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Takotsubo syndrome is a coronary microvascular disease: experimental evidence

Feng Dong [1]*, Liya Yin [1], Hamayak Sisakian [2], Tatevik Hakobyan[1], Lacey S. Jeong[1], Hirva Joshi[1], Ellianna Hoff[1], Selena Chandler [1], Geetika Srivastava[1], Abdur Rahman Jabir[1], Kelly Kimball[1], Yeong-Renn Chen[1], Chwen-Lih Chen[1], Patrick T. Kang[1], Parisa Shabani[1], Lindsay Shockling[1], Thomas Pucci[1], Karlina Kegecik[1], Christopher Kolz[1], Zhenyu Jia[3], William M. Chilian [1], and Vahagn Ohanyan [1]*

[1]Department of Integrative Medical Sciences, Northeast Ohio Medical University, 4209 State Route 44, Rootstown, Ohio 44272, USA; [2]Department of Cardiology, Yerevan State Medical University, Yerevan, Kentron, Armenia; and [3]Department of Botany and Plant Sciences, University of California, Riverside, CA, USA

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See the editorial comment for this article 'Is Takotsubo syndrome just the tip of the iceberg in the clinical spectrum of coronary microvascular dysfunction?', by A. Candreva and C. Templin, https://doi.org/10.1093/eurheartj/ehad264.

Abstract

Background and aims Takotsubo syndrome (TTS) is a conundrum without consensus about the cause. In a murine model of coronary microvascular dysfunction (CMD), abnormalities in myocardial perfusion played a key role in the development of TTS.

Methods and results Vascular Kv1.5 channels connect coronary blood flow to myocardial metabolism and their deletion mimics the phenotype of CMD. To determine if TTS is related to CMD, wild-type (WT), Kv1.5$^{-/-}$, and TgKv1.5$^{-/-}$ (Kv1.5$^{-/-}$ with smooth muscle-specific expression Kv1.5 channels) mice were studied following transaortic constriction (TAC). Measurements of left ventricular (LV) fractional shortening (FS) in base and apex, and myocardial blood flow (MBF) were completed with standard and contrast echocardiography. Ribonucleic Acid deep sequencing was performed on LV apex and base from WT and Kv1.5$^{-/-}$ (control and TAC). Changes in gene expression were confirmed by real-time-polymerase chain reaction. MBF was increased with chromonar or by smooth muscle expression of Kv1.5 channels in the TgKv1.5$^{-/-}$. TAC-induced systolic apical ballooning in Kv1.5$^{-/-}$, shown as negative FS ($P < 0.05$ vs. base), which was not observed in WT, Kv1.5$^{-/-}$ with chromonar, or TgKv1.5$^{-/-}$. Following TAC in Kv1.5$^{-/-}$, MBF was lower in LV apex than in base. Increasing MBF with either chromonar or in TgKv1.5$^{-/-}$ normalized perfusion and function between LV apex and base (P = NS). Some genetic changes during TTS were reversed by chromonar, suggesting these were independent of TAC and more related to TTS.

Conclusion Abnormalities in flow regulation between the LV apex and base cause TTS. When perfusion is normalized between the two regions, normal ventricular function is restored.

* Corresponding author. Tel: +330-325-6535, Fax: +330 325 5978, Emails: fdong@neomed.edu (F.D.); vohanyan@neomed.edu (V.O.)

Structured Graphical Abstract

Key Question

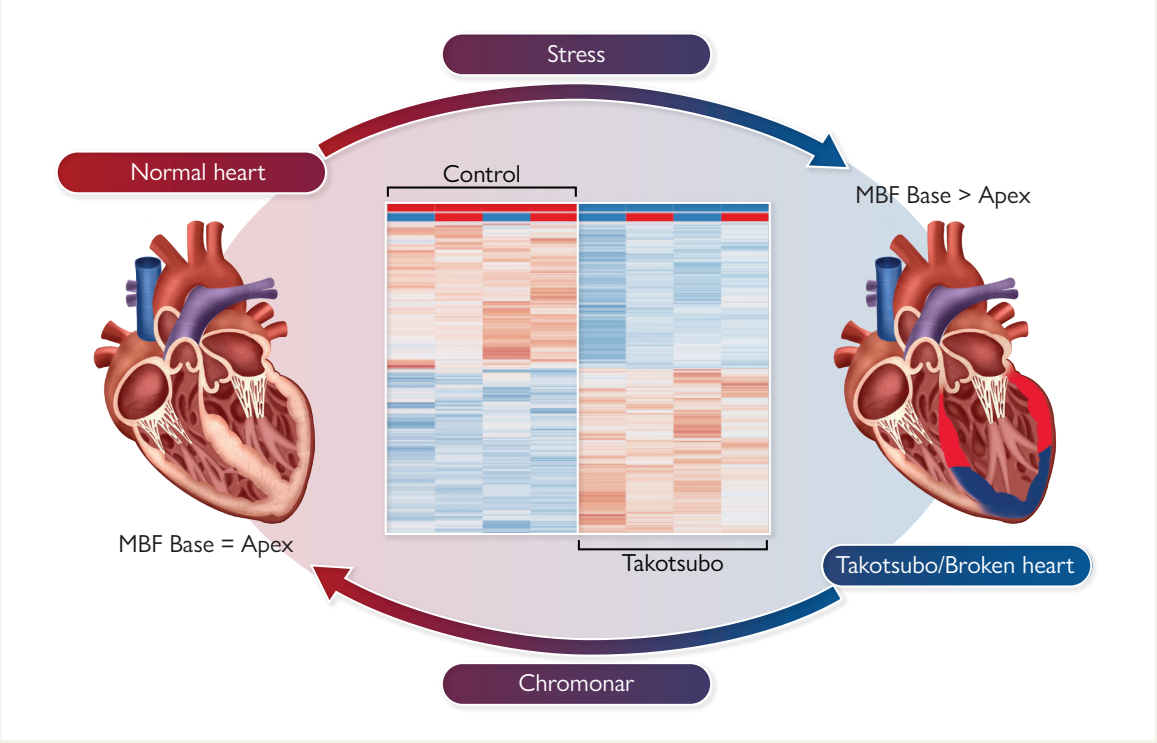
Is Takotsubo syndrome (TTS) caused by impaired coronary vascular regulation?

Key Finding

In mice with coronary microvascular dysfunction, haemodynamic stress-induced TTS was associated with lower myocardial blood flow (MBF) in the apex compared to the base, and downregulation of genes associated with metabolism. Increasing apical MBF restored normal function.

Take Home Message

Takotsubo Syndrome is caused by abnormal coronary blood flow regulation.



This pictorial summary of our study represents the induction of Takotsubo Syndrome by stress resulting in tissue hypoxia in the apex compared to the base of the left ventricle as shown by the blue color. The cause of this difference in tissue hypoxia is related to the lower myocardial blood flow (MBF) in the apex compared to the base. These changes in perfusion and the resultant hypoxia in the apex led to a drastic change in gene expression. The array shown reveals the dramatic changes between control and Takotsubo hearts, in which the expression of several genes appears to be 'flipped' between the two conditions with red–brown color indicating higher expression (Control vs. Takotsubo) and the blue the reverse. The summary also shows the effects of chromonar, a coronary-specific vasodilator, in term of restoring myocardial blood flow to the apex and restoration of normal function.

