

# The Genetics of Congestive Heart Failure

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## KEYWORDS

- Congestive heart failure • Cardiomyopathies
- Genome-wide association studies
- Mendelian cardiomyopathy genetics

Congestive heart failure (CHF) remains the single most common cause of mortality and morbidity in the developed world.<sup>1</sup> With as many as 5 million hospitalizations annually, treating heart failure represents a major component of the health-care budget of every developed nation, and an increasing component of the health-care budgets in many developing nations. The heart failure syndrome is known to represent a final common pathway for a broad range of etiologies, but there is tremendous variation in the propensity to develop CHF after a given insult.<sup>1,2</sup> This variation is thought to result in part from inherited differences in myocardial, vascular, or systemic responses, but the nature of the underlying traits responsible ultimately for the development of heart failure has remained elusive. Other articles in this issue highlight the impressive advances in our understanding of the genetics of the cardiomyopathies, and more recently in the genetics of specific traits within the heart failure syndrome. However, there has been limited progress in the genetic exploration of the key clinical phenotype itself: heart failure. In this article, the author attempts to place the results of genetic studies of cardiomyopathy in the broader context of the clinical syndrome of heart failure, highlighting some of the key questions for future study.

## THE COMPONENT PHENOTYPES OF HEART FAILURE

Several decades of investigation have led to an increased understanding of the mechanisms underlying the later stages of heart failure. Adult cardiac myocytes do not divide in any meaningful numbers, and as a result increased workload leads

not to cardiomyocyte hyperplasia, but rather to cellular hypertrophy and increased ventricular mass.<sup>3</sup> This fundamental response occurs in the face of a broad range of stressors including hypertension, myocardial infarction, or myocardial dystrophy, and has increasingly been associated with energetic defects.<sup>1,4</sup> Although initially adaptive, in most situations the myocardial remodeling pathways eventually become maladaptive. At this stage progressive effects on ventricular shape, substantial changes in most aspects of systolic and diastolic myocardial function, and proarrhythmic effects on calcium cycling or membrane biology occur in a significant subset of patients.<sup>5-7</sup> The remodeling in heart failure is not confined to the myocardium and there is also evidence of profound changes in the biology of many other organ systems.<sup>1</sup>

Early in the development of heart failure, long before the emergence of any symptoms, activation of the sympathetic nervous system can be detected, and adrenergic humoral factors and many other compensatory pathways (labeled generally as neurohormonal activation) are up-regulated.<sup>8-11</sup> The field has been dominated by studies of adrenergic and renin-angiotensin systems, and the natriuretic peptides, but recent work has implicated many other endocrine or paracrine effectors, such as apelin, a host of cytokines, parathyroid hormone, and related peptides.<sup>8,10,11</sup>

Extensive remodeling also occurs throughout the vascular system. Systemic arterial and venous biology is often abnormal as a result of the diffuse atherosclerosis that underlies the most common cause of heart failure: coronary artery disease. However, it is also clear that there are

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perturbations of arterial structure and function, as well as venous capacitance in all forms of heart failure.<sup>12</sup> Two vascular beds merit specific mention: the renal and pulmonary circulations. There is no doubt that once hypoperfusion super-venes, physiologic salt and water retention plays a major role in the genesis of progressive expansion of extracellular fluid volume, worsening elevation of intracardiac pressures, and ultimately the congestion of overt CHF.<sup>1</sup> Whether as a result of systemic abnormalities of vessel biology or as a consequence of other unknown factors, disproportionate renal dysfunction occurs in subset of those with heart failure. This so-called cardiorenal syndrome is a marker of adverse events and is currently the subject of intense investigation.<sup>13–15</sup> In the pulmonary vascular tree, there is also variation in the responses to abnormal myocardial function. For reasons that are obscure, a significant subset of those with elevated left ventricular end-diastolic pressures will develop disproportionate and irreversible pulmonary hypertension.<sup>1,16</sup> This pulmonary hypertension can adversely affect right ventricular function (which is usually also afflicted by diffuse myocardial processes), and is a marker for conspicuously worse outcomes.

