Early signs of cardiomyopathy in delta-sarcoglycan null mice

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Introduction: Delta-sarcoglycan null mice (DSG) develop cardiac and skeletal muscle histopathological alterations similar to those in humans with limb girdle muscular dystrophy. Our previous studies have demonstrated severe cardiac dysfunction in DSG mice at 8 months (Neuromuscular Disorders 21 (2011) 68–73). However it is important to characterize the cardiac phenotype at a younger age so that novel therapeutic interventions can be investigated using this model. In this study we performed cardiac MRI on 12 week old mice compared to wild type (WT) and carried out mRNA analysis and histopathology to correlate with MRI findings.

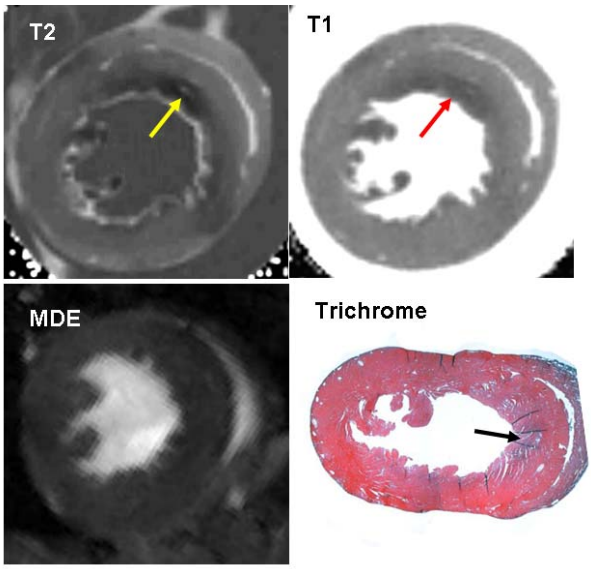

Figure 1. Ex vivo T1 and T2 maps show decreased relaxation times that correlate with fibrotic regions in histology. No delayed enhancement was seen.

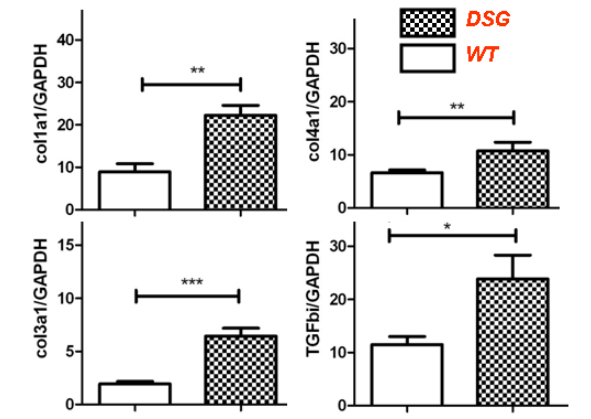

Figure 2. markers of fibrosis were up-regulated in DSG.

Methods: Delayed enhancement, tagging and functional imaging were performed on 12 week old wild type (WT) (n=8, and DSG (n=11) mice. Imaging was performed on a 7 Tesla scanner with prospective ECG gating. A bolus of Gd-DTPA (0.3-0.6 mmol/kg) was given intraperitonealy while the mouse was placed in the scanner bore. Delayed enhancement MR was performed using a T1 weighted (achieved by increasing the flip angle to 30º-40º) cine sequence. Cine imaging was performed in the short axis using a FLASH sequence. Slice thickness=1.0 mm, matrix size=256x256, in-plane resolution=117x117 μm^2. TE/TR=3/5.2ms, Approximately 15-20 cine frames were acquired during the cardiac cycle with a temporal resolution of TR ms. Tagged images were acquired in the mid ventricle. The spatial modulation of magnetization was achieved by a pulse train consist of 5 rectangular 0.1ms RF pulses with flips 10°, 30°, 50°, 30° and 10°. Tagged images were analyzed using HARP (Diagnosoft, CA) software. mRNA was extract from ventricle with trizol, and gene expression was measured by real-time PCR with SyberGreen with a Fast Light Cycler 7700 (Applied Biosystem).

Further, in a sub cohort, hearts were removed after perfusing them with a cardioplegic solution. Ex vivo hearts were then embedded in Tissue equivalent Agarose gel and MR imaging was performed to generate T1 (Saturation recovery, variable TR) and T2 (multi spin echo) maps. In-plane resolution = 125 µm, slice thickness = 1mm.

Results: At 12 wks, the normalized heart weight in DSG mice was not significantly different to that of WT. (5.93 ± 0.22 for WT vs 5.46 ± 0.19 for DSG). There was no difference in the ejection fraction between DSG (66± 10%) and WT (73± 6%). However the circumferential strain of DSG mice (15.3 ± 1.5%) was significantly less than that of the WT 17.7± 2% (p=0.008). No myocardial delayed enhancement was observed but T1 and T2 maps showed decreased relaxation times that correlated with mild diffused fibrosis in histopathology (Figure 1). These results were associated with an increase of the gene expression of collagen 1α1, collagen 3α1, collagen 4α1, active TGFβ and fibroblast marker (α-smooth muscle actin). None of the inflammatory markers tested (T-lymphocytes, macrophages and vascular markers) were positive in WT and DSG mice at 12 weeks.

Conclusion: Although global function is preserved, regional circumferential strain quantified using Tagged MRI show impaired myocardial contractility at 12 weeks in DSG mice. Sub-clinical cardiomyopathy in these mice is associated with fibrosis. *In vivo* High resolution relaxation maps could potentially detect regions of diffused fibrosis accumulation in these mice.