Biomechanical Effects of Hypertrophic Cardiomyopathy Causing Mutations in the Lever Arm of Human Beta-Cardiac Myosin

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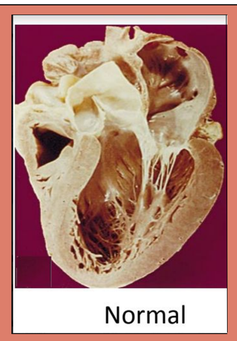
INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a heart disease that affects an estimated 1 in 500 people worldwide. It is caused by genetic mutations that affect the proteins responsible for causing heart muscle to contract (Seidman, 2017). These mutations affect the structure and function of the heart by causing the walls of the ventricles to thicken (see Figure 1), which can be lethal (Colgrave, 2014). The thickening of the wall between the ventricles reduces the amount of blood that can fit in each ventricle, and therefore how much blood can be sent through the body with each contraction. This can result in light headedness, as there is less blood going to the brain. In extreme circumstances, this thickening can block the outflow of blood from the ventricles and requires a surgical procedure to manage. Though medication (such as beta blockers, which slow a patient's heart rate and allow the ventricles to fill up with more blood between each contraction) can be given to patients with HCM to manage symptoms, HCM still poses a serious and potentially lethal threat and there is no known absolute cure (Seidman, 2017).



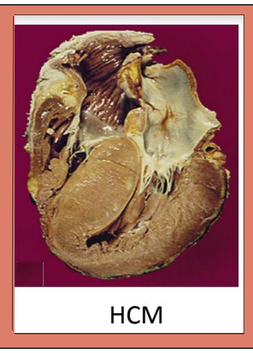


Figure 1: A normal heart (left) has noticeably thinner walls and more space in the ventricles than the heart with hypertrophic cardiomyopathy (right).

MY WORK

I started my work by designing primers which could be used to perform PCR mutagenesis. The primers introduced the hypertrophic cardiomyopathy causing mutation L781P into the MYH7 gene (see Figure 3), which codes for the human beta-cardiac myosin motor. I introduced the plasmids to bacteria and let them grow so that they could produce more of the mutated DNA. I then extracted the DNA from the bacteria and had it to be sequenced to verify the mutation. Because sometimes the bacteria will not take up the mutated plasmid, I had to get the extracted DNA sequenced to make sure that it actually had the desired mutation. Unfortunately, the first bacteria I extracted DNA from did not have the mutation, forcing me to try other colonies from the same plate, and when those didn't have the mutation, forcing me to start over from the PCR step.

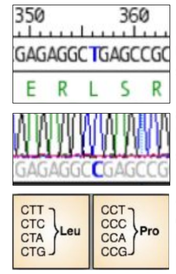


Figure 4: Sections of the gene that I modified. The top shows the original sequence as well as the amino acids they code for. The middle shows the altered sequence with the changed base bolded in blue. The bottom image shows the combinations of three bases that code for both Leucine and Proline. As is shown in the top two images, I changed CTG, which codes for Leucine, to CCG, which codes for Proline.

Once PCR mutagenesis was completed successfully, I had little trouble subcloning the mutant plasmids into the adenoviral vector.

I am currently going through the virus amplification step. Though this should not take too much longer, however I will not have it completed in time for the AAR showcase.

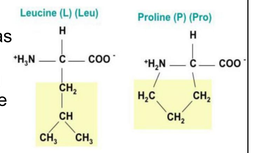


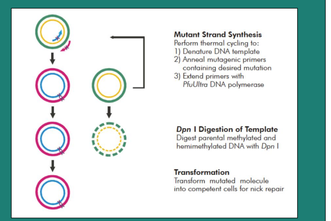
Figure 5: The chemical makeup of Leucine and Proline. All amino acids consist of a hydrogen (top) an amino group (left) and a carboxyl group (right).