Virtually every cell type and every organ has been implicated in heart failure and the picture that is emerging is of a systemic disorder where the entire organism is engaged in a chronic stress response, that encompasses every pathway from insulin regulation of energy balance to innate immunity. The control hierarchy, and in many instances the primary sensor or sensors, for the local and global responses characteristic of CHF are completely unknown. Multiple cellular pathways have been implicated in the myocardial biology of heart failure. Numerous murine models of heart failure have been generated, but few, other than those recapitulating human cardiomyopathy alleles, have shed light on human heart failure mechanisms.<sup>17</sup> At a cellular level a central role for calcineurin signaling has been debated, and several other calcium-regulated pathways have been implicated.<sup>6,7</sup> It has proven particularly difficult to identify the upstream factors responsible for heart failure, as the much of our understanding of the human syndrome has been garnered from symptomatic late-phase or end-stage disease.<sup>1</sup> It is for this reason that genetic approaches to CHF are particularly attractive.

### GENETIC ARCHITECTURE OF HEART FAILURE

Variation in propensity to CHF has been attributed to differences in the extent of myocardial injury, but there is evidence that identical myocardial

insults may lead to disparate outcomes, and also of major inherited and environmental contributions to the risk for heart failure.<sup>1,2</sup> The CHF syndrome is a final common pathway for a vast range of insults including ischemic injury, trypanosomal infection and the mutation of multiple genes.<sup>1,2</sup> Non-ischemic CHF exhibits evidence of familiarity in 30% to 50% of cases, and there are more than 30 known genetic loci, but few cloned genes.<sup>18</sup> In subclinically affected relatives what appears to be a predisposition to ventricular remodeling in response to many different stimuli has been observed.<sup>19</sup> Environmental variables compound the phenotypic pleiotropy seen in such single gene disorders and confound the hope of predictive genetic testing. Therapeutic modulation of neurohormonal abnormalities will delay progression, but often does not prevent the emergence of CHF. In addition to the evidence of a role for genetics, early environmental exposures are now also thought to pattern cardiac biology and responses to injury.<sup>20</sup> Recently, major roles for microRNAs and epigenetic factors have been identified, suggesting that at each step the pathophysiology may be substantially modified by prior environmental or even transgenerational exposures.<sup>20,21</sup> The powerful tools of modern genetics offer the possibility that by defining the inherited contribution we will obtain direct inroads into the environmental and epigenetic modifiers underlying this major cause of morbidity and mortality.

### INSIGHTS INTO HEART FAILURE FROM MENDELIAN CARDIOMYOPATHY GENETICS

The current state of genetic insight into human cardiomyopathies and the component traits of heart failure are outlined in the articles of this issue. The primary causes of rare forms of increased myocardial mass, left and right ventricular dysfunction, and other components of CHF risk have been identified in elegant genetic work with Mendelian families.<sup>2</sup> Similarly, recent work in the epidemiology and genetics of atrial fibrillation has uncovered direct links between CHF and this arrhythmia, at a myocardial and endocrine level.<sup>22–24</sup> Although investigators have identified the primary causes in many instances, a comprehensive understanding of the mechanisms downstream from each of these genes is far from a reality.

A central question in the field is the role, in more common forms of heart failure, of the pathways implicated in single-gene disorders. The selection pressures operating against inherited diseases where reproductive efficiency is reduced would

be predicted to result in high *de novo* mutation rates and tremendous allelic heterogeneity. Indeed, in most inherited human heart diseases this is exactly what has been observed.<sup>2,25</sup> Common forms of heart failure may be hypothesized to result from less penetrant, milder alleles of the cardiomyopathy genes found in extended kindreds with Mendelian disease; alleles that would not be subject to the same negative selection pressures. Large scale re-sequencing efforts for several cardiomyopathy genes have been undertaken in many hundreds of subjects with undifferentiated nonischemic heart failure to test this hypothesis. The results have been rather disappointing to date, and in most instances fewer than 1% of cases have evidence of mutations in the known genes. These data reflect not only the tremendous heterogeneity of inherited forms of heart failure but also serve to emphasize that the genes that result in primary forms of myocardial injury may play little or no role in more common forms of heart failure. It is also conceivable that although the specific genes mutated in rare (and extreme) forms of heart failure may not be mutated in more common variants, the functions of the relevant pathways may be mechanistically important.

