

## FOCUS ISSUE: BIOMARKERS

# Where Genome Meets Phenome: Rationale for Integrating Genetic and Protein Biomarkers in the Diagnosis and Management of Dilated Cardiomyopathy and Heart Failure

Sanaz Piran, MD,\* Peter Liu, MD,† Ana Morales, MS,‡ Ray E. Hershberger, MD‡  
*Toronto and Ottawa, Ontario, Canada; and Miami, Florida*

This review provides the rationale for integrating genomic and protein biomarkers in the evolving diagnosis and management of dilated cardiomyopathy (DCM) and its causal pathway to heart failure (HF), with a larger objective to serve as a template for genomic and phenomic profiling of other cardiovascular disease. DCM is a major cause of HF and accounts for more than half of heart transplantation in adults and children worldwide. DCM may remain asymptomatic for years, but HF and/or arrhythmias, both late manifestations of the disease, ultimately cause significant morbidity and mortality. A significant proportion of DCM has a genetic etiology. DCM can also result from environmental injury such as infection, toxins, or catecholamine excess. While molecular genetic testing can identify those at risk for genetic DCM, epigenetic and sentinel phenomic staging can help to identify those at highest risk in need for intervention. Phenomic staging includes integrating clinical and imaging features, transcriptomics, higher order proteomics and metabolomics interactions, and epidemiological data. This principle can be applied in family members of patients with DCM, where genetic testing and clinical phenotyping are indicated. This will allow the design of specific interventions tailored to individuals sharing similar risks, to alter the natural history of DCM and obviate complications such as HF/arrhythmias. (*J Am Coll Cardiol* 2012;60:283-9) © 2012 by the American College of Cardiology Foundation

Cardiovascular disease occurs as a cumulative consequence of the host's inadequate repertoire, often genetic, to respond to stress or injury. Symptoms typically occur late as a manifestation of failure of compensation. The intermediate stages of disease progression, including inflammation, growth, apoptosis, or autophagy, directly lead to tissue remodeling. This stage is often clinically silent, but offers the optimal opportunity for intervention. Common examples include the atherosclerotic plaque in coronary disease, asymptomatic left ventricular systolic dysfunction, or atrial remodeling prior to symptomatic fibrillation.

This review aims to apply current concepts of genomics and phenomics to the paradigm of dilated cardiomyopathy (DCM) and its progression to heart failure (HF). This includes genetic predisposition, imaging, and proteomics to

characterize DCM in its preclinical stage, and targeted early intervention to prevent complications.

DCM, a disease of the myocardium, is defined by left ventricular enlargement and systolic dysfunction. In familial studies, DCM may be asymptomatic for years (1). Eventual symptoms include HF, arrhythmias or sudden death, or embolus from left ventricular thrombus. In contrast, HF is a symptom complex in which heart function is inadequate to meet physiological demands without presumption of etiology or systolic function.

## Phenome, Genome, and Epigenome

Variation in the genetic repertoire (genome), together with the biological consequences and interactions with the environment, lead to molecular, biochemical, physiological, and clinical manifestations (phenome). We define the phenome here as the high-dimensional phenotype data for the entire organism (2), including not only clinical characteristics, but also information from cells, tissues, organs, and individuals (including epidemiological data), ranging from gene expression (transcriptomics), gene networks (integrative genomics [3]), and higher order proteomics and metabolomics interactions (2). The study of "genomics" in this context means

From \*The Heart and Stroke/Richard Lewar Centre of Excellence and Toronto General Research Institute, University of Toronto, Toronto, Ontario, Canada; †University of Ottawa Heart Institute, Ottawa, Ontario, Canada; and the ‡Cardiovascular Division, University of Miami Miller School of Medicine, Miami, Florida. This work was supported by National Institute of Health award RO1-HL58626 (Dr. Hershberger). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received February 10, 2012; revised manuscript received May 22, 2012, accepted May 23, 2012.

