MR Phenotyping: The importance of utilizing numerous MRI protocols to characterize new mouse mutants

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Introduction MRI has become an important tool for discovering phenotypes of genetically altered mice. A mouse model of disrupted hedgehog signaling pathway was developed and scanned by MRI for phenotypic analysis. To effectively characterize this new mutant, we used *in vivo* manganese-enhanced MRI as well as high-resolution MRI of fixed specimens. Both protocols yielded information regarding morphological and functional alterations in the mutant mice. These included severe hydrocephalus, a malfunctioning blood-CSF barrier as well as abnormalities in the olfactory system. This study illustrates the importance of MRI in diagnosing unknown phenotypes of new mouse mutants.

Materials and Methods A genetic mutation was developed in mice using gene-targeting methods to inactivate a novel gene in the hedgehog signaling pathway. The hedgehog (Hh) signaling pathway is critical for normal development of many tissues throughout the body[1]. Mice carrying the mutation were bred on a CD1/SV129 background strain and submitted to MRI for phenotypic analysis. For this study, we analyzed two homozygous mutants, one female (5 weeks/12 g) and one male (3 weeks/7 g) as well as two littermates that were heterozygous for this specific mutation and used as controls (5 weeks/21 g female and 3 weeks/12 g male). Mice were injected with 20 mg/kg of 0.4% $MnCl_2$ in a 5% dextrose/0.9% NaCl solution and scanned 6 hrs. and 48 hrs. post-injection. These time-points are known to yield complimentary data, each highlighting different anatomical structures in the mouse brain[2]. All MR measurements were performed on a 7 T magnet (Magnex, Oxford, UK) interfaced to a Unity^{INOVA} console (Varian, Palo Alto, CA). 3D T1-weighted images of the brain were acquired (TR/TE = 300/7.7 ms, FOV = 40 x 20 x 20 mm with isotropic resolution of 156 μm^3) of four anesthetized mice in parallel using a multiple-mouse imaging system[3]. 72 hrs. after the $MnCl_2$ injection, two of the mice (one mutant and one control) were fixed using ultrasound-guided perfusion[4]. The mice were perfused with 10% formalin in PBS as a fixative along with 1mM Gd-DTPA (Magnevist, Berlex, QC, Canada) for contrast enhancement. Approximately 24 hrs. after fixation the mice were scanned using a whole-body, high-resolution, 3D protocol (TR/TE=300/11 ms, FOV = 95 x 28 x 28 mm with isotropic resolution of 85 μm^3).

Results *In vivo* MRI revealed enlarged ventricles in both mutants with severe hydrocephalus in the female. Figure 1 shows an image of the female mutant 6 hrs. post-injection. The choroid plexus (CP) is clearly visible at this time point, along with the hippocampus (HC) and the enlarged ventricles (V). We manually segmented the brains and ventricles using AMIRA (TGS, San Diego, CA) and measured ventricular volume in relation to the overall volume of the brain for three of the mice (Table 1). Images from the 48 hr. time point showed increased enhancement in the olfactory bulbs (OB) of the control mouse (Figure 2B) as well as continued hyperintensity in the hippocampus. In contrast, the olfactory bulbs of the mutant mice were undersized and showed a lesser degree of enhancement (Figure 2A). High-resolution *in situ* imaging revealed hyperintense ventricles in the mutant mouse (Figure 3A) but hypointense ventricles (Figure 3B) in the control.

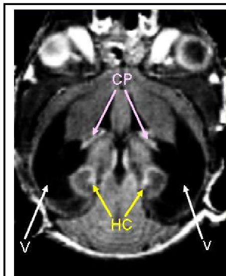

Figure 1: T1-weighted image of 5 week-old mutant mouse, 6 hours after injection of $MnCl_2$. The choroid plexus (CP), hippocampus (HC) and ventricles (V) are clearly visible.

