Molecular genetic analysis of cardiomyopathies inherited as single-gene disorders is now a relatively mature field. Nevertheless, research in this area continues to throw up unexpected complexities that by challenging prevailing paradigms contribute important new insights into myocardial biology and disease processes.

Hypertrophic Cardiomyopathy: More Than a Disease of the Sarcomere?
A common finding in genetic analyses of an inherited condition is that a single clinical entity can be caused by mutations in multiple genes. Similarly, different mutations within a single gene can give rise to surprisingly diverse clinical conditions. Against this background, the genetic complexity that has been shown to underlie hypertrophic cardiomyopathy (HCM) is not unusual. Initially, the emerging picture appeared clear; namely, that HCM was a disease of the sarcomere, attributable to subtle perturbations (mostly missense mutations) affecting a number of components of the cardiac contractile apparatus.1 As further candidate genes were tested, it became apparent that most, if not all, sarcomeric proteins could be mutated to cause HCM.2,3 Although still fitting a single paradigm, these disease-genes encode proteins that contribute to cardiac contractility in very diverse ways — some enzymatic, some structural and others regulatory. Perhaps not surprisingly then, functional analyses have not shown a unifying abnormality of contractility as an explanation of the pathway to the HCM phenotype.4 Although the majority of HCM mutant contractile proteins lead to enhanced contractility, others seem to have the opposite effect by reducing maximum force output.5–7 A proposal has been made that instead, the common abnormality is increased energy cost of force production,4,5,8 and this has been supported by observations of energetic compromise in a mouse model9 and by data on ATP consumption in mutant sarcomeres.10 Testing of positional candidate genes involved in cytoskeletal function implicating candidate genes involved in cytoskeletal function in inherited dilated cardiomyopathy (DCM) built on knowledge of the role of dystrophin mutations in the heart by implicating candidate genes involved in cytoskeletal function and force transmission within the cardiomyocyte. As disease-gene identification depended on candidate gene studies, additional disease genes (desmin, δ-sarcoglycan, and cardiac actin) shared similar characteristics and thus tended to validate the prevailing model of pathogenesis.2,3 However, disease genes for DCM identified through systematic genome-wide linkage analysis have revealed a far wider range of molecular etiologies. These include mutations affecting components of the nuclear envelope (lamin A/C) and a protein of uncertain function (tafazzin).2,3 Perhaps more surprisingly has been the observation that different mutant alleles of the contractile protein genes that underlie HCM are a cause of DCM. Mutations that are specifically associated with DCM without disarray or much hypertrophy have been described in cardiac actin, β-myosin heavy chain, cardiac troponin T, α-tropomyosin, and titin.2,3,14 Thus, abnormalities in force production, rather than transmission, can apparently also cause DCM. Such diversity is predicted by observations from knockout studies in the mouse, where an even wider array of targeted gene ablations produce a DCM-like phenotype. Presumably, this reflects both the limited ways in which the diseased myocardium can remodel and the relatively crude (and hence inclusive) phenotypic definition of DCM. Conceivably, anything that disrupts myocyte architecture or viability sufficient to cause gradual myocyte loss may result in DCM.

Dilated Cardiomyopathy: An End Point for Heterogeneous Pathologies
In analogous fashion, early disease-gene identification studies in inherited dilated cardiomyopathy (DCM) built on knowledge of the role of dystrophin mutations in the heart by implicating candidate genes involved in cytoskeletal function and force transmission within the cardiomyocyte. As disease-gene identification depended on candidate gene studies, additional disease genes (desmin, δ-sarcoglycan, and cardiac actin) shared similar characteristics and thus tended to validate the prevailing model of pathogenesis.2,3 However, disease genes for DCM identified through systematic genome-wide linkage analysis have revealed a far wider range of molecular etiologies. These include mutations affecting components of the nuclear envelope (lamin A/C) and a protein of uncertain function (tafazzin).2,3 Perhaps more surprisingly has been the observation that different mutant alleles of the contractile protein genes that underlie HCM are a cause of DCM. Mutations that are specifically associated with DCM without disarray or much hypertrophy have been described in cardiac actin, β-myosin heavy chain, cardiac troponin T, α-tropomyosin, and titin.2,3,14 Thus, abnormalities in force production, rather than transmission, can apparently also cause DCM. Such diversity is predicted by observations from knockout studies in the mouse, where an even wider array of targeted gene ablations produce a DCM-like phenotype. Presumably, this reflects both the limited ways in which the diseased myocardium can remodel and the relatively crude (and hence inclusive) phenotypic definition of DCM. Conceivably, anything that disrupts myocyte architecture or viability sufficient to cause gradual myocyte loss may result in DCM.

