EXPERIMENTAL STUDY

Destructive Role of TMAO in T-Tubule and Excitation-Contraction Coupling in the Adult Cardiomyocytes

Bu Jin,1,2 MD, Fangfang Ji,3 MD, Anjun Zuo,4 MD, Huiting Liu,1,2 MD, Lin Qi,1,2 MD, Yun He,1,2 MD, Qingyao Wang,1,2 MD and Peng Zhao,1,2 MD

Summary

Heart failure (HF) is a disease with high morbidity and mortality. In patients with HF, decreased cardiac output and blood redistribution results in decreased intestinal perfusion and destruction of intestinal barrier. Microorganisms and endotoxins can migrate into the blood circulation, aggravating systemic inflammation and HF. Trimethylamine N-oxide (TMAO) is highly closed to the occurrence of HF. However, the exact mechanism between TMAO and HF remains unclear.

To investigate the role of TMAO in transverse-tubule (T-tubule) in the cultured cardiomyocytes. T-tubule imaging and analysis detected T-tubule network in cardiomyocytes. Ca2+ handling dysfunction was identified by confocal Ca2+ imaging. Tubulin densification and polymerization were assessed by western blot and immunofluorescent staining of cardiomyocytes.

TMAO induced T-tubule network damage in cardiomyocytes and Ca2+ handling dysfunction in cardiomyocytes under the TMAO stress via promoting tubulin densification and polymerization and therefore Junctophilin-2 (JPH2) redistribution. Mice treated with TMAO represented cardiac dysfunction and T-tubule network disorganization.

TMAO impairs cardiac function via the promotion of tubulin polymerization, subsequent translocation of JPH2, and T-tubule remodeling, which provides a novel mechanism for the relationship between HF and elevated TMAO.

Key words: Tubulin, Heart failure, Junctophilin-2

Heart failure (HF) is an increasingly serious health problem and a major cause of mortality and morbidity worldwide.1,2 In the past decade, the concept of pathophysiology of HF has undergone tremendous changes. People’s understanding of the heart as an endocrine organ has been deepening, resulting in the activation of neurohormonal response and systemic inflammation in multiple organs.3 However, a deeper understanding of the pathophysiology of HF requires the development of more specific and effective treatments.

Intestinal microflora plays a key physiological role in extracting energy from food and controlling local or systemic immunity. However, in addition to these beneficial functions to the host, they can also have negative pathophysiological interactions with the host. For example, the composition of intestinal microflora and microflora seems to be involved in the pathogenesis of many diseases, such as obesity, diabetes, gastrointestinal diseases, cancer, and cardiovascular diseases (CVD), including HF.4,5

Trimethylamine N-oxide (TMAO), which is from gut microbiota-produced metabolites of specific dietary nutrients, has become a key contributor to CVD pathogenesis.5,6 Plasma concentration of trimethylamine N-oxide (TMAO), a gut microbiome-derived metabolite, was elevated in HF patients.7 Changes in composition of gut microbiota, called dysbiosis, can contribute to higher levels of TMAO and the generation of uremic toxins, progressing to both HF and renal impairment. The elevated TMAO level in patients with HF is associated with the New York Heart Association classification, ischemic etiology, and adverse prognosis.8,9 At present, antibiotics, probiotics, probiotics, and symbiotics are tools for regulating the health and pathological conditions of intestinal microflora in clinical practice. These are promising preliminary results in the prevention and treatment of obesity and related metabolic diseases.9,10 However, the role of TMAO in the development of HF remains to be further studied.

Microtubules are ubiquitous cytoskeletal fibers formed by the polymerization of alpha- and beta-tubulin dimers. They regulate a wide range of cellular processes,
including maintaining cell shape, mitosis, and intracellular protein transport. Microtubule polymerizing can lead to redistribution of Junctophilin-2 (JPH2) protein, a member of the junctophilin family, spanning the transverse-tubule (T-tubule) and sarcoplasmic reticulum (SR) membranes, thereby playing an important role in the formation and maintenance of the cardiac dyad and ultimately leading to T-tubule remodeling and Ca\textsuperscript{2+} handling dysfunction in cardiomyocytes. TMAO promotes tubulin polymerization. Based on the above research, we hypothesize that the rise of TMAO may lead to the T-tubule remodeling and Ca\textsuperscript{2+} handling disorder.

