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. 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% MnCl2 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 brain2. All MR measurements were performed on a 7 T magnet (Magnex, Oxford, UK) interfaced to a Unity 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 µm3 of four anesthetized mice in parallel using a multiple-mouse imaging system3. 72 hrs. after the MnCl2 injection, two of the mice (one mutant and one control) were fixed using ultrasound-guided perfusion4. 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).

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 body5. 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% MnCl2 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 brain2. All MR measurements were performed on a 7 T magnet (Magnex, Oxford, UK) interfaced to a Unity 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) of four anesthetized mice in parallel using a multiple-mouse imaging system3. 72 hrs. after the MnCl2 injection, two of the mice (one mutant and one control) were fixed using ultrasound-guided perfusion4. 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).