Diffusion-tensor imaging study of myocardial architecture of Situs Inversus and Situs Solitus mutant mouse hearts

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Target audience: MR researchers, cardiologists, biologists, and animal imaging specialists

INTRODUCTION Congenital heart disease (CHD) is one of the most common birth defects, affecting up to 1% of live births, making it imperative to gain insight into the developmental etiology of CHD. Normal human development results in an asymmetrical arrangement of the organs within the chest and abdomen, as referred to as Situs Solitus (SS). Situs Inversus (SI), a type of laterality defect, is a condition in which the organs of the chest and abdomen are arranged in a perfect mirror image reversal of the normal position. It is widely recognized that the unique architecture of the myocardial fibers and heart chambers determines heart mechanics. The Situs Inversus hearts provide a unique possibility to study regulatory mechanisms for myocardial fiber orientation and mechanics. We have established *Pkd1l1*$^{-/-}$ mutant mice line, in which, some mice exhibit Situs Solitus phenotype, whereas some exhibit Situs Inversus phenotype. Our goal of this study is to elucidate myocardial fiber orientation and mechanical outcomes of Situs Inversus and Situs Solitus mutant mouse hearts.

METHODS ***Animal model:*** Heterozygous *Pkd1l1*$^{-/-}$ mutant mice were maintained in the C57BL6/J background and were intercrossed to generate homozygous offspring. Five mice with Situs Solitus (*Pkd1l1*$^{-/-}$) and 8 mice with Situs inversus totalis (*Pkd1l1*$^{-/-}$) at the age of 8 weeks were selected for echocardiography. The background strain C57BL6/J mice serve as wild-type controls. ***Heart function:*** Cardiac function, wall motion, and strain, strain rate are evaluated by echocardiography at rest, and at stress condition induced by injection of 1.5 mg/kg of dobutamine respectively. The LV functional parameters including ejection fraction (EF), fractional shortening (FS), and cardiac output were calculated based on M-mode Images. Strain and strain rate were quantified in the longitudinal, radial, and circumferential axes from the parasternal long- and short-axis views of left ventricle respectively using Vevo2100 strain software. ***Heart architecture:*** Myocardial fiber orientation and heart architecture is evaluated by *ex vivo* 3D diffusion-tensor imaging (DTI) at a Bruker 11.7-Tesla system with a 10-mm LTR linear micro-coil. 3D DTI was acquired with the following parameters: 100 μm isotropic resolution, b = 1000-1500 s/mm^2, 6 diffusion-encoding directions, Δ = 2 msec and 6 msec, TE = 21 msec, and TR = 1 sec.

RESULTS Situs Inversus and Situs Solitus mutant mouse hearts, despite of complete opposite architecture, show no significant differences in ejection fraction, fractional shortening, and cardiac output (Table 1) both at rest and stress conditions when compared to normal controls. However, strain analysis shows that these 2 phenotypes exhibit significantly different radial strain and circumferential strain (Table 2) at rest. At stress, radial strain between SS and SI mutants continues to be significant difference. DTI (Fig. 1) examination shows Situs Inversus and Situs Solitus phenotypes have opposite myocardial fiber orientation. However, myocardial fiber orientation mapping shows that both Situs Inversus and Situs Solitus phenotypes exhibit aberrant myocardial fiber organization (Fig.2). The Situs Solitus heart, although having the same asymmetry pattern as normal heart, the microscopic myocardial fiber organization is different from the wild type heart. In addition, the endo-cardial and epi-cardial layers seem to display different fiber orientation. Both mutant hearts, regardless their phenotypes, showed much lower Fractional Anisotropy (FA) but much higher Apparent Diffusion Coefficient (ADC), axial diffusivity and radial diffusivity (Fig.3). This indicates that both mutant hearts showed less sheet integrity and the myocardial fibers are less coherent. Interestingly, although the Situs Solitus mutant heart appears phenotypically "normal" in terms of gross architecture, anatomy, and symmetry, our DTI results show that the micro-organization of the heart is far from normal, showing more aberrant myocardial organization similar to those of Situs Inversus mutant heart.