**Methods**

**Adult mouse ventricular myocyte isolation and culture:** Adult ventricular cardiomyocytes were isolated from male C57BL/6 mice (2~3 months) as previously described. Mice were anesthetized by intraperitoneal injection of 100 mg/kg body weight of sodium pentobarbital, and ventricular myocytes were isolated using a modified Langendorff procedure by perfusing a wedge of the left ventricle through a coronary artery with 0.5 mg/mL of collagenase (Worthington type 2) and 0.08 mg/mL of protease (Sigma Type XVI) for 12 to 15 minutes; ≥ 75% Ca\textsuperscript{2+}-tolerant rod-shaped myocytes were used for experiments. After stabilizing in 1.0 mM Ca\textsuperscript{2+} Tyrode solution (in mM: NaCl 137, KCl 5.4, glucose 10, HEPES 10, NaH2PO4 0.33, MgCl2 1, pH adjusted to 7.35-7.45 with NaOH) for 10 minutes, the cell pellet was suspended in Minimum Essential Medium (MEM) with 10% fetal bovine serum (FBS) and plated in 25 mm dishes precoated with laminin (10 μg/mL). The myocytes were cultured in 5% CO\textsubscript{2} incubator at 37°C for 2 hours, and then the medium was changed to FBS-free MEM medium and myocytes were cultured for a period of 24 hours. The structure of cultured cell was shown in Supplemental Figure 1.

**Treatment protocols:** To determine a suitable TMAO concentration, cells were treated with different concentrations of TMAO (300 nM to 10 μM) for 24 hours. Representative images of T-tubules stained with Di-8-ANEPPS (A) and summarized average data of global T-tubule density and TT power from different groups (B and C). n = 25-30 cells for each group. **P < 0.01 versus indicated groups.

![Figure 1](image-url)

**Figure 1.** Effects of TMAO on T-tubule structure in adult murine cardiomyocytes. Isolated adult cardiomyocytes were exposed to increasing concentrations of TMAO (300 nM to 10 μM) for 24 hours. Representative images of T-tubules stained with Di-8-ANEPPS (A) and summarized average data of global T-tubule density and TT power from different groups (B and C). n = 25-30 cells for each group. **P < 0.01 versus indicated groups.
TMAO impairs Ca\(^{2+}\) handling in the cardiomyocytes. A: Representative images of calcium transients in isolated cardiomyocytes cultured for 24 hours from different groups. B-D: Average data of the amplitude of Ca\(^{2+}\) transients, time to peak of Ca\(^{2+}\) transients, and dyssynchronous index, respectively. \(n = 25-30\) cells per group. ***\(P < 0.001\) versus indicated groups.

rode solution at room temperature for 30 minutes. T-tubules from intact hearts were stained with MM 4-64 (2.5 μmol/L, AAT BioQuest, CA) in Ca\(^{2+}\) free Tyrode solution via Langendorff perfusion at room temperature for 30 minutes. And the structure of T-tubules was visualized with confocal microscope (LSM510, Carl Zeiss Microimaging Inc., Germany) on 63× lens.

