Transgenic Analysis of the Role of FKBP12.6 in Cardiac Function and Intracellular Calcium Release

Ying Liu,1,2,* Hanying Chen,2,* Guangju Ji,3,* Baiyan Li,2,4 Peter J. Mohler,5 Zhiming Zhu,2,6 Weidong Yong,2 Zhuang Chen,7 Xuehong Xu,7 Hongbo Xin,1 and Weinian Shou2

1Laboratory for Cardiovascular Diseases, West-China Hospital, Sichuan University, Chengdu, China.
2Riley Heart Research Center, Herman B Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana.
3Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.
4Department of Pharmacology, Harbin Medical University, Harbin, China.
5Department of Internal Medicine and Physiology & Cell Biology, Ohio State University, Columbus, Ohio.
6Department of Hypertension and Endocrinology, Daping Hospital, Third Military Medical University, Daping, China.
*These authors contributed equally.

INTRODUCTION

Calcium release from intracellular stores to the cytoplasm is regulated by calcium release channels, such as ryanodine receptors (RyR) in cardiac, skeletal, and smooth muscle cells. RyR1 and RyR2 are required for the maintenance of skeletal and cardiac muscle calcium homeostasis and excitation–contraction coupling, respectively. The activation and inactivation of RyR is regulated by a number of channel modulators, including FKBP12.6, calmodulin, protein kinase A, and Ca2+/calmodulin dependent kinase (CaMKII).

FK506 binding proteins (FKBPs) are immunophilins that bind to the immunosuppressive drugs FK506 and rapamycin. FKBP12 (also known as fkbp1a) and FKBP12.6 (also known as fkbp1b) are cytoplasmic proteins that share 85% amino acid identity and a similar tissue distribution. Although FKBP12 is highly expressed in cardiomyocytes, FKBP12.6 is the predominant isoform associated with RyR2.1,3-5 Despite their similarities in protein structure, FKBP12 and FKBP12.6 have different roles for normal cardiac function. These differences are clearly illustrated in mice with targeted deletion of FKBP12 or FKBP12.6. Mice homozygous for a null mutation in FKBP12 are embryonic lethal due to aberrant cardiac development.6 In contrast, FKBP12.6-deficient mice display normal cardiac development and are viable. However, once mature, adult FKBP12.6-deficient mice display abnormal cardiac physiology including either enlarged hearts or exercise-induced cardiac arrhythmias and sudden death.8 Furthermore, our recent study demonstrated that FKBP12 is a key regulator for cardiac voltage-gated sodium channel.9

RyR is a large tetrameric calcium release channel and is composed of four identical subunits (560 kDa). The majority of channel modulators interact with N-terminal regulatory “foot” region.1 FKBP12.6 has been shown to regulate RyR2 closure via its interaction with RyR2.10-14 Two independent mouse strains deficient in FKBP12.6 had distinctively different abnormal cardiac phenotypes. The first strain (129SvEv/C57 hybrid or 129SvEv inbred background) displayed sex-dependent cardiac hypertrophy, while the second strain (DBA/lacJ inbred background) displayed sex-distinctively different abnormal cardiac phenotypes. The first strain displayed enlarged hearts or exercise-induced cardiac arrhythmias, while the second displayed exercise-induced cardiac sudden death.8 Furthermore, our recent study demonstrated that FKBP12.6-deficient mice display normal cardiac function and cardiomyocyte calcium release, suggesting that secondary genetic factors may contribute to the final pathogenic

ABSTRACT

FK506 binding protein12.6 (FKBP12.6) binds to the Ca2+ release channel ryanodine receptor (RyR2) in cardiomyocytes and stabilizes RyR2 to prevent premature sarcoplasmic reticulum Ca2+ release. Previously, two different mouse strains deficient in FKBP12.6 were reported to have different abnormal cardiac phenotypes. The first mutant strain displayed sex-dependent cardiac hypertrophy, while the second displayed exercise-induced cardiac arrhythmia and sudden death. In this study, we tested whether FKBP12.6-deficient mice that display hypertrophic hearts can develop exercise-induced cardiac sudden death and whether the hypertrophic heart is a direct consequence of abnormal calcium handling in mutant cardiomyocytes. Our data show that FKBP12.6-deficient mice with cardiac hypertrophy do not display exercise-induced arrhythmia and/or sudden cardiac death. To investigate the role of FKBP12.6 overexpression for cardiac function and cardiomyocyte calcium release, we generated a transgenic mouse line with cardiac specific overexpression of FKBP12.6 using α-myosin heavy chain (αMHC) promoter. MHC-FKBP12.6 mice displayed normal cardiac development and function. We demonstrated that MHC-FKBP12.6 mice are able to rescue abnormal cardiac hypertrophy and abnormal calcium release in FKBP12.6-deficient mice.

