Abstract: Common or sporadic systolic heart failure (heart failure) is the clinical syndrome of insufficient forward cardiac output resulting from myocardial disease. Most heart failure is the consequence of ischemic or idiopathic cardiomyopathy. There is a clear familial predisposition to heart failure, with a genetic component estimated to confer between 20% and 30% of overall risk. The multifactorial etiology of this syndrome has complicated identification of its genetic underpinnings. Until recently, almost all genetic studies of heart failure were designed and deployed according to the common disease–common variant hypothesis, in which individual risk alleles impart a small positive or negative effect and overall genetic risk is the cumulative impact of all functional genetic variations. Early studies used a candidate gene approach focused mainly on factors within adrenergic and renin-angiotensin pathways that affect heart failure progression and are targeted by standard pharmacotherapeutics. Many of these reported allelic associations with heart failure have not been replicated. However, the preponderance of data supports risk-modifier effects for the Arg389Gly polymorphism of β1-adrenergic receptors and the intron 16 in/del polymorphism of angiotensin-converting enzyme. Recent unbiased studies using genome-wide single nucleotide polymorphism microarrays have shown fewer positive results than when these platforms were applied to hypertension, myocardial infarction, or diabetes, possibly reflecting the complex etiology of heart failure. A new cardiovascular gene-centric subgenome single nucleotide polymorphism array identified a common heat failure risk allele at 1p36 in multiple independent cohorts, but the biological mechanism for this association is still uncertain. It is likely that common gene polymorphisms account for only a fraction of individual genetic heart failure risk, and future studies using deep resequencing are likely to identify rare gene variants with larger biological effects. (Circ Res. 2011;108:1270-1283.)

Key Words: adrenergic receptors □ angiotensin-converting enzyme □ cardiomyopathy □ gene polymorphism □ genome-wide association study

An era of genomic cardiovascular medicine is heralded when both the annual American Heart Association meeting and lay television commercials emphasize genetic testing. Long-recommended to screen for rare monogenic mutations that cause familial cardiomyopathies or arrhythmias, routine genetic testing is now being advocated to identify optimal responders for standard pharmacotherapeutics. Genomics studies have uncovered new underpinnings of
Genomics encompasses all of the components of an organismal genome, including DNA sequence and copy number (gene mutations and polymorphisms), mRNA levels and pre-mRNA splicing (transcriptomics), and epigenetic factors that modify gene expression independent of nucleotide sequence (eg, DNA methylation, histone modification, and microRNA). The genetics of heritable cardiomyopathies are described elsewhere in this series. Here, we note that familial cardiomyopathies are usually caused by a mutation in a single gene3,4 (although in ≈5% of familial cardiomyopathy cases, 2 or a few causational mutations have been described in combination5–7). By contrast, the etiology of common heart failure is more complex, and genetics are not the principal causative factor. The prototypical heart failure patient has multiple contributing factors, including clinical conditions that have their own genetic components (eg, diabetes mellitus, hypertension, and coronary atherosclerosis), environmental factors with little genetic input (eg, viral or toxic myocarditis), and an estimated one-third of heart failure risk attributable to genetic influences that account for much of the interindividual variability in this syndrome. Results from the Framingham study indicate that heart failure relative risk is 1.69 if one parent has heart failure, and it increases to 1.92 if both parents have heart failure; parental heart failure was estimated to account for ≈18% of total heart failure risk.8 This familial clustering of heart failure risk differs from Mendelian inheritance of familial cardiomyopathies. The monogenic Mendelian diseases have simple (typically autosomal-dominant) modes of inheritance, whereas inheritance of heart failure risk is more like that of height, determined both by parental characteristics and environmental factors. With multiple clinical, environmental, and genetic determinants, the genetic component of heart failure plays a significant, but minor, role and almost certainly represents the aggregate effect of many different gene events having small individual positive or negative effects.

If it is possible to deconvolute the genomic architecture of heart failure and establish causality between genomic variation and phenotypic differences across a population, then novel insights into disease mechanisms will accrue. Heart failure genomics is translatable to the clinic, linking individual genotype with specific disease characteristics. Although most gene variants do not cause disease, those that change amino acid sequence of encoded proteins (nonsynonymous polymorphisms), pre-mRNA splicing (splicing variants), or mRNA expression level (expression quantitative trait loci) have the potential to alter disease risk, progression, or response to therapy. The preponderance of work in this area has focused on DNA sequence variations, especially SNPs. Newer data describing other genetic and epigenetic factors that may influence heart failure are reviewed first (Figure 1).

**Pre-mRNA Splicing Abnormalities**

SNPs that alter posttranscriptional mRNA processing only recently have been linked to heart disease. Excision of intronic sequences from pre-mRNA at the spliceosome can generate differential splicing of exons, which is the predominant mechanism for producing a diverse proteome from a limited genome and for conferring context-specific expression of protein isoforms. DNA sequence variations within the 5’ and 3’ ends of exon splice sites and in coding regions can alter splicing, which in some instances produces disease3,9,10.