**T-tubule analysis:** The strength of regularity of T-tubules (TT power, also called TT\text{int} (%), and JPH2 (JPH2 power)) was analyzed using AutoTT custom software. Briefly, AutoTT was coded in MATLAB (MathWorks, Natick, MA) and was compiled to be a stand-alone Graphical Interactive application named AutoTTGUl.exe. MATLAB Compiler Runtime (MCR) R2013a (Windows version, 32-bit) is prerequisite to run the AutoTT application. AutoTT is installed by copying AutoTTGUl.exe anywhere on a PC with a Windows operating system. AutoTT is freely available upon request. To run AutoTT, double click the icon.\(^{15}\)

**Protein preparation:** Cardiomyocytes were washed with cold phosphate buffered saline (PBS) for three times and then were suspended and sonicated in lysis buffer (in mM: Tris-HCl 50 [pH 7.4]; NaCl 150; NaF 10; Na3VO4 1; EGTA 5; EDTA 5; 0.2% Triton X-100; 0.5% Na deoxycholate; and 0.1% SDS), containing protease inhibitors (Sigma, P8340). Lysates were centrifuged at 13,000 \(\times\) g 4°C for 15 minutes. The supernatants were kept as whole cell proteins. The protein concentration was determined by BCA assay (Pierce, Thermo Scientific).

**Western blotting:** Whole cell protein samples were electrophoresed on 10% Bis-Tris gels. Proteins were transferred to PVDF (Millipore, Billerica, MA, USA) and probed with primary antibodies recognizing JPH2 (1:1000, Santa Cruz Biotechnology), calpain I (1:500, Cell Signaling Technology), α-tubulin antibody (1:1000, Santa Cruz Biotechnology), anti-β-tubulin antibody (1:1000, T4026, Sigma, St. Louis, MO, USA), and GAPDH (1:5000, G 8975, Sigma) overnight at 4°C. After being washed with PBS solution three times, the binding of the primary antibodies was detected by horseradish peroxidase (HRP)-conjugated second antibodies (1:5000-1:10000 dilution in PBS solution). The immunoreactions were visualized using an enhanced-chemiluminescent detection kit (Pierce,
Figure 3. Cardiomyocytes treated with TMAO display more Ca^{2+} sparks. Representative Ca^{2+} spark images in isolated cardiomyocytes treated with TMAO (A). Mean values of the frequency (B), amplitude (C), duration (FDHM, full duration at half-maximum) (D), and spatial width (FWHM, full width at half-maximum) (E) of Ca^{2+} sparks. \( n = 15-30 \) cells per group. **\( p < 0.01 \), ***\( p < 0.001 \) versus indicated groups. n.s. indicates no significant difference.

Thermo Scientific) science imaging system (LAS-3000, Fujifilm). The bands were quantified using NIH ImageJ software (version 1.43d).

Assessment of free and polymerized tubulin: Free and polymerized tubulin was assessed using a Microtubules/Tubulin In Vivo Assay Kit (Cytoskeleton, Denver, CO). Cells were homogenized in cell lysis and microtubule stabilization buffer containing (in mM) MgCl\(_2\) 5, EGTA 1, GTP 0.1, ATP 1, and PIPES buffer 100 and (in %) glycercol 30, Nonidet-P40 0.1, Triton X-100 0.1, Tween-20 0.1, beta-mercapto-ethanol 0.1, antifoam 0.001, and BME 0.2, pH 7.4, with a protease inhibitor cocktail. Homogenates were centrifuged (100,000 \( g \), 30 minutes, 37°C) to yield supernatants containing free tubulin and pellets containing microtubules, which were resuspended in cold 200 \( \mu \)M CaCl\(_2\).

Immunofluorescent staining of cardiomyocytes: Isolated cardiomyocytes were fixed in 4% Paraformaldehyde (PFA) at 37°C for 15 minutes. For immunofluorescent staining, samples were washed three times with PBS for 10 minutes and followed by permeabilization with 0.3% Triton X 100 in PBS for 30 minutes. Following 1% BSA blocking 30 minutes at room temperature, samples were incubated with anti-\( \beta \)-tubulin antibody (1:100, T4026, Sigma, St. Louis, MO, USA) or anti-JPH2 (1:50, sc-51313; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight, followed by incubation with fluorescent-labeled secondary antibodies at room temperature for 2 hours. Staining was visualized by confocal microscopy with a 63x lens. Microtubule density was quantitated using NIH ImageJ. JPH2 organization (JPH2power) was processed with AutoTT custom software. Mean JPH2 fluorescence around the cell periphery (within \(~4 \mu\)m from cell edge) and mean JPH2 fluorescence inside the cell were quantitated using a home-complied automatic program coded in MATLAB.
I n T H e a r t J

