In vivo and *post mortem* identification of an atrial septal defect in eTbx5-KO mice

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Introduction: In humans, congenital malformations are found in 1 to 3% of newborns and cardiac malformations account for 25% of those. Despite intense research, linkage studies have identified the responsible gene in only a few cases. The advancement of gene targeting in mice has allowed the identification of new candidate genes and confirmed the causative nature and/or mechanism of disease for those gene mutations identified through linkage analysis in human. For example, the ablation (knockout, KO) of the Tbx5 gene in mouse has reproduced the full spectrum of cardiac malformations seen in the Holt Oram syndrome (HOS).[1] The spectrum of cardiac malformations found in HOS is large and ranges from simple arrhythmia to complex structural malformation such as atrial or ventricular septal defect. However, the use of targeted gene in mice is limited by the phenotyping techniques available. Very often, the only way to determine the presence of a cardiac malformation is by dissection of the heart. In this study, we have used high-resolution *ex vivo* and *in vivo* MRI of mice with or without a conditional KO of the Tbx5 gene specifically in endothelial cells (eTbx5-KO).

Experimental Protocol: In the first series of experiment, we determined the feasibility of the technique using wild type and eTbx5-KO mice which had been anesthetized and perfused using a modification on a published protocol.[2] Briefly, Gd-DTPA (Magnevist, Berlex) in neutral formaldehyde 1:20 was perfused through the abdominal aorta for five minutes, followed by 5 minutes of formaldehyde alone. In a second series of experiments, live mice under isoflurane anesthesia were imaged using respiratory and cardiac gating. Typically, 10 repetitions were made for every heart contraction. The images were acquired with a Varian 7T Inova system using a 25 mm Litz coil (Doty Scientific). Imaging parameters of the *post mortem* slab selective 3D gradient echo sequence were TR: 50 ms, TE: 6.2 ms, FOV: $(25.6\ mm)^3$, matrix size: 256^3, NA: 16. *In vivo*, the same sequence was used but the parameters were: TR: 8.7 ms, TE: 4.3 ms, FOV: $(20.5\ mm^3)$, matrix size $(128)^3$, NA: 8. Two animals from each group, i.e., eTbx5-KO (-/-), eTbx5-heterozygote (+/-), eTbx5-wild type (WT) (+/+) were imaged *ex vivo* and one animal from each group was imaged *in vivo*.

Results and Discussions: *Post mortem* results very clearly revealed the atrial septal defect (ASD) in the KO mice and an intact atrial septum in the WT mice (Fig. 1a,c), while a partial defect was seen for the heterozygote mice (not shown). These defects correspond to the observation made after careful dissection of the right atrium in all animals. Although only two animals were imaged in each group, the same observations could be made *in vivo* where a subtle defect could be detected for the KO mouse (Fig 1b).

Conclusion: A genetic cardiac malformation in a mouse that cannot be detected non-invasively by another technique could be clearly identified using *in vivo* high-resolution 3D MRI. These results were confirmed by *post mortem* MRI and dissection of the tissues.

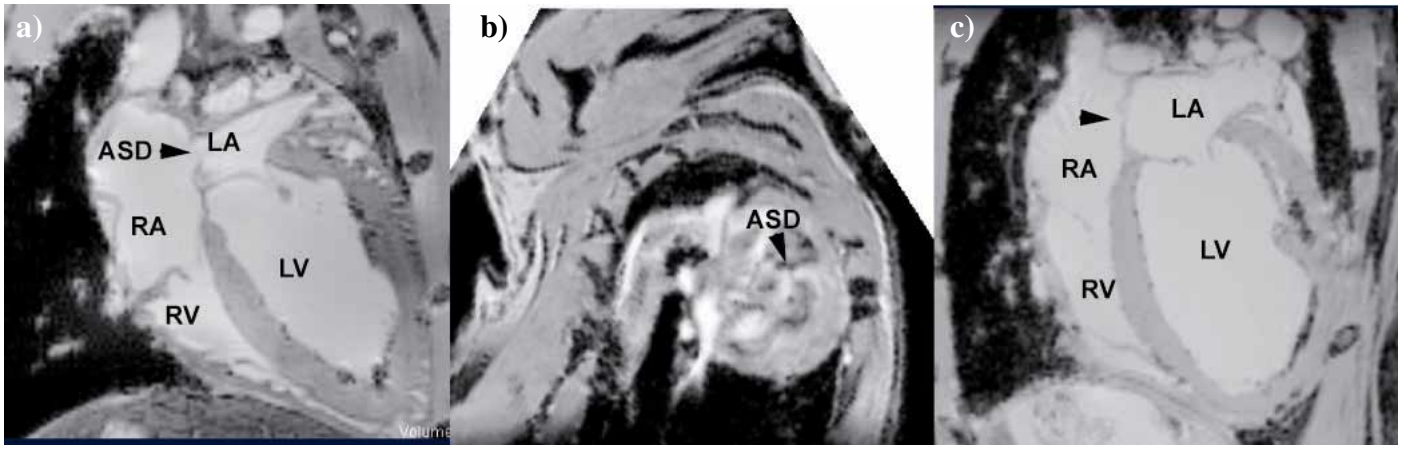

Figure 1. Views from high-resolution 3D volumes of a mouse heart. a) *Post mortem* KO mouse with a clear atrial septal defect (ASD) (arrow) between the right atrium (RA) and the left atrium (LA). The right and left ventricules (RV and LV) and also indicated. b) *In vivo* result from the same KO mouse revealing the subtle ASD. c) *Post mortem* image of a wild type animal showing the integrity of the atrial wall (arrow).

This will allow the non-invasive and longitudinal analysis of cardiac defects and function in mouse models of congenital heart disease.

References : 1- Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S, Conner DA, Gessler M, Nemer M, Seidman CE, Seidman JG, Cell 106, 709-721 (2001). **2-** Johnson GA, Cofer GP, Gewalt SL, Hedlund LW, Radiology 222, 789-793 (2002).