Editorial

Molecular Genetics
Therapy or Terror?

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In the past three decades, the application of information derived from cardiovascular research markedly reduced the death rate from ischemic heart disease, the number one killer in the Western world. This resulted, in part, from the integration and focusing of innovative techniques developed and employed by well-trained investigators enticed from multiple scientific and clinical disciplines. Throughout this interval, it was perhaps appropriate on the basis of priority that another group of cardiac diseases referred to as cardiomyopathies received very little attention. A second, perhaps more legitimate, reason for this lack of attention is that many of these diseases, at least in this country, are inherited and until recently, techniques were not available to identify the underlying genetic defect. The introduction of recombinant DNA techniques in the 1970s followed by their application in molecular genetics drastically changed the potential to explore the molecular basis of these disorders. Previously, unless one knew the protein defect responsible for the inherited disorder, it was almost impossible to identify the gene. The development of linkage analysis makes it possible to map the chromosomal locus of a disease-related gene without knowing the responsible protein and, once mapped, another technique referred to as positional cloning makes it possible to identify the responsible gene. Chromosomal mapping of a disease locus through linkage analysis consists of showing one or more DNA markers of known chromosomal location.

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are co-inherited with the disease (genetically linked) in subsequent affected offspring (more often than by chance). This would indicate the locus of the marker and the disease-related gene are on the same chromosome and in close physical proximity. Knowing the position of the disease-related locus in relation to the marker locus, one can clone the encompassing region (positional cloning) and subsequently identify the responsible gene. Approximately a decade ago, the availability of DNA markers was greatly increased based on polymorphism (distributed throughout the human genome) exhibited by recognition sites for restriction endonucleases (used routinely to digest DNA). To identify these markers, DNA from individuals of a pedigree is digested with multiple enzymes and the fragments of varying lengths are separated by electrophoresis and analyzed after Southern blotting. Coinheritance of the electrophoretic pattern in affected individuals of the pedigree would indicate genetic linkage. Despite the advancement provided by these initial markers, referred to as restriction fragment length polymorphism (RFLPs), they were not evenly distributed (areas of 50,000 bp without markers) and analysis by Southern blotting required 7 to 10 days. The recent discovery of highly polymorphic informative markers based on varying lengths of short tandem repeat DNA sequences distributed throughout the human genome about every 50,000 bp has revolutionized chromosomal mapping location of disease-related genes. The ability to detect these markers with polymerase chain reaction (PCR), which can be performed within hours as opposed to days, has greatly facilitated and accelerated the search for disease-related genes. Theoretically, it is now possible to practically identify any gene responsible for an inherited disease. Following isolation of the gene, the aim is to identify the responsible mutations and develop diagnostic, genetic screening assays so that either before or immediately after birth individuals can be identified as to whether they have the gene and are at risk of developing the disease. This should lead to more appropriate and specific therapies as well as ultimate cure such as gene replacement therapy. It is estimated that more than 100 inherited diseases directly affect the heart, all of which can be expected to be mapped over the next 5 to 10 years.

Hypertrophic cardiomyopathy (HCM) was the first primary cardiomyopathy to succumb to the techniques of modern molecular genetics, being mapped to the chromosomal 14q1 locus in a French-Canadian family in 1989 and subsequently shown to be the locus for several families throughout North America. HCM is the most common cause of sudden death in the young, particularly young athletes. The gene was identified to be BmHC gene in 1990, and in just 2 years families with HCM due to the BmHC gene have been identified throughout the world. BmHC is a relatively large gene comprised of 40 exons spanning 23,000 bp with an mRNA of 6000 bp encoding the BmHC protein of 1936 amino acids having a molecular mass of 220,000 D. The BmHC molecule provides the motor for contractility and comprises about one third of the protein of the heart. The BmHC gene appears highly vulnerable to mutations with a total of 34 different mutations having so far been reported; all but one is of the missense type. A missense mutation refers to a substitution that alters
a single codon that results in the changing of a single amino acid and usually involves a single nucleotide. The missense mutations occur in exons 3 to 23 that span the region that encodes for the globular head of βMHC. Two of the mutations have been shown to be expressed in the myocardial mRNA. In a three-generation family, the mutation Arg270Trp was shown to occur de novo. Both the mutant and normal mRNA and the normal and mutant βMHC protein have been detected in the myocardium of an affected individual, therefore indicating that these mutations are indeed responsible for the disease. The mutation in any one family is the same. However, between families mutations are usually different.