### Abbreviations and acronyms

**BNP** = brain natriuretic peptide

**DCM** = dilated cardiomyopathy

**GWAS** = genome-wide association studies

**HF** = heart failure

**IDC** = idiopathic dilated cardiomyopathy

**MMP** = matrix metalloproteinase

analyzing the genetic code for variants, both common and rare as well as single nucleotide changes or larger structural (copy number variant) changes, and understanding the total impact of gene variants on the phenome. Thus, the phenome integrates higher order interactions or systems biological networks (4) to better define transitional programs to disease.

Epigenetics defines the intersection of the genome, without changes to its nucleotide sequence, with the environment

that leads to phenomic variations. Epigenetic mechanisms most commonly include methylation, acetylation, or nitrosylation patterns of modifications of gene function. Such epigenetic changes may be heritable, and the global changes are incorporated in the *epigenome*. An isogenic murine line with validated identical deoxyribonucleic acid (DNA) sequence can exhibit significantly different phenotypes due to differences in epigenetic modifications (Fig. 1) (5). Other examples include the paternally or maternally inherited predisposition to diabetes or post-natal cardiovascular risk from maternal intrauterine conditions (6).

### DCM and Its Relationship to HF: Phenomics and Genomics Considerations

DCM, when applied without inference to any specific etiology, commonly presents with few phenotypic features that enable differentiating its etiology. Despite the well-established value (7) and now guideline-mandated use of family history as a means to detect genetically based DCM (8) because of familial clustering with Mendelian disease, family history alone is insensitive to detect familial DCM, even when ischemic and other detectable etiologies (aside from genetic) have been ruled out (commonly termed idiopathic dilated cardiomyopathy [IDC]). This is because asymptomatic systolic dysfunction, left ventricular enlargement, or DCM may be present for years with symptoms occurring only late in the causal pathway from (Fig. 2) (9). In addition, the age of onset of DCM varies widely, and ranged from 0 to 75 years in a familial DCM cohort from our group (10). Thus, even in family members genetically at risk to carry a DCM mutation, their DCM may not have yet presented and can only be identified with prospective clinical screening. Family history alone was found to detect 5% of familial disease (11), while clinical screening of relatives has been shown to detect familial DCM in 20% (11) to 48% of cases (see Burkett and Hershberger for review [1]). Combining family history with clinical screening of relatives, and emphasizing echocardiography to assess LV size and function, is essential to identify familial DCM (1,8).

### DCM Genomics: Rare and Common Coding Variants

Most classical Mendelian disease is characterized by familial clustering of the phenotype of interest with a discernible pattern of inheritance, commonly resulting from very rare variants (e.g.,  $\leq 0.1\%$  allele frequency) in coding sequence, thereby to change amino acids (termed nonsynonymous), invoke stop codons, alter splicing, or cause reading frames to shift (12). However, sequencing of genetic DCM has shown that the coexistence of multiple rare variants may also cause DCM (13,14).

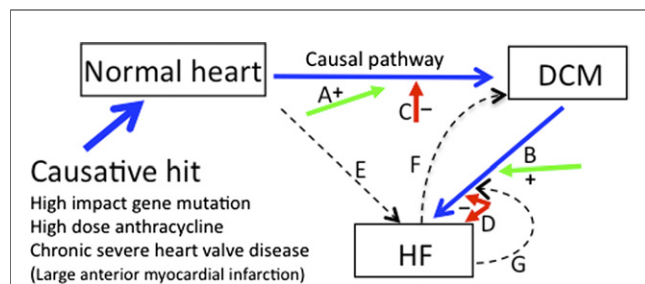
Based on family studies, rare nonsynonymous mutations from >30 genes have been reported to cause nonsyndromic DCM (i.e., isolated DCM not associated with extra cardiac disease; lists of genes are available that cause syndromic DCM [9,15] or mixed phenotypes [e.g., arrhythmogenic right ventricular cardiomyopathy (16)]), even though they account for only ~45% to 50% of genetic DCM (12,15,17,18). The fractional contribution of each gene to DCM varies significantly: truncating variants in *TTN*, encoding titin, accounted for up to 25% of familial DCM (18), although most DCM genes have been shown to have much lower frequencies (e.g., *LMNA* 6%; *MYH7* 4%, *MYBPC3* 4%, *TNNT2* 3%, *MYH6* 3%, *SCN5A* 3%) (12). Most mutations are very rare or novel (19) and are usually specific to 1 individual or family (a “private” mutation). This makes both diagnostic and discovery approaches challenging, as it can be difficult to determine the true contribution of a newly identified variant to disease (12).

