Morphologic abnormalities of Mucopolysaccharidosis Type VII characterized by high resolution MRI in a mouse model

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Introduction: Mucopolysaccharidosis type VII (MPS VII) is a debilitating degenerative lysosomal storage disorder characterized by short stature, mental retardation, and early death. It is caused by a mutation in the gene ß-glucuronidase (GUSB). Murine models of MPS VII have been identified, with similar abnormalities as in human cases: they have skeletal deformities, dwarfism, behavioral abnormalities, as well as early death. Cellular analysis has revealed intracellular vacuolization in many tissues, including the brain (1,2). We hypothesize that the vacuolization seen in the brain may have an effect on the structure of the brain in advanced disease, and evaluate MPS VII mutant and wild-type mice using high-resolution MRI.

Materials and Methods: 26 week old MPS VII mice (n=3) on a C57Bl/6 background and wild-type littermates (n=3) at 26 weeks were anesthetized with intraperitoneal injection of ketamine and xylazine. The animals were perfused with 4% paraformaldehyde and the brains were harvested and postfixed overnight. After fixation, the brains were placed in a plastic NMR tube with proton-free solution (Fomblin, Ausimount, Thorofare USA). A custom-built solenoid coil (20 mm id) was used for all imaging experiments. High resolution structural imaging was performed on the *ex vivo* brains using a 9.4T vertical bore MR scanner (Varian, Palo Alto USA) after incubation of the brains in 2% gadolinium (3) for 4 days at 4°C. A 3D gradient echo pulse sequence was used with TR = 50 ms, TE = 5ms, number of acquisitions = 2, FOV=2 cm x 1 cm x 1 cm, and acquisition image matrix = 512 x 256 x 256, resulting in 39 µm isotropic acquisition resolution. The acquisition time for the 3D imaging was 1 hour and 50 minutes. The images from individual brains were aligned to each other using in-house developed IDL routines. Regions of interest were defined manually in the corpus callosum and hippocampus and volumes were normalized to overall brain volume. Volumetric analysis of the high-resolution images was performed using ITK-SNAP (www.itksnap.org). After imaging, the brains were embedded in OCT and sliced at 20µm thickness on a cryostat. Sections were harvested every 100 µm. Hematoxylin and Eosin staining was performed in the usual fashion. Images were obtained on a Leica dissecting microscope.

Results: Volumetric comparison between GUSB mutant mice and wild-type littermates revealed no significant difference in overall forebrain volume. However, there was a significant decrease in the volume of the corpus callosum and anterior commisure and a small but significant increase in the volume of the hippocampus in the *GusB* mutant mice (Figure 1A,B,E). By histology, the corpus callosum measured 476.2 +/- 6.3 µm in maximim thickness in the wild type and 332.7 +/- 15.9 µm in the mutants (Figure 1C-D, $p < 0.01$), an approximate 30% decrease in the mutants. These data correspond well to the results of the MRI data, where mutant mice have a similar approximately 30% decrease the maximum thickness of the corpus callosum versus wild type.

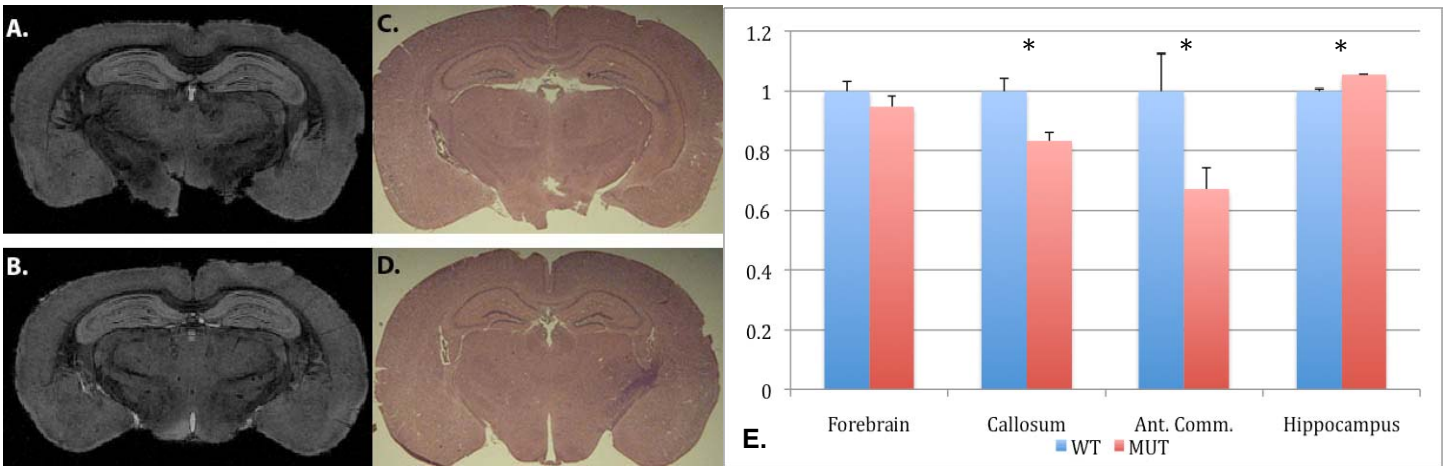

Figure 1: Coronal slices through the corpus callosum and hippocampus of wild type (A) and *GusB-/-* (B) mice from high-resolution MRI. Coronal H&E sections from wild type (C) and *GusB-/-* (D) mice. E. Comparison of regional volumes between wild type and *GusB*-/- mice from high resolution MRI, normalized to the average value for wild type animals. Ant. Comm. = anterior commisure. * $p < 0.05$

Discussion: Using high-resolution microscopic MRI, we identified structural abnormalities in GUSB mutant mice. Such deficiency is known to cause intracellular vacuolization in the brain, which may be accompanied by decrease in axon size or in axon numbers. The slight but significant increase in size of the hippocampus could be a structural manifestation of the nuclear vacuolization with increased cell size. Future evaluation after gene rescue of MPS VII mice could determine whether the normalization seen after gene therapy (4,5) also rescues the structural changes in the brain.

References:

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