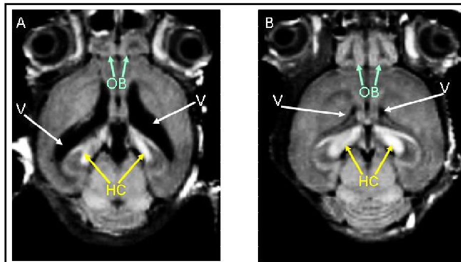

Figure 2: T1-weighted images at equivalent positions from a 3D data set of 3 week-old mutant (A) and control (B) mice, 48 hrs. after injection of $MnCl_2$. Note the decreased enhancement and overall size of the olfactory bulbs (OB) in the mutant mouse. Both images have been scaled identically.

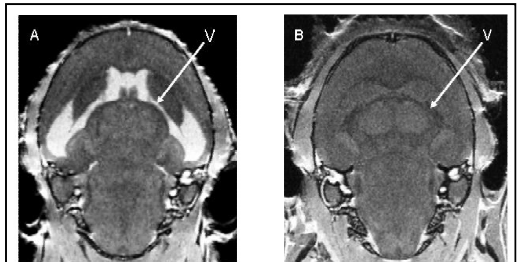

Figure 3: T1-weighted images at equivalent positions from a 3D data set of fixed 5 week-old mutant (A) and control (B) mice perfused with 1mM Gd-DTPA. Note the hyperintense ventricles (V) in the mutant mouse.

Mouse	BV (mm^3)	VV (mm^3)	VV/BV (%)
3 week old male mutant	373	20.5	5.5
5 week old female mutant	448	114	25
3 week old male control	444	1.87	0.42

Table 1: Brain volume (BV), ventricular volume (VV) and ventricular volume as a percentage of total brain volume (VV/BV). The second control animal died during the first scan and so was not included in this analysis

Discussion Due to the abundance of novel genetically altered mice it will become increasingly important to develop MRI protocols for effectively characterizing new and unknown phenotypes such as the one in this study. The mutants were presented to MRI with a pronounced failure to thrive in comparison to their littermates as well as suspected hydrocephalus based on previous histological results. In order to attempt to fully describe this mutation, we employed *in vivo* as well as *in situ* techniques. *In vivo* imaging was required in order to accurately measure the cerebral ventricles which collapse after a mouse is sacrificed (cf. Figure 1 with Figure 3A). Highly enlarged ventricles were seen in both mutants, indicating a connection between the affected gene and hydrocephalus. Using $MnCl_2$ and imaging at two different time points provided the additional benefit of being able to clearly visualize several important anatomical structures. The CP, which plays an important role in regulating CSF volume in the ventricles[5], is well-defined 6 hrs. after injection of $MnCl_2$ while OB generally appear hyperintense 48 hrs. post-injection. The OB of both mutant mice were undersized and displayed a level of intensity less than the control mice, signifying a disruption in the uptake mechanism of the Mn^{2+}. The *in situ* images surprisingly revealed not only morphological but functional information as well. In addition to the blood-brain barrier, which surrounds all blood vessels in the brain, there exists a blood-cerebral spinal fluid (BCSF) barrier as well[6], located at the surface of the choroid plexus. The hyperintensity of the ventricles of the mutant mouse indicates an accumulation of contrast agent in the CSF, probably as a result of a malfunctioning BCSF barrier at the choroid plexus. These results illustrate the utility of employing several MRI protocols when attempting to characterize a new and unknown murine mutant.

References [1]Ingham, PW *et al.* Genes Dev 2001. **15**(23):3059-87. [2]Watanabe T *et al.* Magn Reson Med 2002. **48**:852-59. [3]Bock NA *et al.* Magn Reson Med 2003. **49**:158-67. [4]Zhou YQ *et al.* Lab Invest (*in press*). [5]Segal MB Cell Mol Neurobiol 2000. **20**(2):183-96. [6]Saunders NB *et al.* Cell Mol Neurobiol 2000. **20**(1):29-40.