The gene ontology for DCM is shown (Table 1), with numerous genes encoding sarcomeric, z-disk, or cytoskeletal proteins. However, rare DCM mutations have also been



**Figure 1** Epigenetic Regulation of Coat Color

These 6 mice have identical genomic DNA, as they are littermates from an isogenic line maintained by brother-sister matings for over 30 generations. The difference in coat color reflects variable expressivity of a cryptic promoter upstream from the coat color locus, a manifestation of a transcriptionally active retrotransposon that is epigenetically but variably reset during embryogenesis in each mouse. Adapted, with permission, from Whitelaw and Martin (5).



**Figure 2** Disease Model: DCM and HF

This figure portrays dilated cardiomyopathy (DCM) and heart failure (HF) as separate entities. The causative hit, depicted by a thick blue arrow, includes genetic cause, depicted here as 1 high-probability Mendelian mutation although other genomic models are possible, if not likely (9,12). The causal pathways from normal heart to DCM and from DCM to HF are shown by the 2 other blue arrows. The causal pathway to DCM may take years and is asymptomatic until very late in its causal pathway when HF, arrhythmia, or embolus (from mural thrombus) present. Because of the biological complexity and epidemiological impact of HF, its causal pathway from DCM is shown, although pathways from DCM to arrhythmia and embolus are also relevant. Factors that may accelerate these causal pathways are depicted with green arrows for DCM (A) or HF (B); environmental examples include hypertension and alcohol use, while genomic factors include unfavorable genotypes (risk alleles). Other factors that may delay or arrest progression to DCM or HF are shown in red (C, D); such factors could include favorable environmental factors (e.g., good nutrition, a low-salt diet, low blood pressure, drug therapy with angiotensin-converting enzyme inhibitors or beta-blockers, genomic factors such as a protective alleles). Acute HF (e.g., from a large anterior wall myocardial infarction) is shown with a dotted line (E); in this situation the acute onset of HF may cause DCM subacutely, although the degree that genomics plays a role in DCM resulting from acute HF is uncertain, as most studies have focused on chronic HF. Also, whether chronic HF modulates or exacerbates the DCM causal pathway (F) itself remains poorly defined. The disordered HF physiology also feeds back onto itself, the so-called vicious cycle of HF (G).

identified in genes encoding proteins of diverse function other than these such as *LMNA*, which encodes a protein of the inner nuclear lamina, and others. Collectively these data indicate that DCM, a “final phenotype” (9), results from diverse genomic or phenomic insults via activation of diverse DCM disease-causing cascades.

In contrast to familial DCM, no large systematic studies have been undertaken to examine the genomic basis of sporadic IDC. In our resequencing studies of 11 known DCM genes (20,21) in 312 probands with either sporadic or familial DCM of unknown cause, we observed similar frequencies of possibly or likely disease-causing rare variants. While familial DCM is considered to have a genomic basis (22), whether IDC is principally a genomic disease remains an open question.

Common or complex disease, in contrast to rare variant disease, has been thought to result from the cumulative effect of low penetrance variants that are frequently found in the general population (e.g., usually >5% allele frequency) (23). The first genome-wide association study (GWAS) of common variants in 1,179 DCM patients (24) found 2 single-nucleotide polymorphisms, 1 in *BAG3*, a gene previously identified as having rare nonsynonymous variants causing DCM (25). *BAG3*, as a co-chaperone of heat shock proteins, targets misfolded cellular proteins for recycling via selective autophagy, and thus may be a pathway for causa-

tion or risk whereby if protein quality control is compromised, then cardiomyopathy can result. No additional DCM GWAS have been published, although an earlier pilot association study of 6 resequenced genes suggested that a common variant approach may be fruitful (26), and a peripartum cardiomyopathy GWAS reported a locus on chromosome 12 (27). An HF GWAS has recently been reported (28).

