Purpose: Leber's congenital amaurosis (LCA) is a rare blinding disease with no cure. Due to the relatively slow progression of the disease, LCA type 2 (LCA2), which is caused by mutations in the gene encoding retinal pigment epithelium 65 kDa protein (RPE65), has been considered for gene therapy (GT). Recently, at the Children's Hospital of Philadelphia and the University of Pennsylvania, 12 LCA2 patients received unilateral GT resulting in dramatic enhancement of vision. The remarkable success of GT in LCA2 patients raises questions about the effect of this exciting therapy on the brain. Previous studies on animals with unilateral eyelid closure (UEC) demonstrate vast architectural reorganization of neuronal circuits in both the primary visual cortex (V1) and geniculocortical (GC) fibers. An interesting phenomenon observed in majority of UEC studies was that the shrinkage in arborization of neurons in the primary visual cortex and GC fibers paralleled an increase in plasticity of terminals serving the open eye. Results from follow up studies reinstating vision in the animals by reversing the eyelid sutures showed that both the structural and functional changes of UEC are largely reversible when the deprived eyes are reopened. Based on these results, we hypothesize a similar underlying brain plasticity to occur in patients undergoing retinal gene therapy (regaining sight) as those reported in animal studies with reverse UEC (regaining sight). To test this hypothesis, we utilized fMRI and DTI to assess the functional and structural brain plasticity that may have transpired as a result of gene therapy. To further substantiate this assumption, structural changes were correlated with patients' functional responses and clinical measures.

Methods: Ten unilaterally treated LCA2 patients (8/10 Rt. eye treated) and eleven matched controls underwent fMRI (>1.5 yrs post-surgery) and high resolution (1.7 isotropic) DTI (30 directions) using a 3T MR system utilizing a 32-channel head coil. A DTI population-based atlas was created to perform voxel-based analysis (VBA) and diffusion tensor tractography (DTT). Major fiber bundles crossing the occipital cortex, such as the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, occipito-callosal fibers, GC tracts and optic chiasm were extracted. Voxel-based analyses were performed for FA, axial (AD), radial (RD) and mean diffusivity (MD) diffusion parameters. Integrity of fiber tracts was assessed by comparing the fractional anisotropy (FA) maps of each tract between the LCA2 and control subjects. fMRI was performed to assess functional responses and to correlate visual function with the brain’s structural measures. Clinical measures such as visual acuity, visual field, light sensitivity, and nystagmus were correlated with the main diffusion index (FA) of the primary visual pathways.

Results: The results from VBA showed reduced FA, increased RD and MD, with no changes in the AD maps. As shown in Figure 1, the diffusion changes were primarily located in bilateral visual cortices with the left hemispheric reduced FA within the V1 and along the GC fibers only. Diffusion values for other visual pathways did not deviate from those of demographically matched controls. Diffusion changes observed in the GC fibers were also restricted to the fibers in the left hemisphere (p<0.005) and not the right (p>0.5). As depicted in Figure 2, reduced FA of the left GC but not the right strongly correlated with the degree and frequency of patients' nystagmus. Results from the group averaged fMRI responses depicted symmetrically distributed activation for controls but significantly greater right distributed cortical activations for the LCA2 patients. Pearson correlations showed a strong coefficient (r=0.56) between the FA of the right GC and fMRI avg response of the right visual cortex and (p=0.12) for LCA2. The low significance may be due to a small number of treated LCA2 patients in the same eye (N=8 treated in the right eye).

Conclusions: The diffusion changes observed within the primary visual cortices and along the GC fibers were primarily left lateralized for both the VBA and tractography results. In addition, the left hemispheric reduced FA within the V1 and along the GC fibers correlated negatively with patients' degree and frequency of nystagmus, depicting a relationship between decreased FA and severity of symptoms. These correlations were not observed for the right hemispheric value of FA. Considering that LCA2 is a bilateral degenerative disease, we believe this laterality in damage indeed stems from dramatically reduced right-sided damage that may have transpired from the fact that 8/10 LCA2 patients received their gene therapy in their right eye with subretinal injection delivered to their superior temporal retinal location. Injection of the right eye in the right temporal retinal areas may have then largely strengthened neuronal fibers connecting to the ipsilateral visual cortex which includes fibers of the right GC tracts. Our preliminary results from correlating fMRI and DTT also confirmed the plasticity of the right visual fibers by demonstrating vast amount of visual activations in the right visual cortex as compared to the left. In conclusion, similar to reports from the reversed UEC animal studies our preliminary DTT results from a group of unilaterally treated LCA2 patients suggest that gene therapy may promote re-myelination of axons of GC fibers along with local neurogenesis within the V1 favoring the treated eye. These encouraging outcomes demonstrate that through a multi-modal imaging approach one can dissect mechanisms by which retinal gene therapy mediates changes in brain anatomy and function. Understanding the underlying mechanism of such brain plasticity may aid in establishing a set of neuroimaging biomarkers that would have the potential of predicting GT outcome and therefore help in the selection process to identify patients with optimal response to GT.