Keywords Coronary circulation • Myocardial hibernation • Broken heart syndrome • Stress-induced cardiomyopathy

Translational perspective

Competency in medical knowledge: Takotsubo syndrome is a conundrum in clinical cardiology without any consensus therapy for its treatment. Because this condition appears to be related to abnormal function in the microcirculation of the heart, increasing coronary blood flow may prove to be a therapy that hastens recovery. Translational outlook: The drug chromonar, a selective coronary dilator, increases blood flow to the apex of the left ventricle in Takotsubo syndrome and restores cardiac function.

Introduction

Takotsubo syndrome (TTS), also known as an apical ballooning syndrome, broken heart syndrome, and stress-induced cardiomyopathy, is a heart failure-like syndrome characterized by a temporary systolic left ventricular (LV) abnormality,[1,2] but recently biventricular TTS was characterized.[3] In addition to the systolic contractile dysfunction, the Mayo Clinic criteria for diagnosis include the absence of obstructive coronary disease, electrocardiographic abnormalities (ST-segment elevation and T-wave inversion), the modest elevation of circulating levels of cardiac troponin, and absence of pheochromocytoma and myocarditis.[4,5] The majority of patients present with systolic ballooning of the LV apex with concomitant contraction of the base, resembling a Japanese octopus trap having a bulbous bottom and a long, thin neck.[6] There are reports of a reverse TTS, where the LV apex contracts while the base bulges[7,8] and even a variant with mid-ventricular ballooning.[9] TTS was considered an innocuous condition with temporary LV dysfunction, which resolves sometimes without medical treatment,[10] however, this perception is qualified in light of more recent data, revealing that patients hospitalized with TTS have rates of complications and death similar to patients with acute coronary syndromes.[11,12] Some triggering stimuli for TTS have been identified, including fluctuations in hemodynamics and emotional stress, but nearly one-third of patients have no evident trigger.[11]

Although the incidence of TTS is rising, its underlying mechanism is still unknown. There have been numerous suggestions for the causal mechanism including elevated levels of catecholamines,[13] autonomic dysfunctions with elevations in sympathoadrenal drive,[14,15] endocrine disorders,[16,17] surgical stress,[18,19] and coronary vasospasm.[20,21] A role for the coronary microcirculation in TTS was previously implicated in several clinical studies[22–27] with these groups showing a decrease in coronary vasodilator reserve in patients with TTS. Whether the compromised coronary function is a precipitating event or is the result of the altered ventricular dynamics was not revealed. This conundrum led to our hypothesis that coronary microvascular dysfunction (CMD) is the precipitating cause for TTS, in that an imbalance between myocardial blood flow (MBF) and myocardial work/oxygen demands produces the syndrome.

Previously, we demonstrated that the potassium voltage-gated channel, shaker-related subfamily, member 5, also known as KCNA5 or Kv1.5 channel, in smooth muscle cells of coronary arterioles is vital in coupling MBF to cardiac work.[28] Mice null for Kv1.5 channels have a significant mismatch between myocardial demand and coronary blood flow due to impaired coronary metabolic dilation. Furthermore, reconstitution of the Kv1.5 channel in smooth muscle in the Kv1.5 null mice restored the connection between cardiac work and MBF. If CMD is central to the pathogenesis of TTS, we reasoned that mice null for Kv1.5 channels would demonstrate TTS when subjected to hemodynamic stress. We also reason that increased blood flow to the heart via expression of Kv1.5 channels in smooth muscle or via administration of the coronary vasodilator chromonar would facilitate recovery from TTS. In addition, we completed RNAseq analysis of gene expression in the murine model of TTS and confirmed some of the changes using real-time-polymerase chain reaction (RT-PCR). We also confirmed that some of the changes in gene expression are reversed by increases in blood flow to the left ventricle. Our results are consistent with the conclusion that TTS is caused by abnormalities in flow regulation between the apex and the base of the left ventricle, and that restoration of MBF will restore normal ventricular function (*Structured Graphical Abstract*).

Methods

All procedures were conducted with the approval of the Institutional Animal Care and Use Committee of the Northeast Ohio Medical University in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 85–23, revised 1996). Mice were housed in a temperature-controlled room with a 12:12-h light-dark cycle and maintained with access to food and water *ad libitum*.

Murine models

Wild-type (WT) mice, mice null for Kv1.5 channels (Kv1.5$^{-/-}$), and a double transgenic mouse enabling inducible (doxycycline, tet-on promoter) expression of the reconstituted Kv1.5 channel in smooth muscle (TgKv1.5$^{-/-}$) of either sex were used in this study. To induce expression of the Kv1.5 channels in the TgKv1.5$^{-/-}$ mice, mice were given water with doxycycline (2 mg/mL) ad libitum. All mice were on the S129 background.

Model of takotsubo syndrome

Since many of the triggering stimuli of TTS are known to produce hemodynamic stress on the heart, we postulated that a model of hemodynamic stress would trigger the syndrome in a model of CMD. To induce such stress, we produced transaortic constriction (TAC) via a ligature tied around the aorta to produce a stenosis. This was accomplished in anesthetized mice (3% isoflurane with supplemental oxygen 1 L/min) using an aseptic surgical technique. A horizontal skin incision was made ~ 5–10 mm at the suprasternal notch region, and a ~3–5 mm longitudinal cut was produced in the proximal part of the sternum to allow visualization of the aortic arch. A 6–0 silk suture was situated around the aortic arch between the origin of the right innominate and left common carotid arteries and tied tightly over a 27 G needle. After ligation, the needle was removed, the wounds repaired, and the mice were allowed to recover. A sham procedure was also performed except the aorta was not banded. After completion of the surgery, mice were treated for pain (extended release Buprenex, 0.5–1.0 mg/kg). In Kv1.5$^{-/-}$ the production of TAC induced a TTS phenotype within 7–10 days. Some mice subjected to TAC received a second surgical procedure in which the aortic band was removed to restore normal hemodynamics.

We attempted two other models to induce TTS with i.p. doses of norepinephrine (NE) or isoproterenol. The isoproterenol model induced a high degree of mortality. We observed variability in the NE model with overt systolic apical ballooning in around 50% of the mice, which contrasts with TAC with TTS in 90%–95% of mice and a very predictable time course. Based on this we pursued the TAC model. Although TAC is obviously not the trigger in most patients, the presence of systemic hypertension appears to be one of the best-associated risk factors[29] and it is noteworthy to emphasize the TAC like systemic hypertension both increase cardiac afterload and work. We infrequently observed reverse Takotsubo in mice where the apex contracted during systole with concomitant bulging at the base. However, this observation was rare (<5% of the mice demonstrated) the typical systole apical ballooning. Because of this rarity, we focused on apical ballooning which is reported in this paper. This selection is not designed to be dismissive of other forms of TTS, which appear to have similar complications as the more frequent apical ballooning.[30]

Echocardiographic analysis of cardiac function

Mice underwent an echocardiographic assessment of systolic LV function after induction of TTS via the TAC procedure. Mice were lightly anesthetized with isoflurane and cardiac function was measured using a Vevo 770 or Vevo 2100 (VisualSonics, Canada) using a 12–38 MHz linear transducer. For the evaluation of LV systolic function, we focused on measurements of fractional shortening (FS) in the base and apex of the left ventricle as a means of quantifying systolic bulging, i.e. negative FS, in the apex of hearts of mice exhibiting TTS.