Long-Term Spudich Lab Work Overview

Create Mutagenic Primers

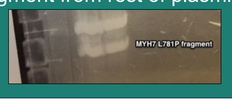
- Design oligonucleotide primers, introducing desired amino acid change (see Figure 4)
- Have primers commercially synthesized

PCR Mutagenesis



Subclone Into Adenoviral Vector

- Digest plasmid containing mutation with restriction enzymes
- Run DNA fragments through agarose gel to separate mutant fragment from rest of plasmid



Transfect Adenoviral Plasmid into HEK Cells

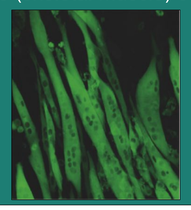
The adenoviral vector plasmid is transfected into the mammalian HEK cells using a lipid-based transfection reagent to get plasmid through cell membrane

Amplify Adenovirus

- The adenovirus created after transfection is used to infect more HEK cells
- Multiple rounds of infection of progressively more cells is needed to obtain sufficient amounts of virus

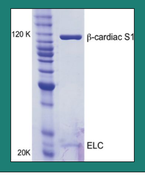
Infect Muscle Cells with Adenovirus

Adenovirus is used to infect myotube (skeletal muscle) cells



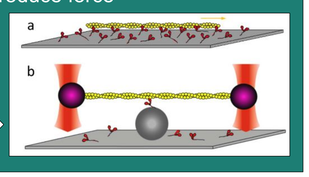
Purify Mutated Myosin from Muscle Cells

Myosin is purified using a combination of affinity and anion exchange chromatography.



Test Motor Function of Mutated Myosin

The mutant myosin is tested for (a) sliding velocity and (b) ability to produce force



RESEARCH GOALS

What the Spudich lab has set out to do is figure out the connection between the mutations in the MYH7 gene and HCM. This gene codes for beta-cardiac myosin, which is expressed in cardiac muscle cells (see Figure 2). The myosin motor is part of the muscle cell that pulls the segments of the cell called sarcomeres together. By connecting to thin filaments made of actin connected to the outer sides of the sarcomere and pulling on them, the myosin motors pull the outsides of the sarcomere closer to the center and contract the muscle. The lever arm is the part of the myosin motor that amplifies the movement of each pull of the myosin motor. Though it has been historically ignored, the lever arm has many of the mutations that cause HCM, which makes us believe that it is an important regulator of myosin function (Spudich 2016).

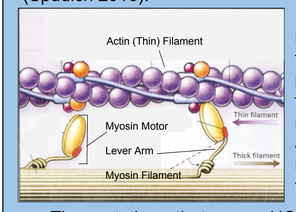


Figure 2: Myosin motors attaching themselves to and pulling the actin filament. The myosin head is attached to the myosin filament by the lever arm.

The mutations that cause HCM are known, but how the mutations affect heart function and cause HCM remains a mystery. By understanding how these genetic mutations affect the contractile proteins of the heart, we may be able to aid in the effort to effectively treat this disease. To achieve this the lab is testing the function of myosin motors that have the HCM-causing mutations in them. I am specifically testing the function of a mutation where Leucine 781 will be switched out for Proline, or L781P for short.

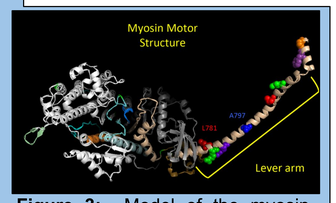


Figure 3: Model of the myosin motor with the myosin head (which connects to the actin filament) shown on the left and the lever arm on the right, as well as the location of Leucine 781 (red).

DISCUSSION, ANALYSIS, AND EVALUATION

Unfortunately, I was not able to complete all the steps to finish my research for the L781P mutation. I am set to finish my work by June or July, though I have been working on the last steps with the mutation A797T (see Figure 3) in the meantime. Eventually, the adenovirus will be ready to infect the the myotube cells, and I will be able to continue work on the L781P mutation. Hopefully, I will have the myosin purified from the myotube cells by mid-June (after AP testing and finals), and the motor function assays will be carried out by the end of the month. However, I have learned that lab work rarely goes as planned, and this projection may not be accurate.

CONCLUSIONS, IMPLICATIONS, AND NEXT STEPS

The purpose of this research is not to come to an immediate or sweeping conclusion with regard to the treatment for hypertrophic cardiomyopathy. It is meant to understand how the mutations in the MYH7 gene affect the function of the myosin motor. With this information, scientists can continue to research the function of myosin in a heart with HCM, though the nature of this research will depend on the results of our tests. The data provided can also be used to screen pharmaceuticals (drugs) to counteract the changes made by the mutations.

ACKNOWLEDGEMENTS / REFERENCES

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Works Cited:

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All Graphics Used (except Figure 4): Adhikari, (2016). Stanford University School of Medicine Department of Biochemistry Retreat Presentation.