### Genetic Influences on Heart Failure Risk

- **ACE** - angiotensin-converting enzyme
- **BEST** - beta-blocker evaluation of survival trial
- **CNV** - copy number variation
- **FRE** - Forster resonance energy transfer
- **GRK** - G-protein receptor kinase
- **GWAS** - genome-wide association study
- **LVH** - left ventricular hypertrophy
- **OR** - odds ratio
- **RAAS** - renin-angiotensin-aldosterone system
- **SNP** - single nucleotide polymorphism

Non-standard Abbreviations and Acronyms

Atherosclerosis, hypertension, and myocardial infarction, expanding our understanding of the pathobiology and epidemiology of these diseases. In contrast, the genetic contributors of common heart failure have been difficult to define (though not through lack of effort; hundreds of genetic studies on heart failure are listed in PubMed). The multifactorial nature of heart failure complicates identification of its genetic risk modifiers, which overlap with those for atherosclerosis, hypertension, and myocardial infarction, which have their own individual genetic components that can indirectly alter heart failure risk. Heart failure risk alleles independent of these clinical risk factors likely determine whether the cardiac response to injury is compensatory or maladaptive, and may be evident only in at-risk populations (eg, after myocardial infarction or with hypertension) or after heart failure has developed (ie, affecting the progression of, rather than risk of, the disease).

Further complicating identification of heart failure risk alleles is intrinsic human genetic diversity; of ≈3 billion bases in the human genome, ≈10 million may be expected to differ between any 2 individuals in the form of single-nucleotide polymorphisms (SNPs), DNA copy number variations (CNVs), and rare mutations. Bioinformatics can be useful to filter the large number of benign polymorphisms from the comparatively few that are functionally significant, but the potential for genetic variation to have functional impact through changing amino acid coding, gene transcription, mRNA splicing, expression or binding of microRNA, and so on is vast, and designation of a gene variant as a risk factor requires not only epidemiological data but also demonstration of dysfunction in cell systems and genetic animal models. This standard has not often been achieved.

Here, major findings that begin to describe a genetic architecture for sporadic heart failure are reviewed in a historical context, but newer findings are highlighted. Some perspectives on how genetic discovery and clinical testing may influence our understanding and management of this challenging disease are also presented.
In most cases, splice variants can only be suggested by DNA sequencing and must be detected or confirmed by studying mRNA. It is important to identify alternatively spliced mRNA isoforms or to detect the absence of alternatively spliced or truncated mRNA eliminated through nonsense-mediated decay because the potential exists to work around RNA splicing abnormalities, as demonstrated by induced exon skipping in the Duchenne muscular dystrophy mouse. Alternative RNA splicing of the cardiac sodium channel (SCN5A), the sarcoplasmic reticular calcium ATPase, brain natriuretic peptide, troponin T, and other sarcomeric genes all have been described in human heart failure. A surprising finding is that digoxin, which has been used to treat heart failure for centuries, is a pharmacological modifier of RNA splicing. This observation suggests that some pathological splicing events might be pharmacotherapeutic targets. The recent development of whole transcriptome microarrays containing probes for every encoded exon permits unbiased analysis of exon-splicing events across the complete transcriptome and should ignite further studies of alternative RNA splicing in heart failure.

**DNA Copy Number Variations**

Whereas polymorphisms affect one or a few nucleotides, DNA CNVs are large sections of DNA (>1 kb) that are deleted or amplified. These genomic rearrangements account for ~12% of the human genome and are increasingly recognized as an important component of interindividual genetic variation. Many CNVs are stable and inherited, whereas others are surprisingly dynamic, arising de novo (as in one individual of a monozygotic twin pair) at repetitive sequences that are “hot spots” for genetic recombination. Interestingly, CNVs and SNPs tend to cluster in different regions of the mouse genome; therefore, CNVs are enriched at loci with comparatively low prevalence of SNPs. CNVs can contribute to disease by altering the “dose,” and therefore expression level, of one or more genes. However, expression level of only a relatively small fraction of genes appears to be modified by CNVs (between 5% and 18%) because of the influences of poorly understood gene–dose compensatory mechanisms. Furthermore, CNVs appear more likely to alter expression of either tissue-specific genes or genes expressed at lower levels, possibly primarily affecting proteins with limited or “niche” functions.

