As we enter into the new millennium, the aura of molecular genetics conjures up an exciting and challenging future for the cardiologist. The prospects for improvement in the diagnosis, prevention, and treatment of cardiovascular disease are limited only by our present knowledge and imagination. Speculations on the future aside, 2 major scientific feats have already affected cardiology in the past decade and will continue to do so in the next century: the Human Genome Project, which is nearing completion, and the ongoing intense effort to identify genes responsible for cardiovascular function and disease. Cardiac myocytes react to physiological and pathological stimuli with a growth response that leads to an increase in sarcomeres generated in parallel, giving rise to hypertrophy or, in sequence, giving rise to dilatation, or the combination thereof. Insights fundamental to ultimate elucidation of this growth process are hoped to be gleaned from understanding nature’s errors (inherited disorders) that represent a paradigm of hypertrophy or dilatation. There have been several surprises, namely, the hypertrophy of familial hypertrophic cardiomyopathy (FHCM) being a compensatory response to defects in sarcomeric structural protein, whereas dilated cardiomyopathy (DCM) appears to be an impaired growth response due to defects in cytoskeletal proteins, including the laminae of the nuclear envelope. The genetic revolution provides the engines of ingenuity to achieve even greater progress in the imminent and distant future.

FHCM as a Paradigm for Elucidating the Left Ventricular Hypertrophic Growth Response to Physiological and Pathological Stimuli

Molecular Basis and Pathogenesis of Hypertrophy in FHCM

Discovery of the first gene responsible for FHCM in 1990 was exciting, but that it encoded \( \beta \)-myosin heavy chain \((\beta-MHC) \), a sarcomeric protein, was unexpected. Because the abnormality in HCM is excessive growth (hypertrophy), a mutation in a growth factor or a growth-signaling pathway would be more expected. \( \beta \)-MHC mutations as a cause for FHCM were detected throughout North America and Europe. A total of 9 genes have now been identified, with multiple mutations responsible for FHCM, and all of the genes encode for sarcomeric structural proteins. The 2 most common genes for the disease are \( MYH7 \) (\( \beta \)-MHC) and \( MYBPC3 \) (cardiac myosin–binding protein C). \( \beta \)-MHC mutations were shown to be present in affected family members but not in unaffected members or the general population. Koch’s postulates were soon satisfied. The human wild-type gene (normal) and the mutant form of several of these genes were expressed either through transgenesis after expression of \( \beta \)-MHC, troponin T, myosin binding protein C mutations, and, most recently, in the rabbit after expression of \( \beta \)-MHC. Most of the genetic animal models also exhibit increased interstitial fibrosis and some alteration in myocardial function; however, very little, if any, hypertrophy is observed in the mouse models. The phenotype of HCM was present only in the animals expressing the mutant gene and not in animals expressing the normal human gene. The heart of the mouse has \( \alpha \)-MHC, whereas in humans 98% of the myosin is \( \beta \)-MHC. The cardiac myocyte of the rabbit also has \( \beta \)-MHC. Expression of the human mutant \( \beta \)-MHC gene in the rabbit exhibits a phenotype that is virtually identical to the phenotype observed in human FHCM, which includes sarcomere disarray, increased interstitial fibrosis, hypertrophy, sudden death, and impaired diastolic function, with normal systolic function.

To decipher the molecular events and their temporal sequence leading to the phenotype required deletion in simpler in vitro models. \( \beta \)-MHC is expressed in the right and left ventricles and in many skeletal muscles. Several studies show that isolated skeletal muscles expressing the mutant \( \beta \)-MHC exhibited impaired contractility. \( \beta \)-MHC and myosin filament formation of the expressed mutant protein were impaired, as determined by in vitro models. Analysis of a 3D crystalline structure of skeletal MHC showed that the \( \beta \)-MHC mutations involved several domains critical to sarcomeric contraction, such as impaired actin binding, ATP generation, or calcium sensitivity. Thus, there is a specific molecular defect induced in the \( \beta \)-MHC molecule to explain the in vitro impaired contractility. Expression of a mutant \( \beta \)-MHC gene in the intact feline cardiac myocyte exhibited sarcomere disarray by 72 hours, and similar results were observed after expression of troponin T in cardiac feline myocytes. Expression of a mutant troponin...
T in adult cardiac rat myocytes and in myotubes was associated with decreased cell shortening and impaired contractility. In addition, myocytes isolated from the heart of α-MHC mutant heterozygote mice exhibited impaired contraction and relaxation. Furthermore, detection of cardiac myocyte shortening by a laser system showed that adult feline cardiac myocytes expressing the mutant β-MHC gene exhibited impaired contractility before the development of sarcomere disarray. Most recent studies have also shown that cardiac contractility is impaired in the transgenic mouse before the development of sarcomere disarray. The mutant gene was shown to be incorporated into the feline myocyte myofibrils and, more recently, into the myofibrils of the heart of transgenic mice and transgenic rabbits. One recent study involved cardiac myosin–binding protein C mutations. Expression of mutated proteins in fetal rat cardiomyocytes was associated with altered expression and incorporation in the sarcomeres, and a novel putative myosin binding site on cardiac myosin–binding protein C was suggested by hydrophobic cluster analysis. One may hypothesize that cardiac myosin–binding protein C mutants act as β-MHC and troponin T mutants to impair cardiac contractility.

