Functional Characterization of Cardiomyocytes in a DCM Mouse model

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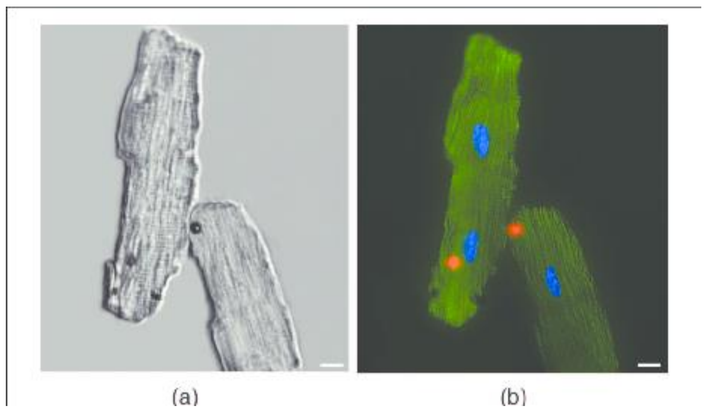
Introduction

Dilated cardiomyopathy (DCM) is a cardiac muscle disorder characterized by ventricular dilation and impaired cardiac contractility, often progressing to heart failure and cardiac death. Rare genetic variants are found in up to 25% of DCM cases. In this study, we investigate a new variant in the TNNC1 gene, which encodes for cardiac troponin C (cTnC), a key calcium-binding protein of the thin filament responsible for initiating sarcomeric contraction. The cTnC protein plays a central role in calcium (Ca^{2+}) handling, as it binds Ca^{2+} ions during systole to activate cross-bridge cycling and releases it during diastole to allow relaxation. The variant studied here was previously identified in a pediatric patient diagnosed with DCM.

Our goal is to study the in vivo functional effects of this variant using a CRISPR/Cas9 mouse model our lab developed, focusing on measuring calcium (Ca^{2+}) transients and contractility at the cellular level. We hypothesize that this mutation alters the Ca^{2+} sensitivity of troponin C, disrupting normal sarcomeric function.

To test this, we isolate primary adult cardiomyocytes from mouse hearts, incubate them with a Ca^{2+}-sensitive dye, and record Ca^{2+} transients and cell shortening during electrical pacing. Some of the parameters that we will analyze include resting and peak intracellular Ca^{2+} ($[Ca^{2+}]_i$), time to peak for $[Ca^{2+}]_i$ and sarcomere shortening.

The results from this study may enhance our understanding of DCM pathophysiology, particularly its impact on Ca^{2+} dynamics, and contribute to the development of targeted therapeutic strategies



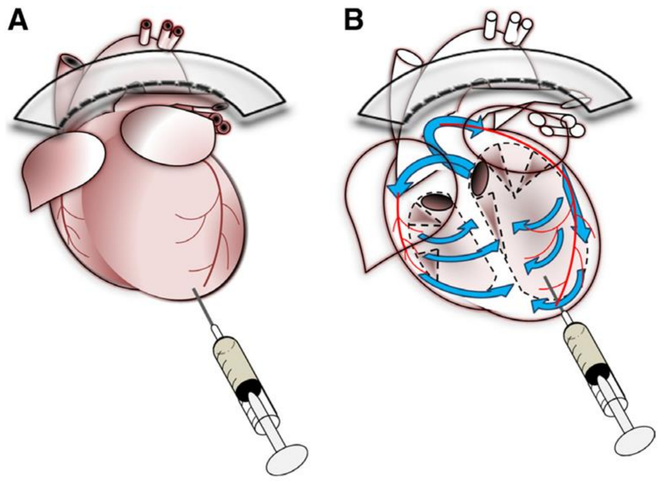
[1]*Figure 1*. Representative image of a cardiomyocyte. **A.)** Shows a cardiomyocyte under a microscope in brightfield, **B.)** Displays the same cardiomyocyte under fluorescence.

Methods

The procedures begin with creating a several solutions; a perfusion buffer, digestion buffer, a calcium solution, and a stop solution. The perfusion buffer will be used to perfuse the mouse cardiomyocyte, the digestion buffer will be used to digest collagen and excess cells, and the stop solution will be used to stop the digestion of the heart to avoid over-digestion. These solutions have their pH adjusted to 7.4 using NaOH. Once the solutions are prepared, a mouse is obtained. This mouse will be anesthetized with isofluorane. The chest of the mouse is then opened, and the heart is clamped from the aorta and cut. The heart is then placed into a petri dish containing 3mL of an EDTA buffer injected with e same EDTA buffer for around 2 minutes with 7ml of the buffer into the left ventricle. It is then placed on a petri. The purpose of the EDTA is to bind to the calcium ions throughout the heart and prevent contraction throughout this isolation process.

The heart is then moved to a petri dish containing 5mL of perfusion buffer and injected in the same hole with 3mL of perfusion buffer. The perfusion buffer is used to maintain a somewhat ideal physiological conditions in the heart.

After, the heart is dipped into a petri dish filled with digestion buffer and has digestion buffer injected for 20 minutes at a rate of 1.6ml/min. Once the heart is flaccid, apply the stop solution to stop further digestion. Next, we tear through the heart with forceps and pipette it, placing it on a filter and letting gravity filter it out. We transfer the pellets to the calcium solution and then record the contractility.



[2]F*igure 2.* Displaying the clamping of the aorta and the injection of the left ventricle. The blue arrows indicate the flow of the injected substances.

Expected Results

While our investigation is still ongoing, our hypothesis was that the calcium transients in the mutant group will be significantly different compared to the wildtype group. We assume that the mutants will demonstrate prolonged and taller peaks compared to the wildtypes, due to an increased myofilament sensitivity to calcium.

In instances where myocardium were put under isometric tension, the left ventricular myocardium with DCM was seen to have delayed peaks. When we measure the calcium transients, we will expect to see delayed peaks and increased amplitudes.[3] This is important to note as this study focused on human hearts.

Other studies have noticed a significant, proportional relationship between the chance of developing a ventricular tachycardia and the sensitivity to Ca2+, and goes as far as to say that sensitivity is a direct cause of the arrhythmia.[4] An important to feature is to note that this was seen in mouse models.

An intact isolated cell should be rod shaped, as seen in Figures 1 and 3. A dead cardiomyocyte will be round with edges, as seem in the small circle in Figure 3.

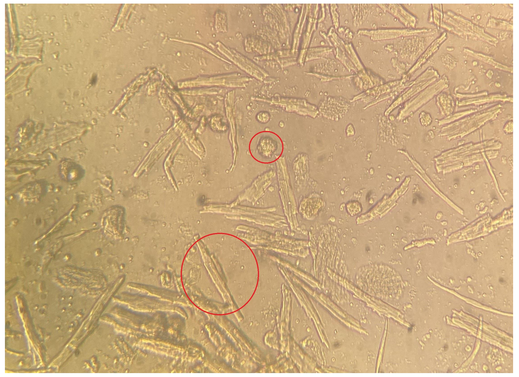


Figure 3. Isolated Cardiomyocyztes. The big circle indicates an in-tact cardiomyocyte and the small circle displays a dead cardiomyocyte.

References

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