ABBREVIATIONS: acta1, skeletal α-actin; αMHC, α-myosin heavy chain promoter; ANF, atrial natriuretic factor; CaMKII, Ca2+/calmodulin dependent kinase; ECG, electrocardiogram; FKBP, FK506 binding protein; LV, left ventricular; LVEDD, left ventricular diastolic dimension; LVESD, left ventricular systolic dimension; p/n, phospholamban; RyR, ryanodine receptor; SERCA2a, sarcoplasmic reticulum Ca2+ ATPase 2a.
outcome. However, there are still many unanswered questions regarding the abnormal cardiac phenotypes in FKB12.6-deficient mice. It has not been tested whether FKB12.6-deficient mice in 129SvEv/C57 background can develop exercise-induced cardiac sudden death or whether the cardiac functional defects seen in FKB12.6-deficient mice (in both strains) are a direct consequence of abnormal calcium handling or an indirect consequence of other altered physiological function(s), such as hypertension or an altered immune system. Resolving these questions will help us to further understand the physiological role of FKB12.6 in heart function and heart failure. In this report, we determined the physiological impact of cardiomyocyte-restricted overexpression of FKB12.6 on cardiac heart failure. We demonstrated that zMHC-FKB12.6 mice were able to rescue abnormal cardiac hypertrophy and Ca$^{2+}$ release phenotype in FKB12.6-deficient mice. These data provide further insight into the role of FKB12.6 in Ca$^{2+}$ release and cardiac hypertrophy.

**MATERIALS AND METHODS**

**Generation of zMHC-FKB12.6 Transgenic and zMHC-FKB12.6/FKB12.6-Deficient Mice**

FKB12.6-deficient mice were generated as described previously and were maintained in 129SvEv/C57BL backgrounds. To generate zMHC-FKB12.6 transgenic mice, an z-myosin heavy chain (zMHC) promoter was used to drive a human FKB12.6 cDNA (coding region), followed by an SV40 early region transcription terminator/polyadenylation site. A myc epitope tag was added in frame to the 5' end of FKB12.6 cDNA. The myc-epitope tag allowed us to distinguish transgenic FKB12.6 from endogenous mouse FKB12.6 and zMHC-FKB12.6 mice were generated as described. To generate mice in which FKB12.6 was only expressed in the myocardium (i.e., FKB12.6 was not present in any other tissue), we first cross-bred zMHC-FKB12.6 +/− mice to FKB12.6 homozygous mutants (FKB12.6 +/−) to generate mice positive for the transgene (i.e., zMHC-FKB12.6) and heterozygous for the FKB12.6 knock-out allele (FKB12.6 +/−). zMHC-FKB12.6 +/− were further cross-bred to FKB12.6 +/− mice to generate zMHC-FKB12.6 +/− : FKB12.6 +/− mice.

**Morphological, Histological, and Echocardiogram Analyses**

Cardiac structure and function of mice were also assessed by histological analysis and echocardiograph (ECG) as previously described. In brief, the heart weight to body weight ratio (mg/g) was measured to the end of the biphasic T wave [Tr+Ts; QTc=QTc= QT/(RR/100)]. For stress tests, mice were forced to run on an inclined treadmill until exhaustion and were then intraperitoneally injected with epinephrine (0.1 or 0.5 mg/kg). Heart rates of ambulatory animals were determined by averaging heart rates over 4 h. The genotypes of the mice were not revealed to the investigator until after all measurements were performed and data were analyzed.
−40 mV. Linescan images were recorded using Lasersharp software (Bio-Rad Laboratories) at an interval of 0.833 ms per line. The images were analyzed using LaserPix version 4.2 software (Bio-Rad Laboratories). Fluorescence profiles were constructed by averaging three pixels bisecting a Ca²⁺ spark for each time point in the scan from the linescan images using LaserPix or a custom developed software.