Measurement of myocardial blood flow

Myocardial blood flow was measured by myocardial contrast echocardiography_ENREF_36. Contrast imaging was performed with a Sequoia 512 (Siemens Medical Systems) via infusion of contrast (microbubbles; 20 μL/min, 5×10^5 bubbles min^{-1}) into the right jugular vein through a PE-50 catheter. This catheter was also used for drug infusion. Long-axis images of the left ventricle were obtained for perfusion imaging. After optimal visualization of the chamber and the ventricular wall, images were collected during a high-energy pulse sequence (used to destroy microbubbles) and for several seconds after destruction to establish refilling of the chamber and ventricular wall. Analysis was done off-line, in which regions of interest in the ventricular wall of the apex and base were positioned within the anterolateral wall in the long-axis view. The supplement contains additional details about this procedure. As we noted previously when compared to measurements of myocardial perfusion obtained using microspheres, the flow measurements with contrast echocardiography were roughly 40% higher.[28]

Measurements of MBF, heart, and arterial pressure were made under basal conditions and during apical ballooning and after the administration of the coronary vasodilator, chromonar. Chromonar can produce maximal coronary vasodilation without affecting systemic hemodynamics.[31,32] MBF was measured in the base and apex of the left ventricle under different conditions.

RNA isolation and analysis

We performed RNA-seq on Kv1.5$^{-/-}$ sham mice and Kv1.5$^{-/-}$ mice exhibiting TTS. The differences in expression observed with RNA seq were confirmed by RT-PCR.[33] Ribonucleic Acid (RNA) was isolated from the apex and base of heart samples using the miRNeasy Mini Kit (Qiagen, Cat No./ID: 217004) according to the manufacturer's instruction. RNA quality was measured with Agilent RNA 6000 Nano Kit (5067–1511). Contaminating Deoxyribonucleic Acid (DNA) was degraded by a 15-min incubation with RNase-free DNase.

RNA-seq was performed as described.[34] RNA-seq libraries were prepared using TruSeq stranded total RNA sample prep kit with Ribo-Zero ribosomal RNA depletion (Illumina) according to the protocols of the manufacturer. NextSeq 500 high output V2 kit (FC-404-2002) was used for sequencing on the NextSeq 550 sequencing system. RNA-seq data analysis with STAR + cufflink combination and gene set enrichment analysis was performed at Case Western Reserve University Computational Biology Core. Real-time-PCR was performed to confirm significant differences observed in RNA expression based on the sequencing analysis. The supplement contains the details of these procedures and the primer IDs.

Statistics

Data were analyzed using Graphpad Prism 9. Results were expressed as mean ± 95% confidence interval. RNA expression between two samples, e.g. apex and base, were compared using Student's *t*-test, where the Bonferroni correction was used to control the family wise type I error rate in multiple comparisons. For multiple comparisons of FS and MBF, a one-way analysis of variance (ANOVA) followed by Šídák's multiple comparison test was used where pairs of pre-selected data sets were compared. A *P*-value of <0.05 (two-sided) was used to establish statistical significance.

For the estimation of sample sizes necessary for hypothesis testing, we used the formula:

$$n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 x \sigma^2 / d^2$$

n, the calculated sample size; $Z_{1-\alpha/2}$, the critical value (associated with type I error rate α under the null hypothesis) on the standard normal distribution curve; $Z_{1-\beta}$, the critical value (associated with type II error rate β under the alternate hypothesis) on the standard normal distribution curve; σ is the standard deviation of the variable being studied; d, the magnitude of the difference that we expect to detect. For α and β we used probabilities of 0.05 and 0.20, with respective $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ values of 1.96 and 0.84 in a standard normal distribution.

Results

Echocardiographic analysis of cardiac function

Figure 1 (top) shows long-axis views of a Kv1.5$^{-/-}$ mouse in end-systole under control conditions (left), 2 weeks after TAC (middle), and 2 weeks after debanding (after 14 days of TAC, right). *Figure 1* (bottom) shows the measurements of FS in WT (bottom left) and Kv1.5$^{-/-}$ (bottom right) mice subjected to TAC (2 weeks) and following debanding (at 2 and 4 weeks). No differences in %FS were noted between the LV apex and base in WT under all conditions. In WT, %FS was equivalent in base and apex under all conditions. In contrast, the Kv1.5$^{-/-}$ mice exhibited ballooning the LV apex, i.e. negative %FS in the apex (−9 ± 4%), with contraction in the base (25 ± 9%). This indicated that when the base contracts during systole, the apex bulges. To show that the bulging is not due to an infarct or an apical aneurysm, a debanding procedure was completed to restore normal afterload, and within a few weeks of debanding systolic contractile function was restored in the apex. *Supplementary Table 1* reports the numerical values of fractional shortening for *Figure 1*.

Regional myocardial blood flow

To gain insight into MBF under baseline conditions and during acute metabolic stress (to induce metabolic hyperemia) we measured MBF during a NE stress test in the base and apex of the left ventricle in WT and Kv1.5$^{-/-}$ subjected to TAC (*Figure 2*). After TAC all Kv1.5$^{-/-}$ presented with TTS and apical ballooning. At baseline and during NE, MBF was equivalent in the apex and base in WT, but in Kv1.5$^{-/-}$ exhibiting TTS, flows were decreased in the apex compared to the base at baseline, and during NE ($P < 0.05$).

Figure 3 shows the effects of chromonar on %FS (top) and MBF (bottom) in Kv1.5$^{-/-}$ mice exhibiting TTS. Treatment with the selective coronary vasodilator, chromonar, not only induced recovery of contractile function (FS) in the apex (control vs. TTS: −19 ± 4% vs. 32 ± 3%), but also increased MBF in the apex and base of the left ventricle. Note, during the NE stress test, differences between MBF in the apex and base were magnified, but chromonar treatment abolished these differences both under basal condition and during NE. It is important to note that these changes occurred during TAC, so the rescue of flow and function occurred despite the continued high afterload induced by TAC. *Supplementary Table 2* reports the numerical values for fractional shortening for the results in *Figure 3* (top panel).

Supplementary data online, *Figure S1* shows FS in the apex and base of Kv1.5$^{-/-}$, TgKv1.5$^{-/-}$, and TgKv1.5$^{-/-}$+Dox mice following TAC. Doxycycline treatment induces the expression of Kv1.5 channels in smooth muscle in the TgKv1.5$^{-/-}$ line, but without Dox treatment, the animals are functionally null for Kv1.5 channels. Supplementary data online, *Figure S2* illustrates the ratio of blood flows between the apex and base in WT, Kv1.5$^{-/-}$ and TgKv1.5$^{-/-}$+Dox under baseline conditions and during NE administration. Note, in the Kv1.5$^{-/-}$ mice, the apex/base flow ratios were lower ($P < 0.05$) than in the other two groups under the three experimental conditions. These observations parallel the FS data shown in Supplementary data online, *Figure S1*.