The availability of high-density genome-wide association studies (GWAS) and whole-genome resequencing data makes it possible to look for CNVs that may be associated with cardiac disease. The first such study described a CNV detected by HapMap data (using the Affymetrix 6.0 microarray) that deleted ~66 000 bases from chromosome 6 and was associated with a modest (~10%) decrease in expression of endothelin-1 (EDN1). Although there are no known genes within the deleted fragment and the EDN1 gene is ~60 kb distant (chromosome 6, 6p24.1), these results show how the expanding database of genome-wide CNV data, when combined with individual whole-transcriptome data, can be used to reveal candidate functional CNVs.

**Epigenetic Factors**

All genetic variation cannot be explained by alterations of DNA sequence. Other mechanisms that produce heritable changes in genes or gene expression are termed epigenetic variations and include DNA methylation, histone modifications, and regulatory noncoding RNA, such as microRNA. Epigenetic mechanisms are the most dynamic of the gene regulatory pathways, differing between tissues, pathophysiological states, and environmental modification. Thus, total interindividual genomic variability must be the aggregate effect of DNA sequence and epigenetic variations.

DNA methylation at clusters of 5′-CG-3′ sequences found in the promoter regions of many genes (termed CpG islands) is a mechanism for gene silencing. An altered DNA methylation signature was recently described in human heart failure and is implicated in tumor necrosis factor α-mediated suppression of sarcoplasmic reticular calcium ATPase 2A expression. The availability of whole-genome screens for DNA methylation mapping can be expected to add further to our knowledge of its role in heart failure.

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**Figure 1. Schematic depiction of epigenetic and genetic mechanisms that may contribute to heart failure.** A simple 2-exon (boxes) gene is depicted with 5′-promoter region (to the left of exons) having CpG island and one cis regulatory element, and 3′-untranslated region (to the right of the exons) having a micro-RNAs binding site. Epigenetic mechanisms: Methylation of CG sequences (top) leads to transcriptional silencing. Histone prevents transcription factors (not shown) from interacting with their cis-binding elements, but methylation and acetylation of histone weakens its interaction with DNA, enabling transcription factor binding and activating transcription. Micro-RNAs binding sites in the 3′-untranslated region recruit transcribed mRNA to RNA-induced silencing complexes for degradation and translational silencing. Genetic mechanisms: Sequence variations (bottom) can alter binding of transcription factors to cis elements (left), amino acid coding (middle), or pre-RNA exon splicing (right). Not shown are DNA copy number variations from large genomic deletions.
Histones are proteins around which DNA is tightly folded within chromatic repeats. Compacted DNA is less accessible to the proteins of transcription complexes and therefore is relatively silent. Modification of histones by acetylation, methylation, and other processes can relax the compacted DNA by releasing the DNA–histone bonds, thus permitting gene transcription. A role for reversible histone acetylation/deacetylation in regulation of cardiac hypertrophy has been recognized for some time, and this subject has been thoroughly reviewed. Kaneda et al. used the technique of differential chromatin scanning to identify genomic regions with differentially acetylated histones and corresponding differentially expressed genes. The same group followed-up with antiacetylated histone chromatin-immunoprecipitation studies that identified specific histone modifications related to genes encoding cardiomyocyte contractile proteins. This is an emerging field, and genome-wide profiling of histone modifications is certain to lead to new insights.

The final class of epigenetic changes is caused by noncoding RNA, especially microRNA that regulate mRNA stability and translation. There has been an explosion of information about microRNA expression in, and effects on, the heart. A detailed examination of this rapidly evolving area is beyond the scope of this article, and the interested reader is referred to one of many recent excellent reviews.

Candidate Gene Studies of Polymorphisms Within Neurohormonal Pathways

Genome-wide SNP detection platforms are a relatively recent development. Early heart failure genetic studies focused on candidate genes suggested by the first 2 clinically successful classes of heart failure therapeutics: β-blockers and angiotensin-converting enzyme (ACE) inhibitors. Our understanding of the roles played by excess neurohormones in heart failure progression was formalized in 1992 by Milt Packer, who defined a mechanism that explained the beneficial effects of β-blockers and ACE inhibitors observed in the early large clinical trials examining heart failure outcome (Figure 2). Accordingly, for 30 years neurohormonal inhibition has been the cornerstone of heart failure management. Early attempts to identify gene variants altering heart failure risk or outcome or both therefore focused on factors within the renin-angiotensin-aldosterone system (RAAS) and adrenergic pathways, “informed” by the pathophysiological significance of neurohormonal pathways in this disease. The conceptual basis that neurohormonal activation is a response to heart failure, and not a primary cause, further suggested that functionally significant gene variants within adrenergic or renin-angiotensin pathways might have greater significance as modifiers of disease progression or response to therapy, rather than causal factors for sporadic cardiomyopathy. However, candidate gene studies have generally fallen out of

