Detection of cardiac developmental failures in Hey-gen knockout mouse embryos with rapid 3D MRI Microscopy

E. Lang[1], A. Fischer[2], M. Gessler[2], and P. M. Jakob[1]

[1]Department of Experimental Physics 5, University of Würzburg, Würzburg, Germany, [2]Department of Physiological Chemistry I, Biocenter, University of Würzburg, Würzburg, Germany

Introduction

Congenital heart defects (CHD) are the most common human congenital malformations, present in about 1% of all life births [1]. Beside other reasons, gene mutations in the Notch signaling pathway and the downstream Hey-gene family lead to CHDs, such as ventricular septum defects (VSD) or valve malformations. Histology is still the gold standard for phenotyping such gene mutations in mice. However, this is an invasive method requiring several days of operator time. And the imaging outcome is rather limited. MRI is noninvasive and can be used to detect cardiac malformations in mouse embryos within a few hours [2]. Here we present a 3D-FLASH imaging method that allows the depiction of a VSD in Hey2 or Hey1/HeyL knockout mouse embryos in approximately 6 hours total.

Materials and Methods

6 of each WT, Hey2$^{-/-}$, and Hey1$^{-/-}$/HeyL$^{-/-}$ 15.5 dpc mouse embryos were screened with. Embryos were fixed in 4% paraformaldehyde doped with 2mM Magnevist (Gadopentetate dimeglumine, Schering, Germany) and individually embedded in 2ml syringes using 1.5% agarose gel. MRI measurements were performed on a Bruker Avance 500 spectrometer (11.7T, 500MHz), equipped with a gradient unit with 0,6T/m, and a quadrature-driven birdcage coil with an inner diameter of 20 mm (Rapid Biomedical, Rimpar, Germany). A 3D-FLASH sequence (TE 5.9ms, TR 50ms, 30º hermite excitation pulse, SW 40kHz) was used. The field of view was 13 x 10 x 10 mm, with a matrix size of 648 x 256 x 256, resulting in a nominal resolution of 20 x 39 x 39 µm. The total experimental time was less than 4h including a four time averaging. Data sets were reconstructed and zero filled to a matrix size of 648 x 512 x 512, leading to an image isotropic voxel size of 20 x 20 x 20 µm. The 3D reconstruction and the surface view were accomplished using a commercial software package (Amira 3.1, Mercury Computer Systems, USA).

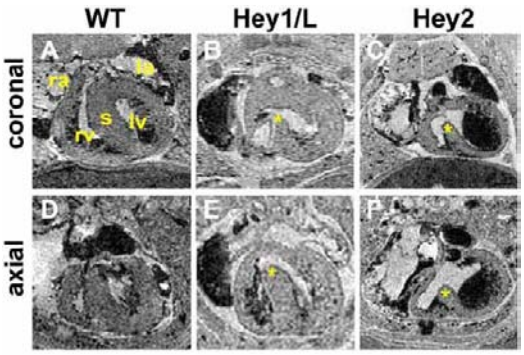

Fig. 1 MRI imaging of wild type (A,D), Hey1/L DKO (B,E) and Hey2-/- (C,F) hearts at E15.5. The membranous VSD (*) is shown in axial and coronal view.

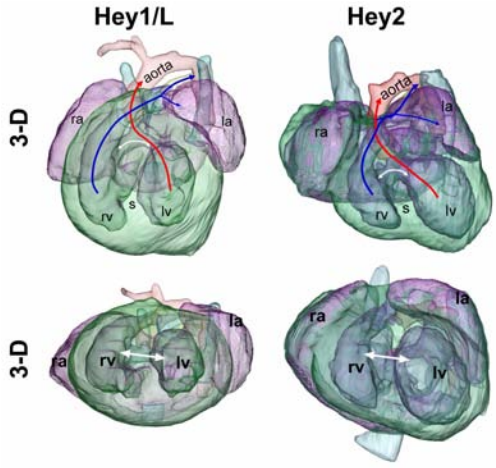

Fig. 2 3D reconstruction of MRI images showing the heart and large vessels of Hey1/L DKO (left colomn) and Hey2-/- (right colomn) embryos in frontal view (upper row) and from the apex (lower row). Arrows denote the blood flow from left ventricle (lv) into the aorta and right ventricle (rv) into pulmonary artery and ductus arteriosus. The VSD is marked by a double-headed arrow.

Results

The acquired images provide sufficient resolution and contrast to distinguish between different tissue types like myocardium, ventricles, atria, aorta, ductus arteriosus, pulmonary artery, and vena cava. The VSD was visible in every knockout embryo. An example of each measured genotype can be seen in Fig.1, showing solely the heart region out of the data set. Atria (ra, la), ventricles (rv, lv), septum (s), and the VSD (*) are marked. The findings were verified by histological examination. The 3D reconstruction in Fig.2 relates the VSD to other parts of the heart. Also, connections between the chambers and blood vessels are easily recognized.

Discussion

The results strongly indicate that 3D-MRI microscopy can be used to screen a large number of fixed mouse embryos in a suitable time. Total operator time is about one hour per embryo, compared to several days of histological examination. Because of the 3D data set and the accomplished resolution of 20µm the VSD can easily be identified and verified from different points of view. In addition, blood vessels and other organs can be illustrated down to their smallest fraction and can be seen in the anatomical context. Thus, this method is also applicable for developmental processes other than the heart.

References

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Acknowledgments

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