

7T MRI and MR Spectroscopy of a Feline Model of Sandhoff Disease After AAV Gene Therapy

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Target Audience. Studies outlined in this abstract will benefit those interested in Magnetic Resonance Imaging as an *in vivo* biomarker of neurodegenerative lysosomal storage diseases and AAV-gene therapy.

Purpose. Sandhoff disease (SD) is a form of GM2 gangliosidosis that is untreatable and fatal by 5 years of age in humans. SD is caused by mutation of the β subunit of the dimeric enzyme hexosaminidase (Hex). We performed intracranial (IC) AAV-mediated Hex gene replacement in a feline model of SD, resulting in a >four-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 and development of non-invasive methods such as MRI and MRS to evaluate amelioration or disease progression are vital to predicting outcomes in humans.

Methods. Monocistronic AAV2/rh8 vectors expressing feline Hex α and β subunits (1:1 ratio) were injected into the left lateral ventricle and bilaterally into the thalami of Sandhoff cats (8.0×10^{12} g.c. total). MRI and MRS data were acquired using a 7 Tesla MAGNETOM scanner (Siemens Healthcare, Erlangen, Germany) at 4 months and 30 months using a 4.5 inch inner diameter (ID) quadrature transmit/receive birdcage coil (RF coil lab, AUMRI center, Auburn AL). Anatomical coronal images were acquired using 3D MPRage (magnetization-prepared rapid gradient echo) with 0.5mm isotropic resolution and TR/TE of 1950/3.5ms, followed by 2D axial T2 TSE images with TR/TE of 3600/87ms and a resolution of $(0.25 \times 0.25 \times 1) \text{mm}^3$. Single voxel spectroscopy (SVS) was then acquired using Stimulated Echo Acquisition Time (STEAM) with Variable Pulse power and Optimized Relaxation Delays (VAPOR) water suppression, TE/TR = 9/4000 ms and 64 averages. In all animals a $(7 \times 7 \times 13) \text{mm}^3$ voxel was placed in the striothalamus that was well defined on the high resolution anatomical images. All metabolite peak integrals were normalized to creatine (Cr). MRI and MRS data were analyzed using the manufactures software (Siemens Healthcare, Erlangen, Germany), EFilm 3.2 software (Merge Healthcare, Chicago) and jMRUI (Java Based Magnetic Resonance user Interface, http://www.mrui.uab.es/mrui/mrui_homePage.shtml).

Results.

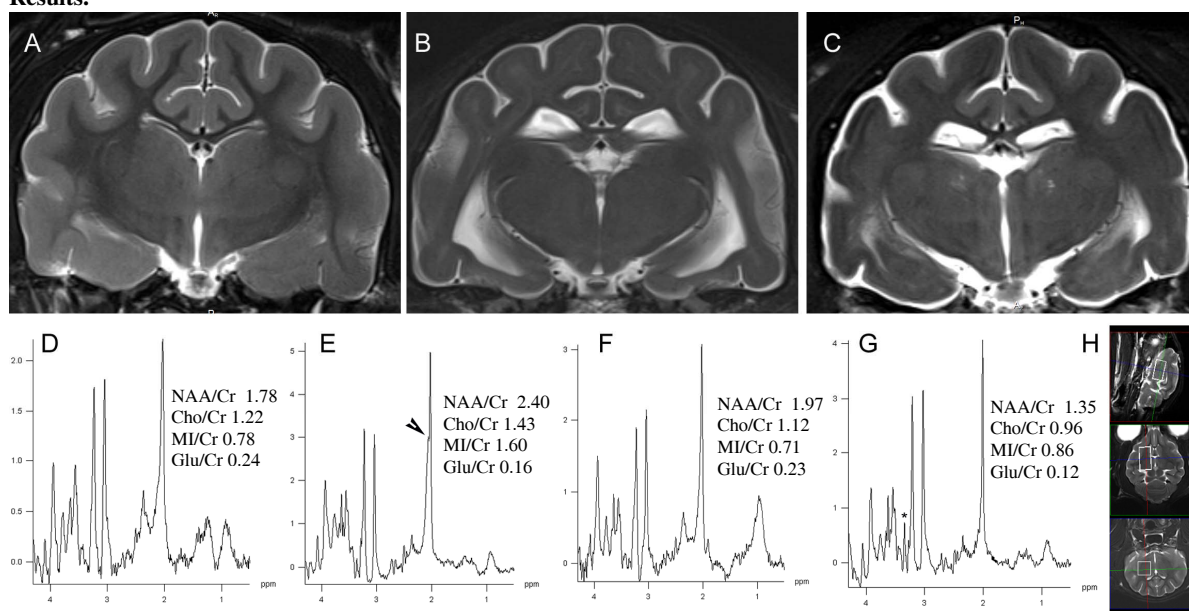


Figure 1 7T MRI and MRS of normal and SD cat brains.

TOP: Representative axial T2 weighted MRIs demonstrate cortical white matter hyperintensity to gray matter at the level of the thalamus in an untreated SD cat (B; 4 mos.) compared to normal (A). The AAV-treated SD cat (C) has normalized white:gray matter intensities. Hyperintense areas are present in the thalamus of the AAV treated SD cat (C) and believed to be the injection site.

Bottom:

Representative MRS revealed increased ratios of N-acetyl-aspartate (NAA)/Creatine (Cr), Choline (Cho)/Cr, decreased Glutamate (Glu)/Cr, and a toxic metabolite, N-acetyl hexosamine (NAHex; arrow) in an untreated SD cat near the humane endpoint (E, 4 mos.) compared to normal (D). At 30 mo. old, an AAV-treated SD cat (G) showed normalization of MI/Cr, reduction of NAA/Cr, Cho/Cr and Glu/Cr, and the presence of taurine (denoted by *) compared to an age matched normal control (F). Voxel placement (H) was consistent between cats and located in the striatum/thalamus.

Discussion. Untreated SD cat brain showed white matter abnormalities and increased NAA/Cr, which is likely caused by summation from buildup of a toxic metabolite previously reported in human SD, NAHex¹. 7T MRI and MRS images in AAV-treated cats show preservation of white and gray matter structures and normalization or even reduction of metabolites after AAV gene therapy. Here we report, for the first time, MRS detection of taurine in GM2 gangliosidosis, which may represent taurine-conjugated GM2 as a novel mechanism for export of water insoluble GM2².

Conclusions. Gene replacement in the feline SD model results in >4 fold increase in lifespan and partial normalization of brain architecture. Metabolite changes present in AAV treated SD cats may help predict outcomes in AAV gene therapy for future human clinical trials.

References: 1. Wilken, B., Dechent, P., Hanefeld, F. & Frahm, J. Proton MRS of a child with Sandhoff disease reveals elevated brain hexosamine. *Eur J Paediatr Neurol* **12**, 56-60 (2008). 2. Li, Y.T., Maskos, K., Chou, C.W., Cole, R.B. & Li, S.C. Presence of an unusual GM2 derivative, taurine-conjugated GM2, in Tay-Sachs brain. *J Biol Chem* **278**, 35286-91 (2003).