Despite the short interval of 2 to 3 years, studies are well underway evaluating the utility of these new findings in patient management. Correlations sought between specific mutations (genotype) and clinical features (phenotype) have provided useful prognostic information for both diagnosis and treatment. The missense mutation leading to the substitution of arginine for glutamine at sequence 403 of the βMHC protein was shown to be associated with high prevalence (number of patients with the gene who develop the disease), early onset of disease, and a high incidence of premature sudden cardiac death. This was observed in five unrelated families studied by three different groups with all showing the mean age of death to be approximately 33 years. Several such correlations became evident. Arg270Trp in four unrelated families had a similar poor prognosis with an average life span of 38 years. The reverse was true for other mutations, namely, Phe351Cys, Leu98Val, and Glu109Lys, which are associated with near-normal life span. However, other mutations, such as Arg99Gln, appear to have an in between prognosis. It soon emerged that mutations which induced a change in the net charge of the βMHC protein, such as Arg99Gln or Arg270Trp, pressure a poor prognosis, whereas mutations that are neutral, such as Phe316Cys or Leu98Val, predict a benign prognosis. In the very elegant study by Fananapazir and Epstein in this issue of Circulation, the investigators have again confirmed the important correlations between specific mutations and prognosis. They report on a mutation in which glycine substitutes for glutamic acid (Gly256Glu) in a large family of 245 kindred that shows essentially a benign prognosis. They confirm that Arg99Gln in those families of North American descent have a high incidence of sudden cardiac death, high penetrance, and early onset of disease. In the mutation, Leu98Val, they report only 8% dead by the age of 50 years, indicating that the prognosis is benign as previously reported. Nevertheless, on the basis of results of this study, the investigators challenge some of the concepts that have been emerging over the genotype-phenotype correlations in HCM. The investigators question the claim that a mutated amino acid with a charge is predictive of a malignant prognosis and suggest that it is no longer tenable. This is based on their findings that the mutation Gly256Glu, which does involve a negatively charged amino acid, glutamic acid, has a benign prognosis. Second, the mutation Val98Met, which has no change in charge, has a relatively poor prognosis in that 50% of the individuals with this mutation were dead by age 50. These two observations clearly challenge the concept that charge is not the only determinant of a poor prognosis, and as pointed out by the investigators, the position of the charge is also likely to be a determinant independent of whether the amino acid is charged or neutral. The other related observation by the investigators is the finding of the mutation, Arg89Gln, in a Korean family that appears not to be associated with a severe prognosis. In all other reported families this mutation is associated with an unfavorable prognosis. As indicated by the investigators, however, it is a small family (eight kindred) and the possibility of sudden death occurring with further observation cannot be excluded. It could, however, employ another intriguing possibility: the deleterious nature of the mutation requires an interaction with other genes found in the Caucasian genotype and not in the Korean genotype. If such should be the case, insights to be gleaned may have important implications for polygenic disorders such as hypertension and ischemic heart disease. Last, the investigators give the impression that the prognosis when stratified for benign or malignant must be all or nothing, which in fact is not the case as clinical studies have documented that certain mutations predict a prognosis between malignant and benign as indicated by the studies on Arg99Gln. In summary, the data in this study support the important observation that there is a definite correlation between specific mutations and the incidence of sudden death as well as the severity of the disease. It is perhaps important to point out that of 35 reported mutations in the βMHC gene, 19 involve a charged amino acid of which 11 of them involve the positively charged arginine. The benign prognosis associated with Gly256Glu may reflect that the amino acid, glutamic acid, is negatively instead of positively charged.

The somewhat surprising statement in this article is the authors’ concluding sentence: “Thus, although it is reasonable to genotype other family members when a mutation is discovered, we feel that until more is known about the correlations between the phenotype and genotype and mutation-specific causes of sudden cardiac death, routine genotyping of HCM patients will add little to their care and divert resources from more useful approaches to the diagnosis and treatment of this disease.” This is contrary to this investigator’s opinion as I believe that every effort should be made to identify all possible mutations in the βMHC gene. This would also include mutations from other gene pools such as Korea and other populations of different racial origin. In contrast to the recommendation by Fananapazir and Epstein, cardiac molecular genetics is on a successful march. In a short interval the following diseases have now been mapped and/or the gene identified: Duchenne muscular dystrophy, X-linked dilated cardiomyopathy, myotonic dystrophy, prolonged QT syndrome, Marfan syndrome, supravalvar aortic stenosis, Holt-Oram syndrome, and familial heart block. Screening for genetic disorders may be fraught with severe disappointment for affected individuals. Also, as we move closer to the ultimate goal of unraveling the etiology and pathogenesis of these disorders, many ethical quandaries will undoubtedly arise without obvious resolution. It is my belief that the ultimate solution is better served with a full knowledge of the facts. To provide a comprehensive genetic diagnosis, one must know most, if not all, of the possible mutations present in the responsible gene.
Fananapazir and Epstein\textsuperscript{17} question the value at the present time of screening families with HCM for the gene and/or its mutations. In my opinion this ignores the need to pursue research and its potential for a therapeutic cure as well as the benefit that might be presently accrued clinically, mentally, emotionally, and economically for families with this disease.

