

Summary and Evaluation of
In vivo Analysis of Troponin C
Knock-in (A8V) Mice:
Evidence that *TNNC1* is a
Hypertrophic Susceptibility
Gene

Name

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Summary Paper

PCB5205-Cell Structure & Function

The troponin complex consists of three proteins: troponin I, troponin T and troponin C which play a crucial role in muscle contraction. Troponin I is an inhibitor of the actomyosin complex.¹ Troponin T is responsible for anchoring of the Tn complex to actin.¹ Troponin C is an essential calcium sensing protein. When a calcium influx is present, calcium binds to the n-terminus of troponin C causing the inhibitory effects of troponin I to be eliminated. Myosin binding sites are then exposed on the actin filament permitting the interaction of actin and myosin and producing the necessary tension needed for muscle contraction.

Hypertrophic cardiomyopathy (HCM) is a rare condition of the heart that causes cardiomyocytes to enlarge, thickening the walls of the ventricles, triggering diastolic dysfunction which further causes the heart to exhaust itself when pumping blood.¹ It has been correlated to mutations found in proteins that are involved in the process of muscle contraction/relaxation and more specifically mutations that effect the binding affinity between calcium and troponin C. The cardiac troponin C mutant focused on throughout this study is known as A8V. This heterozygote variant in TNNC1 was discovered in a male patient out of a large cohort study where a total of four variants were found. The mutation present in cTnC-A8V is consistent with an amino acid change from valine to alanine, causing an increase in the binding affinity for calcium to the n-terminus of troponin C.¹ Even with the knowledge of this mutation, there has previously been a lack of family lineage of this disease, making it difficult to establish whether TNNC1, the gene encoding for Troponin C, could be a hypertrophic cardiomyopathy susceptibility gene.¹

The goals of this study were to establish whether the binding affinity between the n-terminus of troponin C and calcium generate changes in the heart that resemble symptoms of hypertrophic cardiomyopathy, deliver specific evidence that TNNC1 can be defined as a hypertrophic cardiomyopathy susceptibility gene and to explore the mechanisms, both cellularly

and molecularly, that evoke enlargement of the heart due to mutagenesis.¹ The answers to the objectives stated above were discovered through the use of a 34 year old proband who was found to be *TNNC1-A8V^{+/+}* and KI mice who had the *TNNC1-A8V* mutated gene inserted via a “knock-in” system.

Echocardiograms of the proband man displayed values higher than normal in wall and septal thickness and decreased ventricular dimensions in systolic and diastolic intervals.¹ These values proved hypertrophy of the patient with no previous family history for HCM or cardiac problems. In order to further analyze the inheritance effects of this mutant gene, KI-*TNNC1-A8V* mice were used to study the HCM progression.

Cardiac function of the mice was measured via echocardiograms where diastolic and systolic volumes lower than the wild type were observed. The mice also displayed left atrial enlargement and left ventricular hypertrophy consistent with the data from the human male patient. To further measure systolic function, a pressure-volume study was conducted using left ventricular pressure (LVP), +dP/dt (rate of rise of left-ventricular pressure), and -dP/dt (rate of fall of left ventricular pressure). A heart weight by body weight examination indicated a reduced ratio in comparison to the wild-type along with an increased LVP and a decreased +dP/dt and -dP/dt. These results are consistent with diastolic dysfunction, a significant property of hypertrophic cardiomyopathy.¹

To show the differences in tissue arrangement, 16/18-month-old WT, KI-*TnC-A8V^{+/+}*, and KI-*TnC-A8V^{+/-}* mice hearts were stained with masson trichome. The photos yielded myofibrillar disarray and interstitial fibrosis along with hypertrophy of the papillary muscle in the KI-*TnC-A8V^{+/+}* highlighting changes common in HCM.¹

At the cell and molecular level, multiple experiments were performed to view the mechanism behind calcium and its binding affinity and to see how a mutated filament would affect muscle function. To do so, sarcomere length was measured. Sarcomere lengths were discovered to be much shorter, and the cardiomyocytes of the mutant mice promoted an increased contractile percentage consistent with the hypercontractile phenotype.¹ It is possible that under resting conditions mutant myofilaments are pre-active in such a way that the slightest influx of calcium could generate increased muscle contraction.¹ The calcium decay times were shown to be slowed as well as mechanical relaxation. It is possible that the mutation causes the binding affinity for calcium to be higher causing a delay during calcium dissociation from actin.¹ This mechanism was supported using papillary skinned fibers.

