Investigating the β-Adrenergic Response in a *TNNC1* I4M Mouse Model of Dilated Cardiomyopathy

Brittany Mears, Leana Zade, Paula Nieto, Jose R Pinto, Marie Derbes
Department of Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL, USA.

FSU UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM
CENTER FOR UNDERGRADUATE RESEARCH & ACADEMIC ENGAGEMENT

Background

Dilated cardiomyopathy (DCM) is a condition of the myocardium marked by ventricular enlargement and ventricular wall thinning causing systolic disfunction. The estimated prevalence of DCM is about 1 in 250 individuals. Among the most common causes of DCM is genetic variants in the sarcomeric proteins, the sarcomere being the basic unit for striated muscle contraction. Studying sarcomeric function allows a better understanding of how these mutations are likely pathogenic. Cardiac Troponin C (cTnC), encoded by *TNNC1*, is the calcium binding protein of the myofilament which allows contraction to occur. Variants in *TNNC1* are considered a definitive cause of cardiomyopathy. Our lab developed a mouse model carrying a missense mutation in *TNNC1*, the I4M (Isoleucine at position 4, Methionine), this heterozygous mutation was identified in a pediatric patient diagnosed with severe DCM. The β-adrenergic pathway is a key signaling mechanism of the sympathetic nervous system that enhances heart rate during the fight-or-flight response. However, prolonged activation can lead to desensitization, reducing its effectiveness over time. Here, we aim to characterize the cardiac phenotype of this new mouse model and to investigate if its β-adrenergic response is altered.

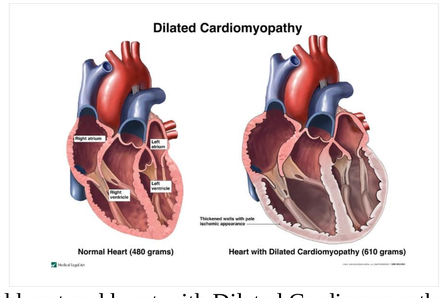

Figure 1. Normal heart and heart with Dilated Cardiomyopathy (U.S. National Library of Medicine, n.d.)

Methods

Mice were shaven, given an anesthetic of isoflurane mixture with oxygen, then place on a table and secured. Then, mice were subjected to the first echocardiogram photo at baseline, observing the heart's ability to pump blood and recirculate that blood to the rest of the body. Once the baseline was established, mice were injected with the β-adrenergic drug dobutamine to assess their heart function under sympathetic nervous system activation by the β-adrenergic pathway. We administered the drug to mice at a dose of 0.75 mg/kg and assessed their cardiac response using echocardiography at baseline and five minutes post-injection. FUJIFILM VisualSonics Inc.'s (VSI) Vevo® F2 Imaging System. Long axis (B-mode), short axis (m-mode) were captured. 8-week-old mice were used, including male and females. WT (n=8): 4 males, 4 females, I4M +/- (n=8): 4 males, 4 females.

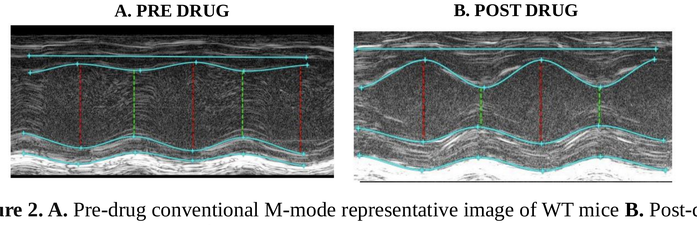

Figure 2. A. Pre-drug conventional M-mode representative image of WT mice **B.** Post-drug M-mode representative image of WT mice

Results

Most of the functional and structural parameters were significantly increased in both groups after dobutamine injection. The key functional parameters ejection fraction (EF%) and fractional shortening (FS%) were significantly increased, with the percentage change being higher in the +/- group.

WT			
0.75mg/kg Dobutamine Echo parameter	Pre	5 min post	P-value
HR (bpm)	411 ± 7	546 ± 16	6.54E-05
Ejection Fraction (%)	56.9 ± 2.9	90.2 ± 1.4	7.15E-06
Fractional Shortening (%)	29.6 ± 2.1	54.4 ± 4.6	0.001
LVID;d (mm)	3.89 ± 0.16	2.70 ± 0.1	2.70E-04
LVID;s (mm)	2.78 ± 0.19	1.12 ± 0.08	1.24E-05
LVPW;d (mm)	0.71 ± 0.06	1.19 ± 0.12	0.008
LVPW;s (mm)	1.07 ± 0.07	1.71 ± 0.09	6.58E-04
LVAW;d (mm)	0.71 ± 0.05	0.88 ± 0.03	0.02
LVAW;s (mm)	1.02 ± 0.06	1.53 ± 0.06	6.13E-04
RWT (mm)	0.37 ± 0.03	0.90 ± 0.10	0.002
CO (mL/min)	15.1 ± 0.9	14.47 ± 1.4	0.70
Stroke Volume (uL)	36.9 ± 2.5	26.5 ± 2.4	0.02

