPE/neural retina exposed to vector within the injection bleb, necessary to estimate an effective "multiplicity of infection" for vector particles per RPE cell, is not identical, or even knowable, in all three studies. This trial and Maguire and coworkers (2008) delivered a 150-μl volume, and might be compared (but see preceding and following information). In Bainbridge and coworkers (2008), 1 ml of vector was delivered subretinally and the resultant bleb was then moved by gas manipulation over an unknown, and perhaps unknowable, retinal area in order to expose the fovea to vector in each patient. Such manipulation of the vector bleb after injection will in effect reduce the vector titer within any repositioned bleb by an unknown amount due to vector binding to RPE cells and photoreceptors in the initial detached area. In addition, as the initial bleb is relocated to its final position, vector binding to newly exposed RPE and photoreceptors will occur as the bleb transits between them. Thus the effective amount of vector in the bleb's final position is difficult to estimate. Third, each of the three vectors was constructed with different regulatory elements controlling expression of the human RPE65 cDNA. Maguire and coworkers (2008) employed a CBA promoter, like the present trial, but inserted a modified Kozak translational initiation sequence before the RPE65 cDNA (Bennicelli *et al*., 2008), unlike the natural initiation sequence used in this trial. Although asserted to optimize transgene expression, side-by-side experiments with a similar vector containing the natural initiation sequence was not reported. Bainbridge and coworkers employed a short RPE65 promoter that, although targeting expression primarily to RPE cells, is of unknown transcriptional strength relative to the CBA promoter-driven expression in RPE cells. Thus, the relative expression strengths of each of the three vectors on a per-vector genome basis cannot be confidently assessed. Fourth, the large differences in visual function at baseline among the cohorts of patients in each trial, as discussed previously, might suggest that the relative availability of RPE cells versus photoreceptors to interact with vector in the subretinal vector bleb could be significantly different from cohort to cohort, particularly if the ratio of

RPE cells to photoreceptors varies progressively with loss of visual function. Thus, it is not currently possible to reliably comment on how the amount of vector delivered relates to any differences in clinical outcome among the three trials.

Retinal complications did occur. These included a full-thickness macular hole in patient 2 of Maguire and coworkers (2008) and foveal thinning defined by high resolution optical imaging in P1 of the current study. Both of these patients had vector injections into areas that included the fovea; and both retinotomy sites were within 1–1.5 mm of the foveal region (Maguire *et al*., 2008; Fig. 1). Other subretinal injections with retinal detachments that included the fovea ($n = 4$) had retinotomies at further eccentricities, near the superior retinal vessel arcade. No measurements of foveal thickness before and after these injections, however, were reported (Bainbridge *et al*., 2008; Maguire *et al*., 2008). Retinotomies near the fovea would appear (on current evidence) to be an ill-advised strategy that may lead to disruption of foveal structure. Although subretinal injection is intricate and complex microsurgery, the procedure has many currently uncontrolled variables that could affect outcomes. This includes the alignment of the fluid stream during vector injection from the small-gauge needles in relation to the fovea, the exact trajectory of the fluid stream, and the degree of frailty of foveal photoreceptors and RPE that are chronically stressed by degenerative retinopathy. Even in normal retinas, there can be incomplete recovery of foveal vision after successful anatomic repair of fovea-off retinal detachments (Burton, 1982; Ross and Kozy, 1998; Schocket *et al*., 2006). Subretinal injections in the proximity of the fovea are expected to cause the largest separation between the neural retina and the RPE, one of the factors related to visual acuity recovery after retinal detachment with foveal involvement (Ross *et al*., 2005). Epiretinal membrane contraction, a common feature of retinal degenerations (Milam *et al*., 1998), has been mentioned as a possible cause of the macular hole in patient 2 of Maguire and coworkers (2008) but is unlikely to be the sole determinant of this complication (Moshfeghi *et al*., 2003).