Thus, the primary genetic defect is impaired contractility, which stimulates the release of a growth factor(s) that leads to interstitial fibrosis and hypertrophy. Despite the presence of the mutant protein in equal abundance in the right and left ventricles, hypertrophy seldom develops in the right ventricle, suggesting that the high pressure of the left ventricle is the stimulus. Furthermore, relief of outflow tract obstruction with septal alcohol injection is associated with regression of hypertrophy and collagen, indicating that the stimulus is increased pressure. Thus, the defective sarcomeric protein triggers the release of growth factors that lead to cardiac hypertrophy and collagen formation, as summarized in Figure 1, a modification of the figure by Marian.

**Problems to Be Resolved**

In the mouse, unlike in human FHCM, there is decreased systolic function, perhaps because there is only minimal hypertrophy, whereas in the rabbit, there is extensive hypertrophy with normal systolic function. Techniques such as subtraction hybridization or DNA microarrays may detect different growth factors that account for the different hypertrophic responses in these 2 models.

The culprit responsible for sudden cardiac death remains unknown in FHCM, as it does for sudden cardiac death in myocardial ischemia and cardiac failure, but for all of these, it is postulated to be fibrosis. The excitement for the future will be elucidating the triggers for the growth response and development of a therapeutic strategy to decrease the incidence of sudden death. We have the genetic animal models, and so the time has come to evaluate known therapies, such as ACE inhibitors and angiotensin II receptor blockers and develop novel drugs, such as specific growth factor inhibitors.

**Recognition That the Cytoskeleton Is a Major Determinant of the Cardiac Growth Response**

The cytoskeleton is a complex set of protein filaments that is very important to generate shape and movement. The sarcomere is thought to represent an evolutionary specialization of filament proteins that originally served functions that were common to early cells, such as cell motility or chromosome movement. One of these sarcomeric proteins, actin, is also involved in the pathogenesis of DCM. In this case, the actin filament proteins that originally served functions that were common to early cells, such as cell motility or chromosome movement. One of these sarcomeric proteins, actin, is also involved in the pathogenesis of DCM. The dystrophin gene, the first gene to be identified by positional cloning, is part of a multisubunit complex that confers a structural link between the extracellular matrix and the actin skeleton. Mutations in the dystrophin gene cause Duchenne muscular dystrophy and its milder allelic variant, Becker muscular dystrophy, which develop profound cardiomyopathies. Dystrophin abnormalities also seem to underlie acquired forms of DCM. Another important component of the extrasarcomeric cytoskeleton is desmin. A subset of skeletal and cardiac myopathies have as a hallmark abnormal deposits of desmin aggregates. These myopathies are characterized by muscle weakness, restrictive cardiomyopathy, cardiomyocyte hypertrophy, cardiac dilatation, conduction blocks, arrhythmias, and heart failure. Mutations were found not only in the desmin gene but also in the α-B crystallin chaperone gene. α-B crystallin interacts specifically with desmin and has a role in desmin intermediate filament assembly. Of particular significance was the finding that a missense mutation in the desmin gene was responsible for DCM, with no discernible clinical involvement of the skeletal or smooth muscles.

Thus, a new concept is emerging from these genetic data. The extrasarcomeric cytoskeleton plays a major role in the growth response of the mammalian heart and in the pathogenesis of cardiomyopathies. Morphological studies show that expression of cytoskeletal proteins is altered in acquired forms of heart failure. Major hopes now reside in the annotation of the human genome sequence and the availability of high-throughput methods for DNA screening.
Recognition of the Role of the Nuclear Envelope in Cardiac and Skeletal Muscle Disease

Phenotypic Variability of Nuclear Envelopathies

The story began in 1902 at the “Clinique Nerveuse de la Salpêtrière,” where 2 neurologists described a myopathy with familial contractures. It was only in 1979 that it was recognized as a distinct clinical entity and called Emery-Dreifuss muscular dystrophy (EDMD). By adulthood, affected individuals invariably develop heart block requiring pacing or severe dysrhythmias sometimes requiring an implantable defibrillator. The disease is transmitted as an X-linked trait (X-EDMD) or as an autosomal dominant trait (AD-EDMD).

