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Genotype, phenotype: upstairs, downstairs in the family of cardiomyopathies

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Commentary

I loved Upstairs, Downstairs . . . you identify with the downstairs people while vicariously enjoying the life of the upstairs people. —Alistair Cooke, Host of Masterpiece Theatre Upstairs, Downstairs was beloved by a generation of Americans who became entranced by this classic story of an upstairs Edwardian family and its downstairs household. The series chronicled the daily repartee and tangled relationships between the upstairs and downstairs contingents, a tale laced with all of the ingredients of human complexity. Despite their diverse perspectives and backgrounds, the lives at 165 Eaton Place became entwined by their core values, conserved traits, and the shared challenges of the day. In terms of human biology, Upstairs, Downstairs was a classic saga of the interplay between genes and environment. In the family of human cardiomyopathies, another complex story is unfolding, this time around the divergent backgrounds and perspectives of clinical cardiology and molecular genetics. In this cardiological version of Upstairs, Downstairs, the theme is Genotype, Phenotype, and the initial storyline revolves around the precept that the primary determinant of the clinical phenotype is the molecular genotype. The clinical viewpoint has been underpinned by noninvasive analyses […]

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Genotype, phenotype: upstairs, downstairs in the family of cardiomyopathies

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I loved Upstairs, Downstairs . . . *you identify with the downstairs people while vicariously enjoying the life of the upstairs people.*

—Alistair Cooke, Host of *Masterpiece Theatre*

Upstairs, Downstairs was beloved by a generation of Americans who became entranced by this classic story of an upstairs Edwardian family and its downstairs household. The series chronicled the daily repartee and tangled relationships between the upstairs and downstairs contingents, a tale laced with all of the ingredients of human complexity. Despite their diverse perspectives and backgrounds, the lives at 165 Eaton Place became entwined by their core values, conserved traits, and the shared challenges of the day. In terms of human biology, *Upstairs, Downstairs* was a classic saga of the interplay between genes and environment.

In the family of human cardiomyopathies, another complex story is unfolding, this time around the divergent backgrounds and perspectives of clinical cardiology and molecular genetics. In this cardiological version of *Upstairs, Downstairs*, the theme is *Genotype, Phenotype,* and the initial storyline revolves around the precept that the primary determinant of the clinical phenotype is the molecular genotype. The clinical viewpoint has been underpinned by noninvasive analyses that can quantitatively assess differences in chamber volume, wall thickness, hypertrophy, systolic versus diastolic dysfunction, and outflow tract obstruction, leading to three clinical subtypes: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and restrictive cardiomyopathy (RCM). The molecular viewpoint has been driven by: 1) the discovery of diverse cardiomyopathic genotypes, resulting in a detailed examination of the differential phenotypic effects of mutations in a myriad of sarcomeric and cytoskeletal genes, 2) cataloguing the effects of missense mutations by the severity of the charge change within a given disease gene, 3) evaluating differences between haploinsufficiency and missense mutations, and 4) correlating these diverse disease genotypes with differences in the severity, time of onset, and diversity of the cardiomyopathy phenotype. The theme of this early script is that there may be a specific set of molecular pathways

Table 1
Molecular defects linked to human cardiomyopathies

Genomic defects	Human defects		
	HCM	DCM	RCM
Sarcomere			
Myosin heavy chain	Missense (17–19)	Missense (20)	
Myosin essential light chain	Missense (21)		
Myosin regulatory light chain	Missense (21)		
Cardiac actin	Missense (22)	Missense (3)	
Troponin-T	Missense/deletion (19, 23)	Deletion (20)	
Troponin-I	Missense (7)		Missense (6)
α-Tropomyosin	Missense (19, 23)	Missense (24)	
Myosin binding protein-C	Missense/deletion (19, 25)		
Titin/titin-related protein			
Titin	Missense (26)	Missense/deletion (27, 28)	
Telethonin (T-cap)	Missense (14)		
Z-disk–associated proteins			
MLP	Missense (14)		
Sarcolemma cytoskeleton			
Dystrophin	Deletion (29–31)		
β-Sarcoglycan	Deletion/duplication (32)		
δ-Sarcoglycan	Missense (33)		
α-Dystrobrevin	Missense (34)		
Metavinculin	Deletion (35, 36)		
Intermediate filaments			
Desmin	Missense (37, 38)		
Lamin A/C	Missense (39)		

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Nonstandard abbreviations used: hypertrophic cardiomyopathy (HCM); dilated cardiomyopathy (DCM); restrictive cardiomyopathy (RCM); troponin I (TNNI3).