RESULTS
Assessment of Cardiac Hypertrophy and Exercise-Induced Cardiac Arrhythmia and Sudden Death in FKBP12.6-Deficient Mice

According to previous reports,7,8 FKBP12.6-deficient mice develop either enlarged hearts after 4 months of age in males or exercise-induced cardiac arrhythmia and sudden death in both sexes depending on the mouse strain backgrounds. To confirm the cardiac hypertrophy phenotype, we systematically compared the size of the hearts in FKBP12.6-deficient males and females (129SvEv/C57B6) at 2 and 5 months of age (Fig. 1A). Consistent with previous findings,7 hypertrophic hearts were seen in 5-month-old FKBP12.6-deficient males, while in both age groups FKBP12.6-deficient females had normal heart size (Fig. 1A). Despite this apparent cardiac hypertrophic phenotype in male adult mutants, as shown previously,7 ECG analysis demonstrated normal cardiac function in these mutant males when compared to controls.

To determine whether this cardiac enlargement in FKBP12.6-deficient males was associated with abnormal cardiac gene expression, we analyzed the expression level of several cardiac markers such as atrial natriuretic factor (ANF), αMHC, βMHC, skeletal α-actin (acta1), sarcoplasmic reticulum Ca²⁺ ATPase 2a (SERCA2a), and phospholamban (pln).21–23 ANF, βMHC, and acta1 are only present in embryonic hearts and are reactivated to persistently higher expression levels in hypertropic hearts.22 SERCA2a and pln are involved in regulating intracellular Ca²⁺ homeostasis and contractile function of cardiomyocytes and are normally found to be down-regulated in hypertrophic and failing hearts.21,23 Intriguingly, although cardiac hypertrophy was seen in FKBP12.6-deficient adult male, mRNA levels of these cardiac hypertrophy markers were not

Fig. 1. FKBP12.6-deficient male mice develop cardiac hypertrophy. (A) Morphological and histological analysis of hearts isolated from 2-month-old and 5-month-old FKBP12.6−/− males, females, and littermate controls. Five-month-old FKBP12.6−/− males have a significantly higher cardiac weight and size, and an increased left ventricular wall and ventricular septum thickness when compare to wild-type littermate controls. In contrast, FKBP12.6-deficient females have normal size hearts. (B) Analysis of cardiac hypertrophic markers using quantitative reverse-transcription (RT)-PCR. There is no significant alteration in αMHC, SERCA, pln, βMHC, ANF, and acta1 expression in 5-month-old FKBP12.6-deficient males. MHC, myosin heavy chain promoter; pln, phospholamban; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase.
elevated (Fig. 1B). This observation suggests that FKBP12.6-mediated signaling is linked to physiological, rather than pathological hypertrophy, consistent with FKBP12.6-deficient male mice never displaying heart failure, a common end stage for the pathological hypertrophic heart.

To determine whether FKBP12.6-deficient males also develop exercise-induced cardiac arrhythmia and sudden death in addition to hypertrophy, we compared conscious ECG parameters in these mutant mice and male littermates (5–6 month old) (Fig. 2A and 2B). Continuous ECG recordings were collected from each mouse. No significant difference was observed in RR intervals (wild type: 94.0 ± 7 ms; FKBP12.6−/−: 89.1 ± 7 ms), PR intervals (wild type: 34.1 ± 1.9 ms, n = 5; FKBP12.6−/−: 33.1 ± 2.4 ms, n = 5, p > 0.05), QRS duration (wild type: 16.7 ± 1.7 ms, n = 5; FKBP12.6−/−: 16.0 ± 1.5 ms, n = 5, p > 0.05), rate corrected QT intervals (QTc; wild type: 59.2 ± 4.2 ms, n = 5; FKBP12.6−/−: 57.8 ± 6.0 ms, n = 5, p > 0.05), or resting heart rate (wild type: 615 ± 59.2 bpm; FKBP12.6−/−: 642 ± 29 bpm, n = 5, p > 0.05). Neither FKBP12.6-deficient nor wild-type mice displayed arrhythmia or syncope during ECG probe implantation or under sedentary conscious conditions.

