# Assessment of metabolic abnormalities from the cerebellar region of the brain of a canine model of mucopolysaccharidosis type I (MPS I) using in vivo 1H MRS

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**Introduction:** Mucopolysaccharidosis type I (MPS I), also known as Hurler syndrome, is a lysosomal storage disease caused by loss of activity of the enzyme α-L-iduronidase, which is responsible for the degradation of mucopolysaccharides in lysosomes[1]. MPS I is characterized by organomegaly, corneal clouding, skeletal deformities, cardiovascular disease, respiratory inadequacies, and varying degrees of CNS involvement including mental retardation and motor activity[2]. Baldo et al reported progressive motor dysfunction in MPS I mice and storage of GAGs in Purkinje cells[3] from the cortex and cerebellum. MRI studies of the canine model of MPS I showed reduced corpus callosum volume in MPS I dogs compared to normal controls[4]. $^1$H MRS provides biochemical information on tissue metabolites and has been used for the study of many brain disorders, such as stroke, tumor, developmental and degenerative and metabolic diseases[5]. However there are no $^1$H MRS studies on MPS I dogs. Hence the purpose of this study was to investigate the metabolic profile in the cerebellar regions of the brain in MPS I using in vivo $^1$H MRS.

**Materials and Methods:**

***Experimental Animals:*** Normal (n =4) and MPS I (n=8) dogs (11 months to 4 years of age) were raised and maintained in the animal colony at the School of Veterinary Medicine under NIH and USDA guidelines for the care and use of animals in research. Dogs were outbred into a colony of mixed-breed dogs to increase their reproductive performance and overall vigor, and all were genotyped for the mutant allele by PCR and restriction enzyme digest[2].

***Animal Preparation for MRI Scan***: Dogs were fasted and received intramuscular atropine (0.02 mg/kg) and hydromorphone (0.1 mg/kg), and then intravenous propofol (6 mg/kg) to induce anesthesia. The animal was intubated with a 6.0 - 10.0 mm endotracheal tube and maintained on 2% to 5% isoflurane and oxygen throughout the procedure. The body temperature was maintained at 37°C by using a water pad with circulating heated water. Physiological monitoring included pulse oximetry and electrocardiography during anesthesia, and vital signs (oxygen saturation and heart rate) were recorded before and during scanning.

***In vivo MRI and $^1$H MRS experiments:*** In vivo MRI and single voxel spectroscopy was performed on a 3 Tesla Tim Trio MR scanner (Siemens, Erlangen, Germany) equipped with a 16-channel phased array coil. Multi-slice spin echo T2-weighted images were acquired for planning the voxel. Single voxel $^1$H MRS was performed using a PRESS sequence by placing a voxel of 8 mm x8 mm x8 mm on the cerebellar region of the brain (Fig. 1) with following acquisition parameters: TR=3000ms, TE=30ms, number of averages=128. An unsuppressed water spectrum was also acquired (number of averages = 8) to compute absolute metabolite concentrations using the LC Model software. Total time to acquire both water suppressed and water unsuppressed spectrum was about 8.12 min.

**Data processing and quantification:** The spectral FID was converted into RDA format and the data was processed using LC-model to measure concentration of the major brain metabolites [N-acetylaspartate (NAA), creatine (Cr), choline (Cho) and myo-inositol (mI)]. NAA, Cho and mI concentration values were normalized to Cr values.

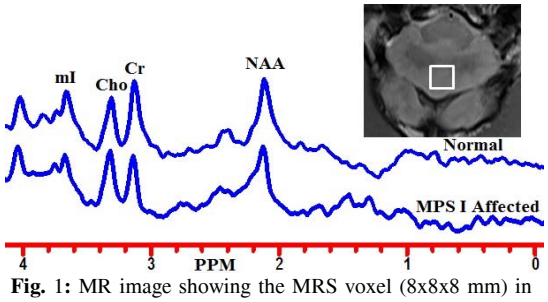


**Fig. 1:** MR image showing the MRS voxel (8x8x8 mm) in the cerebellar region in a MPS I affected dog. The spectrum from the MPS I dogs shows increased tCho and a decrease in NAA compared to the normal dogs.