March 2020 359

Figure 4. TMAO induces T-tubule remodeling in the culture cardiomyocytes. Isolated cardiomyocytes stained with anti-JPH2 antibody after treatment with or without TMAO for 24 hours. A: Representative images; B: average JPH2 power in cultured cardiomyocytes with different treatments; C: semiquantitative analysis of JPH2 distribution between T-tubule and periphery sarcolemma. n =15-20 cells per group. **P < 0.01, ***P < 0.001 versus indicated groups. The expression level of JPH2 was determined by western blot, and GAPDH was used as positive control. D: Representative blot image. Blots were scanned, and optical densities were determined using the Quantity One software. E: Densitometric data from four independent experiments. n.s. means no significant difference.

Confocal Ca²⁺ imaging: Cultured cardiomyocytes were loaded with 5 μM Fluo-4 AM (Invitrogen, Grand Island, NY) in 1.8 mM Ca²⁺ Tyrode solution for 30 minutes. Cells were then washed with dye-free Tyrode solution for 20 minutes for de-esterification before imaging. The Ca²⁺ images were acquired using confocal microscope in line-scan mode along the longitudinal axis of myocytes. Steady-state Ca²⁺ transients were measured in Tyrode solution containing 1.8 mM Ca²⁺ under field stimulation of 1 Hz. Ca²⁺ sparks were recorded 5 seconds after halt of field stimulation. Index of dyssynchronization, defined by the mean absolute deviation of firing time of each scanning pixel (with every 8 pixels binned), was used to evaluate the dyssynchrony of Ca²⁺ transients.

Animal study: In mouse studies, 6 weeks after adding either 1.0% choline or 0.12% TMAO to a chemically defined diet comparable with normal mouse chow (which contains 0.08 g% choline). The cardiac function and T-tubule from normal mice or treated mice were analyzed.

Statistical analysis: Data are expressed as mean ± SE. One-way analysis of variance was used for multiple group comparisons. Student’s t-test was used for two group comparisons. Statistical analysis was carried out using SPSS V15.0 software. P values of P < 0.05 were considered statistically significant.

Results

TMAO induced T-tubule network damage in cardiomyocytes: First, we determined whether TMAO could damage T-tubule structure and the dose at which T-tubule loss and/or disorder developed in a period of 24 hours upon exposure to TMAO. TMAO impaired T-tubule organization in cardiomyocytes in a concentration-dependent manner over the tested concentration range (3 μM to 10 μM, Figure 1A-C). A more significant reduction of global T-tubule density and TT power was observed with 3 μM and 10 μM TMAO. We chose 3 μM TMAO for use in our subsequent experiments based on above results.

Ca²⁺ handling dysfunction in response to TMAO: Next, we investigated whether TMAO could induce Ca²⁺ handling disorders in isolated cardiomyocytes. Cardiomyocytes cultured with TMAO (3 μM) for 24 hours and paced at 1 Hz displayed depressed amplitude, prolonged time to peak, and decreased synchronization (quantified by the index of dyssynchronity) compared with control myocytes (Figure 2A-D). In addition, TMAO treatment resulted in a pronounced increase in the frequency of spontaneous Ca²⁺ sparks and Ca²⁺ spark amplitude, but not other Ca²⁺ index including mean full width at half-maximum (FWHM) and mean duration at half-peak amplitude (FDHM) (Figure 3A-E). These data suggest that...
Figure 5. More rearrangement and densification of microtubules under TMAO treatment in the cardiomyocytes. A, B: Representative images and summary data of β-tubulin immunofluorescent staining in cells treated with or without TMAO. n = 20-25 cells per group, ***P < 0.001 versus indicated groups. C, D: Representative immunoblot and summary data of free (F) and polymerized (P) β-tubulin from different groups. **P < 0.01 versus indicated groups.

