Familial Hypertrophic Cardiomyopathy (FHC) Medicine: The Cloning and Study of Two Novel Mutations in the Regulatory Light Chain (RLC) of Myosin that Cause Familial Hypertrophic Cardiomyopathy (FHC)

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Familial hypertrophic cardiomyopathy (FHC) is a heterogeneous autosomal dominant heart disorder characterized by left ventricular hypertrophy, myocardial disarray, and sudden death. Recent research has identified abnormalities in at least eight related genes important for the contraction of the heart that contribute to FHC. The findings in these studies indicate that the change in the sarcomeric and myocyte structure is indeed caused by mutation-induced alteration of the cardiac contractile function.

The aim of our current project is to clone and study two recently identified novel mutations in the regulatory light chain (RLC) that are believed to have associations with FHC. The main stages involved in this study are: 1) the cloning and expression of the two mutants, N47K and K104E; 2) purification of the proteins; 3) measurements of the far-UV circular dichroism spectra (CD) to examine the secondary structure of the mutants; 4) replacing endogenous RLC in porcine myofibrils with mutated FHC RLC proteins; and 5) performing the ATPase activity assays and comparing the results to wild-type RLC. The replication of the two mutations, N47K and K104E, was successful. The circular dichroism spectroscopy detected decreases in the α-helical contents in the two mutants. Our results show that the ATPase activities, in terms of Ca^{2+} sensitivity in the two mutant groups, were higher than that of the wild type. However, change in the ATPase activities should be verified by further study as significant statistical result is yet to be established.

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Introduction

Familial hypertrophic cardiomyopathy (FHC) is a form of cardiomyopathy involving enlargement and thickening of the heart muscle. The disease is characterized by affecting the left ventricle and the intraventricular septum. It is a disorder of the myocardium, specifically the sarcomeric proteins that show cellular disarray of myofibrils on histologic examination. This enlargement interferes with the function of the heart by: narrowing the outflow of the ventricle, reducing the ability of the heart to relax and fill with blood during the relaxation phase, and reducing the ability of the valves of the heart to function properly. Recent research has identified abnormalities in at least eight related genes that are important in the development of heart muscle cells. The abnormality is known as a *mutation* and may be likened to a spelling mistake in the genetic code make up of DNA. In approximately 50%–60% of families, affected individuals are found to have a mutation in the gene for *myosin*, troponin T, alpha tropomyosin, cardiac myosin binding protein-C, or the essential and regulatory light chains. These are important proteins for the contraction of the heart.

The aim of the current project is to clone and study two recently identified novel mutations in the RLC that are believed to have associations with FHC. From the findings in recent studies, we believe the change in the sarcomeric and myocyte structure is indeed caused by mutation-induced alteration of the cardiac contractile function. The purpose of this investigation is to find the actual cause for these mutations and to find a future method for preventing mutations that can lead to FHC. Overall, we expected to find these mutants to alter the cardiac contractile properties such as the ATPase activity of myosin, and Ca^{2+} dependence of force.

Methods

The main stages involved in this study were:

1) the cloning and expression of the two mutants, N47K and K104E;
2) purification of the proteins;
3) measurements of the far-UV circular dichroism spectra (CD) to examine the secondary structure of the mutants;
4) replacing endogenous RLC in porcine myofibrils with mutated FHC RLC proteins; and
5) performing the ATPase activity assays and comparing the results to wild-type RLC.

Data Analysis

Table 1 presents the calculated values (%) of the HCRLC-WT and the two mutants. The α-helical content of

Table 1. Comparison of α-helical % among the experimental groups

Experimental Groups	α-Helical %	N
Wild-Type (Control)	20.76	5
N47K	19.06	8
K104E	18.05	8

$F = 33.793$, $P = 0.000$
R Squared = .790 (Adjusted R Squared = .766)

Table 2. Summary of ATPase activity level of control and mutated groups

Experimental Groups	pCa_{50} Level	n
Wild-Type (Control)	6.887	9
N47K	6.939	6
K104E	7.025	12

p value for group difference = 0.768.

Table 3. Comparison of molecular weight and pI value among experimental groups

Experimental Groups	Molecular Weight	pI Value
Wild-Type (Control)	18658.1	4.92
N47K	18672.1	5.01
K104E	18659.0	4.78

the HCRLC-WT and its FHC mutants ranges from 18% to 21%. The average percentage of the α-helical content of the HCRLC-WT is 20.76% in 5 replicates. Replacement of asparagines residue with lysine (N47K) shows an average of α-helical content of 19.06% in eight replicates. Similarly, the replacement of the lysine reside with glutamate (K104E) decreases the α-helical content of HCRLC from wild type 20.76 (n=5) to 18.05 (n=8). The difference in the α-helical contents of the three groups is statistically significant (P=.000).

As demonstrated in Table 2, the wild type showed a pCa_{50} value (the common-logarithm value of free Ca^{2+} concentration required for half-maximal activation) of 6.887 (n=9). Reconstitution of the RLC-depleted myosin with mutation N47K, showed an increased in pCa_{50} value to 6.939 (n=4). The reconstitution of RLC-depleted myosin with mutation K104E resulted in pCa_{50} value 7.025 (n=12). The difference in the pCa_{50} values is not statistically significant.

In Table 3, it was noted that the mutations did change the HCRLC's molecular weight and the theoretical pI value. The molecular weight of HCRLC of the wild type is 18658.1, while the theoretical pI is 4.92. The N47K HCRLC, with replacement of asparagines to lysine, has a molecular weight of 18672.1 and a pI of 5.01. The other mutant, K104E, with replacement of positively charged lysine to negatively charged glutamate, has a molecular weight of 18659.0 and pI of 4.78.

RESULTS

From the reproduction of the mutants we were able to study the α-helical content where for N47K had an average of α-helical content of 19.06% in eight replicates. While K104E decreases the α-helical content of HCRLC from wild type 20.76 (n=5) to 18.05 (n=8). On the other hand, in the ATPase activity, the wild type showed a pCa_{50} value of 6.887, while the RLC-depleted myosin with mutations N47K and K104E showed an increased pCa_{50} value at 6.939 and 7.025—a rise of 0.052 and 0.138 units respectively.

CONCLUSION

The replication of the two mutations, N47K and K104E, was successful. The circular dichroism spectroscopy detected decreases in the α-helical contents in the two mutants. The change in the alpha helical contents is statistically significant making evident that the ATPase activities, in terms of Ca^{2+} sensitivity of the two mutant groups, were higher than that of the wild type. However, change in the ATPase activities should be further studied as a significant statistical result is yet to be established.

ACKNOWLEDGMENTS

The authors thank: Danuta Szczesna-Cordary, PhD; Ms. Emma Taylor; Ms. Cynthia Gonzalez; Mrs. Shuk-Shin Ng; Mr. Ishmael Sharpe, BA, MBA; Dr. Joseph H. Davis; Center for Forensic Pathology; and Elena Sgarbossa, MD.