### ***Genome-Wide Association Studies***

Genome-wide association studies (GWAS) offer complementary approaches to the dissection of the genetic basis of heart failure and address common, small effect size alleles at the other end of the spectrum from Mendelian disease. At the core of this technique is the assumption that common diseases result from aggregations of common ancestral gene variants. As the particular combinations of alleles will vary across each unrelated individual, this approach does not use transmission probability, but instead relies on simple association of genotypes with phenotypes within a population.<sup>26,27</sup> By their nature GWASs are designed to detect small population-wide effects operant irrespective of the primary etiology, but these same design features reduce the sensitivity for the detection of even major genetic effects in the face of etiologic heterogeneity. A second limitation of GWAS is population stratification, which can result in the spurious association of a polymorphism with disease, simply because the disease and the unlinked sequence variant are found in the same population subgroup. This limitation can be partly addressed by replicating the findings in large study cohorts drawn from genetically distinct populations. The prior probability that any observed effect is a result of the specific polymorphisms studied is usually extremely low,

resulting in an unacceptably high false-positive rate (through Bayesian inference). Because of the absence of segregation information inherent in these studies, it is also impossible to causally relate specific variants or a definitively bounded segment of DNA to a phenotype. The phenotype in question may result from variations in linked genes, in so-called disequilibrium with the tested polymorphism. These issues are, at least in part, dealt with by using extended haplotypes of markers in large populations.

In the last few years, the completion of the Human Genome Project and the emergence of comprehensive haplotype maps (HapMap) have led to a proliferation of genome-wide association projects. The theoretic advantages of GWAS have all, to some extent, been confirmed including the unbiased assessment of the role of common alleles in disease, the detection of population-wide effects, and the potential for disease pathway entry. Among the most successful GWASs have been those focused on quantitative traits where the allelic architecture likely favors the success of the technique.<sup>28</sup> Many dichotomous traits have also been successfully approached, but here the resolution of the phenotype is emerging as an important variable in determining the power of GWAS. For some traits, particularly where there is little selection pressure on reproductive efficiency (eg, senile macular degeneration or atrial fibrillation), large effect sizes are observed and relatively small studies have sufficed for the detection of genetic loci in the GWAS framework.<sup>23</sup> However, for other traits where there is presumably considerable selection pressure (eg, hypertension) and likely greater genetic and allelic heterogeneity, GWAS have proven less successful despite studies of increasing power.

In heart failure, robust association studies have only just begun to emerge. The EchoGen consortium recently identified several interesting loci for left ventricular (LV) structure and function in a large scale GWAS of echocardiographic parameters in 12,612 subjects. Not all of these loci replicated at genome-wide significance in a replication cohort of 4094 individuals, but at least two novel loci for cardiac chamber dimensions were confirmed. Additional meta-analyses of GWAS data for incident CHF are pending, and larger cohorts may reveal additional loci.<sup>29</sup> The most robust GWAS data for CHF-related phenotypes have been observed for natriuretic peptide levels that are strongly associated with variation at the NPPA-NPPB locus in a population of 14,743 subjects. Specific alleles within the natriuretic peptide gene locus are associated with lower systolic and diastolic blood pressure and reduced

odds of hypertension in a second cohort of 29,717 individuals.<sup>30</sup> These are the first data in humans linking these classic biomarkers of heart failure with variation in blood pressure, the major risk factor for CHF. However, even the NPPA-NPPB locus contains several other genes that may affect the CHF phenotype, including genes for angiotensin interacting proteins.

