MR Investigation of a teratogen-mediated mouse model of cleft lip palate

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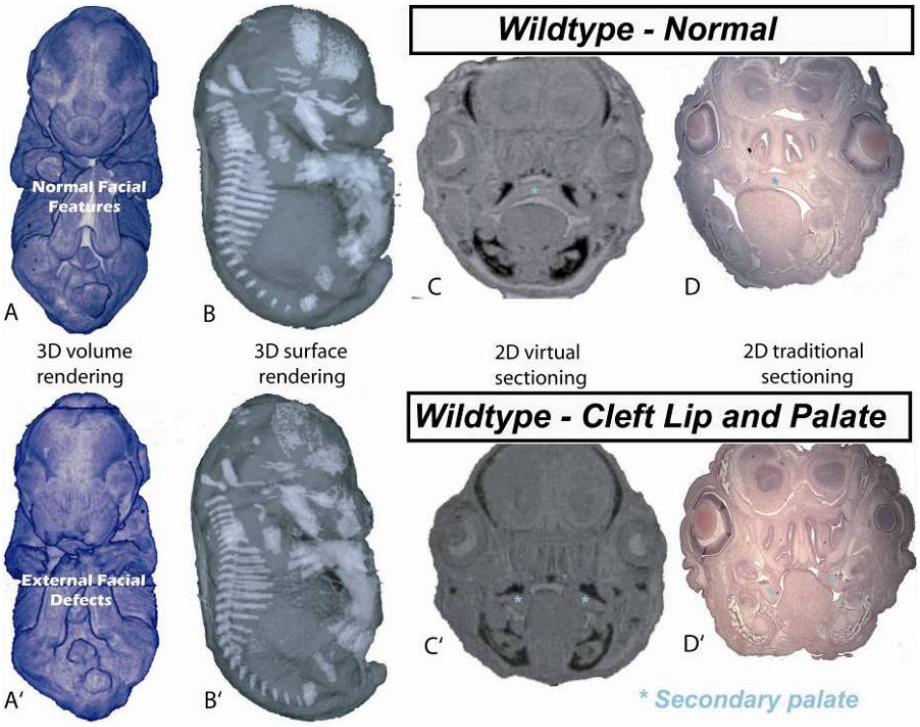
Introduction: Hedgehog (Hh) signaling represents a conserved morphogenetic pathway critical for the development of numerous organs and tissues including the brain and face. Temporally-specific *in utero* exposure to the Hh signaling antagonist, cyclopamine, induces embryonic cleft lip and palate (CLP) defects in the mouse. Clinically, CLP represents one of the most common (approximately 1 in 700 births) and morbid human birth defects, requiring extensive medical intervention. Here, to assess the fidelity of a mouse model of teratogen-mediated CLP to the human clinical condition, we have explored the use of magnetic resonance imaging to qualitatively and quantitatively describe the phenotype of cyclopamine-exposed mouse embryos.

Methods: Wildtype C57BL/6J dams were exposed to cyclopamine or vehicle as described previously (1). Exposed embryos were removed at embryonic day 16.5 and fixed in 10% formalin indefinitely. Four normal and 4 CLP embryos were dehydrated in a 4% sodium chloride solution for 24 hrs. The embryos were then allowed to rehydrate in 5 mM Gadobenate, (1:99 dilution with 0.9% sodium chloride) solution for at least 10 days prior to MR imaging in order to reduce the specimen's longitudinal relaxation time. Gadolinium soaked embryos were placed into a syringe filled with Fluorinert and positioned centrally in a Varian 3cm diameter quadrature coil. Images were acquired at 4.7T using a Varian Inova imaging and spectroscopy system. A 3D gradient echo sequence (Tr=20ms, Te=6.5 ms, Fl=65, FOV= 24x12x12mm, Ma=512x256x256, Nt=32) was used to acquire images with an isotropic resolution of approximately 48 um. Images were then constructed using ImageJ (2) and 3D representations of the data were obtained using Osirix (3). Following MR imaging, embryos were processed, embedded, sectioned (7 µm thick) and stained with Hematoxylin and Eosin by standard protocols.

Results: Soaking fixed embryos in 5mM Gadobenate solution provided specimens exhibiting shortened longitudinal relaxation times suitable for the T1W 3D gradient echo sequence used. The penetration of the contrast agent into the tissue appeared complete in all animals. 3D data was successfully acquired enabling 2D images with a resolution of approximately 48 µm to be reconstructed along any plane for comparison with standard histological slices and analysis. 3D volume rendering successfully visualized the external cleft lip phenotype showing a complete right, and an incomplete left cleft (A, A'). Employing 3D surface rendering, which allows *in situ* visualization of specified structures, we found that the skeletal structures of cyclopamine-exposed embryos were not grossly different from normal embryos (B, B'). H&E stained coronal sections through the face revealed failed fusion of the palatal shelves that form the secondary palate (C, C'). This defect is clearly visible in comparable 2D virtual coronal sections produced from MR acquisition (D, D'). All gross structures of the brain and face were discernible by virtual section.



Discussion: We have successfully used a gadolinium based contrast agent, post fixation, to obtain homogenous relaxation enhancement throughout the embryo. While embryo MRI cannot approximate the inherent resolution of traditional histology, it has significant advantages including the maintenance of structural tissue integrity (compare C and D to C' and D') and the ability to produce virtual sections in any plane. Moreover, successful visualization of major craniofacial structures provides a proof of principle to pursue further phenotypic assessment of other major organs.

References: (1) Lipinski, RJ et al. Toxicol Sci (2008),104,189-197. (2) Rasband, WS, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2008. (3) Rosset, A, Spadola, L, Osman, R. J Digit Imaging. 2004 Sep;17(3):205-216.