TMAO impairs Ca²⁺ handling and disturbs Ca²⁺ recycling. TMAO induced JPH2 redistribution from T-tubules to peripheral plasma membrane, but not degradation in cardiomyocytes: Next, we hypothesized that JPH2 translocation and/or downregulation may play an important role in T-tubule remodeling induced by TMAO treatment. As compared to control myocytes, the organized JPH2 distribution was altered in response to TMAO for 24 hours. As a quantitative index of the regularity of JPH2 staining, we calculated the peak JPH2 power value at the dominant frequency (JPH2power). JPH2 power was reduced in the TMAO treatment group (Figure 4B). In addition, cardiomyocytes treated with TMAO were observed a clear accumulation of JPH2 around the edge of myocytes, suggesting a reorganization of JPH2 protein under TMAO treatment (Figure 4C). However, there were no alterations in the TMAO-treated/control cardiomyocytes on the JPH2, RyR2, DHPR, SERCA2a, and Casq2 protein expression level (Figure 4D-E and Supplemental Figure 2). These data suggest that JPH2 protein translocation is the main potential mechanism of TMAO-induced T-tubule disorder.

TMAO aggravated the assembly of microtubules in cardiac myocytes: Densification of microtubules as a cause of T-tubule remodeling in heart disease and in cultured adult cardiomyocytes via redistribution of JPH2. In patients with HF, plasma levels of TMAO increased. Therefore, we investigated whether the TMAO participate in the microtubule accumulation and E-C coupling dysfunction and HF. To confirm the role of TMAO in microtubule accumulation, cardiomyocytes were treated with TMAO. TMAO treatment exaggerated the densification and aggregation of the microtubule network, as compared to control group (Figure 5A-D).

TMAO damaged cardiac function and T-tubule network in vivo: To determine the role of TMAO in cardiac function in vivo, mice-treated TMAO exhibit decreased cardiac contractile function as compared to the mice treated with vehicle (Figure 6A, B). In addition, TMAO produced T-tubule disorganization and subcellular T-tubule loss as demonstrated by in situ confocal imaging of the left ventricle (LV) (Figure 6C). These results indicated that TMAO could induce cardiac dysfunction and T-tubule remodeling.

Discussion

CVD, including atherosclerosis, hypertension, HF, atrial fibrillation, and myocardial fibrosis, is associated with high morbidity and mortality. Many risk factors are well known, such as smoking, poor dietary habits, obesity, diabetes, and high cholesterol, but these cannot explain the incidence of all CVD. Recent studies have focused on the interaction between intestinal microflora and CVD. The accumulated evidence indicates that intestinal microflora and its metabolites play a key role in the occurrence and development of CVD. Oral microflora can affect the composition of intestinal microflora and contribute to these diseases.

The human gastrointestinal tract is considered to be a huge and diverse ecosystem with trillions of metabolite-secreting microbial communities. Specific diets and in-
**Figure 6.** TMAO undermines cardiac function and T-tubule network. 

**A:** The protocol showed how to treat mice with TMAO and assay of ejection fraction and *in situ* tubule imaging. 

**B:** Representative short-axis echocardiographic images of the left ventricle M-mode recordings from control and TMAO-treated mice. 

**C:** Quantitative evaluation of left ventricular ejection fraction. 

**D:** Representative *in situ* LV T-tubule confocal images after staining with lipophilic marker MM 4-64. 

**E:** Mean values of TT power. *n* = 4-5 mice per group. *P* < 0.05 versus control; TT power indicates strength of regularity of the T-tubule system.

HF is a disease with high morbidity and mortality. About half of the patients died within five years after diagnosis. In patients with HF, decreased cardiac output and blood redistribution lead to decreased intestinal perfusion and destruction of intestinal barrier. Therefore, microorganisms and endotoxins can migrate into the blood circulation, aggravating systemic inflammation and HF. 