The results of this initial wave of GWAS for heart failure and related traits have raised several important challenges. The phenotypes in question include several quantitative traits: biomarker levels, LV structure and function, and the binary diagnosis of incident heart failure. The power of GWAS is most evident in the case of readily quantifiable biomarkers, yet these same phenotypes may be less specific for CHF.<sup>31</sup> In common with many of the most successful genome-wide association studies to date, those in CHF have explained only a small proportion of the heritability. The cost of increasing the size (and power) of many human CHF cohorts for secondary analyses of those loci of borderline statistical significance and for exploring gene-gene or gene-environment interactions is prohibitive.

### ***Detecting Intermediate Effect Size Alleles***

Heart failure, like many human disease syndromes, is highly heterogeneous. Traditional clinical entities often encompass many disorders with distinctive natural histories, environmental contributions, and therapeutic responses. This etiologic heterogeneity compromises epidemiologic investigation, drug trials, and all forms of genetic study. Given the absence of any familial information, GWAS are particularly susceptible to the problems of heterogeneity. Small effect sizes in common pathways far downstream of the causal genes dominate the results, whereas major alleles operant in a small minority are simply not detected. As noted earlier, one of the central barriers to efficient translation of genetic and genomic insights is the reliance on traditional clinical syndromes, a problem best recognized in psychiatric disease.<sup>32</sup> In several clinical fields, efforts have begun to define new phenotypes that resolve heterogeneity by adopting novel approaches to genetic study and to phenotyping.<sup>33–35</sup>

### ***Genetic Approaches to Intermediate Effect Sizes***

One solution to the problems of genetic and phenotypic heterogeneity is the so-called kin-cohort design, which allows the detection of gene effects of a broad range of magnitudes in

a single systematic ascertainment, using proband-based family collections.<sup>36</sup> In this way, the genetic epidemiology of a disorder may be defined; monogenic forms of the disorder identified; and homogeneous populations suitable for other genetic approaches, including nonparametric mapping and association studies, can be collected. This design allows the characterization of family-specific disease features, prioritizes genetic investigation of a condition by the magnitude of the effect, facilitates study of gene-gene or gene-environment interactions, and allows pathophysiologic insights to be gained irrespective of the role of any heritable factors.<sup>36</sup>

This approach has been used to address the genetic basis of atrial fibrillation (AF), a usually paroxysmal arrhythmia known to be a risk factor for CHF. Evidence of a substantial heritable contribution was detected, consistent with a major gene effect in each family, but with markedly reduced penetrance.<sup>37</sup> Multiple genes were mapped (on Chromosomes 3, 11, and 18), and sufficient probands were collected for one of the first GWASs for this condition, which identified a locus on Chromosome 4 in an intergenic region adjacent to the transcription factor *Pitx2* and other candidate genes. In common with similar GWAS for other diseases, this latest locus explains less than 10% of the heritable contribution to AF confirming there are larger heritable effects at work. The biologic mechanism is obscure, but may reflect abnormal atrial patterning.<sup>38</sup>

### **NEW PHENOTYPES FOR HEART FAILURE**

Although much of the focus in human genetics over the last two decades has been on the genetic contributors to complexity, there has been little work to improve the granularity of clinical phenotypes.<sup>32,35,39</sup> Many disease syndromes are rooted in the late 19th century, and the success of randomized controlled trials, although a major advance, has acted as a “lumping” influence over the last two decades. As we begin to explore interindividual variation in the natural history of disease, in drug responses and in drug toxicities (each in the context of the completion of the Human Genome Project), the concept of personalized medicine has emerged.<sup>40,41</sup> Implicit in this construct is a dramatic change in our understanding of the relationship between genotype and phenotype, including quantitative comprehension of gene-gene, gene-environment, and gene-drug interactions. New diagnostic tools are required to discriminate homogeneous disease subsets and to identify causally related, more penetrant endophenotypes. Similarly, a new scale of drug

discovery will be necessary.<sup>39</sup> Formal phenome projects are being proposed for many human clinical syndromes, but the way forward in heart failure, as in other clinical entities, is not clear.

