Long Term MRI and MR spectroscopic evaluation of gene therapy in a feline model of neurologic disease.

Heather L Gray-Edwards[1], Nouha Salbi[2,3], Ashley N Randle[1], Judith Hudson[4], Ronald Beyers[5], Miguel Sena Esteves[6], Thomas Denney[5,7], and Douglas Martin[1,8]
[1]*Scott-Ritchey Research Center, Auburn Univeristy, Auburn, Al, United States,* [2]*Seimens Healthcare, Malvern, PA, United States,* [3]*Auburn Univeristy MRI Research Center, Auburn Univeristy, AL, United States,* [4]*Clinical Sciences, Auburn Univeristy, AL, United States,* [5]*Auburn University MRI Research Center, Auburn Univeristy, AL, United States,* [6]*Neurology, University of Massachusetts, MA, United States,* [7]*Department of Electrical Engineering, Auburn Univeristy, AL, United States,* [8]*Anatomy, Phsiology and Pharmacology, Auburn Univeristy, AL, United States*

Target Audience. Studies outlined in this abstract will benefit those interested in MRI and MRS as an *in vivo* biomarkers of neurodegenerative diseases and AAV-gene therapy.

Purpose. GM1 gangiosidosis is a fatal neurodegenerative disease of children for which there is no cure. AAV gene therapy has shown profound promise in the GM1 cat, with an extension of lifespan approximately five fold [1]. Gm1 is caused by a mutation in the enzyme β galactosidase (βgal). We performed intracranial (IC) AAV-mediated βgal gene replacement in a feline model of GM1, resulting in a >five-fold increase in lifespan and marked attenuation of neurologic signs. With this profound success in IC therapy, human clinical trials are in the planning stages but a non-invasive method to track disease amelioration is required. Here we utilize MRI and MR spectroscopy to reliably track disease progression of GM1 gangliosidosis. Through spatial evaluation of the brain using multiple single voxel spectroscopy measurements we can evaluate brain areas not completely corrected by gene therapy.

Methods. AAV1 and AAVrh8 vectors expressing feline βgal were injected into the thalamus (Th) and lateral ventricle (ICV) or deep cerebellar nuclei (DCN) of GM1 cats at 2-3 months of age. MRI and MRS data were acquired on a 7 Tesla MAGNETOM scanner (Siemens Healthcare, Erlangen, Germany) at 8 months, 12 months and 30 months of age using a 32-channel head coil (Nova Medical, Boston, Mass.). Anatomical coronal images were acquired using 3D MPRage (magnetization-prepared rapid gradient echo) with 0.5mm isotropic resolution and TR/TE of 1910/2.5ms, followed by 2D axial T2 TSE images with TR/TE of 5450/12ms and a resolution of (0.25x0.25x1)mm^3. Single voxel spectroscopy (SVS) was then acquired using a PRESS (Point Resolved Spectroscopy) sequence optimized for 7T with TE/TR = 30/5000 ms , 64 averages and a Variable Pulse power and Optimized Relaxation Delays (VAPOR) water suppression. Using high resolution MRI images voxels were positioned in the thalamus, centrum ovale, parietal cortex, temporal lobe, occipital cortex and cerebellum. T2 was estimated for each group and voxel with the following TEs: 30, 80, 144, 288. MRI data were analyzed with EFilm 3.2 software (Merge Healthcare, Chicago) . MRS data were processed with LC model and internal water scaling (http://www.s-provencher.com/pages/lcmodel.shtml).

