Risk, Genotype, and Cardiovascular Disease

Mark Keating, MD

The sense of loss one experiences after missing a diagnosis, or underestimating the risk, of a life-threatening disorder is difficult to forget. This is particularly true if the disorder affects a young, otherwise healthy individual. Presymptomatic diagnosis of most cardiovascular disease is challenging, even in disorders that are clearly inherited, and risk assessment is frequently inadequate. The possibility of predicting risk of morbidity and mortality from cardiovascular disease at, or even before, birth would be a fantastic addition to our clinical capability. In a series of recent articles, one of which is published in this issue of Circulation, investigators have begun to address the need for improved risk assessment in a specific inherited cardiovascular disease, familial hypertrophic cardiomyopathy, through identification of disease-associated genetic mutations.

See p 345

Familial hypertrophic cardiomyopathy can cause dyspnea, angina, syncope, and sudden death, but the spectrum of symptoms is quite wide, and some individuals show no symptoms at all. The essential pathological features of this disorder are myocardial hypertrophy and myofibrillar disarray, both of which vary in location and extent from one affected patient to another. Diagnosis of hypertrophic cardiomyopathy has been based primarily on electrocardiography, echocardiography, and hemodynamic testing. Risk assessment, therefore, has been limited to those individuals who already manifest clinical features of the disease.

Over the last 3 years, this picture has begun to change. In 1989, Jarche et al, using the technique of linkage analysis, identified the chromosomal location of the familial hypertrophic cardiomyopathy gene in one French-Canadian family on the long arm of chromosome 14. This discovery made genetic testing in asymptomatic members of this family feasible, a matter of considerable importance to them.

The genetic premise underlying linkage analysis is that two genes located on the same chromosomal subunit are physically linked and will be inherited together (coinherited) in subsequent generations. By contrast, genes that are located on separate chromosomes will be inherited independently. Unlinked genes will be coinherited by chance approximately 50% of the time.

Linkage analysis is an attempt to identify genes that are coinherited more frequently than would be expected by chance and, therefore, are likely to be located on the same chromosome.

The identification of cardiomyopathy-linked markers on chromosome 14 allowed presymptomatic diagnosis of individuals in one family. What about other families? Although work addressing this question is ongoing, it has become clear from subsequent linkage studies that many, although not all, families with hypertrophic cardiomyopathy have inherited a mutant gene located on chromosome 14; current data suggest that this disorder is linked to markers on chromosome 14 in fewer than 50% of families. Presumably, the other families have inherited a different mutant gene (or genes) located on another chromosome. The fact that more than one genetic locus can cause hypertrophic cardiomyopathy is known as locus heterogeneity. Locus heterogeneity complicates risk assessment for this disease, because genetic testing provides no predictive information in unlinked families. On the other hand, once additional disease genes have been identified, their characterization will provide valuable information about the molecular biology of myocardial development and homeostasis.

Linkage analysis provided the first clue regarding the molecular basis of familial hypertrophic cardiomyopathy, but the power of this technique is limited. Markers on chromosome 14 are not useful for risk assessment in unlinked families or in sporadic cases caused by new mutations. To solve these problems and to improve our understanding of the disease pathogenesis, it was necessary to identify the disease gene.

Two approaches are available for disease gene identification once the chromosomal location of the gene is known. The first, often referred to as a candidate gene approach, can be fairly rapid. In this approach, a previously characterized gene becomes a candidate for the disease gene on the basis of its chromosomal location and a physiological rationale. If mutations in the candidate gene can be demonstrated in affected family members and are never identified in unaffected individuals or in the general population, support for the involvement of this gene in disease pathogenesis is achieved. If no mutations are identified, on the other hand, the possibility that the disease gene is another, previously unidentified gene must be considered. In that event, the process of disease gene identification involves refined linkage and physical mapping of the genome of interest coupled with the identification of new genes. Each new gene is a candidate for the disease gene, and every candidate must be tested by mutational analysis. This can be a laborious process, but it has led to dramatic successes in several important human genetic disorders.
Fortunately, the candidate gene approach was successful for familial hypertrophic cardiomyopathy. In 1990, Tanigawa et al. and Geisterfer-Lowrance et al. published sequential papers in *Cell* describing mutations in the β-cardiac myosin heavy chain gene that cosegregated with the disease in families, supporting the hypothesis that this gene was responsible for hypertrophic cardiomyopathy in these families. This breakthrough immediately led to hypotheses regarding the molecular mechanism of the hypertrophy and myofibrillar disarray associated with the disease and led to work that has resulted in the recent papers discussed here.

