MR Diffusion Microscopy of the Mouse Pup Heart: Visualization of Myocardial Fiber Reorganization in Congenital Heart Disease Models

J. M. Tyszka[1], D. J. Sahn[2], K. Subramaniam[1], S. E. Fraser[1], and C. W. Lo[3]

[1]Division of Biology, California Institute of Technology, Pasadena, California, United States, [2]Division of Pediatric Cardiology, Oregon Health and Science University, Portland, Oregon, United States, [3]Laboratory of Developmental Biology, National Institutes of Health, Bethesda, Maryland, United States

Introduction Mice have been used extensively for modeling mammalian cardiovascular development and disease (1). Given the structural similarities between the mouse and human heart, and the extensive evolutionary conservation between the mouse and human genome, it is an ideal model system for studying the molecular basis for congenital heart disease. ENU induced mutations causing a wide range of congenital heart disease have been recovered in mice, including mutations causing heterotaxy syndrome. The utility of diffusion tensor imaging (DTI) for visualizing myocardial fiber structure is well established (2) and previous work by Jiang *et al.* (3) demonstrated 3D DTI in fixed normal adult mouse hearts. In this work, we significantly increase spatial and angular resolution of data acquisition in order to compare myocardial fiber organization in the hearts of control and a novel heterotaxy mutant mouse model at E16.5 and E19 (TS24.5 and TS27).

Methods All MR microscopy was performed using an 11.7T Bruker DRX500 spectrometer with Micro2.5 microimaging gradients (maximum gradient strength 1T/m, maximum slew rate 8kT/m/s). All animal procedures were carried out under NIH IACUC approved protocols. The mediastinum and lungs were dissected *post mortem* from two neonatal E19 controls, one E16.5 control and one E16.5 heterotaxy mutant and immersion fixed in 4% paraformaldehyde. Samples were equilibrated in 5mM gadoteridol overnight. Hearts were imaged in pairs under polyfluoropolyether at 10°C. High angular resolution diffusion weighted images were acquired using a conventional 3D Stejskal-Tanner imaging sequence (TR/TE = 100/12ms, 50um isotropic sampling, 1 signal average, 25 diffusion weighted images at b = 1200s/mm^2, 5 reference images at b = 25s/mm^2. Effective diffusion tensor images were calculated following eddy current correction using numerical simulated b-matrices and the FSL FDT toolbox (FMRIB, Oxford University). Fiber tractography and visualization were performed using a customization of Amira (Mercury Computers, Inc).

Results and Discussion High quality dMRI data were obtained with minimal eddy-current and geometric distortion. The SNR efficiency of the Stejskal-Tanner imaging sequence was significantly improved by the use of gadoteridol to shorten tissue T1 globally. Isotropic diffusion weighted structural and fractional anisotropy images allow convenient identification of the myocardial boundaries. Streamline tractography clearly visualized the myocardial fiber organization in all hearts allowing objective comparison of normal and congenital defects in perinatal mouse pup hearts (Figure 1). The heterotaxy mutant displayed marked fiber organization abnormalities associated with dextracardia and the reversal of heart *situs*.

Conclusions We have demonstrated that non-invasive, contrast enhanced MR diffusion microscopy delivers unique, high quality three-dimensional images of myocardial fiber structure relevant to the study of human congenital heart disease in mouse models.

References 1. Lo C, Nabel E, Balaban R. Meeting report: NHLBI symposium on phenotyping: mouse cardiovascular function and development. Physiol Genomics 2003;13(3):185-186. 2. Dou J, Reese TG, Tseng WY, Wedeen VJ. Cardiac diffusion MRI without motion effects. Magn Reson Med 2002;48(1):105-114. 3. Jiang Y, Pandya K, Smithies O, Hsu EW. Three-dimensional diffusion tensor microscopy of fixed mouse hearts. Magn Reson Med 2004;52(3):453-460.

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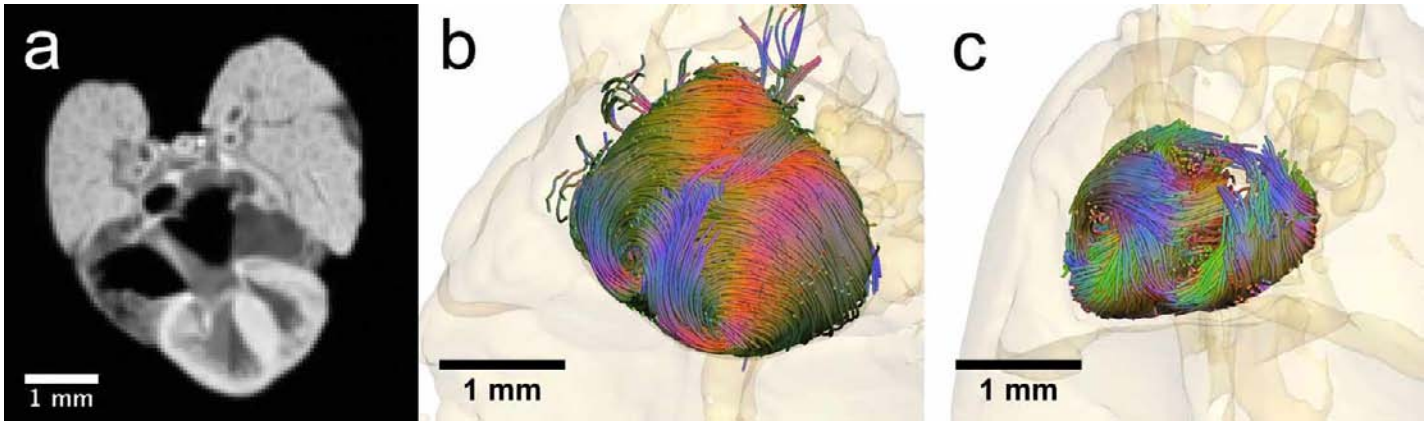

Figure 1: (a) Section from the isotropic mean DWI of an E16.5 control heart and lungs. Nominal sampled resolution is 50um (isotropic). Fiber tractography of the myocardium from (b) E16.5 control and (c) E16.5 heterotaxy mutant displaying dextracardia and abnormal fiber organization.