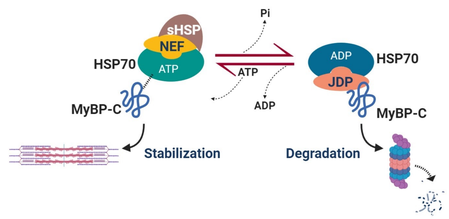
# Hypertrophic Cardiomyopathy: An Investigation of MyBPC and HSP 70 Co-Chaperone Family using siRNA

Taussia Boadi[1], Sharlene Day[2], Jaime Yob[2]

College of Arts and Sciences, University of Pennsylvania C'24[1], Center for Inherited Cardiac Disease, Division of Cardiovascular Medicine and the Cardiovascular Institute[2]

## Introduction

Hypertrophic Cardiomyopathy (HCM) is a genetic, autosomal dominant disorder caused by mutation in myofilament genes. Characterized by left ventricular fibrosis and hypertrophy, HCM is the leading cause of embolic stroke, heart failure, and sudden death in early-mid adult life. Myosin Binding Protein C (MyBPC) is the most mutated gene often with this disorder and is the focus of this research. MyBPC is a substrate of cyclic AMP dependent protein kinases and has numbers functions within the cardiovascular system. More specifically, MyBPC contributes to thick filament structure and helps regulate heart contractions. Mutations in the MyBPC gene are primarily nonsense mutations, or single substitution mutations that result in a premature stop codon. This is unlike other sarcomere genes that usually have missense mutations (single nucleotide substitution). It has been hypothesized that MyBPC is a client of the Heat Shock Protein (HSP) 70 family of chaperone proteins. This is significant because The knockdown of HSC 70 slows the degradation of WT and mutant MyBPC, while an HSP 70 activator accelerates its degradation.



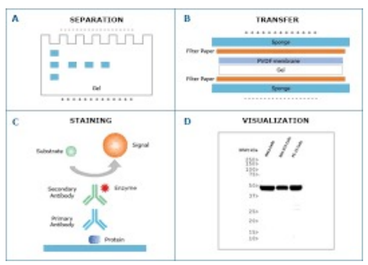

## Project Aims

The main goal of this project was to define a co-chaperone network of MyBPC and determine its other client proteins. We wanted to determine the other clients that the HSP 70 chaperone family interacts with, ultimately allowing us to find a way to modulate co-chaperones specific to MYBPC that won't interfere with the other clients that HSP 70 interacts with.

## Methods

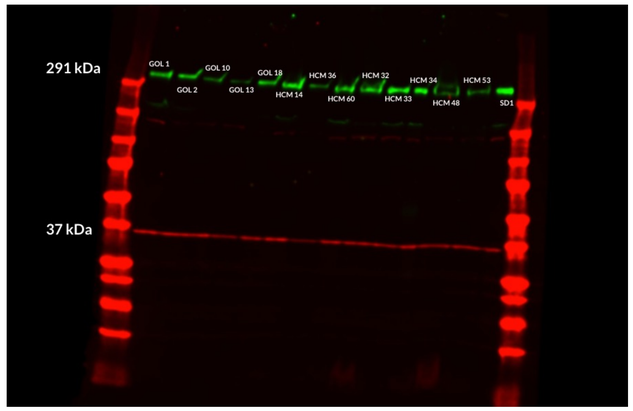
In order to define the co-chaperone network of MyBPC, siRNA was used. siRNA is a noncoding, double stranded RNA molecule that functions as an RNA silencer. In these experiments, siRNA was used to decrease the expression of, or knockdown, certain proteins. Cardiomyocytes were treated with BAG3, MyBPC3, DNAJC18, HSPB 70, HSC 70, and TNNT siRNA.

Following the treatment, the cells were prepared for use in Western Blots, a form of electrophoresis used to separate and identify proteins. The Western Blots were used as a confirmatory tool to ensure that there was protein knockdown. Western Blots were also utilized to determine if certain antibodies were compatible with the human tissue samples in the lab.