Thus, what started as apparently simple has become more complicated. Fortunately, the finding that diverse disease genes can be mutated to cause a given cardiomyopathy may
ultimately help pinpoint the pathogenic pathways by highlighting shared abnormalities. Similarly, different mutant alleles within a single gene that result in contrasting cardiomyopathy phenotypes provide powerful “experiments of nature” to dissect divergent pathways.

A New HCM Disease Gene: Muscle LIM Protein

Into this arena now comes a new disease gene, with the compelling implication of missense mutations in muscle LIM protein (MLP) as a further cause of HCM (see article by Geier et al in this issue15). MLP is LIM-only protein expressed in striated muscles, with expression at high levels in developing and adult hearts. Like other LIM proteins, it has diverse regulatory and cytoskeletal functions. MLP is a key regulator of myogenic differentiation, an effect that must be mediated by binding of the LIM motif to other regulatory proteins, given that MLP does not have a DNA-binding homeodomain.16 MLP is also a costameric protein, involved in part of the cytoskeleton that attaches peripheral sarcomeres to the sarcolemma, linking the β-spectrin network to myofibrillar actin filaments; it is therefore likely to have important mechanical and signaling functions.17

MLP was explored as a candidate gene for human inherited cardiomyopathy on the basis of the murine knockout phenotype. MLP-deficient mice have been described as a murine model of DCM with left ventricular dilatation and depressed systolic function.18 In light of the various disease genes known to induce DCM in humans, the molecular pathology of the MLP knockout could be attributed to a number of possible pathways. These include not only defects of cytoskeletal function (both force transmission and signaling), but also direct effects on cardiomyocyte development and survival. Although Geier et al15 considered MLP as a candidate for DCM in humans, they rightly recognized the potential for overlap in cardiac remodeling pathways and also tested MLP as a candidate gene for HCM. Perhaps surprisingly, MLP missense mutations were found only in probands with HCM, with convincing exclusion of MLP as a significant cause of DCM (no mutations among 400 DCM probands). These findings raise a number of questions: are the genetic data sufficiently robust to prove causality, what is the precise phenotype in affected patients, why is the phenotype that of HCM rather DCM, and what does this mean for our understanding of disease pathways?

Confirmation of a novel disease gene that has been tested as a candidate gene is not always straightforward.19 This approach is less rigorous than that of a genome-wide search for linkage in a large family, and the data are not robust without evidence of co-segregation. A common scenario is the testing of many unrelated probands from small families followed by demonstration of the absence of the mutation in a normal cohort. However, the absence of the mutation from negative controls is necessary, but not sufficient, to establish causality. Similarly, demonstration of functional abnormalities is only definitive if specific patterns of abnormality are known to be expected or if the phenotype is recapitulated in an intact model organism bearing the specific mutation. Fortunately, the cumulative genetic evidence implicating the MLP mutations does seem sufficient to conclude that they have a causal role; although the family sizes are small, they do show significant evidence of co-segregation, and the mutations affect residues that not only are highly conserved but which also occupy critical sites within the zinc finger motif of the LIM domain (including a substitution in 1 of the 4 cysteine residues that anchor the zinc atom).15 Although the genetic data are convincing, MLP mutations are clearly a very rare cause of HCM (3 cases identified from 200 screened); the impact of the finding has biological rather than clinical significance.