Efficacy in these early safety studies, although a secondary outcome, is of high interest to the field of gene therapy and to those seeking treatment for otherwise incurable hereditary retinal degenerations (Bok, 2004). The standard outcome measure in ocular studies is visual acuity (Ferris *et al*., 1982). High-resolution visual acuity is subserved by the fovea, the primate-specific central retinal region with the greatest density of cone photoreceptors and unique neural connectivity (Provis *et al*., 2005). There was no proven increase in ***foveal*** visual acuity in any of the nine patients, despite the fovea being included in the subretinal injections in six of the nine patients. The only study to report improved visual acuity is that of Maguire and coworkers (2008), but these three patients had the lowest visual acuities at baseline of all the studies and the improvements did not increase visual acuity even to the level of the baseline acuities in the other two studies. The fact that a macular hole resulted from surgery but failed to be visually significant confirms the conclusion that any positive change due to treatment was from extremely severe vision loss to less severe vision loss. At lower resolutions (typically worse than 20/200), visual acuity may also be subserved by extrafoveal retina. The Maguire and coworkers (2008) result, although suggested to be a placebo effect due to the low level of vision being measured (Miller, 2008), is more likely real and attributable to para- or perifoveal increases in visual sensitivity.

Another measure of efficacy, dark-adapted sensitivity, targets the characteristic visual deficit in *RPE65*-LCA patients: the >4-log unit reduction in light sensitivity in this disease in humans and in animal models (Jacobson *et al*., 2005; Aguirre *et al*., 2007; Roman *et al*., 2007a). All three patients in the Maguire and coworkers study (2008) self-reported "improved vision in dimly lit environments" but dark-adapted visual measurements were not reported. Bainbridge and coworkers (2008) performed dark-adapted perimetry in all three patients and found increased function only in their patient 3. In the present study, all patients also self-reported an increase in vision under dim light conditions and there was a statistically significant increase in measured dark-adapted sensitivity posttreatment compared with baselines. None of the studies to date have determined whether an increment in sensitivity was mediated by rod or cone photoreceptors or both. The visual cycle subserving rod and cone photoreceptors appears to differ (Travis *et al*., 2007) and it is uncertain how *RPE65* gene replacement will differentially affect this chronically abnormal and key retinoid pathway in rods versus cones.

What is the next step for this clinical research? Conclusions from the previous two studies suggest that any lack of efficacy is due to the advanced stage of retinal disease at baseline and there is thus a need for both higher doses within the fovea and prompt advance to children with *RPE65*-LCA (Bainbridge *et al*., 2008; Maguire *et al*., 2008). More recent work, however, has indicated that there is no strong relationship between age and retinal structure or function until after the fourth decade of life (Jacobson *et al*., 2007, 2008). The present study suggests a need for greater understanding of the positive and negative effects of subretinal injection on retinal structure and visual function. Items worthy of attention in the group of nine patients already treated include the following: identification of the precise retinal location of any positive treatment effects, measurement of the magnitude of the effect across the treated zone, determination of the rod and cone photoreceptor contributions to the observed treatment effects, establishment of the relationship between the magnitude of any effect on baseline measures of photoreceptor and RPE integrity, and full explanation of any treatment failures revealed by sensitive noninvasive testing in this era of sophisticated high-resolution ophthalmic imaging.

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Author Disclosure Statement

B.J.B., W.W.H., and the University of Florida have a financial interest in the use of AAV therapies, and own equity in a company (AGTC Inc.) that might, in the future, commercialize some aspects of this work. The University of Penn-

sylvania, the University of Florida, and Cornell University hold a patent on the described gene therapy technology (United States Patent 20070077228, "Method for Treating or Retarding the Development of Blindness").

References

Acland, G.M., Aguirre, G.D., Ray, J., Zhang, Q., Aleman, T.S., Cideciyan, A.V., Pearce-Kelling, S.E., Anand, V., Zeng, Y., Maguire, A.M., Jacobson, S.G., Hauswirth, W.W., and Bennett, J. (2001). Gene therapy restores vision in a canine model of childhood blindness. Nat. Genet. 28, 92–95.