## Results

Figures 1 &2: Test of FLMN-C (abcam) Antibody on Varying HCM Tissue



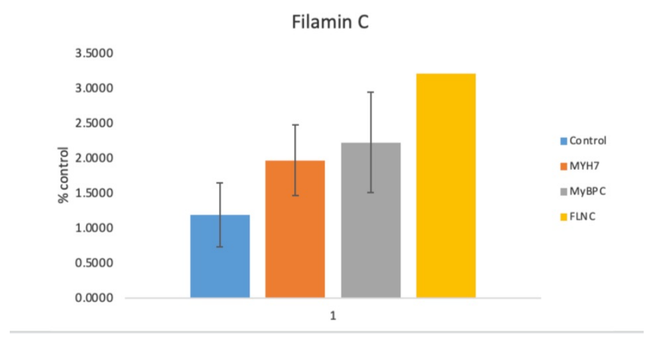
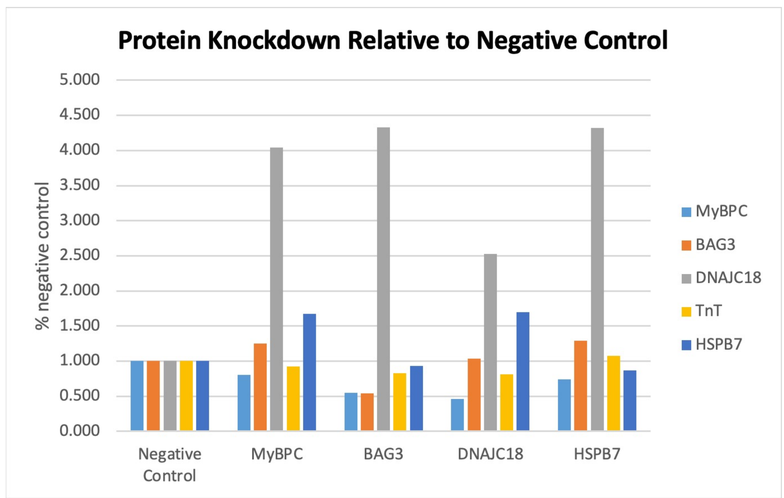




Figure 3: Test of Protein Knockdown using siRNA




## Discussion

Figures 1&2 show the results of a western blot that was used to determine the efficacy of the Filamin C (FLMN C) abcam antibody on various human tissue samples. GAPDH was used to confirm that the Western Blot was successful and is represented by the red bands at 37 kDa. Figure 1 shows the scan of the membrane used in the Western Blot. The bands were quantified in order to obtain raw data. From the raw data, a ratio of FLMNC to GAPDH was created. This ratio was used in order to normalize for the protein loading. The data was then normalized to the control, the GOL samples, providing the percent increase in the amount of FLMNC protein in the other samples over the control, represented in figure 2. The SD1 cells with FLMN C mutations had the highest amount of FLMNC protein, which could be attributed to protein aggregation and lack of degradation in the cells. The standard deviation were not included for the SD1 FLMN C mutated cells because there was only one data point.

Figure 3 shows the quantification for a Western Blot for cells treated with MyBPC, BAG 3, DNAJC18, Troponin T, and HSPB 7 siRNAs. The gels obtained from the western blots were stained with MyBPC, BAG 3, DNAJC18 and HSPB 7 antibodies, represented on the x-axis. Typically, 50% or more knockdown is expected when cells are treated with siRNA. Based on the data in the figure, there was knockdown observed with all the different treatments, but the cells treated with DNAJC18 showed the most significant knockdown. Although there was knockdown, it was not at the expected threshold, indicating that repeat experiments might be necessary to confirm that the siRNA is effective.

## Conclusion and Next Steps

- In the Filamin C antibody test, the SD1 tissue contained the highest amount of FLMN C, which can likely be attributed to protein aggregation. In the future, this FLMN C antibody could be retested multiple SD1 samples to determine if the trend seen with this experiment remains consistent
- While knockdown was observed in the siRNA experiment, it was not to the extend usually expected when using siRNA. As a result, this experiment could be repeated to account of experimental error. Additionally, alternate knockdown methods could be utilized instead of siRNA, as the siRNA might not work as well with the cells the lab uses.

## Acknowledgements

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