AMERICAN ACADEMY™
OF OPHTHALMOLOGY

The Role of the Human Visual Cortex in Assessment of the Long-Term Durability of Retinal Gene Therapy in Follow-on *RPE65* Clinical Trial Patients

Manzar Ashtari, PhD,[1,2,3] *Elena S. Nikonova, BA,*[4] *Kathleen A. Marshall, COT,*[5] *Gloria J. Young, BA,*[1] *Puya Aravand, BA,*[1] *Wei Pan, MS,*[6] *Gui-shuang Ying, PhD,*[6] *Aimee E. Willett, BS,*[1] *Mani Mahmoudian, MD,*[1] *Albert M. Maguire, MD,*[1,2,5] *Jean Bennett, MD, PhD*[1,2,5]

Purpose: Gene therapy (GT) has offered immense hope to individuals who are visually impaired because of *RPE65* mutations. Although GT has shown great success in clinical trials enrolling these individuals, evidence for stability and durability of this treatment over time is still unknown. Herein we explored the value of functional magnetic resonance imaging (fMRI) as an objective measure to assess independently the longevity of retinal GT.

Design: Individuals with *RPE65* mutations who underwent GT in their worse-seeing eye in a phase 1 clinical trial received a second subretinal injection in their contralateral eye in a follow-on clinical trial. Functional magnetic resonance imaging (MRI) was performed longitudinally to assess brain responses of patients with *RPE65* mutations after stimulation of their most recently treated eye before and 1 to 3 years after GT.

Participants: Seven participants with *RPE65* mutations who were part of the follow-on clinical trial gave informed consent to participate in a longitudinal neuroimaging fMRI study.

Methods: All participants underwent fMRI using a 3-Tesla MRI system and a 32-channel head coil. Participants' cortical activations were assessed using a block design paradigm of contrast reversing checkerboard stimuli delivered using an MRI-compatible video system.

Main Outcome Measures: The primary parameters being measured in this study were the qualitative and quantitative fMRI cortical activations produced by our population in response to the visual task.

Results: Functional MRI results showed minimal or no cortical responses before GT. Significant increase in cortical activation lasting at least 3 years after GT was observed for all participants. Repeated measures analysis showed significant associations between cortical activations and clinical measures such as full-field light sensitivity threshold for white, red, and blue colors; visual field; and pupillary light reflex.

Conclusions: Participants with *RPE65* mutations showed intact visual pathways, which became responsive and strengthened after treatment. Functional MRI results independently revealed the efficacy and durability of a 1-time subretinal injection. The fMRI results paralleled those recently reported during the long-term clinical evaluations of the same patients. Results from this study demonstrated that fMRI may play an important role in providing complementary information to patients' ophthalmic clinical evaluation and has usefulness as an outcome measure for future retinal intervention studies. *Ophthalmology 2017;■:1–11* © *2017 by the American Academy of Ophthalmology*

Supplemental material is available at www.aaojournal.org.

Leber's congenital amaurosis (LCA) is a rare blinding disease, usually inherited in an autosomal recessive fashion.[1] It is symptomatic at birth or in the first few months of life and affects approximate 1 in 81 000 people.[2] Leber's congenital amaurosis has been associated with at least 18 different genes.[3,4] The gene encoding retinal pigment epithelium-specific protein 65 kDa (*RPE65*) is involved in one of the more common forms of LCA called LCA2. *RPE65* mutations can also cause retinitis pigmentosa and other early-onset autosomal recessive retinal degenerations.[5,6] Individuals with *RPE65* mutations are good candidates for gene transfer therapy because the degeneration of retinal cells is slow, providing an extended potential time window for intervention. Recent studies in both animal models[7–11] of LCA and in humans[12–17] have demonstrated success in restoring retinal and visual function using measures such as visual acuity (VA), visual fields (VFs), light sensitivity, pupillary light reflex (PLR), mobility, or a combination thereof. There are several clinical trials that have carried out gene therapy for individuals with *RPE65*-mediated disease (see www.clinicaltrials.gov).[18] The program at the Children's Hospital of Philadelphia and the University of Pennsylvania is the first to carry out administration of AAV2-hRPE65v2 to the contralateral eye.

http://dx.doi.org/10.1016/j.ophtha.2017.01.029
ISSN 0161-6420/17

Until now, it was not known whether severe impairment of the visual pathway resulting from congenital or early-onset inherited retinal degeneration would limit the responsiveness of vision processing neurons in the occipital cortex. Recently, we showed that in humans with LCA resulting from *RPE65* mutations, the visual cortex can be made responsive to visual input through unilateral ocular gene therapy, even after prolonged visual deprivation of up to 35 years.[19] In our previous studies, we used dim light stimuli because it is known that young individuals with *RPE65* mutations have some ability to see and navigate under brightly lit conditions.[20–22] Also, to account for variability in the disease stage among study participants and to correlate functional magnetic resonance imaging (fMRI) results with each participant's psychophysical measures, functional analyses were carried out separately for each individual participant.