Lastly, immunoblots were performed to evaluate the importance of calcium related proteins in KI-TnC-A8V hearts. To maintain calcium homeostasis, protein adjustments are necessary and can serve as possible molecular markers for early signs of HCM.¹ Phosphorylation serines, SERCA2 (sarcoplasmic reticulum calcium ATPase) and PLN-p^{Thr17} were found at decreased levels indicating that the sarcoplasmic reticulum fails to buffer calcium quantities.¹ This data proves that calcium handling proteins could be used as a marker for early HCM disease.¹

The mouse model proved consistent data in relation to the rare hypertrophic cardiomyopathy disease. The aforementioned results led to the ultimate conclusion that a TNNC1 mutation can affect the binding affinity of calcium and induce conformational changes that can be used as a marker for the pre-prognosis of hypertrophic cardiomyopathy.

My first opinion of the paper was that the background was weak. The authors did not explain the mechanism behind hypertrophic cardiomyopathy and I feel like more terminology could have been included so that the results section could be understood more easily. The wordiness of the introduction also made it difficult to understand what was being said. Throughout the entire paper, I found myself looking up a bunch of words that I was unsure of mostly because I am not an expert of the heart. Once I got over the wordiness of the paper, I was able to understand the results better, when having them side by side with the figures.

For the most part I found the figures and their legends legible and labeled. I think figure one would have been more helpful if there was a comparison of a normal heart patient so that the differences in the echo- and electro cardiograms could be viewed. I also think that since the authors mentioned that 3,9 and 14-month-old mice were used, they should show all the results of the ages and not just one age (figure 2). In figure 3, the beginning body weight of the mutant mouse was about 5 grams lower than that of the wild-type and the heterozygote and I'm curious if the starting weight would make the heart/weight/body weight difference look more significant than it is. I also believe that even if data was not significant, they still should have included the figures for the right atria. Figure 4G wasn't labeled. Figure 7 C & D could have been blown up a bit for a better visual.

The discrepancies in the mice's ages was my biggest question of all. The one thing I wish they would have touched on more is why the ages of the mice varied so often. In some results, they used 9-month-old mice to display results and others they used 16/18-month-old mice. However, unless I missed it, nothing in the paper mentioned as to why. Under the echocardiography of KI-Tnc-AV8 mice results, the authors stated that significant differences in relative wall thickness (RWT) of the HM mice could be seen as early as 3 months. However,

when looking at age 14 months the heterozygote and HM have about the same RWT. They also state that ejection fraction is higher suggesting hyper systolic function. This is also shown again, only in mice of 3 and 9 months. I personally think this is an inconsistency and that all the results should have been representative of the age ranges they used throughout the study. For example, including the 16/18-month-old mice they used in figure 4 and include their data in table 1 or including all the echocardiograms in figure 2 of the 3 and 9-month-old mice and not just the 14-month-old.

I also didn't agree that they only used one man's data to go off of or why they only mentioned one type of variant when four were found. They failed to explain why this specific mutant was the target choice.

This is also one of the few papers I've seen that went from a background of a few sentences to the results and conclusion and then the introduction. I do understand that some journals have different styles/outlines, however this paper's organization I wasn't a fan of.

The procedures themselves were very well thought out and thorough. If I were more well versed on echocardiograms and terms relating to the heart, I'm sure the paper would have been a smoother read.

Works Cited

1. Martins A, Parvatiyar M, et al. *In vivo* Analysis of Troponin C Knock-in (A8V) Mice: Evidence that *TNNC1* is a Hypertrophic Susceptibility Gene. *Circulation: Cardiovascular Genetics*. 2015; 8(5): 653-664.