Cardiac-specific FKBP12 deficiency alters RyR2 calcium release and causes severe dilated cardiomyopathy in male and female mice

A.D. Hanna, J.M. Oakes, C. Hsu, L. Wong, M.E. Dickinson and S.L. Hamilton, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030, USA.

The 12kDa FK506 binding protein (FKBP12) is a prolyl isomerase expressed in the heart in micromolar concentrations (Guo *et al.,* 2010). Deletion of FKBP12 confers embryonic lethality due to massive cardiac defects (Shou *et al.*, 1998) indicating a vital role for FKBP12 in the heart. FKBP12 binds to the cardiac ryanodine receptor Ca^{2+} release channel (RyR2)(Brillantes *et al.*, 1994) and RyR2 dysfunction is implicated in multiple models of cardiac disease. However, the physiological importance of FKBP12's regulation of RyR2 is unclear from the present evidence, with some studies showing no effect of exogenously added FKBP12 on RyR2 (Guo *et al.*, 2010) and the majority of studies focusing on the 12.6 kDa FKBP12.6.

To elucidate the function of FKBP12 in the heart we generated two strains of mice with enhanced knockdown of FKBP12 in cardiac muscle by crossing mice that expressed Cre recombinase under the control of the cardiac specific, α-myosin heavy chain promotor (Cre^{+}) with mice that had floxed FKBP12. One strain was homozygous for the floxed FKBP12 allele ($Cre^{+}/FKBP12^{f/f}$) and another was heterozygous for the floxed allele and null for the second FKBP12 allele ($Cre^{+}/FKBP12^{f/-}$). Mice expressing Cre recombinase with intact FKBP12 expression ($Cre^{+}/FKBP12^{+/+}$) and littermates with intact ($Cre^{-}/FKBP12^{f/f}$) or partial ($Cre^{-}/FKBP12^{f/-}$) FKBP12 expression were used as controls. All experiments were approved by the Institutional Animal Care and Use Committee. Mice undergoing echocardiography were anaesthetized by isoflurane inhalation and M-mode images were obtained using a GE Vivid 7 Dimension BT05 Ultrasound. For *in vitro* experiments mice were euthanized by cervical dislocation following induction of anesthesia with isoflurane inhalation. To examine whole heart morphology and volume, high resolution X-ray microtomography was performed on paraformaldehyde fixed hearts using a Bruker SkyScan 1272 Scanner. Intact cardiomyocytes were loaded with 10 μM Fluo 4 AM and imaged in line scan mode on a confocal microscope.

At 6 - 8 weeks of age, in the absence of any surgical or drug intervention, deficiency in FKBP12 induced cardiac dysfunction that was indicative of dilated cardiomyopathy. $Cre^{+}/FKBP12^{f/-}$ mice had enlarged left ventricle (LV) diameter and decreased ejection fraction and fractional shortening. By 6 months of age cardiac function had severely deteriorated. Ejection fraction declined to 18.6 ± 2.9% ($Cre^{+}/FKBP12^{f/-}$) and 20.7 ± 4.7% ($Cre^{+}/FKBP12^{f/f}$) in FKBP12 deficient mice, compared to values ranging from 53.2% – 57.9% in our 3 control genotypes that had intact or mild deficiency in FKBP12. Six month old mice also had substantial dilation of the left chamber and decreased LV wall thickness during systole. X-ray microtomography showed that FKBP12 deficient hearts had non-compacted myocardium and enlarged right and left ventricles. Volumetric analysis confirmed that FKBP12 deficient hearts had a 40 - 50% increase in heart volume to body weight ratio compared to littermate controls.

To determine if the depletion of FKBP12 impacted RyR2 function and myocyte Ca^{2+} handling, intact cardiomyocytes were loaded with Fluo4 AM. Myocytes from $Cre^{+}/FKBP12^{f/-}$ mice had dramatically increased Ca^{2+} spark frequency, indicative of greater diastolic Ca^{2+} leak, and decreased Ca^{2+} transient amplitude compared to $Cre^{+}/FKBP12^{+/+}$. These findings are consistent with impaired systolic RyR2 mediated Ca^{2+} release. Caffeine application revealed a 24% decrease in readily-releasable store Ca^{2+} in $Cre^{+}/FKBP12^{f/-}$ myocytes, an indication of partially depleted Ca^{2+} stores.

These findings, obtained in the absence of any surgical or drug intervention, strongly suggest that FKBP12 has a greater regulatory role in overall cardiac physiology then FKBP12.6, the absence of which causes relatively mild cardiac hypertrophy in older male mice (Xin *et al.*, 2002). Future experiments will examine the role of alternate FKBP12 binding partners, such as H-Ras and the TGF-β receptor in the morphological and functional abnormalities seen in our FKBP12 deficient mice

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