Uncovering the genetic bases for cardiac disease has been a central focus of biomedical research for the past 10 yr. The difficulty of the task is compounded by the polygenic nature of most cardiac diseases. That is, the pathologies appear to be due to the (in)action(s) of a number of genes, rendering both their identification and mechanisms of action relatively intractable. Geisterfer-Lowrance et al. (1) first established causality between a defined mutation in a gene encoding a major sarcomeric protein, the β myosin heavy chain (MyHC), and a cardiac disease, familial hypertrophic cardiomyopathy (FHC). FHC is a dominant autosomal disease, resulting in increased left ventricular mass. Its penetrance is highly variable, even within a single pedigree. The work established a paradigm for the field in that a missense mutation in a peptide involved in force generation could lead to a discernible phenotype. Subsequently other mutations in the β MyHC have been identified (for review see reference 2) and a series of investigations established that mutations in genes encoding other sarcomeric proteins such as the myosin binding protein C (3), the regulatory and essential myosin light chains (4), tropomyosin and cardiac troponin T (TnT) (5), can also cause the FHC phenotype.

In this issue of *The Journal*, Watkins and colleagues (6) focus on the cardiac TnT FHC mutation and provide information about the molecular etiology of the disease. The TnT mutation does not result in an amino acid substitution, but occurs at a splice junction and produces a transcript containing, in frame, a premature termination codon. It was originally hypothesized that a null mutation would result (5). In *Drosophila*, alterations in the absolute and relative levels of the sarcomeric proteins can be effected by changing the dosage of a particular gene(s); a similar mutation created a null allele in TnT and resulted in major deleterious effects on both the structure and function of the contractile apparatus (7). The effects of a null allele, altered sarcomeric stoichiometries due to haploinsufficiency, are distinct from those due to a "poison peptide." The missense mutations that occur in the other contractile protein genes all result in altered polypeptides which, after they incorporate into the sarcomere, are dominant over the normal protein encoded by the remaining, wild-type allele.

Watkins et al. (6) devised a series of experiments designed to test whether the mutated TnT functioned as a null allele or produced a poison peptide. Using a newly developed quail myoblast→myotube system, they expressed the mutant TnT and determined the functional consequences. The data show that the mutated TnT locus is not a null allele but rather produces a stable, truncated polypeptide that accumulates in the myotube and is subsequently incorporated into the sarcomere. This protein displays a dominant negative effect on sarcomeric function, as evidenced by greatly diminished force production, even when it is coexpressed with the wild-type sequence. This is consistent with the functional deficits resulting from some of the characterized mutations in the other contractile protein genes such as MyHC (8).

However, it is premature to rule out the possibility that a null allele of a contractile protein gene can cause cardiac disease. Proof-of-principle exists, albeit in nonmammalian striated muscle systems; gene dosage effects can dramatically alter motor function. Although a null mutation in the β MyHC gene has been identified and has been characterized as benign (9), this conclusion is compromised by the very small pedigree coupled with the variable penetrance of the disease. Continued rigorous testing of the alternative possibilities and perhaps a better understanding of the genetic bases for the variable penetrance exhibited can now be performed by creating appropriate animal models using gene targeting in the mouse. Large pedigrees can then be created on different, genetically defined backgrounds and the effects of the various mutations determined in large numbers of inbred animals. Such experiments are now underway in a number of laboratories and should prove illuminating.

The present study underlies the concept that mutations in different genes can lead to a convergent phenotype, in this case, a hypertrophic cardiomyopathy. The structural and functional interactions between the sarcomeric proteins provide a case study of how a mutation in an element of a macromolecular array affects the function of the unit. A detrimental change in any one of a number of critical components compromises the motor unit's function and the resultant deficit in force or power output appears to trigger a common outcome — a hypertrophic response. Understanding the molecular mechanisms of the heart's response(s) to altered motor function remains the next challenge.

Jeffrey Robbins
Division of Molecular Cardiovascular Biology
Children's Hospital Research Foundation

References

1. Geisterfer-Lowrance, A.A., S. Kass, G. Tanigawa, H.P. Vosberg, W. McKenna, C.E. Seidman, and J.G. Seidman. 1990. A molecular basis for familial hypertrophic cardiomyopathy: a β cardiac myosin heavy chain gene missense mutation. *Cell.* 62:999–1006.

2. Rayment, I., H.M. Holden, J.R. Sellers, L. Fananapazir, and N.D. Epstein. 1995. Structural interpretation of the mutations in the beta-cardiac myosin that have been implicated in familial hypertrophic cardiomyopathy. *Proc. Natl. Acad. Sci. USA.* 92:3864–3868.

3. Watkins, H., D. Conner, L. Thierfelder, J.A. Jarcho, C. MacRae, W.J. McKenna, B.J. Maron, J.G. Seidman, and C.E. Seidman. 1995. Mutations in the cardiac myosin binding protein C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nat. Genet.* 11:434–437.

4. Poetter, K., H. Jiang, S. Hassanzadeh, S.R. Master, A. Chang, M.C. Dalakas, I. Rayment, J.R. Sellers, L. Fananapazir, and N.D. Epstein. 1996. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat. Genet.* 13:63–69.

5. Thierfelder, L., H. Watkins, C. MacRae, R. Lamas, H.P. Vosberg, W.J. McKenna, J.G. Seidman, and C.E. Seidman. 1994. Mutations in a tropomyosin and in cardiac troponin T cause hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell.* 77:701–712.

6. Watkins, H., C.E. Seidman, J.G. Seidman, H.S. Feng, and H.L. Sweeney. 1996. Expression and functional assessment of a truncated troponin T that

J. Clin. Invest.

0021-9738/96/12/2433/02 $2.00
Volume 98, Number 11, December 1996, 2433–2434

causes hypertrophic cardiomyopathy. Evidence for a dominant negative action. *J. Clin. Invest.* 98:2456–2461.

7. Fyrberg, E., C.C. Fyrberg, C. Beall, and D.L. Saville. 1990. *Drosophila melanogaster* troponin-T mutations engender three distinct syndromes of myofibrillar abnormalities. *J. Mol. Biol.* 216:657–675.

8. Sweeney, H.L., A. Straceski, L. Leinwand, B. Tikunov, and L. Faust. 1994. Heterologous expression and characterization of a cardiac myosin mutant that causes hypertrophic cardiomyopathy. *J. Biol. Chem.* 269:1603–1605.

9. Nishi, H., A. Kimura, H. Haruhito, K. Yoshinori, K. Adachi, K. Matsuyama, T. Koyanagi, S. Yasunaga, T. Imaizumi, H. Toshima, and T. Sasazuki. 1995. A myosin missense mutation, not a null allele, causes familial hypertrophic cardiomyopathy. *Circulation.* 91:2911–2915.