Large-scale genome-wide searches for genomic variation aside from a GWAS (24) have not been reported in DCM. Three single-gene studies have identified structural variants causing DCM (in *EYA4* [29], *LMNA* [30], and *BAG3* [25]), but to date no genome-wide evaluation of structural variants in a large DCM cohort has been reported. Further, systematic evaluation of 5'- and 3'-untranslated regions, promoter variants, noncoding ribonucleic acids (RNAs), epigenetic impact, or other genomic variation in a large scale study of DCM has not been reported.

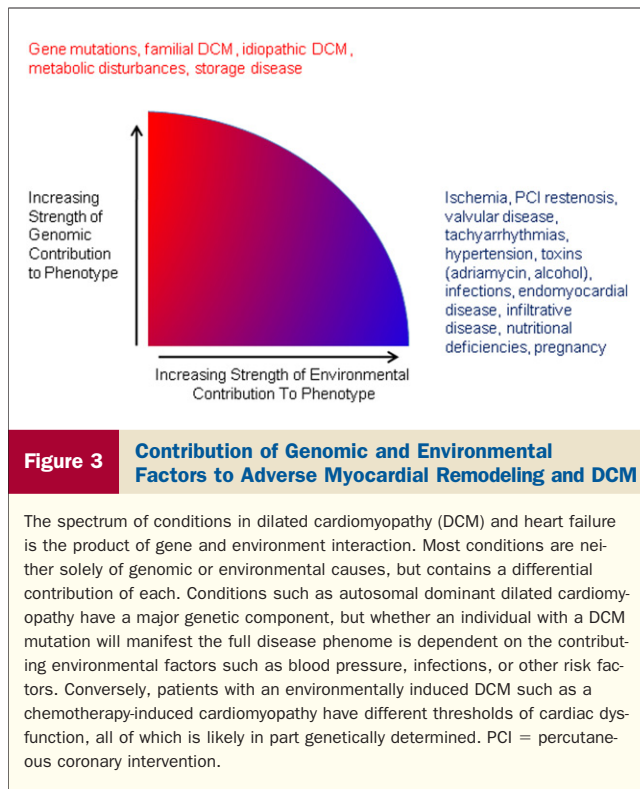
### Where Genomic and Acquired Forms of DCM Meet

DCM is commonly categorized clinically as “ischemic” or “nonischemic,” where nonischemic DCM includes IDC, a diagnosis of exclusion of readily detectable causes. Acquired forms of nonischemic DCM exist, and include toxins (e.g., anthracyclines), infiltrative processes, longstanding mechanical load from valvular or congenital causes, viral infection (e.g., coxsackievirus or parvovirus), nonviral infection (e.g., *trypanosoma cruzii* in Chagas disease), nutritional deficiencies, or a variety of inflammatory processes (9,17,31). Whether any of these environmental factors contribute to genetic DCM risk, and vice versa, the degree that genetic background modulates environmental risk remains an active research area. However, global processes such as ischemia, mitochondrial dysfunction, and chronic tachyarrhythmias (17) may all directly influence the genome to increase the risk for DCM and eventually HF (Fig. 3).