Table 2
In vivo and in vitro cardiac physiological phenotyping in mouse models of cardiac diseases

Anatomical and contractile phenotyping	
Cardiac imaging	
Transthoracic echocardiography	(40)
Transesophageal echocardiography	(41)
Magnetic resonance imaging	(42, 43)
LV pressure analysis	(44)
LV pressure-volume loop analysis	(45)
PA/RV pressure analysis	(46)
LV and RV angiography	(47, 48)
Aortography	(49)
Isolated intact muscle contractility studies	
Isolated working heart system	(50)
Single cell contractility	
Video-edge detection	(10, 51)
Laser diffraction	(52)
In vivo determinations of calcium signaling	
Chemical or genetically engineered fluorescent probes	(53, 54)
Electrophysiological phenotyping	
In vivo electrophysiological analysis	
Surface electrocardiography	(55, 56)
Telemetric electrocardiography	(57, 58)
Transesophageal cardiac pacing	(59)
Open-chest in vivo EP study	(58, 60)
Close-chest endocardial EP study	(60, 61)
Single cell electrophysiological analysis	
Action potential duration	(58, 62, 63)
Patch-clamp analysis	(58, 62)
Measurements of intracellular calcium concentrations	(10, 11, 51)
Assessment of excitation-contraction coupling	(64)
Biomechanical stress assays	
In vivo mechanical loading	
Thoracic aortic banding	(65)
Abdominal aortic banding	(66)
PA banding	(47)
Arteriovenous fistula	(40)
Coronary artery ligation	(67)
In vitro mechanical loading	
Isolated perfused heart system	(50, 68)
Papillary muscle stretch	(14)
Cultured neonatal cardiomyocyte stretch	(14, 69)

LV, left ventricle; RV, right ventricle; PA, pulmonary artery; EP, electrophysiology.

that account for the distinct cardiac phenotypes of the three major forms of cardiomyopathy. Over a decade ago, the Seidmans made the initial, important discovery that mutations in the β-myosin heavy chain can cause HCM (for review, see ref. 1). Subsequent work by this group and others expanded this concept to include other sarcomeric gene mutations, all of which were linked with the HCM subset of cardiomyopathy. At the same time, studies in genetically engineered mice began to uncover a role for cytoskeletal defects in DCM via studies of mice that harbor a mutation in the cardiac Z disk protein MLP (2), and links between other cytoskeletal proteins and familial forms of human DCM were subsequently established (3) (for review, see refs. 4, 5). In short, the early, neat storyline was that sarcomeric mutations always lead to HCM, while cytoskeletal mutations result in DCM, thereby reflecting specific defects in the hardwiring within cardiac muscle cells that govern these two distinct phenotypes. In short, the phenotypic diversity of familial cardiomyopathies appeared to be primarily driven by the disease genotype. From the perspective of molecular cardiologists, the hunt was on for specific signaling pathways that might differentially connect these genetic lesions with DCM versus HCM. However, recent experimental and clinical studies suggest a more complex genotype-phenotype relationship of cardiomyopathies (Table 1). While there is little doubt that the disease genotype is a critical determinant of the clinical phenotype, perhaps the major protagonists and antagonists of this story have yet to enter the stage.

Multiple cardiomyopathy phenotypes from identical sarcomeric genotypes: *TNNI3* mutations lead to HCM and RCM