RNA expression

Figure 4 shows the heat map results of RNA deep sequencing from the LV base and apex of Kv1.5$^{-/-}$ control and Kv1.5$^{-/-}$ + TAC for 7 days

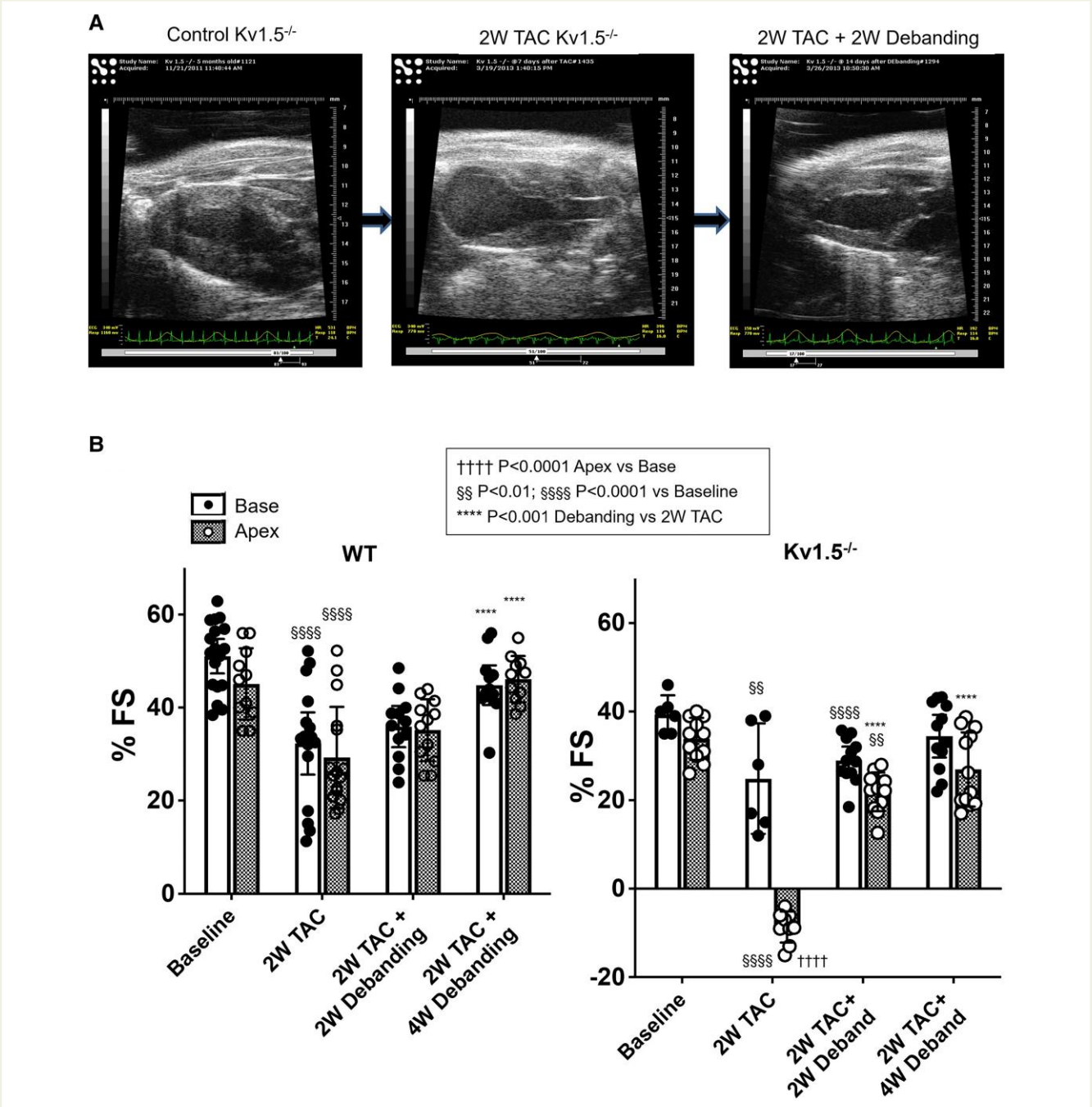


Figure 1 (*A*) B-Mode ultrasound end-systolic images of the left ventricular of Kv1.5$^{-/-}$ mice under control conditions, following transaortic constriction [2 weeks (2W)], and debanding (removal of the transaortic constriction) for 2 weeks. (*B*) Fractional shortening (%FS) of the left ventricular apex and base of wild-type and Kv1.5$^{-/-}$ mice under baseline conditions, 2 weeks after transaortic constriction (2W transaortic constriction), 2 weeks after debanding (2W transaortic constriction + 2W deband, 4 weeks after debanding (2W transaortic constriction + 4W deband). Sample sizes: wild-type-control, base $n = 18$; wild-type -control, apex $n = 11$; wild-type-transaortic constriction, $n = 16$; wild-type-2W transaortic constriction + 2W deband, $n = 12$; 2W transaortic constriction + 4W deband base, $n = 12$; 2W transaortic constriction + 4W deband apex, $n = 11$; Kv1.5$^{-/-}$-control base, $n = 6$; Kv1.5$^{-/-}$-control apex, $n = 11$; Kv1.5$^{-/-}$ −2W transaortic constriction + 2W deband, $n = 12$; Kv1.5$^{-/-}$ −2W transaortic constriction + 4W deband, $n = 12$. If base and apex are not mentioned, the sample sizes are equal for the two regions in the group. The data sets were analyzed by a one-way ANOVA followed by Šídák's multiple comparison test. ANOVA, analysis of variance; CI, confidence interval.

(with TTS). Differences in expression were evident in the Kv1.5$^{-/-}$—TAC vs. Kv1.5$^{-/-}$ controls.

Figure 5 shows the results of a gene set enrichment analysis (GSEA) in Kv1.5$^{-/-}$ mice under control conditions or following TAC (7 days), this analysis showed significantly downregulated genes involved in pathways of fatty acid metabolism, oxidative phosphorylation, and significantly upregulated genes involved in pathways of hypoxia (Kv1.5$^{-/-}$ + TAC) compared to Kv1.5$^{-/-}$ Controls.

