Approaching Cardiac Development in Three Dimensions by Magnetic Resonance Microscopy

T. Mesud Yelbuz, MD, PhD*; Xiaowei Zhang, MS, MD*; Michael A. Choma, BS; Harriett A. Stadt, HTL; Marzena Zdanowicz, DVM; G. Allan Johnson, PhD; Margaret L. Kirby, PhD

Cardiac neural crest (CNC) ablation in embryonic chicks leads to conotruncal anomalies of the heart as a result of altered cardiac looping. Altered looping results from failure of the myocardium from the secondary heart field to be added to the outflow tract. Various imaging techniques have been applied to visualize embryonic heart development. However, morphological abnormalities frequently cannot clearly be identified or appreciated in 2 dimensions, particularly those involving misorientation of cardiovascular structures and changes of myocardial volume. We present here 3-dimensional (3D) reconstructions of the embryonic chick heart at looping stages in sham-operated and CNC-ablated embryos acquired by magnetic resonance microscopy (MRM) using a new dual-contrast method for specimen preparation that combines perfusion fixation and immersion in fixative with a macro-molecular gadolinium-based contrast agent. In contrast to previous techniques, this method provides imaging not only of the cardiac chambers and vessel lumens but also of internal and external cardiac structures, such as the ventricular wall, myocardial trabeculations, cardiac jelly, and endocardial cushions. Furthermore, it allows morphovolumetric analysis of hearts at different stages. There is an excellent correlation between images obtained from MRM and those obtained by routine histology (Figure 1). 3D MRM can represent anatomic information ranging from sectional images along arbitrary anatomic planes to volumetric reconstructions of the heart (Figure 2 and Movies I through IV). A great advantage of 3D reconstructions is that rotation of the image around any axis is possible without the limitations associated with interpretation based on a single plane, as shown in Figure 3 and in Movies V and VI. MRM was performed on a 9.4-T magnet using custom-designed radiofrequency coils (Helmholz pair and solenoid coils), resulting in image resolutions of 25- and 31-μm³ isotropic voxels for 2 stages of chick embryos (stage 22 [day 4] and stage 28 [day 5.5 to 6]). The 3D imaging data were postprocessed with ImageJ (NIH). The movies were created in VoxelView (Vital Images) and Matlab (The MathWorks), and the 3D images and slices in Slicer Dicer (PIXOTEC, LLC).

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Figure 1. Comparison of histological sections with MRM of 2 embryos. A and C, Histological sections of a stage 22 and stage 28 chick embryo, respectively. B and D, Corresponding MRM images. There is an excellent correlation between images obtained from MRM with those obtained by routine histology. AAA indicates aortic arch arteries; a, atrium; ec, atrioventricular endocardial cushions (arrowheads); ey, eye; o, outflow tract; and v, presumptive ventricle. Arrows in panels A and C indicate trabeculated myocardium; bars = 0.5 mm.

*Both authors contributed equally to this work.

From the Neonatal Perinatal Research Institute, Division of Neonatology (T.M.Y., H.A.S., M.Z., M.L.K.), and Center for In Vivo Microscopy (X.Z., G.A.J.), Duke University Medical Center, Durham, NC; Department of Biomedical Engineering (M.A.C.), Duke University, Durham, NC; and Department of Pediatric Cardiology and Intensive Care Medicine (T.M.Y.), Hannover Medical School, Hannover, Germany.

Movies are available in the online-only Data Supplement at http://www.circulationaha.org.

Correspondence to Dr Talat Mesud Yelbuz, Children’s Hospital, Medical School Hannover, Department of Pediatrics III (Pediatric Cardiology and Intensive Care Medicine), Carl Neuberg Strasse 1, 30623 Hannover, Germany. E-mail yelbuz.mezud@mh-hannover.de

The editor of Images in Cardiovascular Medicine is Hugh A. McAllister, Jr, Chief, Department of Pathology, St Luke’s Episcopal Hospital and Texas Heart Institute, and Clinical Professor of Pathology, University of Texas Medical School and Baylor College of Medicine.

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Figure 2. Visualization of the embryonic heart by MRM along arbitrary anatomic planes. Panel A shows a frontal and panel B a sagittal section through the outflow limb, presumptive ventricle, and left atrium of a sham-operated stage 22 chick heart. Compare the trabeculated (arrows) and outflow (arrowheads) myocardium in the image planes shown here with images of CNC-ablated embryos in same planes presented as "flip-book movies" in the Online Data Supplement (Movies I and II [sham-operated] and Movies III and IV [CNC-ablated embryos]). C, Using the 3D MRM data set, 3 orthogonal plane images have been created in a sham-operated stage 28 chick embryo. Left and right atrium, presumptive ventricle, and outflow limb are visualized easily. Compare also with panels C and D in Figure 1, which show histological (C) and MRM sections (D) of the same heart in the sagittal plane. o indicates outflow tract; as, atrial septum; la, left atrium; ra, right atrium; and v, presumptive ventricle. Bar=0.2 mm (A and B) and 1.0 mm (C).

Figure 3. 3D MRM reconstructions of chick hearts from sham-operated (A through D) versus CNC-ablated embryos (E through H) at stage 22 shown from the frontal (A and E), right lateral (B and F), left lateral (C and G) and cranial (D and H) views. In sham-operated embryos, the presumptive ventricles appear U-shaped in the frontal plane, with the outflow limb arising almost vertically from the presumptive ventricle, followed by a curving bend toward the midline (A). Note the ventral and cranial displacement of the presumptive ventricle and its trabeculated myocardium, the degree of trabeculation in the outflow tract, and the abnormal configuration of aortic arch arteries of the CNC-ablated embryos as compared with sham-operated embryos. Animated 3D reconstructions of both sham-operated (Movie V) and CNC-ablated (Movie VI) embryos are presented "on a rotating plate" in the Online Data Supplement. I, 3D reconstruction of a stage 22 embryo with peripheral vessels (paired dorsal aortae, etc.) shown from the left lateral view. J, 3D reconstruction of the same embryo with aortic arch arteries and peripheral vessels (paired dorsal aortae, etc.) shown from the cranial view. AAA indicates aortic arch arteries; dAo, dorsal aortae; i, inflow limb of the heart tube; ivc, inferior vena cava; la, left atrium; ra, right atrium; o, outflow tract; sv, sinus venosus; svc, superior vena cava; v, presumptive ventricle. Bar=25 µm (A through H), 50 µm (I and J).