In 1994, the gene responsible for X-EDMD, STA, was discovered. Its product was called emerin. It was a surprise to find that emerin is a nuclear integral membrane protein. In 1999, the lamin A/C gene, LMNA, was identified as the disease gene of AD-EDMD. LMNA encodes lamins A and C, which derive from alternative splicing at the 3’-end of the gene. Lamins A and C are components of the nuclear envelope and are located in the lamina (Figure 2A). Very unexpectedly, in the majority of affected members of one of the French families analyzed in that study, the disease was confined exclusively to the heart and was associated with integral proteins of nuclear envelope. LBR indicates lamin B receptor; LAP2, lamina-associated polypeptide 2; MAN1 is a recently characterized polypeptide recognized by specific autoantibodies (MAN from initials of patient). Image kindly given to us by R.M. Barton and H. Worman. B. LMNA mutations and their position on protein structure of lamins A and C. Lamin proteins display a common central α-helical rod domain (coils 1a, 1b, and 2), flanked by nonhelical domains at amino- and carboxy-terminal ends. Mutations in bold type cause dilated cardiomyopathy with conduction defects; mutations in plain type cause autosomal dominant EDMD; underlined mutations cause limb-girdle muscular dystrophy type 1B; mutations in italics cause partial lipodystrophy.
The year 2000 is adding a very intriguing complexity to the role of \textit{LMNA} products in the pathogenesis of human diseases. First, it was found that mutations in this gene cause another muscular dystrophy, the limb girdle muscular dystrophy type LGMD1B. The LGMD1B form is inherited as an autosomal dominant trait. It is slowly progressive, with age-related atrioventricular cardiac conduction disturbances and DCM and absence of early contractures\textsuperscript{50} (Figure 2B). We identified \textit{LMNA} mutations in 3 very-well-characterized LGMD1B families, demonstrating that LGMD1B and AD-EDMD are allelic disorders.\textsuperscript{51} In addition, a deletion was identified in a family with DCM and skeletal muscle abnormalities.\textsuperscript{52} Second, missense mutations were reported to be implicated in Dunningan-type familial partial lipodystrophy (FPLD)\textsuperscript{53} (Figure 2B). Patients with FPLD are born with extremities, trunk, and gluteal region after the onset of normal fat distribution but then lose subcutaneous fat from their extremities, trunk, and gluteal region after the onset of puberty. The elegant rationale to consider \textit{LMNA} as a candidate gene for this disease was that there is an analogy between the highly specific anatomic site involvement in AD-EDMD and FPLD.

\textbf{What Mechanisms Link the Nuclear Envelope to Cardiac and Skeletal Muscle Diseases and to Lipodystrophies?}

Thus, rare mutations in \textit{LMNA}, a ubiquitously expressed gene that encodes nuclear structural proteins, cause inherited disorders of cardiac and skeletal muscles and of adipose tissues. The mutations are distributed all along the gene, without distinct regions being specific to a disease (Figure 2B). Functional knockout of the mouse gene that encodes lamin A/C induces a postnatal cardiac and skeletal muscular dystrophy.\textsuperscript{55} The mice also lack adipose tissue. The lamins have been implicated in mediating DNA replication, chromatin organization, spatial arrangement of nuclear pore complexes, nuclear growth, and anchorage of nuclear-envelope proteins. Because both muscle cells and fat cells derive from the mesenchymal stem cell, a general model was recently proposed by Wilson.\textsuperscript{56} \textit{LMNA} mutations would selectively affect the differentiation, maintenance, repair, or regulation of cells in the mesenchymal stem cell. Another theory is that forces generated during skeletal or cardiac muscle contraction or the specific metabolic activity of the adipose tissue might render these tissues especially sensitive to nuclear damage produced by the mutation. Finally, the mutations could alter the interactions between lamins A/C and putative tissue-specific partners.

\textit{ARVD, a Model to Understand the Right Ventricular Response to Injury and Its Associated Right Ventricular Failure and Sudden Death}