TMAO is associated with the severity of HF. When the mice were fed with TMAO, the pulmonary edema and cardiac enlargement in transverse aortic constriction mice increased significantly. The exact mechanism between TMAO and HF is not yet clear. Therefore, in our present study, we firstly established the relationship between TMAO and T-tubule remodeling using a TMAO treatment model in the isolated cardiomyocytes. The major findings of our study are as follows: (1) TMAO impairs T-tubule organization, (2) TMAO disturbs the Ca²⁺ handling, (3) JPH2 translocation in cardiomyocytes under TMAO stress, and (4) TMAO treatment in mice exhibited decreased ejection fraction and T-tubule disorganization. Taken together, our data suggest a novel mechanism by which TMAO induced tubulin densification, translocation of JPH2, subsequent T-tubule remodeling, and ultimately Ca²⁺ handling dysfunction.

It has been reported that cardiac T-tubules are physical extensions and orderly invaginations of the surface membrane that are continuous with the extracellular space and extend deep into the interior of mammalian ventricular cardiomyocytes. Critical to excitation-contraction (E-C) coupling function is the spatial relationship between T-tubules and the SR, where Ca²⁺ release channels or ryanodine receptors (RyRs) are located. Early studies demonstrated that T-tubule remodeling disrupts the precise communication between the voltage-gated L-type Ca²⁺ channels located mainly on the T-tubule membrane and Ca²⁺ release channels (RyR2) on the SR, which is important for the rapid electric excitation, initiation, and synchronous triggering of SR Ca²⁺ release. Evidence from isolated ventricular myocytes and intact hearts suggests...
that T-tubule remodeling is a significant and common event in advanced HF of different etiologies and results in dysynchronous Ca\(^{2+}\) release and therefore decreased contractility and aggregated Ca\(^{2+}\)-dependent arrhythmias.\(^{31,32}\) With loss of T-tubule integrity, the systolic Ca\(^{2+}\) transient results from a “fire-diffuse-fire” sequential recruitment of Ca\(^{2+}\) release sites from the cell edge to the center and hence mark a spatiotemporal heterogeneity in systolic Ca\(^{2+}\).\(^{32,33}\) T-tubule disruption, which removes the L-type hence mark a spatiotemporal heterogeneity in systolic Ca\(^{2+}\) handling in adult hearts.\(^{12,32}\) Herein, we expand these polymerization. It has been reported that JPH2 is required for the non-physiological cardiac dimers, including more SR fragments without stress,\(^{13}\) which is in line with our findings that JPH2 mislocalization mediates translocation of JPH2 in response to cardiac pressure.\(^{39}\) Traditional JPH2 knockout is fatal in embryos, whereas JPH2-deficient embryonic myocytes have defective cardiac dimers, including more SR fragments without T-tubule coupling proteins.\(^{40,41}\)

Previous studies have shown that tubulin densification mediates translocation of JPH2 in response to cardiac stress,\(^{1}\) which is in line with our findings that JPH2 mis-tracking in response to TMAO due to increased tubulin polymerization. It has been reported that JPH2 is required for maintaining T-tubule structural integrity and preserving Ca\(^{2+}\) handling in adult hearts.\(^{12,32}\) Herein, we expand these findings by showing that TMAO-induced JPH2 translocation caused by increased tubulin assembly contributes to T-tubule remodeling. In summary, our data provide compelling evidence for how TMAO might impair cardiac function via the promotion of tubulin formation and subsequent translocation of JPH2. These findings suggest reducing TMAO may be a promising cardioprotective action, such as improving the quality of intestinal flora or preventing intestinal flora disorder.

**Disclosure**

**Conflicts of interest:** none.

**References**

1. Hunt SA, Baker DW, Chin MH, et al. ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the adult: executive summary A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the International Society for Heart and Lung Transplantation; endorsed by the Heart Failure Society of America. Circulation 2001; 104: 2996-3007.
2. Kinugawa K, Sato N, Inomata T, Yasuda M, Shimakawa T, Fukuta Y. A prospective, multicenter, post-marketing surveil-

\(^{11,13,33}\)
23. Nagatomo Y, Tang WH. Intersections between microbiome and heart failure: revisiting the gut hypothesis. J Card Fail 2015; 21: 97-80.