I4M			
0.75mg/kg Dobutamine Echo parameter	Pre	5 min post	P-value
HR (bpm)	433 ± 12.31	557 ± 10.7	1.00E-03
Ejection Fraction (%)	37.1 ± 1.8	75.6 ± 5.7	1.00E-03
Fractional Shortening (%)	17.8 ± 1.0	45.3 ± 4.9	0.002
LVID;d (mm)	4.27 ±0.09	3.34 ± 0.25	7.00E-03
LVID;s (mm)	3.61± 0.09	1.79 ± 0.26	2.18E-04
LVPW;d (mm)	0.63 ± 0.04	0.96 ± 0.15	0.05
LVPW;s (mm)	0.79 ± 0.04	1.47 ± 0.15	4.56E-03
LVAW;d (mm)	0.70 ± 0.04	0.88 ± 0.08	0.04
LVAW;s (mm)	0.89 ± 0.04	1.32 ± 0.17	0.04
RWT (mm)	0.30 ± 0.02	0.63 ± 0.14	0.05
CO (mL/min)	13.7 ± 1.2	19.6 ± 2.2	0.06
Stroke Volume (uL)	31.5 ± 2.3	35.3 ± 4.1	0.41

Table 1: Numerical values of *in vivo* echocardiography of control (left panel) and I4M mice (right panel) pre and 5 minutes post-injection with 0.75 mg/kg Dobutamine. Values represent mean ±S.E.M. for 8 control and 7 I4M +/- mice with Dobutamine. P-values vs. pre-injection using a two-way ANOVA analysis with Šídák's multiple comparisons test.

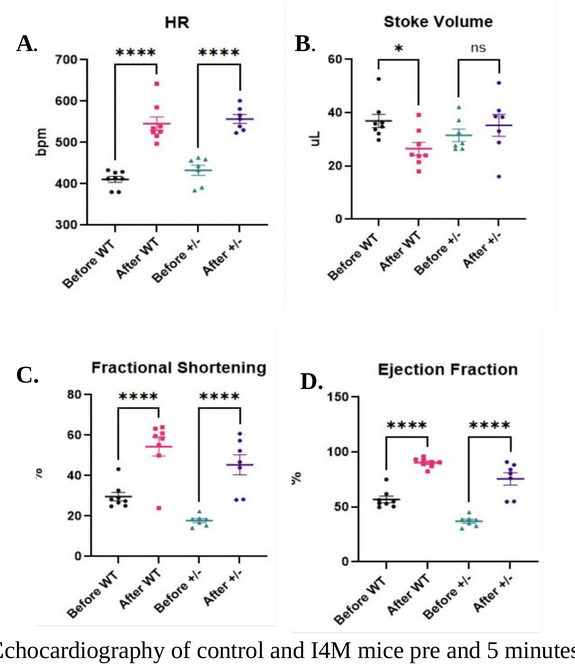

Figure 3. Echocardiography of control and I4M mice pre and 5 minutes post-injection with 0.75 mg/kg Dobutamine **A.** Heart Rate (HR). **B.** Stoke volume. **C.** Fractional Shortening. **D.** Ejection Fraction. Values represent mean ±S.E.M. for 8 control and 7 I4M +/- mice with Dobutamine. P-values vs. pre-injection using a two-way ANOVA analysis with Šídák's multiple comparisons test.

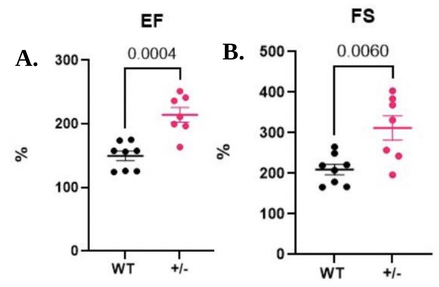

Figure 4. Percent change between control and I4M mice pre and post-injection. **A.** Ejection fraction (EF). **B.** Fractional Shortening (FS)

Conclusions

Genetic causes account for 30-40% of DCM and is associated with genes that encode a heterogeneous group of molecules that partake in force generation and transmission, cytoskeletal and nuclear architecture, sarcomere integrity, electrolyte homeostasis, transcription, and mitochondrial function (Paris 2019). renin-angiotensin-aldosterone (RAA) system inhibition and β-adrenergic blockade present to markedly attenuate or reverse left ventricle (LV) remodeling in patients with heart failure and LV dilation2. Both WT and mice with the I4M mutation were shown to be affected by the β-adrenergic drug, dobutamine. This is indicated by the results and response that was recorded during echocardiography. The indication of the drugs ionotropic effect on both groups gives insight to its effect and proper ability to impose the β-adrenergic response in the cardiomyocytes. The results of stoke volume and heart rate show no significance in their comparison response between the two groups. There is a small difference between WT and mutated mice indicated in the results for the fractional shortening and ejection fraction; however, these differences are still considered insignificant, with a P-value less than or equal to 0.005. Overall, these results showed that both the I4M +/- and WT mice responded to the drug. We aim to further compare the drug response between both groups using statistical tests to assess if there was a significant difference in the drug response.

References

1. U.S. National Library of Medicine. (n.d.). Dilated cardiomyopathy: Medlineplus medical encyclopedia. MedlinePlus. https://medlineplus.gov/ency/article/000168.htmf
2. Paris, V. D. (2019, May 18). *Pathophysiology*. Dilated Cardiomyopathy: From Genetics to Clinical Management [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK553848/
3. U.S. Department of Health and Human Services. (n.d.). *Types*. National Heart Lung and Blood Institute. https://www.nhlbi.nih.gov/health/cardiomyopathy/types