Acland, G.M., Aguirre, G.D., Bennett, J., Aleman, T.S., Cideciyan, A.V., Bennicelli, J., Dejneka, N.S., Pearce-Kelling, S.E., Maguire, A.M., Palczewski, K., Hauswirth, W.W., and Jacobson, S.G. (2005). Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Mol. Ther. 12, 1072–1082.

Aguirre, G.K., Komaromy, A.M., Cideciyan, A.V., Brainard, D.H., Aleman, T.S., Roman, A.J., Avants, B.B., Gee, J.C., Korczykowski, M., Hauswirth, W.W., Acland, G.M., Aguirre, G.D., and Jacobson, S.G. (2007). Canine and human visual cortex intact and responsive despite early retinal blindness from *RPE65* mutation. PLoS Med. 4, e230.

Aleman, T.S., Cideciyan, A.V., Sumaroka, A., Windsor, E.A., Herrera, W., White, D.A., Kaushal, S., Naidu, A., Roman, A.J., Schwartz, S.B., Stone, E.M., and Jacobson, S.G. (2008). Retinal laminar architecture in human retinitis pigmentosa caused by *rhodopsin* gene mutations. Invest. Ophthalmol. Vis. Sci. 49, 1580–1590.

Bainbridge, J.W., Smith, A.J., Barker, S.S., Robbie, S., Henderson, R., Balaggan, K., Viswanathan, A., Holder, G.E., Stockman, A., Tyler, N., Petersen-Jones, S., Bhattacharya, S.S., Thrasher, A.J., Fitzke, F.W., Carter, B.J., Rubin, G.S., Moore, A.T., and Ali, R.R. (2008). Effect of gene therapy on visual function in Leber's congenital amaurosis. N. Engl. J. Med. 358, 2231–2239.

Bennicelli, J., Wright, J.F., Komaromy, A., Jacobs, J.B., Hauck, B., Zelenaia, O., Mingozzi, F., Hui, D., Chung, D., Rex, T.S., Wei, Z., Qu, G., Zhou, S., Zeiss, C., Arruda, V.R., Acland, G.M., Dell'Osso, L.F., High, K.A,, Maguire, A.M., and Bennett, J. (2008). Reversal of blindness in animal models of Leber congenital amaurosis using optimized AAV2-mediated gene transfer. Mol. Ther. 16, 458–465.

Bok, D. (2004). Gene therapy of retinal dystrophies: Achievements, challenges and prospects. Novartis Found. Symp. 255, 4–16, 177–178.

Booij, J.C., Florijn, R.J., ten Brink, J.B., Loves, W., Meire, F., van Schooneveld, M.J., de Jong, P.T., and Bergen, A.A. (2005). Identification of mutations in the *AIPL1*, *CRB1*, *GUCY2D*, *RPE65*, and *RPGRIP1* genes in patients with juvenile retinitis pigmentosa. J. Med. Genet. 42, e67.

Brantly, M.L., Spencer, L.T., Humphries, M., Conlon, T.J., Spencer, C.T., Poireir, A., Garlington, W., Baker, D., Song, S., Berns, K.I., Muzuczka, N., Snyder, R.O., Byrne, B.J., and Flotte, T.R. (2006). Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 α_1-antitrypsin (AAT) vector in AAT-deficient adults. Hum. Gene Ther. 17, 1177–1186.

Burton, T.C. (1982). Recovery of visual acuity after retinal detachment involving the macula. Trans. Am. Ophthalmol. Soc. 80, 475–497.

