Supplementary Information:

Supplementary fMRI Results

The following is a detailed explanation of the results for each RPE65 subject before and after gene therapy.

NP02 fMRI Results

NP02 was 30 years old when he received his FO administration to his previously untreated eye (left eye). Longitudinal fMRI results for baseline and year 1-3 of NP02 are presented in the first row of Figure 1. As depicted in this figure, fMRI results for NP02 at baseline presented with cortical activations primarily distributed within the bilateral extrastriatal cortex with no activation in or around the striatal cortex (primary visual cortex). At one year after second eye injection NP02 showed highly significant and widespread, bilateral activations in response to high and medium stimuli at the same statistical threshold (fdr<5%, corrected p-value (pc)<0.004, cca ≥ 100 mm2). Most importantly, NP02's one year fMRI results presented with significant bilateral increases in cortical activations within the primary visual cortex (medial aspects of occipital lobes). While this important pattern of activations within the primary visual cortex continued into years 2 and 3 the amount of extrastriatal activation decreased, resembling the overall cortical activation pattern for NP02 closer to the activation patterns observed in normal sighted controls.[19, 26] Overall, NP02 presented with more activation in the right hemisphere at the one year FO exam, and the contribution to each hemisphere became relatively equal by second and third year FO examinations.

CH08 fMRI Results

CH08 was 12 years old when he received his FO subretinal injection to his previously untreated left eye. Longitudinal fMRI results for baseline and year 1-3 for CH08 are presented in the second row of Figure 1. As depicted in Figure 1, similar to NP02, fMRI results for CH08 at baseline presented with cortical activations primarily distributed within the bilateral extrastriatal cortex with minimal activation in or around the striatal cortex (primary visual cortex). fMRI results at one year time point for CH08 showed a dramatic increase in the

amount of medial and lateral visual cortex activations. Both medial and lateral activations continued to increase in distribution and magnitude for year 2. At year 3, while the degree of medially located activations remained elevated, the extrastriate activation declined bringing the overall cortical activation for CH08 closer to the sighted control activation patterns.[19, 26]

CH09 fMRI Results

CH09 was 11 years old at the time of his administration to his previously untreated eye and the only subject who received FO subretinal injection to the right eye. Longitudinal fMRI results for baseline and year 1-3 for CH09 are presented in the third row of Figure 1. As shown in Figure 1, fMRI results for CH09 at baseline reveal minimal bilateral cortical activations within the primary visual cortex and the extrastriatal cortex. At one year FO time point, CH09 showed considerable increases in the amount of activations and widespread bilateral visual response distribution in both striatal and extrastriatal cortex. A similar pattern of activations was observed at the year 2 FO fMRI exam. No FO year 3 fMRI analysis was carried out for CH09 due to excessive motion.

CH10 fMRI Results

CH10 was 14 years old when he received his FO administration to his previously untreated left eye. Longitudinal fMRI results for baseline and year 2 and 3 for CH10 are presented in the fourth row of Figure 1. The first year fMRI examination for CH10 was not possible because of his newly acquired dental braces. As shown in Figure 1, CH10's cortical activations at baseline mostly appeared to be distributed within the bilateral extrastriatal cortex with no activations in or around the striatal cortex (primary visual cortex). At year 2 and 3 FO fMRI examinations, CH10 showed considerably higher amounts of cortical activation in and around the calcarine fissure (primary visual areas) with a slight increase in his extrastriate activations.

CH11 fMRI Results

CH11 received her FO subretinal injection to her previously treated left eye at the age of 26. The results of her longitudinal fMRI are presented in Figure 1. As depicted in the first row of Figure 1, CH11 showed no cortical activation, regardless of the level of contrast presented to her untreated eye prior to administration. At one year FO fMRI, CH11 presented with highly significant and widespread bilateral activations and continued to show similar levels of cortical activations at FO years 2 and 3. As shown on the left and right inflated cortices, activation in the medial aspect of the visual cortex was symmetrically distributed in both hemispheres and upper and lower banks of the calcarine fissures. In summary, CH11's visual cortex was completely unresponsive to high- and medium-contrast stimuli at FO baseline. However, her fMRI results for years 1-3, in response to high- and medium-contrast stimuli showed dramatically increased areas of activation in the occipital cortex and were bilaterally distributed.