To test the FKBP12.6-deficient male for exercise and stress-induced arrhythmia (Fig. 2C and 2D), we subjected the mutant mice and wild-type controls to a strenuous exercise protocol followed by intraperitoneal injection of epinephrine, as previously described. We did not observe differences between the two groups of mice. Again, both groups had elevations in heart rate, but neither FKBP12.6-deficient mice nor wild-type littermates displayed syncope or polymorphic ventricular arrhythmia (n = 5 mice/genotype). These observations indicate that the 129SvEv/C57B6 strain of FKBP12.6-deficient mouse does not develop exercise-induced arrhythmia and sudden death.

**Generation and Analysis of MHC–FKBP12.6 Transgenic Mice**

We generated transgenic mice in which FKBP12.6 expression was driven by a cardiomyocyte-specific alpha myosin heavy chain (αMHC) promoter (Fig. 3A). The αMHC promoter has a transient burst of activity in embryonic heart around E9.5–10.5. This cardiac specific promoter is reactivated during early postnatal life and remains persistently high into adulthood. Five transgenic lines carrying the MHC–FKBP12.6 transgene were generated with similar FKBP12.6 expression levels and with normal cardiac development and function. Western blot analysis demonstrated that FKBP12.6 expression in MHC–FKBP12.6 hearts was about seven- to ninefold higher than endogenous FKBP12 (Fig. 3B). MHC–FKBP12.6 mice developed normally with no cases of premature death. The heart weight versus body weight ratio in MHC–FKBP12.6 mice was normal in both sexes (Fig. 3). Histological analysis demonstrated that MHC–FKBP12.6 hearts were normal in both ventricles and atria from 1 to 12 months of age. To determine whether MHC–FKBP12.6 mice have altered cardiac function, we performed ECG and Doppler analysis (Table 1). Consistent with our histological observations, MHC–FKBP12.6 (n = 6, male, 4 months old) transgenic hearts had normal left ventricular end diastolic and systolic diameter (LVEDD and LVESD), left ventricular mass, percent fraction shortening, and Vcf when compared to littermate controls (n = 4). These data suggest that overexpression of FKBP12.6 does not significantly alter cardiac structure and function.

**Rescuing Cardiac Hypertrophy Phenotype by MHC–FKBP12.6 Transgenic Mice**

FKBP12.6 is ubiquitously expressed. One important question was whether the abnormal cardiac hypertrophy seen in FKBP12.6-deficient mice in 129SvEv/C57B6 background was directly caused by the loss of FKBP12.6 expression in cardiomyocytes. In fact, noncardiac defects were also found in FKBP12.6-deficient mice, such as hypertension and renal hypertrophy. Our next goal was to determine whether this cardiac hypertrophy was secondary to noncardiac or noncardiomyocyte defects in FKBP12.6-deficient mice. To test this, we generated αMHC–FKBP12.6/ FKBP12.6−/− (129SvEv/C57B6) compound mice in which FKBP12.6 transgene was only present in cardiomyocytes. Heart size and cardiac morphology were compared between littermate adult males (5 months old) with different genotypes, αMHC–FKBP12.6/ FKBP12.6−/−, FKBP12.6−/−, and wild type. As shown in Fig. 3D, cardiomyocyte-specific expression of FKBP12.6 could prevent cardiac enlargement in FKBP12.6-deficient adult males. This

**Fig. 2.** Assessment of exercise induced arrhythmia and sudden death in FKBP12.6-deficient males. ECG traces of littermate control wild-type (A) and FKBP12.6-deficient males (B) under resting condition. ECG traces in wild-type (C) and FKBP12.6−/− males (D) following exercise and intraperitoneal injection of epinephrine. There was no significant difference between ECG traces of wild-type and FKBP12.6−/− mice under both resting and exercise–epinephrine administration.

Fig. 3. Generation of MHC-FKBP12.6 transgenic mice and MHC-FKBP12.6/FKBP12.6−/− mice. (A) Schematic diagram of the construct to generate MHC-FKBP12.6 transgenic mice. Myc-tagged FKBP12.6 was driven by MHC promoter. (B) Western blot analysis using antibodies against myc-tag and FKBP12 (recognizing both FKBP12.6 and FKBP12) confirmed overexpression of FKBP12.6 in MHC-FKBP12.6 transgenic hearts. The level of GAPDH was used as loading control. (C) MHC-FKBP12.6 transgenic adult mice had normal cardiac morphology. (D) Myocardial restricted overexpression of FKBP12.6 rescued hypertrophic heart in FKBP12-deficient males. Comparison of male FKBP12.6−/− hearts to male FKBP12.6−/− or wild-type (WT) hearts and male MHC-FKBP12.6/FKBP12.6−/− hearts.