Dejneka, N.S., Surace, E.M., Aleman, T.S., Cideciyan, A.V., Lyubarsky, A., Savchenko, A., Redmond, T.M., Tang, W., Wei, Z., Rex, T.S., Glover, E., Maguire, A.M., Pugh, E.N., Jacobson, S.G., and Bennett, J. (2004). *In utero* gene therapy rescues vision in a murine model of congenital blindness. Mol. Ther. 9, 182–188.

Donsante, A., Vogler, C., Muzyczka, N., Crawford, J.M., Barker, J., Flotte, T., Campbell-Thompson, M., Daly, T., and Sands, M.S. (2001). Observed incidence of tumorigenesis in long-term rodent studies of rAAV vectors. Gene Ther. 8, 1343–1346.

Felius, J., Thompson, D.A., Khan, N.W., Bingham, E.L., Jamison, J.A., Kemp, J.A., and Sieving, P.A. (2002). Clinical course and visual function in a family with mutations in the *RPE65* gene. Arch. Ophthalmol. 120, 55–61.

Ferris, F.L., III, Kassoff, A., Bresnick, G.H., and Bailey, I. (1982). New visual acuity charts for clinical research. Am. J. Ophthalmol. 94, 91–96.

Fu, T.M., Dubey, S.A., Mehrotra, D.V., Freed, D.C., Trigona, W.L., Adams-Muhler, L., Clair, J.H., Evans, T.G., Steigbigel, R., Jacobson, J.M., Goepfert, P.A., Mulligan, M.J., Kalams, S.A., Rinaldo, C., Zhu, L., Cox, K.S., Guan, L., Long, R., Persaud, N., Caulfield, M.J., Sadoff, J.C., Emini, E.A., Thaler, S., and Shiver, J.W. (2007). Evaluation of cellular immune responses in subjects chronically infected with HIV type 1. AIDS Res. Hum. Retroviruses 23, 67–76.

Grimm, D., Kern, A., Rittner, K., and Kleinschmidt, J.A. (1998). Novel tools for production and purification of recombinant adenoassociated virus vectors. Hum. Gene Ther. 9, 2745–2760.

Hernandez, Y.J., Wang, J., Kearns, W.G., Loiler, S., Poirier, A., and Flotte, T.R. (1999). Latent adeno-associated virus infection elicits humoral but not cell-mediated immune responses in a nonhuman primate model. J. Virol. 73, 8549–8558.

Hogan, M.J., Kimura, S.J., and Thygeson, P. (1959). Signs and symptoms of uveitis. I. Anterior uveitis. Am. J. Ophthalmol. 47, 155–170.

Hogan, M.J., Alvardo, J.A., and Weddell, J.E. (1971). Retina. In *Histology of the Human Eye*. (W.B. Saunders, Philadelphia, PA) *p*. 491.

Huang, Y., Cideciyan, A.V., Papastergiou, G.I., Banin, E., Semple-Rowland, S.L., Milam, A.H., and Jacobson, S.G. (1998). Relation of optical coherence tomography to microanatomy in normal and *rd* chickens. Invest. Ophthalmol. Vis. Sci. 39, 2405–2416.

Jacobson, S.G., Aleman, T.S., Cideciyan, A.V., Sumaroka, A., Schwartz, S.B., Windsor, E.A., Traboulsi, E.I., Heon, E., Pittler, J., Milam, A.H., Maguire, A.M., Palczewski, K., Stone, E.M., and Bennett, J. (2005). Identifying photoreceptors in blind eyes caused by *RPE65* mutations: Prerequisite for human gene therapy success. Proc. Natl. Acad. Sci. U.S.A. 102, 6177–6182.

Jacobson, S.G., Acland, G.M., Aguirre, G.D., Aleman, T.S., Schwartz, S.B., Cideciyan, A.V., Zeiss, C.J., Komaromy, A.M., Kaushal, S., Roman, A.J., Windsor, E.A., Sumaroka, A., Pearce-Kelling, S.E., Conlon, T.J., Chiodo, V.A., Boye, S.L., Flotte, T.R., Maguire, A.M., Bennett, J., and Hauswirth, W.W. (2006a). Safety of recombinant adeno-associated virus type 2-*RPE65* vector delivered by ocular subretinal injection. Mol. Ther. 13, 1074–1084.