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Figure 2. Postulated effects of common polymorphisms on neurohormonal activation in heart failure. Schematic representation of neurohormonal signaling in heart failure. Myocardial injury in the form of cardiac damage or hemodynamic stress compromises forward cardiac output, activating compensatory increases in renin-angiotensin-aldosterone system (RAAS) and catecholamine release from sympathetic nerves. Angiotensin-converting enzyme (ACE) DD genotype increases ACE expression, and CLCNKA Gly83 may prime renin secretion, thus magnifying the RAAS response and causing reactive left ventricular hypertrophy with secondary myocardial impairment. The deletion polymorphism of presynaptic α1c adrenergic receptors increases norepinephrine release from sympathetic nerves, and the Arg 389 variant of myocardial β1-adrenergic receptors increases receptor signaling. Both effects sensitize the heart to catecholaminergic toxicity. Opposing these effects is the Leu41 gain-of-function GRK5 polymorphism that accelerates β-receptor desensitization. (Illustration by Cosmocyte/Ben Smith).
favor because of perceptions that investigator bias and underpowered studies generated too many false-positive associations. Many of the original case-control associations between ACE or adrenergic receptor polymorphisms and heart failure were of marginal significance in small experimental populations and have not been independently replicated. Some of the stronger associations are discussed.

Adrenergic Signaling Pathway Polymorphisms in Heart Failure

The Arg389 β1-Adrenergic Receptor Polymorphism May Be Beneficial in Heart Failure

β-Adrenergic receptors are highly polymorphic, and many candidate gene studies have been performed to evaluate the association of genetically variant adrenergic receptors with heart failure risk, outcome, and response to β-blocker therapy. This topic was recently comprehensively reviewed.40 The major myocardial/cardimyoocyte β-adrenergic receptor subtype is the β1-receptor, comprising ~80% of the β-receptors in normal myocardium.41 β1-Receptors are responsible for most of the positive chronotropic, inotropic, and lusitropic effects of catecholamines. β2-Adrenergic receptors are the dominant subtype in vascular smooth muscle and are less common than β1-adrenergic receptors in normal myocardium. For this reason, polymorphisms of β1-adrenergic receptors were considered more likely to impact myocardial contraction and heart failure, and polymorphisms of β2-adrenergic receptors were considered more likely to impact hypertension.

The most studied β1-adrenergic receptor polymorphism is the Arg389Gly variant located within a predicted fourth intracellular loop, a region important for receptor coupling to intracellular signaling molecules. Arg occurs at the position analogous to human amino acid 389 in every species from which sequence data are available (the only known exception is the human Gly389 variation), suggesting that this highly conserved amino acid is functionally important. The agonist-analogous to human amino acid 389 in every species from which sequence data are available (the only known exception is the human Gly389 variation), suggesting that this highly conserved amino acid is functionally important. Arg389 receptors recapitulate dose-dependent cardiac toxicity in vivo. These findings indicate that increased signaling through Arg389 receptors may contribute to heart failure risk.

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is essential for G-protein coupling and signal transduction. The combination of gain-of-function β1-adrenergic receptor Arg389 with α2c del 322-325 receptor therefore might confer a cardiotoxic “double whammy” of increased adrenergic signaling (because of the more efficient β-receptor and loss of normal α2c-receptor–mediated synaptic inhibition of catecholamine delivery; Figure 2). Small et al initially reported that the combination of homozygosity for the α2c receptor del polymorphism and the Arg389 β1-adrenergic receptor polymorphism increased heart failure risk 10-fold in blacks. However, this was a small study in which only 17 blacks (2 controls and 15 with heart failure) had the combination of genotypes reported to increase heart failure risk, and this particular gene-gene-phenotype interaction has not been confirmed. An adequately powered study of 1121 black and 740 white subjects from the Dallas Heart Study found no significant associations between the 2 adrenergic receptor polymorphisms and ventricular contractile performance, ventricular dimension, and circulating levels of brain natriuretic peptide. Likewise, case-control studies from South Africa and Japan failed to confirm the original associations. However, when a small study retrospectively examined the effects of the β1-adrenergic receptor position 389 and α2C receptor del polymorphisms on echocardiographic cardiac function in 6 months after initiation of metoprolol in β-blocker naïve heart failure patients, patients homozygous for Arg389 (n = 23) showed greater improvement than Gly389 carriers (n = 31), and Arg389 homozygotes who also had the α2C receptor del polymorphism (n = 7) had even greater improvement in left ventricular ejection fraction. The latter results are based on secondary subgroup analyses with small numerical values. They have not been replicated and should be regarded as hypothesis generating. Thus, the totality of available evidence does not indicate that the α2C del polymorphism, with or without the Arg389 β1-receptor variant, constitutes a significant independent risk factor for de novo development of heart failure.