Table 1. Echocardiograph Analysis

<table>
<thead>
<tr>
<th></th>
<th>LVEDD (mm)</th>
<th>LVESD (mm)</th>
<th>A wall (mm)</th>
<th>P wall (mm)</th>
<th>HR (bpm)</th>
<th>h/r</th>
<th>FS%</th>
<th>Vcf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (n=4)</td>
<td>2.98 ±0.07</td>
<td>1.25 ±0.09</td>
<td>0.56 ±0.03</td>
<td>0.58 ±0.02</td>
<td>700 ±6</td>
<td>0.38 ±0.01</td>
<td>58 ±2.59</td>
<td>14.16 ±0.67</td>
</tr>
<tr>
<td>MHC-FKBP12.6 (n=6)</td>
<td>3.12 ±0.06</td>
<td>1.56 ±0.14</td>
<td>0.61 ±0.02</td>
<td>0.64 ±0.03</td>
<td>699 ±17</td>
<td>0.40 ±0.01</td>
<td>50 ±3.75</td>
<td>11.55 ±0.96</td>
</tr>
<tr>
<td>*P value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

LVEDD, left ventricular diastolic dimension; LVESD, left ventricular systolic dimension; HR, heart rate; h/r, eccentricity index that is derived from taking the mean wall thickness/radius of ventricular end diastole chamber dimension; FS%, percent fraction shortening; Vcf, velocity of circumferential fiber shortening.
observation strongly suggested that cardiomyocytes were responsible for the development of cardiac hypertrophy in FKBP12.6-deficient male.

**Cardiac Expression of FKBP12.6 Rescues Abnormal Calcium Release**

Previously, we had shown that Ca\(^ {2+}\) spark and calcium transients were altered in FKBP12.6-deficient cardiomyocytes.\(^7\) These findings were consistent with the probability of RyR2 opening being increased in the absence of FKBP12.6 or pharmacological dissociation of FKBP12.6 from RyR2 channel complex.\(^8,27\) To evaluate Ca\(^ {2+}\) release in cardiomyocytes isolated from αMHC-FKBP12.6 transgenic mice, we measured Ca\(^ {2+}\) sparks in the cardiomyocytes derived from MHC-FKBP12.6 transgenic hearts. We observed normal Ca\(^ {2+}\) release in αMHC-FKBP12.6 cardiomyocytes when compared to wild-type controls (Fig. 4).

To determine whether or not the altered Ca\(^ {2+}\) release in FKBP12.6-deficient cardiomyocytes was a direct consequence of FKBP12.6 ablation in cardiomyocytes, we measured the characteristics of Ca\(^ {2+}\) sparks in cardiomyocytes isolated from αMHC-FKBP12.6/ FKBP12.6\(^-/-\) (male) and compared them to Ca\(^ {2+}\) sparks in cardiomyocytes isolated from sex-matched littermate FKBP12.6\(^-/-\), αMHC-FKBP12.6, and wild-type control mice (Fig. 4). These parameters included the frequency and amplitude of the Ca\(^ {2+}\) spark, fluorescence magnitude versus average prestimulus fluorescence, full width at half maximum spark size, spark rising time (ms), and half decay time (ms). For all parameters measured, αMHC-FKBP12.6/ FKBP12.6\(^-/-\) cardiomyocytes had normal Ca\(^ {2+}\) sparks. These data indicated that cardiomyocyte-specific expression of FKBP12.6 was able to rescue abnormal calcium release in FKBP12.6-deficient cardiomyocyte (Fig. 4), which further demonstrated a direct association of FKBP12.6 in regulating calcium release.

