

Genetic Polymorphisms and Oxidative Stress in Heart Failure

Heart failure results from various known cardiovascular diseases, such as coronary artery disease, or can be the result of an idiopathic dilated cardiomyopathy. It is of utmost importance for diagnostic, preventive, and therapeutic purposes to understand the cellular events that trigger the cascade of functional and structural changes that result in the development and progression of heart failure. Progress in unraveling the genetic background in both ischemic and nonischemic cardiomyopathies has been slow compared with that for monogenic diseases, such as some forms of hypertrophic cardiomyopathy or familial dilated cardiomyopathies. It is likely that susceptibility to and risk of progression of heart failure are both influenced by many genes acting in concert or independently. Among the diverse subcellular mechanisms implicated in the pathogenesis and progression of heart failure, reactive oxygen species play a major role. The search for genetic polymorphisms in clinical association studies in order to identify genotypes susceptible to develop and affect the progression to heart failure has been the focus of many investigations over the past several years. In this review, the authors summarize the current data in support of the role of various polymorphisms of genes related to oxidative stress in the susceptibility to develop heart failure, and its progression. (CHF. 2002;8: 157–164, 172) ©2002 CHF, Inc.

Fadi M.F. Alameddine, MD;¹
A. Maziar Zafari, MD, PhD,^{1,2}

From Emory University School of Medicine, Department of Medicine, Division of Cardiology, Atlanta, GA;¹ and Atlanta Veterans Administration Medical Center, Division of Cardiology, Decatur, GA²

Address for correspondence:

A. Maziar Zafari, MD, PhD, Assistant Professor of Medicine, Emory University School of Medicine, Division of Cardiology, 1639 Pierce Drive, WMB 319, Atlanta, GA 30322

E-mail: azafari@emory.edu

Heart failure (HF) remains a significant health problem with a 5-year mortality of 50%; it afflicts nearly 4.7 million Americans, with 550,000 new cases each year.¹ End-stage HF is the end result of a diverse range of diseases, including hypertension, valvular heart disease, infiltrative diseases, diabetes mellitus, viral myocarditis, metabolic diseases, genetic mutations, and coronary artery disease.

All failing hearts have in common a temporary or permanent decline in contractile function. The factors that lead to progression of HF from mildly symptomatic to a severely decompensated stage, leading to death or the need for cardiac transplantation, are not well defined, and substantial interindividual variability in progression is observed. Therefore, it is of utmost importance to better understand the cellular events that trigger the cascade of functional and structural changes that result in development of ischemic and nonischemic cardiomyopathies and HF, as well as the compensatory changes that take place to preserve cardiac function.

During the past decade, there has been much interest in characterizing the role of genetic polymorphisms in ischemic cardiomyopathy (ICM) and nonischemic cardiomyopathy (NICM), polygenic diseases leading to HF. ICM and NICM cannot be distinguished by clinical findings alone, although the pathogenesis is etiologically different. Among the diverse subcellular mechanisms implicated in the pathogenesis and progression of HF, the generation of reactive oxygen species (ROS) plays a major role. These oxygen free radicals are involved in many biological processes, including cardiac cell injury. ROS can be stimulated by mechanical forces, inflammatory cytokines, vasoactive agonists, during prostaglandin biosynthesis, by catecholamine auto-oxidation, and by repetitive episodes of ischemia and reperfusion. In a canine model, in vivo infusion of ROS was found to cause myocardial systolic dysfunction.² Plasma malondialdehyde-like activity, a marker of lipid peroxidation, is increased in patients with ICM and NICM, and appears to correlate with severity and chronicity of symptoms, and inversely with left ventricular ejection fraction (LVEF) and exercise capacity.^{3–5} It has been shown that production of oxygen-derived free radicals is four-fold increased in polymorphonuclear leukocytes of pa-