In the current issue of the *JCI* (6) Mogenson et al. reinforce this notion by clearly documenting that a single mutation within the *troponin I* (*TNNI3*) gene can lead to either HCM or RCM within the same family. In a series of patients with RCM, a number of independent *TNNI3* mutations were uncovered, again suggesting that *TNNI3* mutations cannot only lead to HCM, as previously described (7), but also RCM. Previous studies have shown that sarcomeric mutations in the tropomyosin, troponin T, titin, and β-myosin heavy chain gene can lead to either DCM or HCM (Table 1). Taken together, it appears that mutations in a given sarcomeric gene can lead to a spectrum of cardiomyopathic phenotypes, often overlapping between the clinical subsets of DCM, HCM, and RCM. Although there is little doubt that the disease genotype plays a critical role in initiating the cardiomyopathic process, the ultimate clinical phenotype undoubtedly represents the integrated effect of multiple interacting factors. This view is supported by a host of circumstantial evidence, including the poor penetrance of many cardiomyopathic genotypes, the influence of hemodynamic stress (pressure, volume, etc.) on disease progression (8), the secondary effects of the loss of cardiac myocyte survival and subsequent replacement fibrosis (9), strong modifying effects of calcium cycling (10, 11)

and calcium signaling (12), and clear evidence of genetic background effects in gene-targeted mouse models of cardiomyopathy (13).

Resolving cardiomyopathy phenotypes and disease pathways with refined physiological technology: the MLP story

Part of the difficulty in attempting to define the molecular pathways that link the myriad number of sarcomeric and cytoskeletal mutations with specific forms of cardiomyopathy stems from our relatively primitive understanding of the precise physiological phenotype of these and other cardiac muscle diseases. Given the vast diversity of human cardiomyopathic genotypes, as well as the inherent difficulty in assessing the physiological function of cardiac muscle cells from a large number of distinct patients and their families, it is likely that many of the major insights will arise at the interface of mouse models and human disease. For example, recent studies have identified a missense mutation in the *muscle LIM domain protein* (*MLP*) gene that is associated with human DCM that has arisen as a result of a founder effect in a Northern European population (14). Parallel studies in *MLP*-deficient mice indicate that MLP plays a critical role as part of a Z disc-telethonin-titin complex that is an essential component of the cardiac muscle stretch sensor (14). The human *MLP* mutation disrupts this complex, indicating that the pathway that links the disease genotype with the cardiomyopathy phenotype is related to a primary defect in the cardiac muscle stretch sensor pathway (14). Accordingly, it may become possible to develop a more functional approach to the classification of human cardiomyopathies on the basis of a detailed phenotypic analysis of mouse model systems. It will become critical to continue to develop new, high-resolution, high-throughput technology to resolve cardiac muscle physiological phenotypes at the whole organ, intact muscle, and single cell level.

As noted in Table 2, an arsenal of physiological technology has already been developed by multiple laboratories, which can now be coupled with the growing power of mouse genetics and well-characterized gene-targeted model systems. Background effects in various mouse strains have been clearly observed and attempts to map and clone these modifiers are ongoing, a task made easier by the mouse genome project. The generation of hypomorphic alleles that correspond to known human cardiomyopathy genotypes could be especially informative. Conditional mutagenesis will be valuable in assessing whether the onset of cardiomyopathy in the postnatal setting actually reflects subtle, developmental effects of the sarcomeric and cytoskeletal gene mutations on chamber morphogenesis or function and a fleet of CRE recombinase mouse lines have now been well validated to restrict the mutations to specific cardiovascular lineages, i.e., atrial, ventricular, atrioventricular nodal, epicardial, endothelial, and neural crest (for review, see ref. 15). Genetic complementation via germline gene modification or Adeno-associated virus-mediated transcoronary gene transfer should be informative (10, 16), capitalizing on candidate genes uncovered in surrogate systems, i.e., in vitro cardiac myocyte assays, zebrafish, fly, and mouse models.

Phenotype, Genotype

Of course, without parallel advances in functional cardiac phenotyping, attempts to use these models to dissect specific molecular pathways for this multifaceted disease are likely to remain superficial. Ironically, the next episodes of the continuing story on the family of cardiomyopathies may rely more on innovative strategies for precision phenotyping, as opposed to simply expanding the number of genetically engineered mouse model systems per se. It is highly likely that a re-analysis of existing gene targeted mouse models of cardiomyopathy with more refined phenotyping will uncover unsuspected physiological mechanisms of direct relevance to cardiac muscle diseases. High-throughput patch clamp arrays, in vivo expression of calcium reporter genes in intracellular micro-domains of living cardiac muscle cells, novel strategies to monitor conduction system function, and new technology to assay cardiac muscle stretch sensor and effector pathways are likely to be featured. Consult your local listing for the sequel to the cardiomyopathy story, *Phenotype, Genotype*.

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