Phenotyping a Novel Mouse Model of Congenital Heart Disease using µMRI

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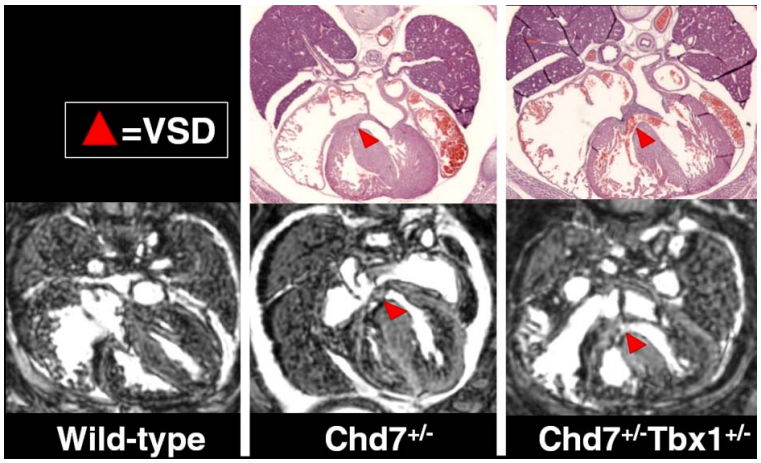
Introduction CHARGE and DiGeorge syndromes are conditions with incidences of 1 in 10,000 and 1in 4000 respectively, and are strongly associated with haploinsufficiency of specific genes (CHD7 and TBX1). These conditions are both partly characterised by presence of cardiovascular defects. Knockout mouse models are an important tool in genetic studies, allowing genes implicated in congenital heart conditions to be identified and characterised. µMRI is an emerging technique for high resolution cardiac phenotyping in a reduced time compared to conventional histology, enabling the acquisition of 3D images of multiple embryos in a single scan[1]. Given the phenotypic overlap of these conditions we sought to examine the effect on cardiac morphology in novel double-knockout mouse embryos ($Chd7^{+/-}Tbx1^{+/-}$)[2], performing an initial assessment of these mice using MRI.

Methods *Study Design:* 18 embryos (1 wild-type, 7 $Chd7^{+/-}$, 2 $Tbx1^{+/-}$ and 8 $Chd7^{+/-}Tbx1^{+/-}$) were imaged and examined for cardiac abnormalities. *Embryo Preparation:* 16.5 dpc embryos were dissected from the mother in warm Hanks solution and fixed for at least 2 weeks in a solution of 4% formaldehyde-PBS with 8mM Gd-DTPA (Bayer-Schering AG). The embryos were then embedded in 1% agarose gel (doped with 8mM Gd-DTPA) in 50ml centrifuge tubes. *Imaging:* Performed on a Varian 9.4T VNMRS system with 33mm quadrature birdcage coil (RAPID Biomedical GmbH), using a 3D gradient echo sequence (TE/TR/FA/NSA=9ms/20ms/60°/7, FOV=27x27x27mm^3, voxel size=52x52x52µm^3). Acquisition time was 10 hours 12mins. *Image analysis:* Datasets were zero-filled to 26x26x26µm^3 and reviewed in Amira visualisation software (v5.2, Visage Imaging Inc.). *Histology:* Embryos identified with abnormal hearts by MRI, were then histologically examined by H&E staining.

Results

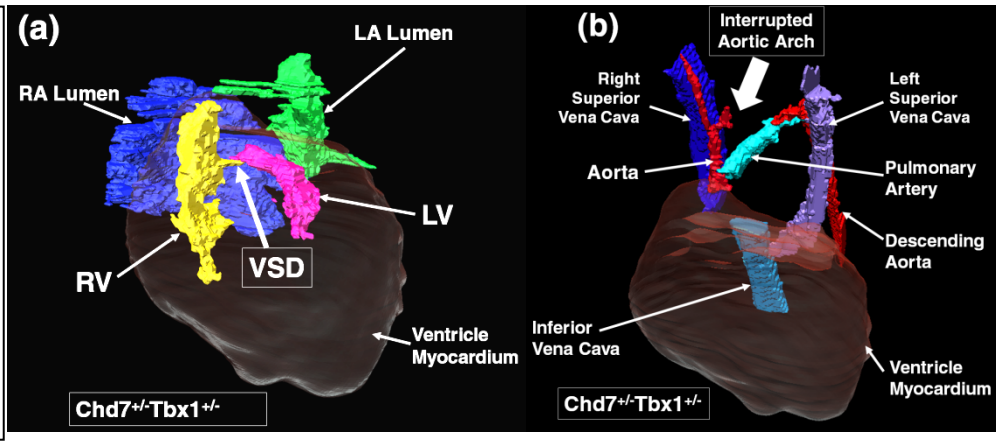


Of the 18 embryos scanned for MR analysis, we identified abnormal (thin or patent) ventricular septa (VSDs) in 6 embryos. Of these, 1 was a $Chd7^{+/-}$ heterozygote (an incidence of 1/7 in this study, see Fig. 1). VSDs were seen in 5/8 $Chd7^{+/-}$Tbx1 embryos (Fig. 2a). In one double heterozygote we also observed an interrupted aortic arch, in addition to a VSD, which was readily seen by MRI (Fig 2b).

Figure 1: Axial sections through example embryo datasets showing the presence of ventricular septal defects (indicated by red triangles) in both $Chd7^{+/-}$ embryos and double $Chd7^{+/-}$ $Tbx1^{+/-}$ heterozygotes.

Figure 2: (a) Volume rendering of a heart of a $Chd7^{+/-}Tbx1^{+/-}$ double heterozygous embryo. (RA: right atrium, RV: right ventricle, LA: left atrium, LV: left ventricle) A clear ventricular septal defect can be seen between left and right ventricles.

(b) Volume rendering of the same embryo showing great vessel structures only . An interrupted aortic arch was identified in this embryo (arrow).



Conclusion Using µMRI we have successfully identified cardiac abnormalities in genetically-modified embryos. A single $Chd7^{+/-}$ embryo was found to have an abnormal ventricular septum. A relatively high incidence of VSDs was observed in $Chd7^{+/-}Tbx1^{+/-}$ compared to $Chd7^{+/-}$mice, indicating a possible interaction between these two genes. An interrupted aortic arch was identified in one $Chd7^{+/-}Tbx1^{+/-}$ embryo. All abnormal findings were later confirmed by histology. Our study indicates that µMRI is an effective technique for cardiac phenotyping, producing no false-positives in this study.

References [1]Cleary JO, Price AN et al. *NMR Biomed* 2009, 22(8): 857-866, [2]McCue K, Randall V et al., 06-P038 *Mech. of Dev.* 2009, 126: S131

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