tients with HF compared with controls.⁶ Imbalance between the production of ROS and the cellular and extracellular antioxidant defense mechanisms can result in increased oxidative stress and myocardial dysfunction. It has been shown that patients with chronic HF have reduced antioxidant activity and vitamin C concentrations in plasma.⁷ Superoxide dismutase (SOD), one of the first-line defense enzymes against superoxide-mediated damage, increases H_2O_2 levels by dismutation of the superoxide anion (Figure). Accumulated H_2O_2 may lead to production of the highly reactive hydroxyl radical, for which no physiologic defense exists. As a result, catalase and glutathione peroxidase become crucial antioxidant enzymes regulating H_2O_2 levels. Singlet oxygen (1O_2) is formed when molecular oxygen loses one of its unpaired electrons. Finally, a major interaction of superoxide is with nitric oxide (NO), resulting in the production of peroxynitrite. The role of the NO pathway and oxidative stress in HF is discussed in a separate review of this issue.

Molecular Genetics in Clinical Association Studies

Understanding the genetic heterogeneity of complex polygenic diseases like ICM and NICM is challenging. One of many achievements in medical genetics in the past decades has been the ability to visualize sequence differences directly in DNA. These differences are called polymorphisms, and they underlie the diversity of humans. Genetic polymorphisms have many alternative forms, and can serve as genetic markers of disease. Two techniques are available to measure DNA sequence length at polymorphic sites: polymerase chain reaction and Southern blotting. Medically useful and meaningful polymorphisms alter function or expression of the protein encoded by the gene.

The process of mapping the responsible genes for a complex disease such as HF is confounded by the genetic heterogeneity of the disease, incomplete penetration of the disease-causing allele, presence of phenocopy (false-positive), high frequency of the disease-causing alleles in the society, and the effect of environmental factors. Despite such complexity, several new methods have been developed and applied to localize and identify the genes responsible for complex traits.

Approaches to mapping the responsible gene for a complex trait can be classified into the following three categories. First, candidate gene analysis is based on a priori knowledge of the candidate genes, with the aim to demonstrate that a disease-associated allele is more common in cases than in controls.

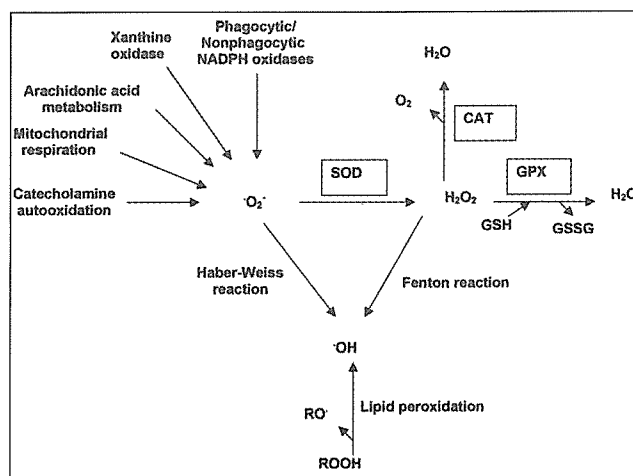


Figure. Reactive oxygen species (ROS) and antioxidant enzymes. CAT=catalase; GPX=glutathione peroxidase; GSH=reduced glutathione; GSSG=oxidized glutathione; H_2O_2 =hydrogen peroxide; O_2^- =superoxide; OH=hydroxyl radical; RO•=lipid radical; ROOH=lipid peroxide; SOD=superoxide dismutase

It assesses the role of the known genes and their functional variants in susceptibility to cardiac disease. A positive association does not mean causality but probable linkage disequilibrium (nonrandom segregation) with the actual mutation. A major problem with association studies is the high frequency of spurious results.

Second, genome-wide search is based on the principle of linkage disequilibrium and thus postulates that affected individuals share alleles in a chromosomal region that contains the susceptibility gene at a greater frequency than would be expected by chance alone. Several variations of linkage disequilibrium have been developed and used to map the susceptibility genes for complex traits. These include sib-pair linkage analysis looking at affected siblings, and transmission disequilibrium testing looking at heterozygote parents and their offspring.