The β2-Adrenergic Receptor Ile164 Polymorphism and Heart Failure Risk

Compared to β1-adrenergic receptors that play the major role transducing cardiac catecholaminergic effects, β2-adrenergic receptors play a subordinate role regulating cardiac function. Accordingly, most studies of common β2-receptor polymorphisms have not found major effects on cardiac function or heart failure risk. However, in one of the first series of studies to integrate cell, mouse, and human data, Liggett et al described a powerful negative association between the rare (prevalence <1%) β2-adrenergic receptor Ile164 polymorphism and heart failure outcome. This polymorphism impairs β2-receptor signaling in cell systems, transgenic mice, and human subjects, and heart failure subjects carrying a single Ile164 allele had a 1-year survival of only 42% compared with 76% for those without the variant allele. The observation that the β2-receptor Ile164 polymorphism adversely impacts heart failure outcome has been supported by one clinical study, but not another. Intriguingly, β2-receptor haplotype analysis in a study of almost 15,000 healthy American men detected a directionally oppo-
subo cardiomyopathy and thought to be caused by chronic catecholamine excess. GRK5 Leu41 was more common in the Takotsubo patients than in controls (21% versus 6%). This is a small candidate gene study of a relatively rare condition and therefore is subject to the usual caveats. However, these findings are consistent with the tissue culture, transgenic mouse, and clinical data that point to involvement of GRK5 Leu41 in cardiac syndromes characterized by an excess of circulating catecholamines.

**Angiotensin-Converting Enzyme in/del Polymorphism and Heart Failure**

Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase that converts angiotensin I to angiotensin II and proteolytically inactivates bradykinin. It has been recognized for some time that circulating ACE levels vary widely between individuals, but tend to be correlated between family members. This familial concordance in ACE levels suggested a genetic component that was identified as a common 287 bp inserted (I) or deleted (D) Alu repeat fragment within intron 16 of the ACE gene (located at 17q23). Approximately half of normal interindividual variation in plasma ACE levels in humans may be determined by ACE in/del genotype.

Two del alleles (ACE DD genotype) are associated with higher plasma ACE activities, ID genotype with intermediate activity and II genotype with lower ACE activities. The DD genotype is also associated with increased ACE activity in human lymphocytes and myocardium. It is important to note that the ACE in/del polymorphism itself is almost certainly not the cause of the change in plasma ACE activity, and that it may instead serve as a readily measurable marker for one or more other ACE polymorphisms with which it is in tight functional linkage-disequilibrium. The molecular and cellular mechanisms for increased ACE expression associated with the ACE del polymorphism are unknown.

After the discovery of the ACE in/del polymorphism and its associated effects on ACE activity, the ACE DD genotype was implicated in myocardial infarction and ischemic and nonischemic cardiomyopathies (with variable results in hypertension). The results of these high-profile studies helped to develop the notion that increased RAAS activation conferred by the ACE DD genotype could adversely impact many cardiovascular syndromes (Figure 2), and literally hundreds of clinical association studies have since been performed examining almost every conceivable cardiovascular end point.

The preponderance of evidence now indicates that ACE in/del genotype has little impact on coronary artery disease or hypertension, but the results with cardiac hypertrophy and heart failure are more suggestive of a meaningful association. Raynolds et al described a higher frequency of DD genotype in ischemic (39%) and idiopathic (36%) cardiomyopathies than in control subjects (24%). Disproportionate representation of DD genotype in ischemic cardiomyopathy could have been attributable to an allelic association with myocardial infarction, but the association with nonischemic or idiopathic cardiomyopathy suggested an independent risk modifier effect for heart failure. This early finding was not confirmed in subsequent studies. Rather than causing heart failure, ACE DD may provide a genetic background on which other causes of heart failure have more severe consequences, as suggested by its interaction with the clinical heart failure risk factors of hypertension and smoking.

Evidence is more convincing that the ACE DD genotype can accelerate progression of preexisting heart failure progression or adversely impact heart failure survival or both. The first study to directly suggest a modifier, rather than causal, role assayed ACE in/del genotype in a subset of subjects from a large Swedish heart failure cohort and assessed echocardiographic metrics and survival as a function of ACE genotype (DD, ID, and II). ACE D allele frequency was not an independent risk factor for heart failure (allele frequency in controls = 0.56; allele frequency in heart failure = 0.57); however, among heart failure subjects with DD genotype, left ventricular mass was greater and survival time was decreased. This important finding links the ACE genetic polymorphism and its effects on ACE activity to left ventricular hypertrophy and heart failure prognosis. A follow-up report from this group further suggested that a gene–gene interaction between ACE DD genotype and a polymorphism in the 3′ untranslated region of the angiotensin II type 1 receptor gene contributed to heart failure phenotype.