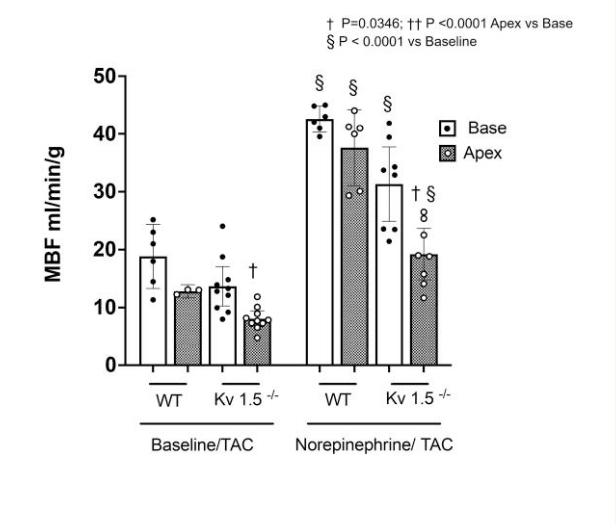


Figure 2 Myocardial blood flow in the left ventricular base and apex of wild-type and Kv1.5$^{-/-}$ mice (presenting with Takotsubo syndrome) after transaortic constriction surgery. MBF was measured under baseline conditions and during a norepinephrine stress test. Sample sizes: wild-type -control base, $n = 6$; wild-type -control apex, $n = 3$; wild-type -NE, $n = 6$, Kv1.5$^{-/-}$-Control, $n = 10$; Kv1.5$^{-/-}$-NE, $n = 8$. The data sets were analyzed by a one-way ANOVA followed by Šídák's multiple comparison test. ANOVA, analysis of variance.

To confirm the RNA sequencing we analyzed RNA expression from significantly changed genes in the pathway analyses (*Figure 6*). To increase the scientific rigor, we analyzed RNA expression from different animals (Kv1.5$^{-/-}$, Kv1.5$^{-/-}$—TAC, and $^{-/-}$, Kv1.5$^{-/-}$—TAC + chromonar), to confirm the sequencing data. Chromonar reversed many of the changes in gene expression back toward the untreated, naïve control. However, not every gene had its expression returned to baseline, which we believe is due to the continued presence of TAC. TAC creates a high afterload independent of chromonar. Importantly, we would like to emphasize that despite the continued presence of TAC we could restore normal contractile function of the apex, e.g. and restored expression of many genes to control levels. *Figure 7* illustrates differences in mRNA expression between the base and apex of Kv1.5$^{-/-}$ with TAC (presenting with TTS phenotype) and Kv1.5$^{-/-}$+TAC treated with chromonar. Note, chromonar treatment reversed many of the differences in gene expression between base and apex, e.g. UCP3, PFKb1, Mir-133a-2, but had less effect on others, e.g. Ltb4r2, Acaa2, Postn, Lox. The continued dysregulation in the expression of the genes could be related to the presence of TAC and the persistently increased afterload and/or a lingering effect of TTS.

Discussion

Our results support the conclusion that altered flow regulation in the heart precipitates TTS. This conclusion is based on several key observations. First, during TTS, MBF in the apex was less than in the base of the left ventricle; in contrast to the normal heart, where perfusion to the apex and base are similar. These lower levels of perfusion in the LV apex during TTS appeared to be causal in the apical ballooning because when myocardial perfusion was increased following chromonar treatment or via genetic re-expression of the Kv1.5 channel in smooth muscle, normal systolic function in the apex was restored.

Takotsubo syndrome was also associated with alterations in gene expression in the base and the apex of the left ventricle within the same heart and comparisons of expression in the apex from control and TTS hearts (both Kv1.5$^{-/-}$). Pathways associated with metabolism were decreased in the TTS hearts compared to the control mice. In contrast, pathways activated by hypoxia were elevated in TTS, these changes occurred in the base of the left ventricle. Pharmacological coronary hyperemia with chromonar restored many of these changes to levels seen in Kv1.5$^{-/-}$ controls, but several changes persisted. We propose these persistent changes are due to the maintenance of the ventricular afterload with not only can induce hypertrophy but also lead to heart failure if maintained for weeks. To place these results and conclusions in perspective, we will discuss an overview of TTS, regulation of coronary blood flow, myocardial adaptations to low flow, and study limitations.

Overview of takotsubo syndrome

A summary of the literature reveals little consensus about the causes of TTS. One of the hypothetical causes relates to excessive sympathoadrenal drive, elevations in circulating catecholamines, and augmented β-adrenergic signaling in the heart.[35–37] Another hypothesis speculated there is a switch in G-protein coupling leading to the ballooning phenotype.[36] Even in induced pluripotent stem cell-derived cardiac myocytes from patients with TTS, β-adrenergic signaling was enhanced.[38,39] Yet, it is reported that beta-adrenergic antagonists do not improve mortality, development, or recurrence in patients with TTS.[11,40–42] Moreover, some authors speculated that the augmented sympathetic activity was a response to the impaired ventricular function, rather than the cause.[43] Perhaps, the cause of TTS during sympathoadrenal excitation is not mediated via β-adrenergic signaling in cardiac myocytes; rather though the hemodynamic consequences of such activation. Hypertension, a consequence of sympathoadrenal excitation, has the strongest association with TTS.[44] Patients presenting with TTS also have endothelial dysfunction as evidenced by impaired flow-mediated vasodilation and endothelial oxidative stress,[45,46] which are also associated with hypertension. Interestingly, hyperthyroidism is also associated with TTS,[17,47,48] and this endocrine disorder has well-known systolic hypertension. If our thesis is correct, impaired vasodilation during a metabolic challenge, i.e. increased cardiac work, may be the precipitating event triggering TTS. We would be remiss to not mention the role of gender in TTS as the majority of cases occur in postmenopausal women,[49,50] leading to the speculation that reduced estrogen levels are central to the syndrome. However, women who have had an oophorectomy did not show an increased incidence of TTS,[51,52] which led to the speculation that ageing plays a key role in this process. Our purpose in the above overview is to reinforce the idea that there is no completely accepted consensus about TTS and that additional work is needed to more fully understand this clinical conundrum.

Regulation of coronary blood flow

The coupling of MBF to myocardial oxygen consumption, a process termed metabolic dilation/hyperemia, involves the production of metabolites that appear to activate voltage-gated potassium channels.[53–56] We reported previously that Kv1.5 channels, a member of the Shaker-related family of potassium channels, play a key role in connecting MBF to metabolism[28,57,58] Although there have been numerous candidates postulated as mediators of coronary metabolic dilation,[59] one putative factor, H_2O_2, has been found to open Kv1 family ion channels.[56,60] These observations by no means have ended the pursuit of a more complete understanding of how blood flow is regulated in