In the study described in this issue of *Circulation*, Epstein and colleagues used the technique of single-strand conformation polymorphism (SSCP, Reference 9) analysis to identify two mutations in the β-cardiac myosin heavy chain gene in affected members of two unrelated families. In a similar study published recently in the *New England Journal of Medicine*, Watkins et al. described seven mutations in 12 families. In this case, a ribonuclease protection assay, rather than SSCP, was used to screen for sequence anomalies in patient DNAs. Both assays permit rapid screening of large segments of DNA. If an abnormality is identified through screening, the specific mutation can be characterized by direct sequencing. To date, eight β-cardiac myosin heavy chain mutations have been associated with familial hypertrophic cardiomyopathy.

Myosin is the primary contractile protein in thick filaments of myofibrils. Each molecule of myosin consists of six polypeptides: two identical heavy chains and two pairs of light chains. Myosin biosynthesis results in the assembly of these polypeptides into two structural domains, the globular head and the rodlike tail. The head of myosin contains both the ATPase and actin binding domains that are critical for the generation of force. All of the myosin mutations identified in hypertrophic cardiomyopathy thus far have been missense mutations; missense mutations are single-base-pair changes leading to the substitution of one amino acid for another in the encoded protein. Interestingly, no mutations have been identified in the region of the gene that encodes the ATPase, actin binding, or myosin light chain binding domains. Possibly mutations in these areas would be incompatible with life. Instead, hypertrophic cardiomyopathy mutations have encoded amino acid substitutions in the head or head–rod junction. Each of the altered amino acids identified in these studies has been highly conserved during evolution, suggesting that these residues are also important for the function of the protein. Their precise function is unclear, but their identification provides important clues for future studies into the structure/function relation of the β-cardiac myosin heavy chain protein. It is also unclear how these myosin mutations lead to disease. The mechanism may involve abnormal myofibrillar assembly, reduced force generation, and secondary myocardial hypertrophy. Continued careful cataloging of specific myosin mutations will lead to testable hypotheses about the pathogenesis of this disorder.

In both studies, investigators took their work one step beyond mutational analysis; they examined the phenotype of their genotypically defined hypertrophic cardiomyopathy families. Genotype is defined as the genetic constitution of an individual, and, of course, it varies from one individual to another. Phenotype, on the other hand, is the physical appearance of an individual and results from genotypic and environmental factors. Examination of the phenotypic characteristics of individuals within disease families and comparison of their genotypes showed that certain β-cardiac myosin heavy chain mutations carry greater risk of morbidity and mortality than others; that is, certain genotypes have more serious prognostic implications than others. The reason for these substantial risk differences is not apparent but presumably involves the specific site and type of the mutation. In particular, mutations that alter the charge of the substituted amino acid appear to have a more serious phenotypic effect than more conservative substitutions. If these findings are confirmed by subsequent studies, it will be possible to use genetic testing to assess the risk of sudden death from hypertrophic cardiomyopathy in an individual at, or even before, birth. Currently, genetic testing for familial hypertrophic cardiomyopathy is available only through research centers; eventually, however, genetic testing will become generally available and risk assessment will have important clinical use.

In future studies, it will be important to identify and characterize additional genes that induce hypertrophic cardiomyopathy, as these genes will be critical for comprehensive genetic testing. Molecular genetic studies, however, are just the beginning of the story. Important questions about the biochemistry, cell biology, and physiology of familial hypertrophic cardiomyopathy need to be addressed if we are to learn all that we can from this experiment of nature. With time, the application of molecular biology to human disease will replace at least some of our clinical uncertainty with accurate disease prediction and, ideally, disease prevention.

Acknowledgment

I am indebted to Mark Leppert for his critical reading of this manuscript.

References

Risk, genotype, and cardiovascular disease.
M Keating

*Circulation.* 1992;86:688-690
doi: 10.1161/01.CIR.86.2.688

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/86/2/688.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/