Subsequent studies described deleterious associations between ACE DD genotype and cardiac function or heart failure prognosis after myocardial infarction and in a Chinese cohort of subjects with chronic heart failure. Two more reports from the same study, but at different follow-up periods, observed decreased survival of ACE D allele carriers in ischemic and nonischemic heart failure. Unexpectedly, the latter 2 reports also described a pharmacogenomic interaction between ACE genotype and β-blocker therapy. Heart failure subjects carrying an ACE D allele had worse transplant-free survival, but ACE DD heart failure patients derived the greatest survival benefit from β-blocker and ACE inhibitor therapy.

Because angiotensin II is a potent stimulus for left ventricular hypertrophy (LVH), the link between ACE DD genotype, ACE, angiotensin II levels, and heart failure severity or progression suggested that ACE DD genotype also could modify the LVH response (or that of hypertrophic cardiomyopathy). The first study to critically examine the ACE DD-LVH association using a case-control study design found a disproportionate representation of DD genotype among LVH subjects. Similar findings suggested that DD genotype can be a modest risk factor for echocardiographic LVH and for exercise-induced hypertrophy in military recruits. A pathological study relating ACE in/del genotype to autopsy heart weights of 443 subjects that excluded known valvular or myocardial disease found that DD genotype was a modest independent predictor of heart weight but also determined that the conventional LVH risk factors of hypertension and age were more powerful predictors. Collectively, these findings suggest that DD genotype can contribute to LVH in the context of other hypertrophy stimuli, which is similar to the risk-modifier effect described by the heart failure studies. This paradigm has received further support from a number of relatively small studies showing that ACE DD genotype in healthy individuals is not sufficient to cause LVH, but that ACE DD measurably increases LVH.
the context of predisposing factors such as essential hypertension or end-stage renal disease.\textsuperscript{119,120}

**Genome/Subgenome-Wide Studies of Heart Failure**

Unlike candidate gene studies described above that examined 1 or 2 genes for a disease association, GWAS take advantage of microarray platforms to genotype hundreds of thousands or millions of common SNP distributed across the entire human genome. The theoretical advantage of GWAS is that the unbiased approach of scanning the whole genome will identify previously unsuspected genotype–phenotype associations.\textsuperscript{121} Although GWAS approaches are limited, however, to discovery of associations with common polymorphisms that typically have small individual effects,\textsuperscript{122} Furthermore, a positive GWAS result will only establish a location at the genome within which putative causative risk alleles must still be defined through resequencing. Notwithstanding these limitations, GWAS have identified risk loci for a number of complex diseases, including hypertension, myocardial infarction, atherosclerosis, and diabetes.\textsuperscript{123–127} Given the successes of GWAS with these heart failure antecedents, it is noteworthy that success has been limited for the syndrome of heart failure itself.

The first heart failure GWAS study was performed as part of the Framingham Heart Study and used the Affymetrix 100K Gene Chip, a relatively low-resolution array by current standards. One SNP showed a trend for association with heart failure, rs740363 ($P = 8.8\text{E}-6$).\textsuperscript{128} This finding has not been independently replicated and is of unknown significance because no functional gene variant was implicated.\textsuperscript{128}

A multilocus analysis of 2.4 million HapMap imputed polymorphisms in >20 000 subjects identified 2 loci associated with heart failure, rs10519210 (15q22, containing the USP3 gene encoding a ubiquitin-specific protease) in subjects of European ancestry and rs11172782 (12q14, containing the LRIG3 gene encoding a leucine-rich, immunoglobulin-like domain containing protein of uncertain function) in subjects of African ancestry.\textsuperscript{129} In a companion article using the same population and genotyping results, mortality analysis of the subgroup of individuals who had heart failure implicated an intronic SNP in the CMTM7 gene (CKLF-like MARVEL transmembrane domain containing 7).\textsuperscript{130} These recent findings await confirmation in independent studies and further evaluation to identify the causal variants marked by the array SNPs. However, the data support the idea that multiple different genetic factors are likely to influence heart failure susceptibility (disease risk) and outcomes (disease progression).

These results illustrate both the promise and pitfalls of a pure GWAS approach to heart failure genomics. The potential for whole-genome approaches, because they are completely agnostic, is that unexpected gene–disease associations will lead to novel mechanistic insights. The risk of absolute mechanistic agnosticism is that a true genetic signal will be lost within the whole-genomic noise under the rigorous statistical procedures necessary to exclude false-positive associations. Furthermore, when a statistically significant association is identified, too frequently the SNP has only a tenuous functional relationship. Such SNPs presumably mark linked gene variants within the same locus that are causal and must be identified in a more targeted manner. Thus, a positive GWAS result will generate a list of candidate genes for resequencing.

