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Phenotypic Plasticity of Sarcomeric Protein Mutations*

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Abstract

The practice of medicine today is founded primarily on the phenotypic characteristics of diseases. The recognition of common forms of cardiomyopathies as hypertrophic, dilated, restrictive, and arrhythmogenic right ventricular cardiomyopathies typifies the phenotype-based approach to diseases. The approach clearly has had numerous positive impacts on the diagnosis, prognostication, prevention, and treatment of various diseases. The advent of the beta-blockers and inhibitors of the renin-angiotensin-aldosterone pathway in the treatment of systolic heart failure are testaments to the clinical utility of the phenotype-based approach. Despite the enormous impacts, however, the phenotype-based approach has considerable shortcomings. For example, the approach has failed to offer a cure for many diseases including systolic heart failure, in which pharmacologic interventions reduce mortality by approximately 20% to 30% and prolong survival by a few months (1–3).

Accordingly, the annual incidence of heart failure, the common “end” phenotype of many cardiovascular diseases, has increased to 550,000 cases (4). It affected approximately 5.2 million individuals in the U.S. alone and accounted for approximately 1,100,000 hospitalizations and 57,000 deaths in 2004 (4). The inherent problem of the phenotype-based approach to the practice of medicine is that it does not provide sufficient information on the cause and/or the pathogenesis of the disease. To cure a disease, it is necessary, at least in most cases, to understand the cause and the pathways involved in the pathogenesis of the phenotype.

The dawning of modern molecular genetics has raised the possibility of a switch from a phenotype-based to a genotype-based approach to medicine. Elucidation of the molecular genetic basis of various cardiovascular diseases, although yet incomplete, has led to the categorization of the phenotypes according to their genetic etiology. For example, hypertrophic cardiomyopathy (HCM) is considered a disease of sarcomeric proteins, dilated cardiomyopathy (DCM) a disease of cytoskeletal proteins, and long QT syndrome a disease of ion channels. The clear advantages of the genotype-based as opposed to a phenotype-based approach are in early diagnosis of those at risk, before and independent of the clinical phenotype as well as in the accurate diagnosis of the true phenotype from phenocopy conditions. The clinical significance of the first advantage is evident by the possibility of interventions to prevent the evolving phenotype. The utility of an accurate diagnosis and distinction from a phenocopy state is well illustrated in certain circumstances, such as Fabry disease, which could be clinically indistinguishable from HCM caused by mutations in sarcomeric proteins (5,6). Enzyme replacement therapy with alpha-galactosidase, the enzyme responsible for Fabry disease, has been shown to impart considerable clinical benefit in management of patients with Fabry

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disease, while the conventional treatment offered for true HCM would render no significant benefit in such patients (7).

The genotype-based approach, however, is compounded by the genetic heterogeneity of each specific phenotype. It is now evident that a large number of mutations in different genes, albeit largely within the same class, could cause the same phenotype. Moreover, mutations in one gene could cause multiple phenotypes, as best illustrated in the case of lamin A/C, whereby mutations can cause 13 different diseases, including DCM, conduction defects, Emery Dreifuss muscular dystrophy, familial partial lipodystrophy, premature aging, axonal neuropathy, and insulin resistance (8). Likewise, it is also intriguing that mutations in the same gene and even the same mutation in different background cause disparate phenotypes (8). In the case of cardiomyopathies, such phenotypic plasticity was best illustrated by Dr. Seidman's group, who reported that mutations in the *MYH7* and *TNNT2*, encoding beta-myosin heavy chain and cardiac troponin T, respectively, could cause either HCM or DCM, the opposite ends of the spectrum of phenotypic responses of the heart to injury, stress, or mutations (9). The report by Kubo et al. (10) in this issue of the *Journal* expands on the phenotypic plasticity of sarcomeric protein mutations. In a study of 1,226 patients from 688 families with the clinical diagnosis of HCM, Kubo et al. (10) detected a restrictive phenotype, resembling restrictive cardiomyopathy (RCM) in 19 individuals from 16 families. The phenotype was characterized by mild or no left ventricular hypertrophy, preserved left ventricular systolic function, restrictive mitral inflow pattern on Doppler echocardiography, enlarged atria, and a high rate of mortality (10). Interestingly, 8 probands had mutation either in the *MYH7* or *TNNI3*, encoding cardiac troponin I. The findings that HCM could present, yet uncommonly, with a restrictive phenotype resembling RCM and the high mortality rate associated with a restrictive physiology are noteworthy and have clinical implications. The phenotypic plasticity of sarcomeric protein mutations reported by Kubo et al. (10) is also in accord with previous reports from Dr. McKenna's group, Dr. Seidman's group, and others (9,11,12). Collectively, the data show mutations in sarcomeric proteins can present phenotypically as HCM, DCM, or RCM.