In most cases we are studying heart failure at rather limited resolution. Detailed cell biology, or even histology, is rarely available and current clinical classifications are based on chamber dimensions and wall thicknesses. Although these phenotypes have been extremely useful in clinical management and in the study of Mendelian disorders, there is extensive heterogeneity in clinical outcomes and in drug responses. Even in single gene disorders, the existence of overlap syndromes where families contain members who are each affected with a different morphologic class of cardiomyopathy (hypertrophic, restrictive, dilated or right ventricular) suggests that our current classification schemes cross important biologic boundaries. Individual genes or pathways may be causally involved in one form of heart failure, yet irrelevant in other forms of the same syndrome that are indistinguishable using current clinical techniques. To move beyond this impasse, novel phenotypic classifiers of heart failure and rigorous genetic approaches to identify genes across the entire range of effect size are required. Ultimately, much more powerful strategies than those currently employed will be necessary if we are to reach a quantitative understanding of disease etiology, far less than the gene-gene, gene-environment, or pharmacogenetic interactions implicit in personalized medicine.

### EXAMPLES OF RATIONAL PHENOTYPE EXPLORATION IN HUMANS

There are many potential approaches to the definition of robust quantitative phenotypes in the causal chain leading to heart failure. Even a reevaluation of traditional clinical phenotypes can result in new insights. For example, characteristic EKG features of the major long QT loci were uncovered only as the genes were being cloned, approximately 50 years after the description of the original syndrome,<sup>42</sup> while the subtle EKG phenotypes of the Brugada syndrome went unnoticed until families were studied.<sup>43</sup> Similarly, evaluation of other phenotypes in the context of an extended family can offer novel insights. Tissue Doppler has proven useful in identifying pre-clinically affected individuals with hypertrophic cardiomyopathy, but has not been systematically explored in the relatives of those with other forms of inherited heart failure.<sup>19,44</sup> The neurohormones of the renin-angiotensin system and the natriuretic peptides are perturbed late in CHF, but other

biomarkers may be discriminating in the appropriate context. For example, specific biomarkers of anabolic-catabolic balance, such as parathyroid hormone (PTH), PTH-related peptide (PTHrP), leptin, or ghrelin may be perturbed in discrete CHF subsets before any other abnormalities. These are but speculative examples of phenotypes that may be useful. Exploring the pathways already implicated in specific cardiomyopathies with innovative functional genomics, metabolomics, or other technologies will be a vital part of broadening the phenome of human heart failure. For example, unbiased metabolomics and small molecule profiling of patient-derived cellular samples (endothelial, fibroblast, and skeletal muscle) are already feasible.<sup>45,46</sup>

Novel endophenotypes not only enable the identification of distinctive disease subsets but also open the potential to detect abnormalities that might antedate, by many years, the onset of heart failure. The evaluation of endophenotypes requires strategies designed to reduce the influence of etiologic heterogeneity, because the specific subclinical traits of interest may vary widely across a single disease entity and from family to family. The kin-cohort design offers a robust assessment of candidate endophenotypes of any sort.<sup>36</sup> It is possible to validate such endophenotypes in at-risk relatives of those with inherited heart failure, and then apply such quantitative metrics not only as a means of extending Mendelian kindred for mapping and cloning the responsible genes but also to resolve heterogeneity.