In our initial report, treated and untreated eyes within the same *RPE65* participants were compared to assess the efficacy of gene therapy. Although there is a high degree of symmetry in disease progression between the 2 eyes, lack of baseline fMRI data for the initial injection made it difficult to reach a definitive conclusion on the magnitude and timing of the reported functional improvements. In the follow-on phase 1 clinical trial, the same participants who originally received a subretinal injection to their worse-seeing eye were candidates to receive administration of the AAV2-hRPE65v2 vector to their previously untreated contralateral eye. Neuroimaging results from 3 adults with *RPE65* mutations in the follow-on study subsequently were reported comparing the baseline cortical response of the contralateral eye with short-term effects of retinal gene therapy on the human visual cortex.[23] This report demonstrated that the visual cortex is extremely responsive to the stimulation of the photoreceptors via retinal gene therapy. As compared with baseline, participants with *RPE65* mutations showed significant cortical activations at 1 and 3 months after gene therapy administration. The follow-on study also demonstrated that prior exposure to the AAV2 vector did not result in any adverse effects to the second administration of AAV2-hRPE65v2 because of potential immunologic complications.[23] The current study went beyond examining the short-term effects of retinal gene therapy and evaluated the human brain responses in a large population of participants with *RPE65* mutations over a 3-year period. We hypothesized that fMRI results would be similar to those recently reported for the *RPE65* follow-on clinical trial[1] and that the fMRI results would demonstrate independently the long-lasting effects of a 1-time retinal gene therapy.

Methods

Study Participants

Participants were enrolled and evaluated as described (see ClinicalTrials.gov identifier NCT01208389; http://www.med.upenn.edu/carot/) at baseline and 1 to 3 years after surgical administration of AAV-hRPE65v2 to the fellow eye in the follow-on study. Although participants with *RPE65* mutations in the initial phase 1 clinical trial had received different doses and volumes of AAV2-hRPE65v2 in their first eye,[17,24] all participants received the high dose of 1.5E11 vector genomes in 300 μl for the follow-on study (Table 1). (The approximate location of the subretinal injection for the contralateral eye of all participants with *RPE65* mutations is presented in a recent report outlining a 3-year longitudinal clinical outcome of the follow-on clinical trial).[1] Overall, all participants with *RPE65* mutations except for 1 (patient CH10) received their subretinal injection as close as possible to the superior macula location.[1]

Seven of the original 10 participants who participated in the phase 1 neuroimaging study[19] were evaluated in the follow-on second eye study (Table 1). From the 10 original participants, patient CH13 was not eligible for intervention because of glaucoma in the contralateral, uninjected eye. Patient CH06 elected not to continue with the neuroimaging study. Longitudinal fMRI results from patient NP01 also are not included in the current report because patient NP01 had a history of smoking, and chronic smoking is known to abate cortical blood flow and has a dramatic effect on the fMRI cortical activations.[25] Patients NP03 and NP04 did not participate in any neuroimaging studies. After providing a complete description of the study, written informed consent (and when necessary, parental consent and child assent) was obtained from all participants for the longitudinal neuroimaging study. The Institutional Review Board of the Children's Hospital of Philadelphia approved all study procedures. All participants were assessed clinically as part of their qualification to enter the clinical trial for retinal gene therapy.[16,17,24] This study complied with the Health Insurance Portability and Accountability Act.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) scans were obtained at Children's Hospital of Philadelphia on a research-dedicated 3-Tesla Siemens Verio system using a 32-channel head coil (Siemens Medical Systems, Erlangen, Germany). All scans were carried out by a single operator and were monitored to be free of artifacts at the time of acquisition.

Functional Magnetic Resonance Imaging Sequence. Functional data were acquired using blood oxygenation level-dependent imaging, acquiring 3-mm isotropic resolution (matrix, 64×64; repetition time/echo time, 3000/30 ms) with a total acquisition time of 4:39 minutes. To permit T1 saturation, 3 additional volumes were acquired at the beginning of the fMRI experiment, but were not used in image analysis. A transistor-transistor logic pulse was used to start the stimuli automatically in sync with the start of fMRI acquisition. An MRI-compatible response device (a button that the participant pushed when recognizing the stimulus) was used to record participant responses. Participants were instructed to press the button once when the checkerboard first appeared.

Functional Magnetic Resonance Imaging Paradigm. In the past, while using simple contrast reversing checkerboard stimuli, we have been successful in showing the efficacy of gene therapy in this participant population.[19,23] Similar to our earlier study,[19,23] the current report used dim stimuli that went unperceived by most of the participants at baseline before retinal intervention. The purpose of this dim stimuli presentation was to assess the ability of participants in perceiving dim light after gene therapy. The fMRI paradigm consisted of 15-second blocks of flickering (8-Hz) black-and-white checkerboards, which consisted of 3 contrasts of high, medium, and low, interleaved with 15 seconds of blank (black) screens.[19,23] Participants were asked to fixate on a yellow cross in the center of the checkerboard patterns or, if they could not see the cross, to look straight ahead to their central vision. Participants additionally were asked to press the response button immediately after presentation of the visual stimuli and to hold the response