CH12 fMRI Result

CH12 was 46 years old at the time of her FO administration. She received her FO subretinal injection in her previously untreated left eye and the results from her longitudinal fMRI are presented in Figure 1. As shown in the second row of Figure 1, CH12 was highly unresponsive to the high and medium-contrast stimuli and did not present with any cortical activations before her subretinal administration even at a more relaxed statistical threshold. At the one year time point following her gene therapy, CH12 showed a significant bilateral cortical response to the checkerboard stimuli for high- and medium-contrast. At 2 and 3 years time points, CH12's cortical response to the same stimuli dramatically increased presenting with bilateral activation primarily concentrated in the extrastriate visual cortex with no activation within the primary visual cortex. In summary, CH12's visual cortex was completely unresponsive to high- and medium-contrast stimuli at FO baseline. At 1-3 years subsequent to administration to her contralateral eye (left eye) CH12 presented with extended activations primarily distributed to the extrastriatal visual cortex.

NP15 fMRI Results

NP15 was 14 years old when he received his retinal administration to his previously untreated left eye. Unlike all other participants, fMRI results from FO baseline for NP15 showed significant cortical activations within the bilateral striatal and extrastriatal cortex. As shown in Figure 1, the amount and significance level of cortical activations for NP15 increased over time particularly within the primary visual areas (in and around the calcarine fissure) bilaterally. NP15 did not participate for the second year FO neuroimaging study. In summary, NP15's cortical responses to the same stimuli were more than other participants at FO baseline. Moreover, his FO fMRI results after receiving subretinal injection showed significant increases in cortical response to the same high and medium stimuli at 1 and 3 year FO as compared to FO baseline.

Supplementary Method:

fMRI pre-processing

Pre-processing of data included slice scan time correction, 3D motion correction, spatial smoothing, and temporal filtering. Sinc interpolation was used for scan time correction to ensure that all voxels in the volume represented the signal simultaneously. A high-pass temporal filter of 2 cycles/run was applied to remove signal drift. Spatial smoothing was performed using a 3 mm full-width at half-maximum (FWHM) Gaussian filter. In addition to real time monitoring of the subjects' motions, all functional data sets were additionally processed using the motion correction algorithm implemented in BrainVoyagerQX that calculates head translation (in millimeters) and rotation (in degree) for each volume in relation to the first volume, in order to account for excessive motion. Since the subjects' motions were monitored at the time of data acquisition, using real time fMRI, none of the subjects showed excessive motion based on offline analyses (≥0.6 mm). Statistical analyses were performed using the general linear model (GLM) as implicated in BrainVoyagerQX. Each condition was analyzed by specifying a design matrix defined as blocks with checkerboard presentation versus blocks with a blank black screen, followed by application of the hemodynamic response function and correction for multiple comparisons using the false discovery rate (fdr). Since the RPE65 subjects demonstrated the largest response to the high- and medium-contrast conditions,[19, 23] fMRI results from these stimuli were used to correlate with the

subjects' clinical measures. Only the fMRI results from the high- and medium-contrast stimuli are correlated with the various measures of subjects' clinical evaluation over time.

Quantification of the fMRI activations of the overall visual cortex and the medial surface

The longitudinal qualitative evaluation (see Figure 1) clearly attests to the efficacy and durability of a one time administered retinal gene therapy. The significance of relationships between subjects' clinical measures and fMRI cortical activations must be examined when considering the utility of fMRI as a potential outcome measure for subjects who undergo retinal intervention. To examine such relationships, cortical fMRI activations were quantified for the entire areas of the visual cortex within the left hemisphere (LH), right hemisphere (RH) and total hemisphere (TH) of the visual cortex (LH+ RH) (see Figure S1). Using the Patch of Interest (POI) tool in BrainVoyagerQX29, RH, LH and TH clusters of activations (areas > 100 mm2) were automatically extracted and the areas of clusters of activations were then calculated and outputted by the POI program as separate patches of interest for each hemisphere (see Figure S1). fMRI quantification was carried out separately for the clusters of activations restricted to the primary visual cortex located within the medial surface of the visual cortices for the left medial (LM), right medial (RM) and total medial (TM). Quantification of the medial activations was performed by manual outlining of all clusters of activations located within the medial surface and extracting the volume using the Volume of Interest (VOI) tool as implicated by the BrainVoyagerQX (Supplementary Figure S2). Note that POI and VOI tools in BrainVoyagerQX export values in area (mm2) and volume (mm3) respectively.