**Table 1** DCM Gene Ontology

Sarcome	Cytoskeleton	Z-disc	Nuclear envelope
<i>ACTC1</i>	<i>DMD</i>	<i>TCAP</i>	<i>LMNA</i>
<i>MYH7</i>	<i>DES</i>	<i>CSRFP3</i>	<i>TMPO</i>
<i>MYH6</i>	<i>LDB3</i>	<i>ACTN2</i>	<b>Gamma secretase activity</b>
<i>MYBPC3</i>	<i>SGCD</i>	<i>MYPN</i>	<i>PSEN1</i>
<i>TNNT2</i>	<i>PDLIM3</i>	<i>ANKRD1</i>	<i>PSEN2</i>
<i>TNNC1</i>	<i>VCL</i>	<i>NEBL</i>	<b>Transcription factor</b>
<i>TNNI3</i>	<i>RYAB</i>	<i>NEXN</i>	<i>EYA4</i>
<i>TPM1</i>	<i>ILK</i>		<b>RNA binding</b>
<i>TTN</i>	<i>LAMA4</i>		<i>RBM20</i>
<b>Ion channel</b>	<b>Mitochondrial</b>		<b>Co-chaperone, heat shock proteins</b>
<i>ABCC9</i>	<i>TAZ/G4.5</i>		
<i>SCN5A</i>	<b>Sarcoplasmic reticulum</b>		<i>BAG3</i>
	<i>PLN</i>		

DCM = dilated cardiomyopathy.



A particular illustrative case is the coxsackievirus, an etiological agent for human myocarditis. Coxsackievirus in the host can produce an enteroviral protease 2A that can cleave the dystrophin complex in the myocyte, resembling the phenotype seen in patients with a genetic deficiency of dystrophin (32). Furthermore, murine dystrophin dysfunction facilitates the release of virus from infected cells (33) and pre-existing cardiomyopathy up regulates the coxsackie-adenoviral receptor to facilitate viral entry (34). This illustrates a cooperative feedback system to facilitate the development of DCM (35).

### Evolution of DCM to HF

Regardless of cause, whether genomic, environmental, or both, the underlying mechanisms leading from DCM to HF involve multiple staged processes (Fig. 2). The progression from DCM to HF may start with major perturbations of cytoskeletal-sarcomeric stability leading to contractile inefficiencies cumulative in time, followed by metabolic dysregulation, progressive cell death, cardiac remodeling, inflammatory activation, and fibrosis (17,36). This commonly leads to progressive cardiac dilatation involving both left- and right-sided chambers; and eventually, to death from advanced HF or arrhythmia. While we consider DCM to be one principal cause of HF, considerable clinical and basic data support the concept of multiple overlapping feedback loops shared by different etiologies (Fig. 2). Once the HF cascade has been activated, multiple factors may accelerate the causal pathways. Despite a potential genomic etiology for DCM, its progression and responsiveness to

treatment may vary depending on the molecular network involved. This underscores the opportunities of combining genetic risk evaluation with biomarkers specific for pathway activation as an integrated analysis for risk stratification, pathway classification, and tailoring of intervention.

In addition, patients with DCM, whether from principally genomic or acquired factors or components of both, may also harbor risk alleles (rare or common variants of variable penetrance) that in combination predispose the patient toward more aggressive cardiac remodeling (9).

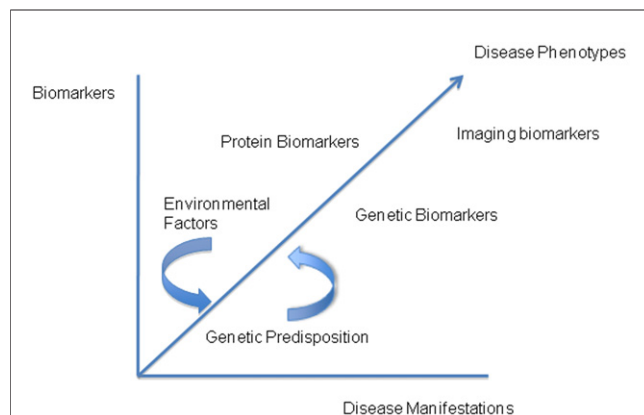
### Measuring Environmental Imprints Reflected by Systems Biology Markers and Imaging Phenotypes

