MRI Tagging of In Vivo Ventricular Function Reflects Histological and Cellular Changes in α-Dystrobrevin Knockout Mice

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The dystrophin-glycoprotein complex (DGC), a membrane-associated structural protein complex that links the intracellular cytoskeleton to the extracellular matrix, plays an important role in the mechanical coupling and force transmission of myocyte contraction in heart. The disruption in DGC components leads to a variety of skeletal muscular diseases, as well as cardiomyopathy. In addition to maintaining structural integrity, DGC also plays a signaling role by binding the signaling protein, nNOS, through syntrophin and α-dystrobrevin. Deficiency of dystrophin or α-dystrobrevin will lead to displacement of nNOS from the sarcolemma (1). The disruption of DGC structure also influences the activity of stretch-activated channels (SAC) and calcium leak channels. nNOS signaling, SACs and calcium leak channels together with aging related cellular remodeling can have profound impact on the myocyte contractility and calcium handling of myocyte. Our MRI tagging studies observed an unexpected increase in left ventricular contraction in young mdx mice (dystrophin deficiency), and α-dystrobrevin knockout ($adbn^{-/-}$) mice, while the adult mouse displayed either decreased (mdx) or compromised ($adbn^{-/-}$) cardiac function. However, the underlying mechanism is still unknown. In this work, we will explore the cellular basis for the ventricular functional changes in the young and adult $adbn^{-/-}$ mouse by assessing the myocyte contractility and calcium transients.

Methods

MR imaging Two months old $adbn^{-/-}$ mice (n=5) and age matched control mice (n=7) were scanned on a Varian 4.7T scanner with a 2.5 cm surface coil. Eleven $adbn^{-/-}$ mice of 9~10 month old and 9 age-matched controls were scanned on a Bruker 7T scanner with a volume RF coil. Tagged images were acquired from apex to base with 1 mm slice thickness. The tagging sequence used a SPAMM1331 sequence applied twice immediately after the ECG trigger, yielding a two-dimensional tag grid in imaging plane. The tagging sequence was followed by gradient-echo cine sequence with the following imaging parameters: TR, R-R interval; TE, 2.2~3 ms; field of view, 4 cm × 4 cm or 3.5 cm × 3.5 cm; tagging resolution, 0.6 mm. 15 frames were acquired per cardiac cycle.

Data analysis Images were analyzed with finite element method. Epicardial and endocardial borders were traced interactively using B-spline interpolation with 8 control points. Intersecting tag points were tracked semi-automatically with HARP-based approach. Subsequently, strains were calculated by 2D homogenous strain analysis.

Cardiomyocyte shortening and Ca^{2+} transient Ventricular myocytes were isolated with enzymatic digestion. Both 2-3 month-old and 9-10 month-old mice were characterized. The cell length during electric stimulation was recorded with a Phillips video camera and a Crescent video-edge detector connected to an Olympus IX-71 inverted microscope. Myocytes were loaded with 1 μM indo-1 AM, and the intracellular Ca^{2+} was measured by indo-1 emission fluorescence ratio of 410 nm to 480 nm.

Histology Histology was performed on hearts from mice of ages 3, 6, 9, and 12 months with Masson's Trichrome staining for the identification of fibrotic lesions.

Results

Global function was similar between $adbn^{-/-}$ mice and the controls for both age groups. Histological analysis revealed that there were no cardiac lesions in 3 and 6 month old $adbn^{-/-}$ mice. While older $adbn^{-/-}$ heart displayed obvious fibrotic lesions. For mice 9 and 12 months of age, 7 out of 9 mice showed lesions ranging from patches of fibrotic lesions to transmural fibrosis.

Young $adbn^{-/-}$ mice exhibited significantly enhanced regional deformation from MRI tagging studies. The magnitude of maximum circumferential strain was increased throughout the ventricle in the $adbn^{-/-}$ mice (Figure 1A). However, no difference is observed in the circumferential strain of the old $adbn^{-/-}$ hearts compared with the controls.

Isolated cardiomyocytes were characterized to elucidate the cellular changes responsible for alterations in regional cardiac function. In contrast to in vivo ventricular function, both young and old group shows an increase in myocyte contractility and calcium transients (Figure 1B&C). Myocyte contractility, represented by maximal fractional shortening, increased from -6.7±1.0% to -8.0±1.5% in young $adbn^{-/-}$ mice ($p<0.01$) and from -9.8±2.0% to -11.5±1.8% in old $adbn^{-/-}$ mice ($p<0.01$). Calcium transient, represented by indo-1 emission ratio, increased from 0.32±0.07 to 0.39±0.09 in the young group ($p<0.01$) and from 0.33±0.05 to 0.38±0.06 in the old group ($p<0.05$). In addition, the half relaxation time during calcium uptake was also increased in $adbn^{-/-}$ mice in both age groups (Figure 1D).

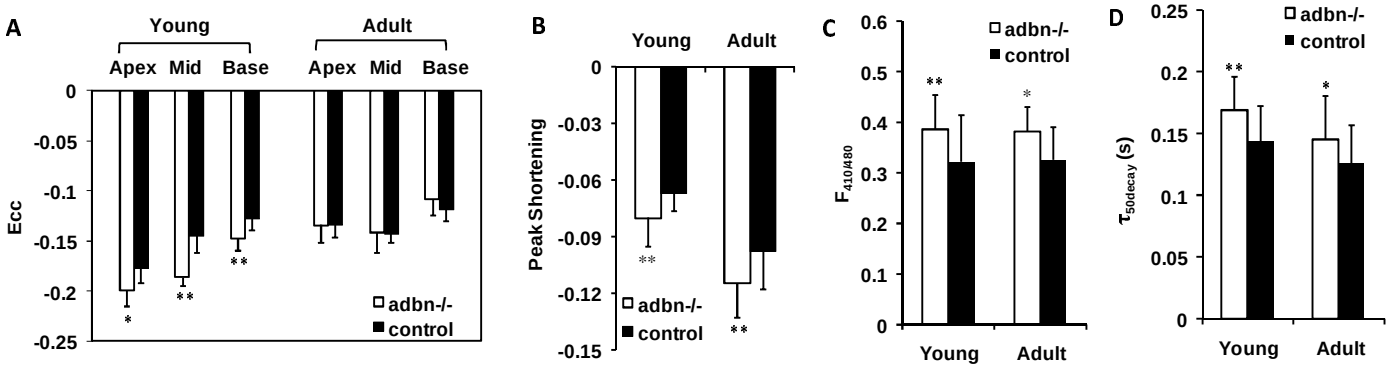

Figure 1. A. maximum circumferential strain by MRI tagging; B. Maximum myocyte fractional shortening; C. Amplitude of calcium transient; D. Relaxation time constant of the calcium transient.* P<0.05, **P<0.01

Conclusion and Discussion

In young $adbn^{-/-}$ mouse, increased circumferential strain was associated with increased myocyte contractility and calcium transients. However, in the adult mice, there was no difference in the circumferential strain despite increased myocyte contractility and calcium transients. It is possible that the development of cardiac lesions, as well as aging-associated remodeling, lead to a compromised ventricular function in vivo. However, it is still not understood the molecular mechanisms responsible for increased calcium transient and myocyte contractility. While the abolishment of nNOS inhibition on calcium channel may play a role, it is also possible that the SACs and the calcium leak channels were altered in $adbn^{-/-}$ mouse as has been shown in the mdx mouse.

References:

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