Jacobson, S.G., Boye, S.L., Aleman, T.S., Conlon, T.J., Zeiss, C.J., Roman, A.J., Cideciyan, A.V., Schwartz, S.B., Komaromy, A.M., Doobrajh, M., Cheung, A.Y., Sumaroka, A., Pearce-Kelling, S.E., Aguirre, G.D., Kaushal, S., Maguire, A.M., Flotte, T.R., and Hauswirth, W.W. (2006b). Safety in nonhuman primates of ocular AAV2-*RPE65*, a candidate treatment for blindness in Leber congenital amaurosis. Hum. Gene Ther. 17, 845–858.

Jacobson, S.G., Cideciyan, A.V., Sumaroka, A., Aleman, T.S., Schwartz, S.B., Windsor, E.A., Roman, A.J., Stone, E.M., and Macdonald, I.M. (2006c). Remodeling of the human retina in choroideremia: Rab escort protein 1 (*REP-1*) mutations. Invest. Ophthalmol. Vis. Sci. 47, 4113–4120.

Jacobson, S.G., Aleman, T.S., Cideciyan, A.V., Heon, E., Golczak, M., Beltran, W.A., Sumaroka, A., Schwartz, S.B., Roman, A.J., Windsor, E.A., Wilson, J.M., Aguirre, G.D., Stone, E.M., and Palczewski, K. (2007). Human cone photoreceptor dependence on RPE65 isomerase. Proc. Natl. Acad. Sci. U.S.A. 104, 15123–15128.

Jacobson, S.G., Cideciyan, A.V., Aleman, T.S., Sumaroka, Windsor, E.A., Schwartz, S.B., Heon, E. and Stone, E.M. (2008). Photoreceptor layer topography in children with Leber congenital amaurosis caused by RPE65 mutations. Invest. Ophthalmol. Vis. Sci. June 6 [Epub ahead of print].

Ladas, J.G., Wheeler, N.C., Morhun, P.J., Rimmer, S.O., and Holland, G.N. (2005). Laser flare-cell photometry: Methodology and clinical applications. Surv. Ophthalmol. 50, 27–47.

Lorenz, B., Gyurus, P., Preising, M., Bremser, D., Gu, S., Andrassi, M., Gerth, C., and Gal, A. (2000). Early-onset severe rod–cone dystrophy in young children with *RPE65* mutations. Invest. Ophthalmol. Vis. Sci. 41, 2735–2742.

Maguire, A.M., Simonelli, F., Pierce, E.A., Pugh, E.N., JR., Mingozzi, F., Bennicelli, J., Banfi, S., Marshall, K.A., Testa, F., Surace, E.M., Rossi, S., Lyubarsky, A., Arruda, V.R., Konkle, B., Stone, E., Sun, J., Jacobs, J., Dell'Osso, L., Hertle, R., Ma, J.X., Redmond, T.M., Zhu, X., Hauck, B., Zelenaia, O., Shindler, K.S., Maguire, M.G., Wright, J.F., Volpe, N.J., McDonnell, J.W., Auricchio, A., High, K.A., and Bennett, J. (2008). Safety and efficacy of gene transfer for Leber's congenital amaurosis. N. Engl. J. Med. 358, 2240–2248.

Milam, A.H., Li, Z.Y., and Fariss, R.N. (1998). Histopathology of the human retina in retinitis pigmentosa. Prog. Retin. Eye Res. 17, 175–205.

Miller, J.W. (2008). Preliminary results of gene therapy for retinal degeneration. N. Engl. J. Med. 358, 2282–2284.