While the nucleotide sequence of an individual's genome is generally stable, gene expression depends on the interactions with the environment and epigenetic influences (e.g., the state of DNA methylation, acetylation, impact of micro-RNAs) all part of the epigenome. The transcriptome is the unique set of genes expressed or transcribed under such specific conditions. Indeed recent data suggest that transcriptomic signatures can be used in prognosticating patients with new onset HF (37). The resulting diversity of proteins produced by a cell or organ can now be characterized by high throughput gel electrophoresis or mass spectrometry as the *proteome*, and the metabolic processing profiles determined by mass spectrometry, the *metabolome* (38). Finally, the ultimate product of genetic and environment interaction that determines the protein-protein interactions that direct measurable structural, signaling, functional, and remodeling parameters contributes to the *phenome*. These parameters will rapidly progress when environmental influences on the genetic repertoire create further stress, abnormal protein production and accumulation, and ultimately structural instability and dysfunction of the heart chambers (Fig. 4). These processes generally will be very advanced before symptoms occur.

Common imaging tools that are useful for patients with DCM include ultrasound or magnetic resonance imaging to assess cardiac dimensions, systolic and diastolic function, and evolving tissue characterization. Positron emission tomography and to a lesser extent, conventional radioisotope imaging, can delineate the metabolic activities in the heart, and differentiate areas of viability from areas of scar.

### Protein Markers of DCM

Proteomic analysis can currently examine all the proteins produced by the heart in detail (e.g., membrane, cytosol, microsomal, or nuclear fractions) down to the femtomolar level (39). This approach has allowed identification of alterations in key proteomic pathways in DCM, and complements well other genomic and systems biology tech-



**Figure 4** The Role of Biomarkers Reflecting Disease Phenotype and the Interplay of Genetic and Environmental Factors During Disease Progression

A variety of biomarkers (including genetic, protein, and imaging) can be deployed to delineate disease progression in dilated cardiomyopathy. Genetic biomarkers can identify an individual's genetic predisposition. It is, however, the environmental imprints on the genetic background that produces abnormal unique proteins that foster disease progression in the susceptible individual. The latter can be detected as proteomic or metabolomic markers. As the disease progresses further in the susceptible individual, cardiac structural and functional alterations can be detected through imaging technologies. All of this can take place before the onset of symptoms, thus setting the stage for early intervention.

niques. Further analysis of post-translational modifications, their cellular location and function provide not only biological insights, but also are candidates for diagnostic biomarkers and potential therapeutic targets (40). For example, analysis of the phospholamban mutation in human and mouse identified major alterations in endoplasmic reticulum stress, calcium signaling pathways, cytoskeletal transitions, and activation of stress-inflammatory-apoptotic programs (36).

While protein biomarker candidates are numerous in this post-systems biology era, only a handful have been validated to enter clinical decision making. The validated protein markers in DCM and HF to date include those that provide information on stress (natriuretic peptides, ST2), cell deaths or turnover (hsTroponin), collagen turnover (PIIINP or C1TP), and matrix dynamics (serum matrix metalloproteinases [sMMPs] and possibly Galectin-3), among others. These markers appear at different time points in the natural history of DCM evolving to HF, conferring differential advantages for early detection, prognosis, or therapeutic targeting.

Both brain natriuretic peptide (BNP) and ST2 appear to reflect ventricular stress, and can be used to predict risk of decompensation. There is substantial experience with natriuretic peptides in DCM as well as in HF (41). BNP or proBNP levels increase the accuracy of diagnosis of HF in the emergency department (42), and is cost-effective (43,44). Encouraging data also suggest that BNP can be used to prognosticate patients at the time of hospital discharge and to help assess targets for therapy in HF (45).

ST2 is a member of interleukin-1 receptor family, is secreted by cultured monocytes subjected to mechanical

stress, and is induced and released by stretched cardiomyocytes (46). In patients with HF, an increased ST2 level is an independent predictor of death and need for heart transplantation, beyond BNP (47). In the PRIDE (N-terminal proBNP Investigation of Dyspnea in the Emergency Department) study, Januzzi, Jr. et al. (47) showed that ST2 levels were useful in diagnosing acute HF in comparison with N-terminal proBNP, and were strongly predictive of mortality at 1 year, particularly elevated in patients with systolic HF (48). ST2 has just been approved by the Food and Drug Administration for clinical use.