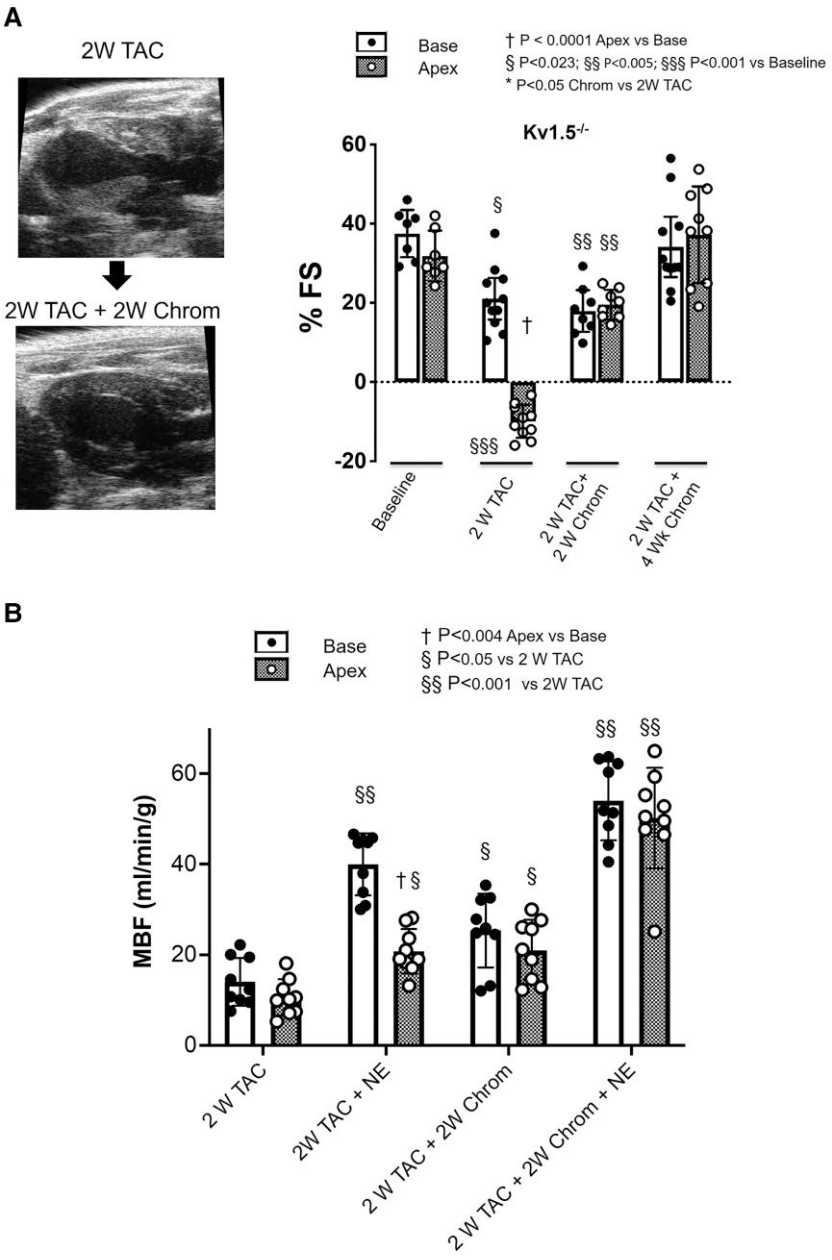


Figure 3 (*A*). Left: B-mode ultrasound end-systolic images of the left ventricle of Kv1.5$^{-/-}$ mice following transaortic constriction or transaortic constriction + chromonar (chrom). Note, despite the transaortic constriction, chromonar eliminated the Takotsubo phenotype. Right: fractional shortening (%FS) of the left ventricular apex and base of Kv1.5$^{-/-}$ mice under baseline conditions ($n = 7$), 2 weeks after transaortic constriction [2W transaortic constriction ($n = 11$ base; $n = 10$ apex)], 2 weeks of chromonar treatment after transaortic constriction [2W transaortic constriction + 2W chrom ($n = 8$)] and 4 weeks of chromonar treatment after transaortic constriction [2W transaortic constriction + 4W Chrom ($n = 11$ base; $n = 9$ apex)]. (*B*) Myocardial blood flow in the left ventricular apex and base of Kv1.5$^{-/-}$ mice following 2 weeks of transaortic constriction ($n = 9$), 2 weeks of transaortic constriction + Norepinephrine [NE ($n = 9$)], 2 weeks of chromonar treatment after transaortic constriction [2W transaortic constriction + 2W chrom ($n = 9$)] and 2 weeks of chromonar treatment after transaortic constriction + NE [2W transaortic constriction + 2W Chrom + NE ($n = 9$)]. The data sets were analyzed by a one-way ANOVA followed by Šídák's multiple comparison test. ANOVA, analysis of variance.

the heart but provide a perspective for the Takotsubo phenotype observed in mice null for Kv1.5 channels.

One aspect of the previous discussion bears significantly on the present findings, vis-à-vis, the role of Kv1.5 channels in facilitating the connection of MBF to myocardial oxygen consumption. Our previous investigation of Kv1.5 null mice revealed this redox-sensitive ion channel facilitates the connection of flow to metabolism in the heart. Although the knockout is global, the phenotype of impaired metabolic hyperemia is based on loss of the channels in vascular smooth muscle. We assert this because, in our previous study,[28] re-expression of Kv1.5 channels in smooth muscle in the global knockout rescued the impaired metabolic dilation. We believe this coronary insufficiency, which occurs

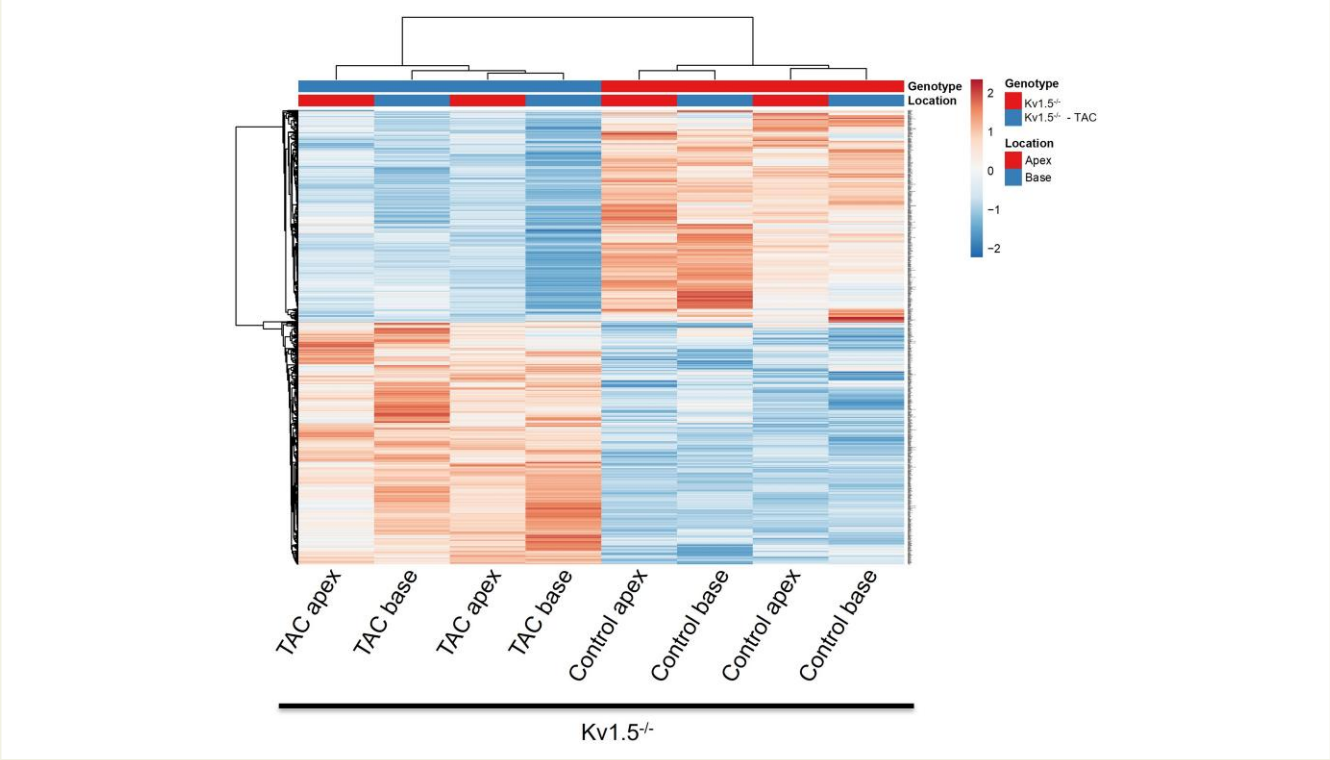


Figure 4 Heatmap with significantly different genes between apex and base of kv1.5$^{-/-}$ mice under control conditions and after transaortic constriction to produce Takotsubo syndrome. Rows are centered and unit variance scaling is applied to each gene. Rows and columns are clustered using correlation distance and average linkage. $n = 2$ per group.