Morimura, H., Fishman, G.A., Grover, S.A., Fulton, A.B., Berson, E.L., and Dryja, T.P. (1998). Mutations in the *RPE65* gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. Proc. Natl. Acad. Sci. U.S.A. 95, 3088–3093.

Moshfeghi, A.A., Salam, G.A., Deramo, V.A., Shakin, E.P., Ferrone, P.J., Shakin, J.L., and Fastenberg, D.M. (2003). Management of macular holes that develop after retinal detachment repair. Am. J. Ophthalmol. 136, 895–899.

Nussenblatt, R.B., Palestine, A.G., Chan, C.C., and Roberge, F. (1985). Standardization of vitreal inflammatory activity in intermediate and posterior uveitis. Ophthalmology 92, 467–471.

Pang, J.J., Chang, B., Hawes, N.L., Hurd, R.E., Davisson, M.T., Li, J., Noorwez, S.M., Malhotra, R., McDowell, J.H., Kaushal, S., Hauswirth, W.W., Nusinowitz, S., Thompson, D.A., and Heckenlively, J.R. (2005). Retinal degeneration 12 (*rd12*): A new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). Mol. Vis. 11, 152–162.

Poirier, A., Campbell-Thompson, M., Tang, Q., Scott-Jorgensen, M., Combee, L., Loiler, S., Crawford, J., Song, S., and Flotte, T.R. (2004). Toxicology and biodistribution studies of a recombinant adeno-associated virus 2-α-1 antitrypsin vector. Preclinica 2, 43–51.

Provis, J.M., Penfold, P.L., Cornish, E.E., Sandercoe, T.M., and Madigan, M.C. (2005). Anatomy and development of the macula: Specialisation and the vulnerability to macular degeneration. Clin. Exp. Optom. 88, 269–281.

Redmond, T.M., Yu, S., Lee, E., Bok, D., Hamasaki, D., Chen, N., Goletz, P., Ma, J.X., Crouch, R.K., and Pfeifer, K. (1998). Rpe65 is necessary for production of 11-*cis*-vitamin A in the retinal visual cycle. Nat. Genet. 20, 344–351.

Redmond, T.M., Poliakov, E., Yu, S., Tsai, J.Y., Lu, Z., and Gentleman, S. (2005). Mutation in key residues of *RPE65* abolishes its enzymatic role as isomerohydrolase in the visual cycle. Proc. Natl. Acad. Sci. U.S.A. 102, 13658–13663.

Roman, A.J., Schwartz, S.B., Aleman, T.S., Cideciyan, A.V., Chico, J.D., Windsor, E.A., Gardner, L.M., Ying, G.S., Smilko, E.E., Maguire, M.G., and Jacobson, S.G. (2005). Quantifying rod photoreceptor-mediated vision in retinal degenerations: Dark-adapted thresholds as outcome measures. Exp. Eye Res. 80, 259–272.

Roman, A.J., Boye, S.L., Aleman, T.S., Pang, J.J., McDowell, J.H., Boye, S.E., Cideciyan, A.V., Jacobson, S.G., and Hauswirth, W.W. (2007a). Electroretinographic analyses of Rpe65-mutant *rd12* mice: Developing an *in vivo* bioassay for human gene therapy trials of Leber congenital amaurosis. Mol. Vis. 13, 1701–1710.

Roman, A.J., Cideciyan, A.V., Aleman, T.S., and Jacobson, S.G. (2007b). Full-field stimulus testing (FST) to quantify visual perception in severely blind candidates for treatment trials. Physiol. Meas. 28, N51–N56.

Ross, W., Lavina, A., Russell, M., and Maberley, D. (2005). The correlation between height of macular detachment and visual outcome in macula-off retinal detachments of 97 days' duration. Ophthalmology 112, 1213–1217.

Ross, W.H., and Kozy, D.W. (1998). Visual recovery in macula-off rhegmatogenous retinal detachments. Ophthalmology 105, 2149–2153.

