Quantification of Mutant Protein in Heterozygous Hearts of a *TNNC1-Ile4Met* Dilated Cardiomyopathy Mouse Model

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Introduction

Dilated Cardiomyopathy (DCM) is a myocardial disease that causes systolic dysfunction and can lead to heart failure (Johnston et al.,2019). This disease can be caused by genetic variants in the sarcomeric proteins, such as troponin C (cTnC) (Landim-Vieira et al.,2020). Here we study a missense mutation in *TNNC1*, which encodes for the mutation cTnC I4M, that has been associated with DCM in a pediatric patient (Landim-Vieira et al.,2020). Using a cTnC-DCM mouse model created through CRISPR/Cas9, we investigated the protein profile of the key sarcomeric proteins such as the troponin complex, tropomyosin, actin, and myosin light chains. Previous studies have shown that mutations in *TNNC1* cause an increase in myofilament Ca2+ Sensitivity which leads to hypercontractility (Landim-Vieira et al.,2020). The cTnI-I4M mice, when homozygous, do not yield, indicating that 100% of the mutant protein is lethal at the embryonic level. We aim to characterize the protein expression levels of sarcomeric proteins of a TnC mouse model vs a wild-type (WT) using myofibrils and whole cell extract from mouse hearts. We used the traditional 1D SDS-PAGE gel electrophoresis in conjunction with protein identification by mass spectrometry (MS). After electrophoresis, gels were stained with Coomassie brilliant blue and imaged for densitometry analysis. MS results showed the ratio of mutant to wildtype of the cTnC protein in the heterozygous mice offering explainations of the possible pathogenesis observed. Next, we want to compare the amount of mutant cTnC protein in myofibril extraction vs whole cell extract. This work on the analysis of the key sarcomeric proteins in the role of DCM pathogenesis can provide new insights into cellular mechanisms involved in cardiac dysfunction.

Materials & Methods

Whole cell extract --> RIPA buffer
Determination of Protein Concentration --> Pierce BCA
SDS-PAGE and Coomassie staining --> 12% SDS

Discussion

We isolated whole-cell lysate and myofibrils from the left ventricles of wild-type and heterozygous mice. First, we performed Coomassie Blue staining to assess protein levels, as this dye binds to proteins. As expected, myofibril extractions showed fewer bands compared to whole-cell lysates.

We then conducted Western blot analysis to identify the location of TnC, TnT, and TnI in our samples. However, we were unable to distinguish the mutant protein using either Western blot or Coomassie staining. To overcome this, we performed LC-MS to quantify the ratio of mutant TnC to wild-type protein. Our results indicate that ~60% of the TnC in the myofibril samples is the mutant form.

Next, we will perform LC-MS on whole-cell lysates to determine whether the mutant-to-wild-type ratio differs from that in the myofibril fraction.

Results

Myofibril Extract

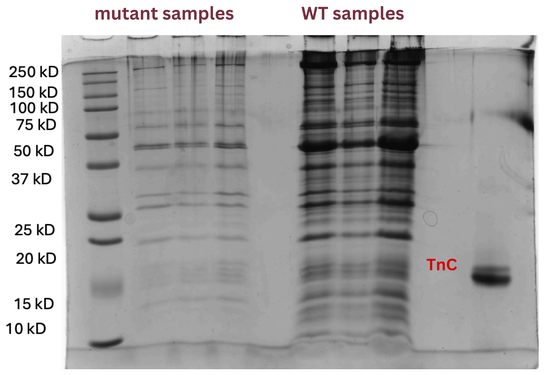

Figure 1. 12% SDS-PAGE Coomassie blue stained myofibril extract proteins

Whole Cell Extract

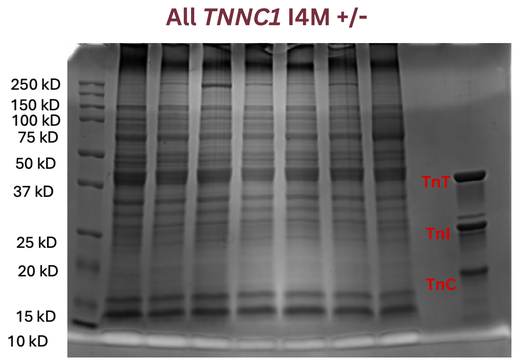

Figure 2. 12% SDS-PAGE Coomassie blue stained oxidized whole cell extract mutant proteins

Western Blot

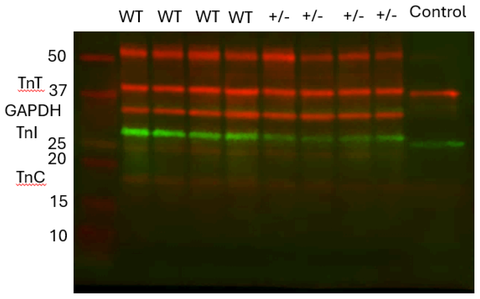

Figure 3. Western Blot showing precise location of Troponin Complex

Liquid Chromatography-Mass Spectrometry (LC-MS)

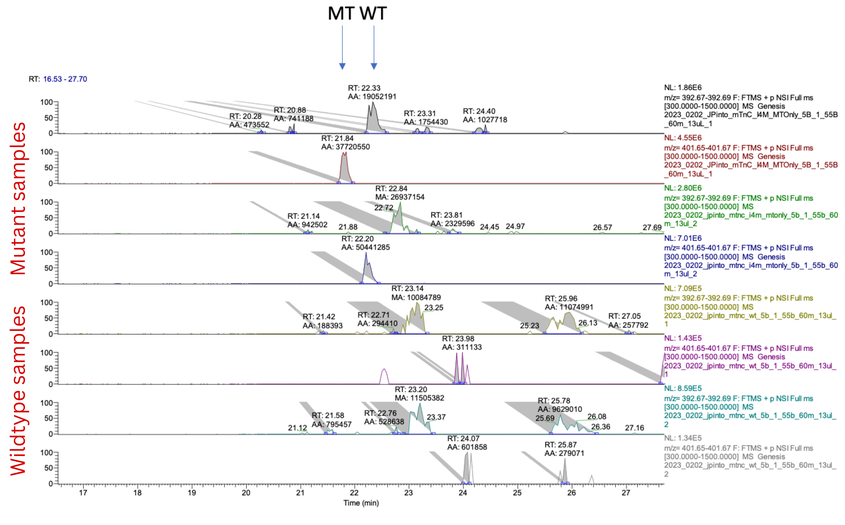

Figure 4. Mass Spectrometry analysis shows expression of Mutant and Wildtype protein across samples

Sample	MT/(MT+WT) Avg	SD
186	8.02%	0.06%
179	66.35%	0.93%
180	65.84%	0.24%
244	61.54%	0.48%
247	60.11%	0.32%
248	63.66%	1.30%
240	62.82%	0.64%
241	65.50%	0.50%
242	66.80%	0.43%

Figure 5. Mass Spectrometry analysis quantification of mutant protein expression in myofibril samples

References

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Landim-Vieira, M., Johnston, J. R., Ji, W., Mis, E. K., Tijerino, J., Spencer-Manzon, M., Jeffries, L., Hall, E. K., Panisello-Manterola, D., Khokha, M. K., Deniz, E., Chase, P. B., & Pinto, J. R. (2020). Familial dilated cardiomyopathy associated with a novel combination of compound heterozygous TNNC1 variants. Frontiers in Physiology, 10, Article 1612. https://doi.org/10.3389/fphys.2019.01612