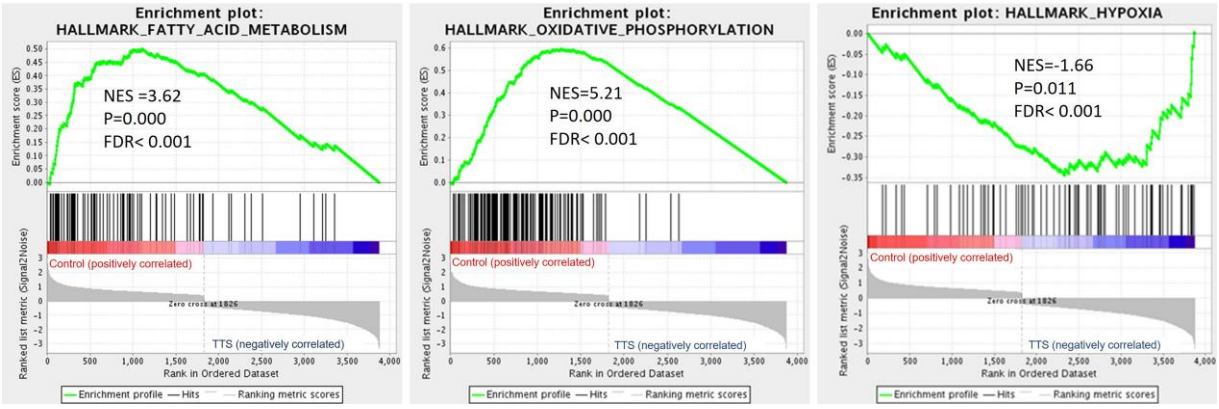


Figure 5 Enrichment plots of gene expression associated with pathways of fatty acid metabolism (left), oxidative phosphorylation (middle), and hypoxia (right). Comparisons were made between control Kv1.5$^{-/-}$ and those subjected to transaortic constriction exhibiting Takotsubo syndrome. Genes associated with the pathways of fatty acid metabolism and oxidative phosphorylation were downregulated in Takotsubo syndrome (vs. control), but the pathway associated with hypoxia was upregulated in Takotsubo syndrome. $n = 2$ per group.

in Kv1.5$^{-/-}$ mice, leads to the phenotype of TTS. Several observations support this contention. First, after imposition of the hemodynamic challenge by TAC in Kv1.5$^{-/-}$ mice, MBF in the apex of the Kv1.5$^{-/-}$ mice was less than in the base. This difference between base and apex was observed under basal conditions during TAC as well as heightened metabolism to produce metabolic hyperemia with NE. Second, these differences in flow between the apex and base, when eliminated with the vasodilator chromonar, restored normal contractile function to the apex; essentially eliminating the Takotsubo phenotype. Third, in Kv1.5 null mice, expression of the Kv1.5 channel in smooth muscle (Kv1.5$^{-/-}$-RC), increased MBF to the left ventricle and eliminated differences between the base and apex. Importantly these mice did not develop TTS, suggesting that the loss of metabolic coupling is a key to the appearance of the phenotype. Fourth, no such differences between MBF (basal or NE) to the base and apex were observed in WT mice corresponding to no differences in regional ventricular function.

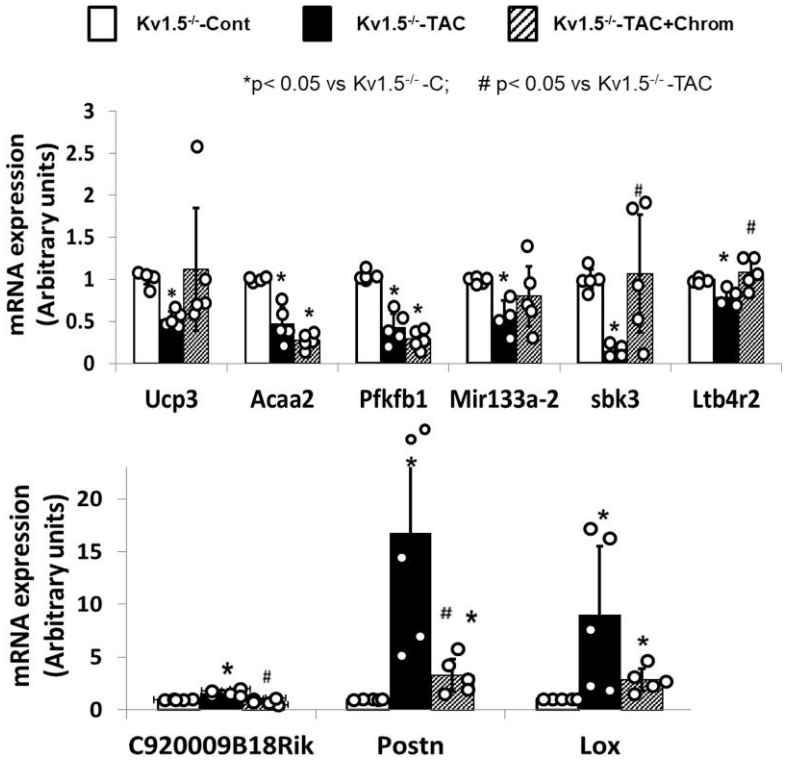


Figure 6 Real-time-polymerase chain reaction results of genes associated with metabolism (Ucp3, Acaa2, Pfkfb1) signaling, mitochondrial-localized kinases (Sbk3), cardiac function (Mir133a-2), apoptosis (C920009B18Rik), hypoxia (lox), and interstitial remodeling (postn). Chromonar restored or partially restored the expression of C920009B18Rik, Postn, Lox, Ucp3, and Sbk3 but did not alter the expression of Mir133a-2, Acaa2, or Pfkb1. Data are mean ± 95% CI ($n = 4$–5 per group). *$P < 0.05$ vs. corresponding Kv1.5$^{-/-}$-control group; # $P < 0.05$ vs. corresponding Kv1.5$^{-/-}$-transaortic constriction (Takotsubo syndrome) group. The data were analyzed by a one-way ANOVA followed by Šídák's multiple comparison test. ANOVA, analysis of variance; CI, confidence interval.

Taken together these observations suggest that an impairment in flow regulation, leading to blood flow insufficiency, in the area where the ballooning occurs causes TTS.