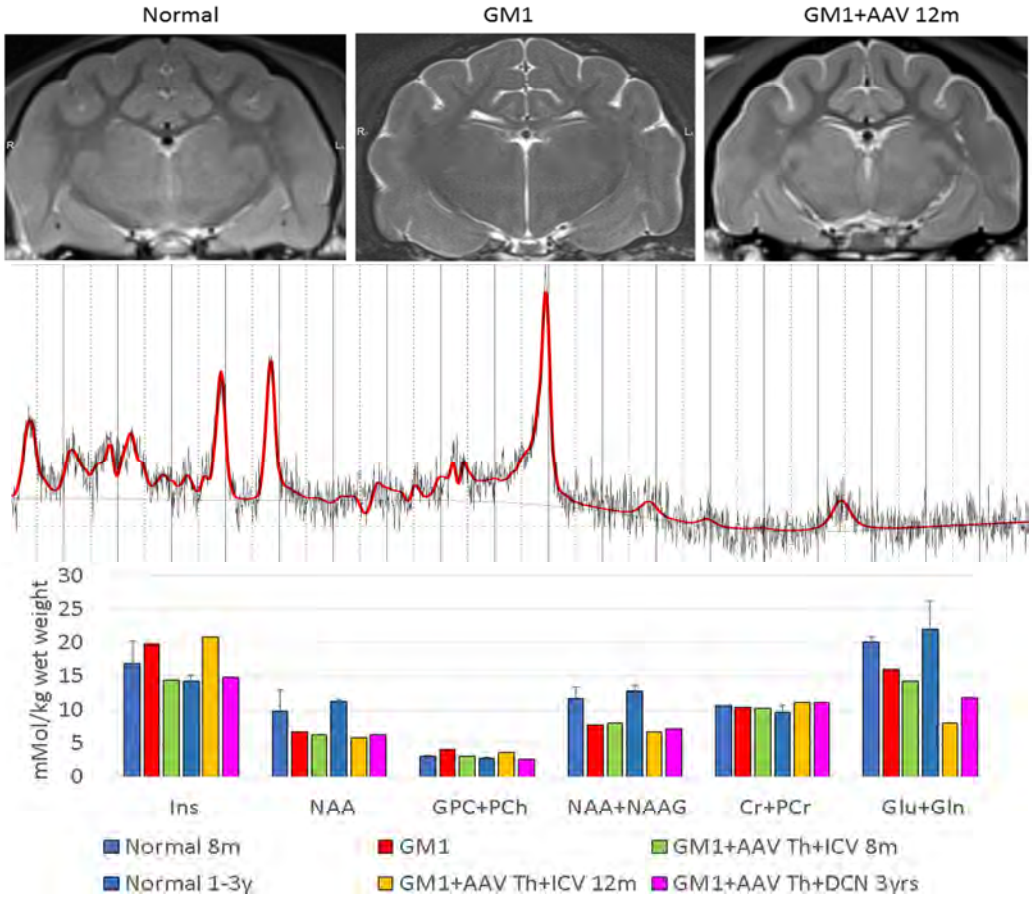

Figure 1. *7T MRI and MRS of normal and GM1 cat brains.* **Top:** Representative axial T2 weighted MRIs demonstrate cortical white matter hyperintensity to gray matter at the level of the thalamus in an untreated GM1 cat comparted to normal. AAV gene therapy normalizes gray: white matter intensities. **Middle:** Representative LC model output and spectra fit. **Bottom:** Representative MRS voxel analysis (cerebellum). Myoinsoitol (Ins) levels increase in GM1 cat and are normalized after AAV gene delivery in the cerebellum of the Th+ICV cat at 8 months but not at 12 months. In the Th+DCN group Ins levels remain normalized 3 years later. N-acetyl-aspartate (NAA) and glutamate+glutamine (Glu_Gln) levels decreased in the untreated GM1 cat as compared to normal control, but gene therapy failed to normalize NAA levels regardless of delivery route.

Results and Discussion. GM1 gangliosidosis results in increased Ins, reduced NAA +/- NAA-glycine, +/- elevated glycerophosphocholine and phosphocholine (GPC+PCh) and reduced glutamine/glutamate (Glu+Gln), although metabolite levels vary depending upon brain area. The cerebellum shows the most pronounced metabolite changes at humane endpoint in the untreated GM1 cat, which correlates with histopathology. After AAV gene therapy, the most normalized areas are the parietal cortex and thalamus, and intermediate areas are the occipital cortex and temporal lobe. The cerebellum is best corrected after direct injection, (MRS findings correlate with post mortem assays), suggesting that cerebellar injection is superior to lateral ventricular injection for treatment of the cerebellum.

1. McCurdy, V.J. *et al.* Sustained normalization of neurological disease after intracranial gene therapy in a feline model. *Sci Transl Med* **6**, 231ra48 (2014).