**Table 1:** Metabolite concentrations with respect to Cr showing differences in metabolite/Cr ratio in Normal and MPS I dogs.

| Group | Metabolites | | |
|---|---|---|---|
| | Cho/Cr | NAA/Cr | mI/Cr |
| Normal (n=4) | 0.26±0.02 | 0.97±0.16 | 1.20±0.14 |
| MPS I (n=8) | 0.31±0.03 | 0.72±0.14 | 1.21±0.19 |
| p value | **0.02** | **0.04** | 0.88 |

**Statistical analysis and results:** Independent Student's t-test was performed between normal and MPS I dog using SPSS. We observed significantly reduced NAA/Cr ratio in MPS I dogs (Table 1 and Fig 2). We also observed significantly elevated Cho/Cr ratio (Table 1 and Fig 2) and a slight increase in mI/Cr ratio in MPS I dogs compared to normal dogs.

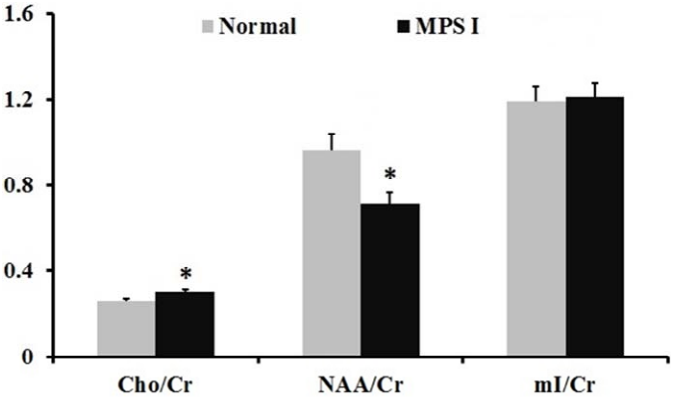


**Fig. 2:** Bar graph showing the mean±SEM metabolite/Cr ratio of normal (n=4) and MPS I (N=8) dogs from cerebellar region of the brain. Asterisk (*) represents significant differences ($p<0.05$).

**Discussion:** We observed significantly elevated Cho and reduced NAA in MPS I dogs compared to normal dogs which may suggests demyelination, gliosis or axonal injury. Takahashi et al also found an elevated Cho/Cr ratio in the WM of patients with MPS and suggested that this was a result of myelin damage, increased glial proliferation, or elevated cell membrane synthesis[6]. NAA is considered to be a marker of neuronal loss or degeneration, and experimental and clinical studies suggest that it could also be a marker of neuronal damage. Its decrease, therefore, could be due to subclinical metabolic neuronal dysfunction rather than loss[7]. Our studies indicate that $^1$H MRS might be a sensitive technique for detecting and quantifying the neuroaxonal injury status in this model. We focused on the cerebellum in this study as ataxia and motor function deficits have been reported in this model. However, since MPS I effects the brain globally, future studies will involve whole brain EPSI based MRS studies to map the metabolic abnormalities in the entire brain in this model.

**References:** [1] Roubicek M, et al. Am. J. Med. Genet. 1985; 20: 471-481. [2] Dierenfeld AD, et al. Sci Transl Med. 2010 ;2:60ra89. [3] Baldo G, et al. Behav Brain Res. 2012;233:169-75. [4] Vite CH, et al. Comp Med. 2013; 63:163-73. [5] Condon B. EPMA J. 2011; 2: 403-10. [6] Takahashi Y, et al. Pediatr Res 2001;49:349-55. [7] Inglese M, et al. AJNR 2005; 26:2037-42.

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