Modest elevations of the myofibrillar proteins troponins T and I in the serum have been found to be sensitive markers of myocyte injury in patients with HF without ischemic heart disease (49). Both cardiac troponin I and T are markers of poor prognosis and independent predictors of death (50). The recently available high-sensitivity troponin is detectable in 92% of HF patients (51), in contrast to the traditional troponin being detectable in only 10%. Impressively, the high-sensitivity troponin predicts early onset HF and increased mortality in the longitudinal Cardiovascular Health Study cohort study in asymptomatic patients over a decade of follow-up (52) and is probably important for intermediate follow-up, but it is not related to etiology of HF.

Progressive cardiac dilation also involves matrix remodeling and increased collagen turnover. The level of plasma procollagen type III in patients with HF is an independent predictor of adverse outcomes (53). There is also a correlation between the propeptide serum collagen type I and degree of myocardial fibrosis on biopsies taken from patients with hypertension (54). Similarly soluble MMPs such as sMMP9 also correlated with cardiac dilatation and a fall in ejection fraction (55). Plasma MMP-2, -7, -8, and -9 are elevated in the pediatric patients with DCM in comparison with healthy controls.

Recently approved galectin-3 is a protein produced by activated macrophages under stress, for which plasma levels have been reported to predict adverse outcomes in patients with HF and can provide prognostic stratification for both systolic and nonsystolic HF (56). It has also been shown that the combination of galectin-3 with N-terminal proBNP was the best predictor for prognosis in subjects with acute HF (56). Some of the novel biomarkers have potential utility in helping tailor therapy in acute HF. For example, early data for endoglin indicate that it may be useful as a noninvasive measure of left ventricular end-diastolic pressure and its levels decrease with diuresis, potentially providing an opportunity to titrate volume removal (57). Neutrophil gelatinase-associated lipocalin is useful as an early marker of worsening renal function, which can be helpful in determining timing and aggressiveness of diuresis (58,59).

Fc(gamma) receptors IIa on cardiomyocytes have potential functional importance in DCM, and contribute to the negative inotropic effects of autoantibodies (60).

## Integrating Genomic and Proteomic Markers Into DCM Management

The combination of genomic and proteomic markers can help in our understanding the human DCM refocusing to earlier at-risk asymptomatic stages where better categorization and risk stratification can help with personalized risk management. The combinatorial genetic, proteomic, and image phenotyping tools also help to devise tailored intervention strategies according to genetic categories and host response patterns. Attempts to characterize different types of cardiomyopathy in advanced state have proven to be challenging, as there are more similarities than differences at that stage (35). End-stage ischemic and nonischemic cardiomyopathies share many molecular and cellular pathways in common, with only small differences in transcriptome (61). The earlier intervention thus followed may have much more impact on the natural history of the disease and prove cost-effective in the long run.

This approach will be most valuable for family members of affected probands with DCM, where patients with inherited genetic risk can be better risk stratified by imaging and protein markers to determine the appropriateness and intensity for intervention at an early stage.

### Summary

The rationale for more comprehensive genomic and phenomic knowledge of DCM and its progression to HF is compelling because of the enormous public health impact of these conditions. Major gaps in knowledge include the remainder of genomic and epigenetic cause of DCM, that when discovered will not only enlarge our insight into the pathways to the final DCM phenotype but will also aid the discovery of additional genomic, proteomic, and metabolic biomarkers to detect very early DCM and HF, inform prognosis, and guide the development of novel therapeutics.

**Reprint requests and correspondence:** Dr. Peter Liu, Toronto General Hospital, University Health Network, NCSB 11-1266, 200 Elizabeth Street, Toronto, Ontario, M5G 2C4, Canada. E-mail: peter.liu@utoronto.ca OR Dr. Ray E. Hershberger, Ohio State University College of Medicine, 304 Biomedical Research Tower, 460 West 12th Avenue, Columbus, Ohio 43210. E-mail: Ray.Hershberger@osumc.edu.

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**Key Words:** biomarker ■ cardiomyopathy ■ genomics ■ personalized medicine ■ proteomics.