\textbf{Increased Awareness of ARVD as a Cause for Sudden Death}

Arrhythmogenic right ventricular dysplasia (ARVD) is relatively new as a diagnostic entity and was not included in the WHO classification of cardiomyopathies until 1996. Reports since the days of Osler in 1905\textsuperscript{57} of patients with partial replacement of the right ventricular myocardium by fat or fibrous tissue were probably Uhl’s anomaly rather than ARVD. In 1978, Frank et al\textsuperscript{58} referred to the new entity as RV dysplasia. In 1982, Fontaine et al\textsuperscript{59} added the term arrhythmogenic because arrhythmias and sudden death seem to be major clinical manifestations. ARVD is a familial cardiomyopathy of unknown pathogenesis characterized by a gradual loss of myocytes and replacement by fatty and fibrous tissue, which, as it progresses, leads to dilatation of the ventricle and impaired function. The clinical course is characterized by arrhythmias, sudden death, and heart failure. The prevalence of the disease in Italy is 1:5000,\textsuperscript{60} and it accounts for 22.4% of deaths in athletes. In Olmsted County, Minnesota, histological features in keeping with ARVD were observed in 9 of 54 sudden death victims 23 to 40 years old.\textsuperscript{61} Thus, in this study, ARVD accounted for 17% of sudden deaths in the young. Despite the diagnostic difficulties, ARVD is now established as a major cause of sudden death in the young, and the rate of sudden death is 2.5% per year, frequently without prior symptoms.

\textbf{What Are the Implications to Be Cleaned From Studying ARVD?}

Although ARVD, like idiopathic or familial DCM, is associated with a dilated cardiac chamber, it has distinguishing features with broad biological, physiological, and pathologic implications relating directly to the cardiac response to injury and the subsequent development of cardiac failure. Whereas familial or idiopathic DCM is manifested in the left ventricle, with involvement of the right ventricle being secondary and occurring at a later stage, ARVD is a mirror image, with the disease initiating exclusively in the right ventricle, and may only much later involve the left ventricle. In familial HCM, all of the responsible genes identified so far encode sarcomeric proteins, which are equally distributed throughout the right and left ventricles. Thus, restriction of the phenotype to the left ventricle in FHCM is not because of expression of a chamber-specific gene but rather from an interaction with the environment, such as the increased pressure load, which exceeds by several-fold that of the right ventricle. The 2 genes identified as being responsible for familial DCM, actin and desmin, are also present throughout the heart, yet the initial phenotype is in the left ventricle. These observations would suggest that the restriction of the ARVD phenotype to the right ventricle is more likely due to a unique stimulus of the right ventricle rather than a chamber-specific gene. The other intriguing aspect of ARVD is the possibility that myocardial cells die and are replaced by fatty-fibrous tissue due to apoptosis.\textsuperscript{60,62} Confirmation of the involvement of the apoptosis system would immediately provide a new framework to direct the development of specifically targeted therapies.

Although the genetic basis for FHCM has rapidly evolved (9 genes) and that for familial DCM is beginning to emerge (6 loci and 4 genes), a gene responsible for the autosomal dominant form of ARVD is yet to be discovered. Although no gene has yet been identified, 3 loci, 1q42\textsuperscript{63} 1p42,\textsuperscript{64} and 2q32,\textsuperscript{65} have been mapped in Italian families. Recently, 2 loci, 3p23\textsuperscript{66} and 10p12-p14,\textsuperscript{67} have been mapped in North...
American families. Identifying the responsible genes has significantly improved the diagnosis of FHCM and is likely to improve the diagnosis of familial DCM even more. A family with a recessive form of ARVD was identified on the Greek island of Naxos. The recessive form of the disease appears to be quite distinct from the dominant form, being associated with palmoplantar keratoderma and woolly hair. The gene responsible for this disease resides on chromosome 17q21 and most recently was shown to encode for plakoglobin. Plakoglobin is one of the proteins involved with cell-to-cell adhesions and plays a major role in maintaining myocyte integrity. Whether this will provide a clue to the genes responsible for other loci remains to be determined.

Familial Atrial Fibrillation: A Cornerstone of Our Understanding of Atrial Conduction

The identification of several genes for ventricular tachycardia of the long-QT syndrome and Brugada syndrome is in the process of enhancing our understanding of the normal physiology of ventricular conduction and the pathophysiology of arrhythmias. It is likely that genetic defects exist in a host of genes involved with ventricular conduction and arrhythmias. We know very little about atrial conduction and supraventricular arrhythmias, including atrial fibrillation (AF), paroxysmal tachycardia, and Wolff-Parkinson-White syndrome. AF is the most common form of arrhythmia affecting humans; it is associated with extensive morbidity and mortality and accounts for >33% of all strokes in patients >65 years old.

The first chromosomal locus responsible for AF was mapped to chromosome 10q21 in 1997. The region containing the locus has since been narrowed from 27 to 0.6 cM, and thus, the gene is expected to be identified in the near future. It is hoped that isolation of the first gene on 10q21 will identify a pathway from which other candidate genes can be derived analogous to that of the sarcomeric genes of FHCM.

Once the genes and the mutations have been identified, the collaboration of the cardiologist will again be necessary to further define the precise mechanisms and provide the infrastructure for prevention and treatment.

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