**Fig. 4.** Analysis and comparison of Ca\(^ {2+}\) sparks in cardiomyocytes. (A) Line-scan mode recording of voltage-clamped cardiomyocytes. Prestimulation and voltage ramp protocol from −70 to −40 mV are shown in the top panel. The line-scan mode recording of Ca\(^ {2+}\) sparks in cardiomyocytes isolated from wild-type (WT), FKBP12.6\(^-/-\) mice (KO), MHC-FKBP12.6 (transgenic, TG), and MHC-FKBP12.6/ FKBP12.6\(^-/-\) (KO+ TG) hearts. The kinetic profiles taken from the line-scan recording in various cells are indicated by red lines and shown in the bottom panel. Typically, the Ca\(^ {2+}\) sparks in FKBP12.6\(^-/-\) cells display long tails indicating delayed inactivation of RyR2. (B) Summary of characteristics of Ca\(^ {2+}\) sparks in cardiomyocytes with different genotypes. The kinetics of Ca\(^ {2+}\) sparks including the frequency and amplitude were completely restored in the FKBP12.6\(^-/-\) mice rescued by cardiomyocyte-restricted FKBP12.6 transgenic mice. F/Fo, fluorescence magnitude divided by average prestimulus fluorescence; FWHM, full width at half maximum spark size; rising time (ms), taken from point of rise to peak F/Fo; half decay time (ms), exponential fit of rapid phase of F/Fo decay. (\(^*\)P<0.05, all compared to WT).
channel RyR2 in cardiomyocyte. Taken together, our data provide additional confirmation and insight on the role of FKBP12.6 in regulating cardiomyocyte calcium release and cardiac function.

**DISCUSSION**

We have carefully analyzed a cardiac hypertrophy phenotype: a total of 173 FKBP12.6 mutants and littermate control mice (88 males and 65 females in two age groups) were analyzed. We also analyzed ECG records of FKBP12.6-deficient mice under resting and extreme-exercise conditions. No exercise-induced cardiac arrhythmia and sudden death were seen in FKBP12.6-deficient mice. We also generated FKBP12.6 transgenic mice and assessed the impact of up-regulated FKBP12.6 expression on cardiac function. For the most part, our data were consistent with a recent finding in an inducible FKBP12.6 transgenic line, except that we did not observe significant alteration in Ca\(^{2+}\) release in the cardiomyocytes isolated from MHC-FKBP12.6 transgenic mice, which could be due to two different transgenic expression systems having been used in the studies. What we observed was the consequence of chronic up-regulation of FKBP12.6, which is different from the temporarily controlled up-regulation of FKBP12.6 in the inducible transgenic system. By crossing FKBP12.6-deficient mice to MHC-FKBP12.6 transgenic mice, we demonstrate that cardiomyocyte-restricted overexpression of FKBP12.6 prevents cardiac hypertrophy in FKBP12.6-deficient mice. This observation strongly indicates a direct impact of FKBP12.6 on cardiomyocyte and cardiac function.

The regulatory role of FKBP12.6 in cardiac function and calcium release in cardiomyocytes via RyR2 has been extensively studied in the past decade. It is believed that FKBP12.6 stabilizes the RyR2, and altered calcium release in cardiomyocytes confirms these findings and demonstrates the essential role of FKBP12.6 in regulating RyR2 channel function. Given the altered Ca\(^{2+}\) release phenotype in FKBP12.6-deficient mice and the important role of Ca\(^{2+}\)-mediated signaling in cardiac hypertrophy, the mouse model may serve as an interesting in vivo model for testing novel compounds that impact on various disease states of the heart.

**ACKNOWLEDGMENTS**

We wish to thank Dr. Shaoliang Jing and Mr. William Carter of Indiana University Mouse Core for their superb assistance in generating MHC-FKBP12.6 transgenic mice, and Drs. Michael Rubart-Von Der Lohé and Pascal Lafontant for comments. This work was supported in part by National Institutes of Health (WS) and Riley Foundation for Children (WS), the NSF of China (81070095 to H.X.) and the NBRP of China (2007CB512100 to H.X.).

**DISCLOSURE STATEMENT**

No competing financial interests exist.

**REFERENCES**

12. Xiao B, Sutherland C, Walsh MP, Chen SR: Protein kinase A phosphorylation at serine-2808 of the cardiac Ca2+ release channel (ryanodine receptor) does not dissociate 12.6-kDa FK506-binding protein (FKBP12.6). *Circ Res* 2004;94:487–495.


Address to correspondence to:
Weinian Shou, PhD
Riley Heart Research Institute
Department of Pediatrics
Indiana University School of Medicine
Indianapolis, IN 46033
E-mail: wshou@iupui.edu

Hongbo Xin, MD, PhD
Laboratory for Cardiovascular Diseases
West-China Hospital
Sichuan University
Chengdu, China
E-mail: hongboxin@yahoo.com