24. Organ CL, Otsuka H, Bhushan S, et al. Choline diet and its gut microbe-derived metabolite, trimethylamine N-oxide, exacerbate pressure overload-induced heart failure. Circ Heart Fail 2016; 9: e002314.

25. Han J, Wu H, Wang Q, Wang S. Morphogenesis of T-tubules in heart cells: the role of junctophilin-2. Sci China Life Sci 2013; 56: 647-52.

26. Zhang C, Chen B, Wang Y, et al. MG53 is dispensable for T-tubule maturation but critical for maintaining T-tubule integrity following cardiac stress. J Mol Cell Cardiol 2017; 112: 123-30.

27. Guo A, Zhang C, Wei S, Chen B, Song LS. Emerging mechanisms of T-tubule remodelling in heart failure. Cardiovasc Res 2013; 98: 204-15.

28. Lyon AR, MacLeod KT, Zhang Y, et al. Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. Proc Natl Acad Sci U S A 2009; 106: 6854-9.

29. Al-Qusairi L, Weiss N, Toussaint A, et al. T-tubule disorganization and defective excitation-contraction coupling in muscle fibers lacking myotubularin lipid phosphatase. Proc Natl Acad Sci U S A 2009; 106: 18763-8.

30. Frisk M, Koivumäki JT, Norseng PA, Maleckar MM, Sejersted OM, Louch WE. Variable t-tubule organization and Ca2+ homeostasis across the atria. Am J Physiol Heart Circ Physiol 2014; 307: H609-20.

31. Poláková E, Sobie EA. Alterations in T-tubule and dyad structure in heart disease: challenges and opportunities for computational analyses. Cardiovasc Res 2013; 98: 233-9.

32. Reynolds JO, Chiang DY, Wang W, et al. Junctophilin-2 is necessary for T-tubule maturation during mouse heart development. Cardiovasc Res 2013; 100: 44-53.

33. Lipp P, Bootman MD. Which Ca2+ channels control cardiac E-C coupling? J Physiol 1998; 508: 331.

34. Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. Proc Natl Acad Sci U S A 2006; 103: 4305-10.

35. Louch WE, Mørk HK, Sexton J, et al. T-tubule disorganization and reduced synchrony of Ca2+ release in murine cardiomyocytes following myocardial infarction. J Physiol 2006; 574: 519-33.

36. Brette F, Sallé L, Orchard CH. Quantification of calcium entry at the T-tubules and surface membrane in rat ventricular myocytes. Biophys J 2006; 90: 381-9.

37. Brette F, Sallé L, Orchard CH. Differential modulation of L-type Ca2+ current by SR Ca2+ release at the T-tubules and surface membrane of rat ventricular myocytes. Circ Res 2004; 95: e1-7.

38. Reuter H, Pott C, Goldhaber JJ, Henderson SA, Philipson KD, Schwinger RH. Na(+)-Ca2+ exchange in the regulation of cardiac excitation-contraction coupling. Cardiovasc Res 2005; 67: 198-207.

39. Landstrom AP, Kellen CA, Dixit SS, et al. Junctophilin-2 expression silencing causes cardiomyocyte hypertrophy and abnormal intracellular calcium-handling. Circ Heart Fail 2011; 4: 214-23.

40. Guo A, Wang Y, Chen B, et al. E-C coupling structural protein junctophilin-2 encodes a stress-adaptive transcription regulator. Science 2018; 362.

41. Garbino A, Wehrens XH. Emerging role of junctophilin-2 as a regulator of calcium handling in the heart. Acta Pharmacol Sin 2010; 31: 1019-21.

Supplemental Files

Supplemental Figures 1, 2
Please see supplemental files; https://doi.org/10.1536/ihj.19-372