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Supplementary Materials for

Plasticity of the human visual system after retinal gene therapy in patients with Leber's congenital amaurosis

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Supplementary Materials

Materials and Methods

Vision Testing and Ocular Examination

Multiple age-adapted tests of visual function were performed as part of the approved clinical trial protocol, including evaluation of visual acuity, visual field, pupillometry, and light sensitivity testing (dark adaptometry)(*46*). In addition, amplitude and frequency of nystagmus was evaluated independently for the left and right eyes.

Neuroimaging Methodology

3D T1 Weighted (MPRAGE) Imaging: A 3D isotropic structural high resolution T1 sequence was acquired with inversion preparation pulse (IR-Prep: TR = 2080 ms, TE = 2.54 ms, BW =180 Hz/Px, matrix size = 320x320, FOV = 256x256 mm2, 192 axial slices, slice thickness = 0.8 mm, inversion time = 1200 ms with Flip Angle =8°, NEX = 1, Echo Spacing= 7.8, iPAT = 2 and scan time = 7:04 minutes). This sequence was obtained for visual activation localization and the generation of group-averaged inflated hemispheres (www.brainvoyager.com).

Diffusion Tensor Imaging (DTI): The DTI sequence employed in the present study was a hybrid between the standard bipolar scheme, a monopolar Stejskal-Tanner implementation, and a modification of the latter (Siemens Medical Systems). The sequence was used with a total of 30 non-parallel diffusion gradient directions and a diffusion sensitization b-factor of 1000 s/mm^2 and four b0 images for the acquisition of 80 contiguous isotropic (1.7 x 1.7 x 1.7 mm^3) slices through the whole brain with no gaps. Sequence parameters for diffusion tensor imaging were: TR = 1100 ms, TE = 76 ms, matrix size = 128 x 128, FOV = 220x220 mm^2, bandwidth of 1446 Hz/Px, number of excitation NEX = 1 and acceleration factor (iPAT)=2 with a total acquisition time of 7:14 minutes.

fMRI Acquisition Parameters: Functional data were acquired using blood oxygenation level-dependent (BOLD) imaging, acquiring 3 mm isotropic resolution (matrix, 64 × 64; TR/TE, 3,000/30 ms) with a total acquisition time of 4:39 min. To permit T1 saturation, three additional volumes were acquired at the beginning of the fMRI experiment, but were not used in image analysis. A transistor-transistor logic (TTL) pulse was used

to automatically start the stimuli in sync with the start of fMRI acquisition. An MRI compatible response device (a button that the subject pushed when recognizing the stimulus) was used to record subject responses. Subjects were instructed to press the button once as soon as they noticed the appearance of the checkerboard.

fMRI Preprocessing Steps: All functional data from individual subjects and group averaged results were processed using BrainVoyager- QX (www.brainvoyager.com). 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 rule out 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 blank black screen, followed by application of the hemodynamic response function and correction for multiple comparisons using the false discovery rate (fdr). Since the LCA2 subjects demonstrated the largest response to the high contrast condition(*19*, *34*), only the fMRI results from the high contrast stimuli (high contrast – rest) are correlated to the diffusion tractography results.

Real Time fMRI: The research MR system at the Children's Hospital of Philadelphia is equipped with fMRI software that allows real time monitoring of the patients' performance during fMRI experiments as well as their translational and rotational head motions(*56*). Using the real time feature, fMRI acquisition with ≥0.6 mm translational or ≥0.6 degrees for rotational movement was terminated, the subject informed to stay still, and the experiment restarted.

Diffusion Tensor Tractography: Tractography was performed on a population-based diffusion tensor template constructed from diffusion tensor images of LCA2 patients and matched controls using DTI-TK (*51*, *52*). We used a different technique of registration (DTI-TK) from the voxel based analyses (PipeDream) to maximize the independence of the tractography results. Specific fibers of interest were extracted from the diffusion tensor template using DTIStudio, which is a program based on the *fiber assignment by continuous tracking* (FACT) method (*53*). Fibers were selected by initiating a seed pixel in the anatomy of choice using the "OR" operation function of DTIStudio. From this seed point a line is propagated which follows the principal eigenvector in 3D contiguous space from voxel to voxel (*53*). A threshold of 0.15- 0.20 for fractional anisotropy value (*53*) and a turning angle of 41°(*54*, *55*) were used. A subset of projections that were not part of the tracts of interest were excluded using the "NOT" operation in DTIStudio.

Table S1. LCA2 patient demographics

Subject	**Age at MRI (years)**	**MRI: Time Post Intervention (years)**	**Sex**	**Treated Eye**	**Visual Acuity (LogMAR)**		**Visual Fields at MRI (sum total degrees at each meridian)**		***RPE65* Mutations**
					Right	**Left**	**Right**	**Left**	
CH06	23.59	2.95	F	Right	1.74	1.36	337	303	IVS1+5g>a/L341S
CH08	12.75	1.99	M	Right	1.44	0.72	741	454	F530fs/F530fs
CH09	11.34	1.45	M	Left	0.76	1.03	303	929	R124X/Lys297del1aggA
CH10	14.06	2.03	M	Right	1.25	1.07	1257	1283	IVS1+5g>a/Phe530del1ttc
CH11	27.98	2.00	F	Right	0.76	0.65	1304	1012	V473D/V473D
CH12	47.40	1.53	F	Right	2.5	3.0	51	44	K303X/W431C
CH13	37.92	0.49	M	Right	1.61	1.53	523	112	IVS1+5g>a/IVS1+5g>a
NP01	29.63	3.4	F	Right	1.60	1.77	122	76	E102K/ E102K
NP02	30.45	4.1	M	Right	1.64	1.0	45	43	E102K/ E102K
NP15	13.30	0.84	M	Right	0.6	0.47	1371	1208	D167W/H313R

Table S1. Demographic summary for LCA2 patients including clinical data for visual acuity and visual fields at the time of MRI.