Sandberg, M.A., Brockhurst, R.J., Gaudio, A.R., and Berson, E.L. (2005). The association between visual acuity and central retinal thickness in retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci. 46, 3349–3354.

Schocket, L.S., Witkin, A.J., Fujimoto, J.G., Ko, T.H., Schuman, J.S., Rogers, A.H., Baumal, C., Reichel, E., and Duker, J.S. (2006). Ultrahigh-resolution optical coherence tomography in patients with decreased visual acuity after retinal detachment repair. Ophthalmology 113, 666–672.

Sieving, P.A., Caruso, R.C., Tao, W., Coleman, H.R., Thompson, D.J., Fullmer, K.R., and Bush, R.A. (2006). Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc. Natl. Acad. Sci. U.S.A. 103, 3896–3901.

Simovich, M.J., Miller, B., Ezzeldin, H., Kirkland, B.T., McLeod, G., Fulmer, C., Nathans, J., Jacobson, S.G., and Pittler, S.J. (2001). Four novel mutations in the *RPE65* gene in patients with Leber congenital amaurosis. Hum. Mutat. 18, 164.

Snyder, R.O., and Flotte, T.R. (2002). Production of clinical-grade recombinant adeno-associated virus vectors. Curr. Opin. Biotechnol. 13, 418–423.

Snyder, R.O., and Francis, J. (2005). Adeno-associated viral vectors for clinical gene transfer studies. Curr. Gene Ther. 5, 311–321.

Song, S., Scott-Jorgensen, M., Wang, J., Poirier, A., Crawford, J., Campbell-Thompson, M., and Flotte, T.R. (2002). Intramuscular administration of recombinant adeno-associated virus 2 α-1 antitrypsin (rAAV-*SERPINA1*) vectors in a nonhuman primate model: Safety and immunologic aspects. Mol. Ther. 6, 329–335.

Streilein, J.W. (2003). Ocular immune privilege: Therapeutic opportunities from an experiment of nature. Nature Rev. 3, 879–889.

Takahashi, Y., Chen, Y., Moiseyev, G., and Ma, J.X. (2006). Two point mutations of *RPE65* from patients with retinal dystrophies decrease the stability of RPE65 protein and abolish its isomerohydrolase activity. J. Biol. Chem. 281, 21820–21826.

Thompson, D.A., Gyurus, P., Fleischer, L.L., Bingham, E.L., McHenry, C.L., Apfelstedt-Sylla, E., Zrenner, E., Lorenz, B.,

Richards, J., Jacobson, S.G., Sieving, P.A., and Gal, A. (2000). Genetics and phenotypes of *RPE65* mutations in inherited retinal degeneration. Invest. Ophthalmol. Vis. Sci. 41, 4293–4299.

Travis, G.H., Golczak, M., Moise, A.R., and Palczewski, K. (2007). Diseases caused by defects in the visual cycle: Retinoids as potential therapeutic agents. Annu. Rev. Pharmacol. Toxicol. 47, 469–512.

Yzer, S., van den Born, L.I., Schuil, J., Kroes, H.Y., van Genderen, M.M., Boonstra, F.N., van den Helm, B., Brunner, H.G., Koenekoop, R.K., and Cremers, F.P.M. (2003). A Tyr368His *RPE65* founder mutation is associated with variable expression and progression of early onset retinal dystrophy in 10 families of a genetically isolated population. J. Med. Genet. 40, 709–713.

Zaiss, A.K., and Muruve, D.A. (2008). Immunity to adeno-associated virus vectors in animals and humans: A continued challenge. Gene Ther. 15, 808–816.

Zolotukhin, S., Potter, M., Zolotukhin, I., Sakai, Y., Loiler, S., Fraites, T.J., Jr., Chiodom, V.A., Phillipsberg, T., Muzyczka, N., Hauswirth, W.W., Flotte, T.R., Byrne, B.J., and Snyder, R.O. (2002). Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods 28, 158–167.

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