By conventional clinical criteria, the phenotype in the 3 families is typical of HCM, with a characteristic range of symptoms, ECG changes, and extent/pattern of hypertrophy among individuals (including classical asymmetric septal hypertrophy with outflow obstruction). Penetrance by ECG and echo criteria was incomplete, and an attractive aspect of the study was the application of more sophisticated indices of subtle clinical dysfunction through the use of tissue Doppler imaging. An issue which may come increasingly to the fore as “non-sarcomeric” disease genes are proposed for HCM is whether histological criteria need to be met to define the phenotype. The hallmark feature of myocyte disarray has been present in diverse contractile protein disease-gene groups, with the result that the more recently implicated contractile protein genes have usually been defined as HCM genes without histological confirmation of the phenotype. The observation that clinically typical features of HCM in families with AMPK mutations are associated with atypical histology12 suggests that a histological classification is additionally informative. It is then a matter of semantics how these similar phenotypes, or phenocopies, are labeled; the important issue is the way in which differences in phenotype are used to dissect differences in disease pathway. Unfortunately, no histological data were available for the individuals described in the current report.15

There are a number of reasons why the MLP mutant phenotype in humans may be different from that described in the mouse. Obviously, this may just reflect different downstream pathways and adaptations in the different species; there are numerous precedents for mouse models of human disease where the phenotype is, at best, only partly recapitulated. Additionally, both the nature of the mutation and the gene dosage differ. The knockout mice are homozygous for a null allele (ie, a complete ablation of MLP), whereas the patients with HCM are heterozygous for missense alleles. Some of the contractile protein mutations that have been modeled to mimic HCM in mice are associated with cardiac hypertrophy in heterozygotes but with DCM in homozygous animals.20 Alternatively, it may be that the oversimplification of defining cardiomyopathy phenotypes in a dichotomous way masks underlying similarities. Whereas some LIM−/− mice present in adulthood with the DCM phenotype, others present with an early phenotype characterized by a major increase in heart weight (2 to 4-fold) associated with histological hypertrophy before rapid progression to heart failure in the second post-natal week. Further, DCM is not typically associated with substantial distortions of cell architecture, and yet sarcomeric disorganization was prominent in the MLP−/− mice.18 Thus, in these animals, the phenotype could just as
reasonably have been characterized as HCM with rapid progression to the dilated phase. Comparable oversimplification of phenotype designation may also be problematic clinically, as newer disease genes may blur the margins between HCM and DCM in humans.

What can be inferred from the MLP mutant alleles in terms of HCM disease pathways? With a pleiotropic protein such as MLP, a number of potential disease pathways immediately present themselves. The 3 mutations described all occur in the LIM-1 domain of MLP that is responsible for binding to α-actinin in the z-line of the sarcomere; preliminary analyses by Geier et al.\(^\text{15}\) indicate that one of the MLP mutations does indeed reduce affinity for α-actinin. Thus, these mutations have the potential to directly disrupt sarcomeric organization and integrity and so may simply be analogous to HCM mutations already described in titin and in the LMM region of the myosin rod.\(^\text{21}\) Disrupted sarcomeric integrity could diminish the efficiency and economy of force production in much the same way as mutations within the force-generating apparatus itself. Alternatively, a hypertrophic pathway based on disordered intracellular calcium homeostasis is equally plausible. One of the ways in which it is envisaged that energy compromise could mediate hypertrophy is through failure to meet the extreme ATP requirements of the SERCA-2 calcium re-uptake pump, resulting in elevated cytosolic calcium and calcium-dependent hypertrophic signaling. In MLP\(^{-/-}\) mice, the [Ca\(^{2+}\)] transient was markedly increased, raising the same possibility of calcium-dependent hypertrophic signaling.\(^\text{22}\) Further, genetically engineered enhancement of sarcoplasmic reticulum calcium re-uptake by βARK-1 inhibition or by phospholamban ablation can prevent the development of the cardiomyopathic phenotype in MLP\(^{-/-}\) mice.

What can we expect in the future? Almost certainly, greater complexity is yet to come. A significant proportion of HCM families appear not to have mutations in genes encoding known components of the cardiac sarcomere. Thus, new disease genes will likely signify additional hits in the disease pathway; current models predict that these may include proteins involved in energy metabolism or calcium handling in the myocyte. A proliferation of new genes can be expected for DCM, perhaps not reflecting a single pathway as such but rather a set of pathologies with a common endpoint. Fortunately, complexity generates more numerous clues and improved tools for defining disease processes.

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Genetic Clues to Disease Pathways in Hypertrophic and Dilated Cardiomyopathies
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