The molecular basis of phenotypic plasticity of sarcomeric protein mutations is unknown. It is likely to be multifactorial and partly determined by the impact of the mutant protein on sarcomere structure and function, the unit of contraction and relaxation in the striated muscle. Complex mechanisms govern assembly and function of the sarcomeres. The complexity affords the mutant proteins the opportunity to impart a diverse array of effects spanning from the assembly of the filaments, protein-protein interactions to Ca^{2+} sensitivity of the actomyosin interaction. The motor molecule of the actomyosin interaction is myosin heavy chain, which exists in a hexameric complex comprised of 2 myosin heavy chain proteins, and 2 regulatory and 2 essential light chains. The partner for MyHC in contraction and relaxation is cardiac alpha-actin, which is a component of the thin filaments. Association and dissociation of MyHC and actin is regulated by a series of proteins including the troponin-alpha-tropomyosin complex. Other constituents, such as obscurin, myosin binding protein C, titin, and Z-disc proteins also play important roles in the regulation of sarcomere structure and function. Electrical depolarization of the cell membrane opens the L-type calcium channels and allows Ca^{2+} influx, which incites the dynamic interactions between the sarcomeric proteins. The influx opens the ryanodine receptors and releases Ca^{2+} from sarcoplasmic reticulum. The released Ca^{2+} binds to cardiac troponin C and induces conformational changes in the troponin-alpha-tropomyosin complex. The change displaces cTnI, the inhibitory protein of the complex, from actin and allows association of actin and MyHC. In the relaxed state, the globular head of MyHC is bound to adenosine triphosphate (ATP). Upon binding to actin, MyHC hydrolyzes ATP to adenosine diphosphate (ADP) and inorganic phosphate. The ADP-bound MyHC globular head generates the working stroke by displacing the actin filament by several nanometers and generating several piconewton force (13). Uptake of Ca^{2+} by the sarcoplasmic reticulum reverses the process, and replacement of ADP by ATP returns the

sarcomeres to the relaxed state. Thus, the phenotypic plasticity of mutant sarcomeric proteins could be partly explained by the effect of mutant proteins on different structural and regulatory components of the force generation and relaxation complex. In support of this hypothesis, mutations in cTnT, known to cause HCM in humans, enhance Ca^{2+} sensitivity of myofilament force-generation and ATPase activity (14,15). In contrast, those leading to DCM impart opposite effects (16–18). Likewise, our preliminary data show cTnI, the inhibitory protein of the acto-myosin interaction, exhibits a lower affinity for cardiac alpha-actin in the presence of the cTnT-Q92, known to cause HCM (19). The opposite is the case in the presence of cTnT-W141, responsible for DCM in humans (19). Thus, the existing data could provide some explanations for the contrasting phenotypes of HCM and DCM arising from mutations in the same gene, which are primarily based on the differential effects of the mutant proteins on force generation and ATPase activity. However, there is a paucity of information to explain the molecular mechanisms responsible for RCM phenotype arising from mutant sarcomeric proteins. Speculations include impaired ATP-mediated dissociation of myosin from actin, leading to impaired myocardial relaxation and a restrictive physiology. Alternatively, mutant protein could affect function of titin isoforms, implicated in myocyte stiffness (20), and phosphorylation of sarcomeric proteins.

The clinical phenotype of a genetic disorder arises from more information than is contained in the causal deoxyribonucleic acid sequence or the mutation. The levels of complexity that govern evolution of the phenotype in cardiomyopathies can not be solely restricted to differential interactions between the constituents of the sarcomeric proteins. Such differential interactions alone are insufficient to explain phenotypic plasticity resulting from the same mutation in a given gene (8,11). The point is exemplified by a previous work also from Dr. McKenna's group showing mutations in *TNNI3* causing either HCM or RCM in members of the same family (11). One could speculate on the potential determinants of the ensuing phenotypes in such situations to include the effects of the modifier genes, epigenetic factors, and post-transcriptional and translational modifications of expressed proteins.

Genes contain information that is essential for the development of the phenotype but not necessarily complete. Any perturbation in the gene structure is expected to convey a phenotype, sometimes quite subtle, as in the case of complex phenotypes and occasionally drastic, as in the case of single-gene disorders with Mendelian pattern of inheritance. The mutation is the instigator but not the sole determinant of the clinical phenotype. The final phenotype is determined not only by the causal mutation but also by the modifier genes, each exerting a modest effect, epigenetic factors, which link the gene to the phenotype, and the environmental factors. Thus, while advances in molecular genetics of cardiovascular diseases are gradually changing our classical understanding of the disease and the phenotype-based approach to the practice of medicine, they are unlikely to be sufficient to trigger a full switch from a phenotype-based to genotyped-based medicine. The research emphasis must be not only in identification of the causal genes and mutation but also on delineating the molecular mechanisms involved in the pathogenesis of the phenotype. Only then can a comprehensive molecular approach, integrating the genetic, epigenetic, transcriptomic, and proteomic profiles, be developed to propel medicine toward the molecular-based diagnosis, prevention, and treatment, targeted to specific genes or pathways involved in the pathogenesis of the phenotype.

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