Subgenome arrays represent an intermediate approach between single candidate gene and whole-genome GWAS studies that takes advantage of simultaneous multigene SNP evaluation on microarrays without completely ignoring well-established biological foundations of cardiovascular disease. The ITMAT Broad Care cardiovascular SNP array includes 50 000 SNPs in 2000 genes prioritized based on their likelihood of being involved in cardiovascular disorders according to accepted pathophysiological concepts. Ancestry-informative markers are also included. In the first published study to use this platform, Cappola et al\textsuperscript{131} performed a 2-stage case-control analysis of SNP allele frequency in advanced white systolic heart failure, identifying 2 SNPs that are significantly associated with heart failure in both the primary discovery cohort and an independent replication cohort. The top SNP was rs1739843, located at 1p36 in the second intron of the HSPB7 gene that encodes a small cardiovascular heat shock protein. This SNP association was similar in strength for ischemic and nonischemic cardiomyopathies and, importantly, was also the top polymorphism associated with idiopathic dilated cardiomyopathy in a new European study.\textsuperscript{132} Thus, within the past year, rs1739843 has been associated with heart failure in 2 separate United States university-based heart failure referral programs (University of Cincinnati and University of Pennsylvania\textsuperscript{133}), in 2 separate cohorts from Germany (Berlin and multiple centers\textsuperscript{134}), and in 2 separate French cohorts,\textsuperscript{132} i.e., in 6 independent heart failure populations on 2 continents.

Because the heart failure-associated HSPB7 SNP is intronic, pooled resequencing of the entire HSPB7 gene in the Cincinnati and Pennsylvania cohorts was undertaken to see if one or more functional HSPB7 coding polymorphisms was responsible for the SNP association detected by microarray. Resequencing demonstrated that HSPB7 is highly polymorphic and of 19 common SNPs, 12 were associated with heart failure (including the seminal rs1739843 SNP reported by ITMAT Broad Care array).\textsuperscript{135} All 12 heart failure-associated HSPB7 SNP are either intronic or synonymous, however; therefore, none suggested a clear mechanism for the heart failure effect. Additionally, all 12 SNPs were in tight linkage-disequilibrium. These results suggested 2 possibilities: either rs1739843 or one of the linked SNPs affects HSPB7 expression (i.e., it is an expression quantitative trait locus) or the HSPB7 SNP are telegraphing the location of a functional polymorphism in a different gene at 1p36.

Recently, we examined both of these possibilities and identified a functional polymorphism that may explain the association between rs1739843 and heart failure.\textsuperscript{136} First, to determine whether the HSPB7 SNP marked an expression quantitative trait locus, we measured HSPB7 mRNA expression in left ventricular myocardium of 111 heart failure subjects of European ancestry. Neither microarray nor quantitative reverse-transcription quantitative polymerase chain reaction showed any difference in HSPB7 mRNA levels according to rs1739843 genotype, nor were there differences in HSPB7 exon splicing. Thus, the data do
not suggest an expression quantitative trait locus mechanism for the SNP–heart failure association. Accordingly, we examined the other possibility, that rs1739843 and the other 11 heart failure-associated HSPB7 SNP marked one or more functional heart failure risk alleles within the broader 1p36 genomic region. HapMap data shows the CLCNKA coding exons identified 51 SNPs, including 40 nonsynonymous polymorphisms, most of which are rare. Case-control analyses demonstrated a significant heart failure association for one common CLCNKA SNP, rs10927887, encoding a Gly substitution for Arg at amino acid 83. The rs10927887 SNP was overrepresented in heart failure (allele frequency ∼6% versus 49% in controls) in 2 independent white study cohorts (combined n = 5659 subprojects) showed that the polymorphism increased heart failure risk in an additive manner, with an odds ratio of 1.27 per allele copy (thus increasing heart failure risk by 54% in homozygotes) independent of age, sex, or hypertensive status. Given that the population lifetime attributable risk for heart failure in the general population is 20%,134 with an incidence rate reported as 6 per 1000 patient-years,135 it would be expected that the lifetime heart failure risk of a population homozygous for the CLCNKA risk variant would increase to ∼30% (compared with homozygous wild-type CLCNKA), and the incidence rate would increase to ∼6 per 1000 patient years. It will be interesting to see if the combined heart failure risks of hypertension (that, by itself, doubles heart failure risk in men and triples it in women134,135) and CLCNKA Gly83 interact in an additive or synergistic manner to even further predispose to heart failure.

