

The Effects of TnC Mutations on Calcium Dissociation Rates

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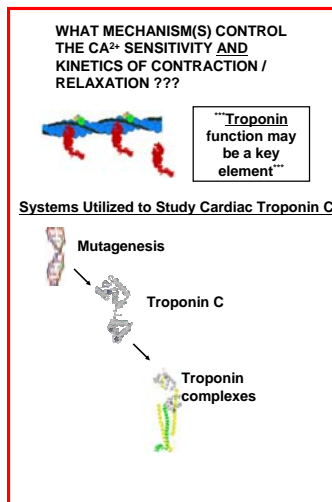


Figure # 1

MOTIVATION AND BACKGROUND

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease that can cause sudden death in athletes and young people. Mutations in sacromeric genes are found in those with this disease. The mutations used in this experiment (C84Y and E134D) were found through analysis of 1025 patients with HCM from the Mayo clinic who displayed left ventricle hypertrophy and left ventricular outflow obstruction*. Those patients with the E134D and C84Y mutations showed no family history of the disease concluding that these mutations may be sporadic.

The purpose of this study was to examine the effects of E134D and C84Y mutations on the calcium binding properties of troponin C.

*A Functional and Structural Study of Troponin C Mutations Related to Hypertrophic Cardiomyopathy The Journal of Biological Chemistry Vol.284, pg.19099

Figure # 2

METHODS

- Mutations of cTnC(HcTnC) were produced through the process of PCR
- DNA sequences were inserted into pET3d expression plasmid, and sequenced for verification.
- TnC proteins are expressed in *E.coli*.
- Tnc's are then passed over several columns for purification
- After mutants have been purified by column chromatography, they are then labeled with fluorescent probes. By testing the mutants in a stopped flow apparatus, the rates of calcium dissociation from the mutants can be measured (figure #9).

Figure # 3

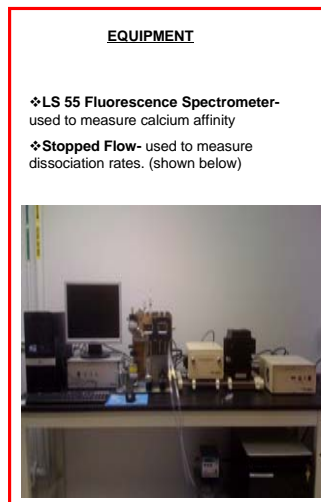


Figure # 4

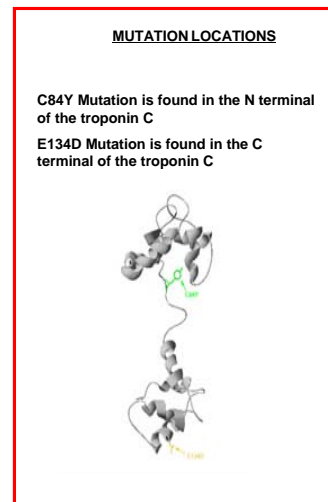


Figure # 5

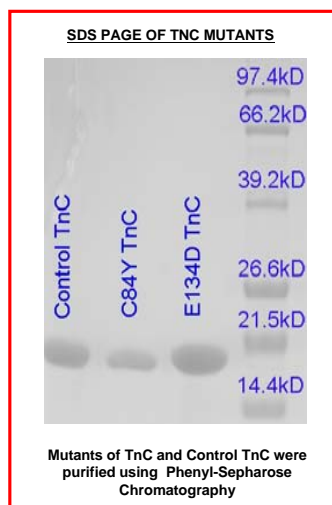


Figure # 6

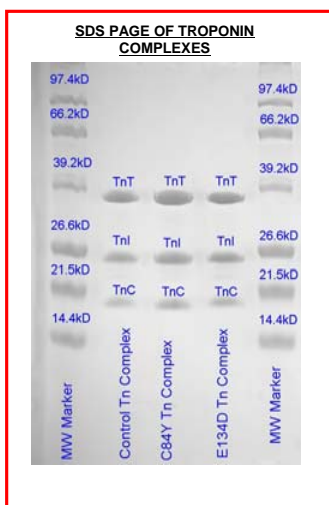


Figure # 7

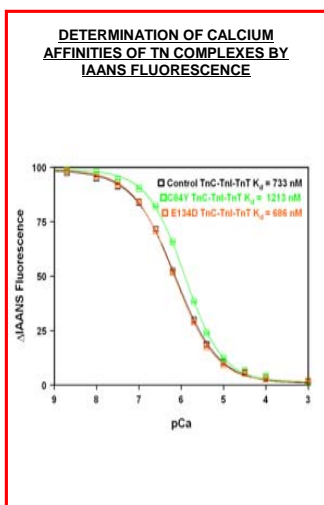


Figure # 8

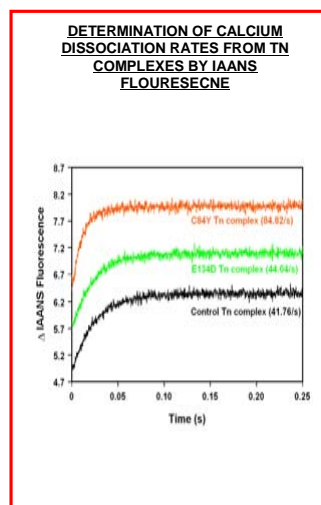


Figure #9

SUMMARY / CONCLUSIONS

Compared to the wild type, E134D had an insignificant difference in both in both the affinity and rates of calcium dissociation from the troponin complex. C84Y not only had a 1.6-fold lower affinity than the control troponin complex, it also had 2-fold faster rate of calcium dissociation from the troponin complex. These differences in calcium binding properties may help explain why C84Y mutation is associated with disease. Future plans include determining the effects of E134D and C84Y mutations on calcium binding properties of troponin C after reconstitution of these mutants into the thin filaments or cardiac muscle.

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