I shall discuss the last issue first. HCM is a disease in which only 50\% of the offspring is affected because the gene is passed on to only 50\%. Furthermore, even in the 50\% with the gene the disease is usually not evident, either from symptoms or echocardiographic assessment, until adolescence. Because this disease is generally regarded as having a potential for sudden death, children with this disease are usually prohibited from participating in sports throughout childhood. Is it preferable that all such children and their parents should suffer the mental and emotional anguish when only 50\% are even likely to develop the disease? Would you rather know early in life so that activities can be planned accordingly or after their formative years when participating in an activity they would have preferred is now too late? Screening to determine whether they have the mutation would provide, in my opinion, a more satisfactory answer. It might be argued, however, that because most of the people who die suddenly do so only after they have echocardiographic evidence of hypertrophy, one could simply follow them yearly or until hypertrophy develops and then recommend more restrictive activity. This approach still carries with it the emotional and mental anguish as well as the inconvenience and high cost of serially testing some one who is not even likely to develop the disease. These reasons alone, in my opinion, justify the search for every possible mutation as well as other responsible genes. Second, it is already evident that several of these mutations are predictive of a prognosis that is informative and can be determined before the development of the disease. Consider the individual who has the mutation Arg\textsuperscript{20}Gln who was adopted or has no previous history and is asymptomatic with the disease. Would that individual not qualify for an implantable permanent defibrillator? Such devices are already being implanted in some of the patients with HCM at our institution as well as that of Fananapazir and Epstein. Furthermore, information travels rapidly from the medical profession to the lay population and the need to know and satisfy curiosity can be very demanding for those who are born into a family with HCM. In our experience, we are frequently sought out by members of families with HCM to perform genotype typing and to provide full disclosure, albeit confidential, concerning their disease and the status of their children. Certainly, obligatory screening is not recommended because treatment cannot be implemented in everyone. Let us, however, not hold back on searching for the facts, particularly when it is already evident that considerable benefit can be derived, even if in only 50\%. Just as with other diseases, ethical considerations will arise for which we have no answers.

There is another equally important reason not to halt the search for mutations in $\beta$MHC and other genes responsible for HCM. The time is rapidly approaching when more definitive therapy will be available; perhaps even for those adults who already have the disease. We must realize that because HCM is an autosomal dominant disease, there is one normal gene (allele) and one abnormal gene (allele) and that the time is rapidly approaching when it will be possible to inhibit the mutant allele and permit only the normal allele to transcribe its normal protein. Although it is not a proliferating organ, the heart "turns over" every few weeks or months because practically all proteins of the heart have half lives of hours and certainly not more than days. Because the heart replaces itself even in the adult, if the mutant allele is inhibited and only the normal protein is translated it is quite conceivable that within months to a year the new heart would be made from normal $\beta$MHC and devoid of the abnormal. Whether this is achieved through antisense mRNA, triple-helix inhibitor, or other as yet unknown genetic weapons, it is very likely that such therapy is forthcoming in the early part of the 20th century. It is also conceivable that within the next decade gene replacement therapy or other means will be possible. It is also likely that the mutant protein does not directly induce hypertrophy but is the primary abnormality that stimulates secondary compensatory growth. Other means of inhibiting or modulating cardiac growth prior to the development of excessive hypertrophy may be of benefit.

Third, we must not forget what William Harvey said in 1657:

"Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of cases of rare forms of diseases. For it has been found that in almost all things, that what they contain of useful or applicable nature is hardly perceived unless we are deprived of them, or they become deranged in some way."

Identifying the mutations responsible for hypertrophic cardiomyopathy has not yet provided important clues to understanding cardiac growth but it still may as we discover more of the mutations in the $\beta$MHC and other genes responsible for this disease. The three dimensional structure of $\beta$MHC protein has just been derived from X-ray crystallography,\textsuperscript{19} which, together with the functional information to be gained from point mutation, should rapidly accelerate structure function analysis of this important molecule. Myosin is the motor driving contractility of the heart and is also the major motor for contractility in all muscles. HCM is now known to be genetically heterogeneous with three other loci having been mapped (1q, 11q, 15q)\textsuperscript{19-21} and other loci yet to be identified\textsuperscript{22}; however, no other genes have yet been isolated. Ultimately, not only will all of the molecular defects be identified but so will the pathogenesis of the resulting biochemical, pathological, and clinical abnormalities. These should come together to provide clues fundamental to our understanding of cardiac growth and usher in a new era providing us with the first important key to the cascade that modulates cardiac growth. In contrast to the conclusion by Fananapazir and Epstein, I would strongly recommend that we relentlessly pursue the search to identify all mutations in the $\beta$MHC gene and other genes responsible for HCM. We must continue the march forward not only...
for the sake of individuals who may be impaired as a result of their unfortunate random hit by the mutagen, but also for its other potential implications that may be displayed as a result of this unconventional pathway not so often tread. The long-term response of the heart to all forms of injury whether it be hypertension or myocardial infarction is increased muscle mass (hypertrophy) and/or dilatation. Nature has modeled pure forms of these two responses in the form of familial HCM (hypertrophy) and familial dilated cardiomyopathy (dilatation). Elucidation of the pathogenesis of these diseases should provide insight into cardiac development, its architectural design, and the remodeling response to acquired injury.

References

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