Table 1

Measurement	SIT (*Pkd1l1*$^{-/-}$)	SS (*Pkd1l1*$^{+/+}$)	*P*
Ejection fraction, %	63.27±4.41	66.62±5.25	0.21
Fractional shortening, %	33.59±3.03	36.08±4.22	0.22
Cardiac output, ml/min	15.05±2.96	13.11±2.34	0.19

Table2

Measurement	At Rest		p-value	At Stress		p-value
	SIT (*Pkd1l1*$^{-/-}$)	SS (*Pkd1l1*$^{+/+}$)		SIT (*Pkd1l1*$^{-/-}$)	SS (*Pkd1l1*$^{+/+}$)	
Short axis						
Radial strain	25.80±9.57	40.59±7.40	**<0.01**	39.07±8.61	51.74±4.50	**<0.01**
Circumferential strain	19.68±3.36	27.25±3.58	**<0.01**	34.83±9.34	39.18±4.68	0.27
Radial SR	9.45±1.85	11.68±2.33	0.05	17.61±2.61	17.81±3.00	0.9
Circumferential SR	8.13±1.82	9.97±2.45	0.13	26.14±4.08	24.82±6.94	0.82

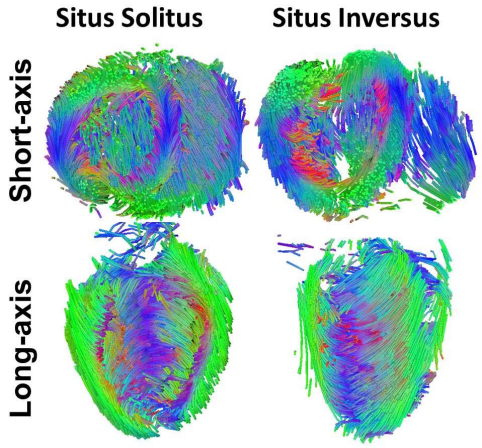

Fig1. DTI tratography of Situs Solitus and Situs Inversus mutant mouse hearts

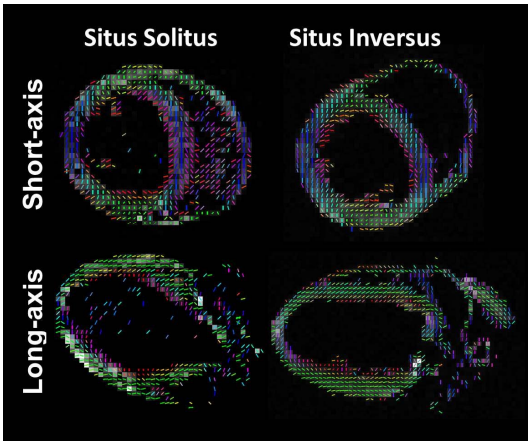

Fig2. DTI fiber orientation mapping of Situs Solitus and Situs Inversus mutant mouse hearts

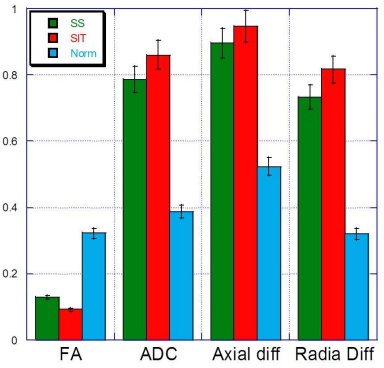

Fig3. Fractional Anisotropy (FA) Apparent Diffusion Coefficient (ADC), axial diffusivity and radial diffusivity of a Situs Solitus (green) and Situs Inversus (red) and normal (blue) heart.

CONCLUSION Situs Inversus and Situs Solitus mutant mouse hearts exhibited different myocardial fiber organization, which can be depicted in detail with DTI. DTI is an ideal imaging tool to evaluation myocardial fiber organization, which can provide a new insight into the underlying mechanics of cardiac function and motion