Most literature regarding the treatment of TTS recommends the therapies associated with heart failure management.[61] Vasodilators are not recommended, presumably because the hypotension induced by the drug will further increase sympathoadrenal drive and potentially worsen the condition. Importantly, chromonar does not have this side effect. Specifically, Opherk et al. found in humans, chromonar could induce maximal coronary dilation without changing arterial pressure or heart rate, which contrasted with dipyridamole that induced hypotension and a compensatory increase in arterial pressure.[32] However, the basis for the strong preference for chromonar to vasodilate only coronary blood vessels is still unknown. The drug was once approved for the treatment of obstructive coronary disease but was discontinued because of its effect in producing coronary steal.[62] Nevertheless, a coronary-specific vasodilator may have potential in the treatment of TTS in patients without obstructive coronary disease.

Myocardial adaptations to low flow

An intriguing aspect of the RNAseq results pertains to the molecular changes in gene expression that provide insight into the TTS phenotype. First, in a comparison of transcripts between the control Kv1.5−/− and those subjected to TAC exhibiting TTS, pathways associated with metabolism and oxidative phosphorylation are downregulated in TTS. These decreases during the TTS phenotype resemble a condition described decades ago as 'hibernating myocardium'.[63,64] Myocardial hibernation challenged the concept that during an imbalance between myocardial oxygen demand and supply, where supply is insufficient, irreversible damage and compromised mechanical function will occur if the flow is not restored. Myocardial hibernation occurs during a chronic decrease in perfusion to the ventricle, but the level of flow is still sufficient to maintain the viability of the tissue.[64] As long as perfusion remains inadequate, cardiac function is impaired. Interestingly, myocardial hibernation was thought as a protective mechanism in that the reduction of myocardial demands, matched the reduced level of flow to limit ischemia and necrosis. Although we did not perform an entire transcriptomic analysis of mice subjected to chromonar treatment, some of the RNA transcripts associated with metabolism were changed toward control levels during the pharmacological coronary hyperemia. We also note that chromonar did not restore every transcript to control levels—which is to be expected as the hemodynamic challenge produced by TAC is still present. TAC is often used as a model to produce heart failure and LV hypertrophy[65,66] and importantly chromonar will not affect ventricular afterload, only coronary blood flow.

Another interesting facet of the RNAseq results was the upregulation of genes associated with hypoxia in TTS. This upregulation occurred in the base and not in the apex, which seems counter-intuitive given that the apex has reduced perfusion compared to the base.

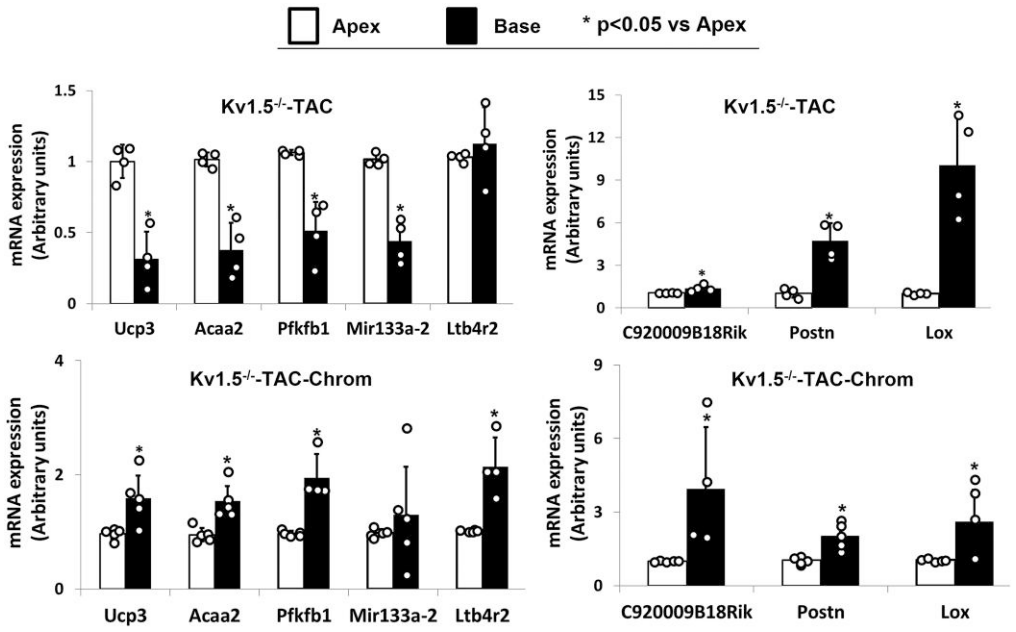


Figure 7 Real-time- polymerase chain reaction data for apex vs. Base in hearts from Kv1.5$^{-/-}$- transaortic constriction and Kv1.5$^{-/-}$- transaortic constriction plus chromonar (chrom) groups. Data are mean ± 95% CI ($n = 4$ to 5 per group). *$P < 0.05$ vs. apex. The data sets were analyzed by a one-way ANOVA followed by Šídák's multiple comparison test. ANOVA, analysis of variance; CI, confidence interval.

However, if the concept of the apex being in a state of hibernation is correct, down-regulation of function to match the low flow would restore oxygen balance and ameliorate hypoxia. We postulate the base is hypoxic because of increased levels of cardiac work (working without a functional apex), and the limitations in metabolic dilation associated with the loss of Kv1.5 channels.

Study limitations

One limitation of our study pertains to our model of TTS, which occurs in a murine model null for the Kv1.5 channel. Because the knock-out is global, there could be many cell types involved in the TTS phenotype; however, re-expression of the channel in vascular smooth muscle prevented the development of TTS. This result from re-expression of the channel in smooth muscle lessens the concern about off-target effects of a global knockout. Another limitation is that we precipitated the TTS with transaortic condition to simulate hemodynamic stress, which is different than most of the 'triggers' described for the human population.[36,67] However, we would argue that if ischemia is the consequence of the trigger, due to impairments in coronary regulation,[2] this could explain why so many different triggers have been reported, and why endothelial dysfunction is now viewed as a risk factor for TTS.[68] Nevertheless, we would be remiss to not mention that TAC is a very different stressor than the reported triggers in patients. Finally, another limitation pertains to our use of FS to evaluate the extent of apical ballooning. This measurement can be subjective in terms of the location in the apex where the FS is measured. A different, and perhaps, better approach would have been the assessment of systolic LV strain, which unfortunately we did not complete.

Conclusions

Our results support the concept that TTS is the result of CMD and insufficient myocardial perfusion. Interventions designed to increase blood flow in the apex of the heart, pharmacologically via chromonar or genetically via expression of Kv1.5 channels in smooth muscle, restore function even in the face of the increased hemodynamic challenge that provoked the condition. We also speculate that the coronary vasodilator, chromonar, may hasten recovery in patients afflicted with TTS.

Supplementary data

Supplementary data is available at *European Heart Journal* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding authors.

Conflict of interest

Drs. Chilian, Ohanyan, and Yin are co-founders of KromTherapeutics, and have filed a patent for the use of chromonar in the treatment of Takotsubo Syndrome. The remaining authors have nothing to disclose.

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