Comparative functional analysis of recombinantly expressed wild-type Arg83 and polymorphic Gly83 human CIC-Ka channels uncovered diminished current amplitude in the Gly variant channels, with decreased sensitivity to extra-cellular chloride concentration. Thus, the Gly83 CIC-Ka renal chloride channel variant encoded by rs10927887 is a functional heart failure risk polymorphism that is tightly linked to, and marked by, the HSPB7 rs1739843 SNP associated with ischemic and dilated cardiomyopathy cohorts in the United States, Germany, and France. The CLCNKA polymorphism marked by the HSPB7 SNP found by cardiovascular gene-centric microarray provides an example of how unbiased subgenome SNP association studies can be the foundation for unexpected discoveries. Variant renal chloride channels were not considered as candidate heart failure risk alleles, and the pathological basis for this genetic association must still be elucidated. However, the studies of recombinant variant ClC-Ka channel in cultured cells demonstrating a ∼50% decrease in channel current suggest a testable hypothesis that the CLCNKA variant decreases the set point for activation of the RAAS after myocardial injury or stress. The basis for this hypothesis is the previous report of a similar, but rare, loss-of-function CIC-Ka mutation described as one-half of a biallelic lesion that caused congenital Bartter syndrome in one individual.136 Most forms of Bartter syndrome, a salt-wasting nephropathy, are caused by mutations in other proteins that affect renal sodium chloride handling.137 However, common to all genetic lesions causing Bartter syndrome is hyper-reninemic hyperaldosteronism, ie, autonomous activation of the RAAS system.138 Clinical, experimental, and genetic data (some of which were reviewed) show that RAAS contributes to the development and progression of heart failure. Thus, it is possible that the RAAS system is intrinsically primed in individuals with the Gly83 CIC-Ka variant, providing a subclinical genetic first “hit” that predisposes them to have heart failure after a second “hit” damages the myocardium or impairs cardiac function. This hypothesis needs testing in proper animal models, but if it is correct it establishes a genetic modifier affecting the cardiorenal axis in sporadic heart failure.

**Future Directions**

The majority of alleles described as genetic risk modifiers for sporadic heart failure are common, at least in the affected subpopulations. Frequent alleles have not been subjected to suppressive selection, as would be expected either for disease-causing or for major disease-modifying gene variants. Accordingly, these common polymorphisms have modest individual effects, typically increasing disease risk by only 20% to 40%.122 This contrasts with the many rare causative mutations that have been described in familial hypertrophic and dilated cardiomyopathy. The inverse relationship between the prevalence of a gene variant and its pathological impact is a general rule (Figure 3). A corollary to this paradigm is that less common gene variants may have a greater impact on individual disease risk, without causing disease outright. Identifying such rare disease modifiers is a challenge because whole-genome sequencing techniques applied to large numbers of affected individuals are necessary for their detection, and the majority of silent polymorphisms
in any individual genome complicate purely statistical approaches to determining pathological significance in a multifactorial disease like heart failure. By way of example, comparing the tumor and nontumor genomes within multiple individuals with the same type of cancer is a logical approach to determine the genetic causes of that cancer. Presumably, the cancer-causing gene mutations will be common among tumor genomes, but the silent interindividual variation will not and can be bioinformatically filtered out. Notwithstanding the compelling rationale behind such an approach (which is being funded on a massive scale by the National Cancer Institute), initial results have shown surprising sequence diversity in tumor genomes, likely because multiple independent biological processes must be perturbed for malignant transformation, and because many different genetic mutations can produce any given individual biological result.\cite{139,140} This problem is amplified in diseases with complex etiologies, such as heart failure, which have only a fractional genetic component.

Addressing this question may be accomplished using next-generation sequencing technologies aims to detect rare gene variants of familial cardiomyopathy genes in heart failure syndromes that are not (typically or entirely) mono- genic. Whereas conventional genetic epidemiology seeks to identify a genetic marker for at-risk individuals, this approach begins with a genotype that mimics the phenotype of interest (as for the familial cardiac hypertrophy or heart failure genes) and looks for differences in allele frequencies between cases and controls. This approach assumes that hypertrophy or heart failure may be modified by confounding external environmental or unidentified genetic factors, but that genotype–phenotype associations, in these studies the same genetic disease. Hundreds of mutations in other sarcomeric genes also produce hereditary hypertrophic cardiomyopathy. Thus, it is not the mutation per se that confers a disease phenotype, but rather the cumulative biological impact of the mutations. If the same paradigm applies to sporadic heart failure, then gene–gene interactions will play significant roles in this disease. Although the results require confirmation, the combination of gain-of-function Arg389 β1-adrenergic receptor and loss-of-function deletion poly- morphism for the presynaptic α2c adrenergic receptor, which strikingly increases sympathetic tone at the myocardium, reportedly compound the modest heart failure risk profile conferred by either polymorphism alone. Similar functionally synergistic gene–gene interactions likewise would be expected to multiply small individual allelic effects. It will be interesting, for example, to see if there is an interaction between the ACE del and CLCNKA Gly83 polymorphisms, both of which are hypothesized to affect RAAS activity.

Sources of Funding
Supported by National Institutes of Health grants R01 HL078771 and RC2 HL102222.

Disclosures
None.

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The Genomic Architecture of Sporadic Heart Failure
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Circ Res. 2011;108:1270-1283
doi: 10.1161/CIRCRESAHA.110.229260
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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