### INTEGRATIVE BIOLOGY

To define all of the major genetic and epigenetic contributors to the etiology of heart failure, systematic strategies to resolve distinctive forms of the syndrome, to identify the causal genes, and to explore new phenotypes must be deployed. Fortunately, many of these efforts are entirely complementary. Known human heart-failure genes offer inroads into the genetic pathways that might be perturbed, and animal modeling of these specific mutations is leading to the discovery of major modifier genes. New quantitative phenotypes, once rigorously validated, will be the focus of next generation human GWAS and will also be accessible in genetic model organisms. Screenable animal or cellular models of human heart failure could be used to systematize the discovery of new phenotypes. Ideally this effort would encompass unbiased assessment of multiple phenotypic axes including diverse organ systems or tissues.<sup>32,35,39,47</sup> Recently, the zebra

fish has emerged as a screenable vertebrate, but at present the detection of basal phenotypes requires careful observation or directed rational assay design.<sup>35</sup> Modeling genetic defects, such as the dystrophin mutations causing X-linked dilated cardiomyopathy, not only allows surrogate fish phenotypes to be defined but might also detect new aspects of the disease.<sup>48,49</sup> Combining in vivo screening of existing drugs with such faithful animal models is one potential approach to the systematic discovery of pathway probes and could lead to the development of provoked phenotypes.

The explosion of genomic and post-genomic technologies (eg, transcript profiling, proteomics, and metabolomics), when combined with the challenges of interpreting the resultant large-scale datasets, has led to the reemergence of the concept of the organism as a system.<sup>50–53</sup> This reemergence has not only fostered a resurgence in classical integrative fields, such as physiology, but the sheer size and diversity of the available datasets have forced the development of new analytic strategies. These computational algorithms must define the basic relationships between disparate types of data, characterize the hierarchies of these relationships, and ultimately generate quantitative models to simulate and predict cellular or organismal phenotypes. Computational approaches have been developed to enable the identification of groups of variables that change as clusters, the functional annotation of genes, metabolites or proteins, and the classification of interactions based on shared phenotypes across multiple assays. The combination of such analytic strategies with large empiric datasets is beginning to revolutionize the rate of biologic discovery. In many instances, such systems approaches have not only validated established interactions but have predicted quite unsuspected behaviors. Taken together, these data outline how systematic approaches to a human phenotype, even if it is paroxysmal, can begin to unravel apparent complexity.<sup>35,47</sup>

## SUMMARY

Genetic analyses to date suggest there may be substantial unidentified etiologic heterogeneity underlying many common disorders, and that this heterogeneity must be resolved if the genetic basis of these traits is to be understood. These challenges have emerged at a time when systematic functional genomics analyses are changing our approach to biologic problems. Although the application of such techniques to GWAS interpretation remains limited, work to date has been

promising. The relationship between common alleles and disease is complex. However, initial efforts suggest that combining traditional genetics with higher-resolution phenotypes, implementing tissue-specific functional genomics in an approach known as *integrative genomics*, may elucidate not only the genes responsible at individual GWAS loci but also offer pathway entry points. The pathways can then be explored for further mechanistic insights and ultimately for drug discovery. Phenotype-driven modeling in model organisms has proven powerful for pathway dissection, and unbiased phenotypes are emerging in these fields. Large-scale in vivo modeling of vertebrate phenotypes is now feasible in the zebra fish. For each phenotype or disease entity, modeling must seek a compromise with robust recapitulation of complex vertebrate biology and accessibility to functional genomics for different tissues or organs, but with the scalability necessary for rigorous exploration of gene-gene and gene-environment analyses.

New diagnostic resolution might lead immediately to earlier diagnosis, introducing the possibility of screening for an underlying diathesis toward CHF, and possibly the preventative use of agents, such as beta-blockers, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers. Comparative biology and integrative approaches in humans will lead to new rational candidate approaches to diagnosis and therapy,<sup>54</sup> and even to empiric screening for disease markers or provoked phenotypes. New disease subsets would offer the potential for improved resolution in genetic and pharmacogenetic studies, as a result of larger effect sizes.<sup>55</sup> By analogy with recent work in oncology (though the clonality of neoplasia may be a unique advantage), new drug-response classifiers could lead to smaller, less expensive drug trials. The discovery of provoked, functional endophenotypes that integrate not only genetic but also epigenetic and environmental factors, may offer better disease or drug-response classifiers than genetics alone.<sup>32,34</sup>

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