Finally, microarray technology is based on the principle that genes are subject to differential expression. It is designed to reveal the temporal and spatial changes in gene expression and thus to provide insight into cellular functions and underlying mechanisms in disease pathogenesis. DNA microarrays are micron-range-sized spots of genomic DNA, cDNA, or oligonucleotides arrayed on glass slides by robotic microfabrication techniques ("gene chips"). Messenger RNA is purified from normal and failing hearts and used to label the chips using a fluorescent biotin-binding protein.⁸ Levels of expression are therefore monitored simultaneously, as levels of fluorescence overlying each DNA spot, for as many genes as are printed on the slides. This technology is

used not only for sequencing and gene expression profiling, but also for detection of polymorphisms. Microarray-based assays allow rapid comparative sequence analysis of intra- and interspecies genetic variation. It allows us some degree of mechanistic speculation, yet one has to caution against using these data to predict the functional importance of genes. Moreover, it is important to remember that some genes may be regulated at the translational rather than the transcriptional level, which would preclude detection by DNA microarrays.⁹

Candidate Genes

The genetic factors that predispose toward ICM and NICM are poorly understood, and it is likely that susceptibility to and risk of progression of HF are both influenced by many susceptibility and modifier genes acting in concert as well as independently.

Oxidative stress describes the imbalance between the production of ROS and antioxidant defense mechanisms, in favor of the former. Thus, pro-oxidant and antioxidant enzymes that generate or reduce levels of ROS represent genes which affect various cellular and signaling mechanisms involved in myocardial homeostasis.

This review discusses the role of clinically meaningful polymorphisms in candidate genes related to oxidative stress and HF in ICM and NICM (Table). We have not included catalase and glutathione peroxidase in our discussion, since no data have been reported in clinical association studies regarding the role of potential polymorphisms of these important antioxidant genes with susceptibility to or progression of HF.

Superoxide Dismutase. The superoxide anion is one of the ROS that contributes to the deterioration of the decompensating myocardium, and SOD is the principal scavenger enzyme (Figure). Humans have three genes encoding SOD1, SOD2, and SOD3. These antioxidant enzymes localize to the cytosol, the mitochondria, and the extracellular space and contain copper-zinc, manganese, and iron, respectively. The dismutation reaction catalyzed by the SODs makes use of the fact that superoxide is both an oxidant and a reductant, eager to get rid of its extra electron, or to take on another. SOD uses one superoxide radical to oxidize another radical. SOD3 or extracellular SOD accounts for the majority of the SOD activity of the plasma.

The *SOD2* gene is located on chromosome 6q25, and consists of five exons.¹⁰ It is polymorphic at the 16th position. Like most mitochondrial proteins, manganese-containing SOD (MnSOD), or SOD2, is synthe-

sized in the cytoplasm as a precursor containing a leader signal that is removed during the processing to a mature enzyme. The Ala16Val polymorphism changes the conformation of the leader signal with subsequent loss of the α -helical structure, and hence lowers mitochondrial processing efficiency.¹¹

A recent study found that the SOD2-VV genotype of the leader peptide is more frequent in Japanese patients with idiopathic dilated cardiomyopathy (IDC) than in controls (odds ratio [OR], 2.3).¹² Individuals with the SOD-VV genotype may be predisposed to develop IDC because of reduced SOD levels and the subsequent increase in superoxide anion, which may cause myocyte dysfunction.

In contrast to carriers of the Ala16Val polymorphism, subjects carrying the Arg213Gly substitution of the *SOD3* gene have about 10-fold higher plasma SOD levels than subjects without the mutation.¹³ It is possible that this mutation has protective effects and may play a role in decreased susceptibility to myocardial dysfunction.

Endothelial NO Synthase. NO is synthesized from L-arginine and molecular oxygen by a family of three enzymes, NO synthases (NOS). In the endothelial cell, NO is synthesized by endothelial NOS or NOS III that is localized to the 7q35-7q36 region of human chromosome 7, comprises 26 exons that span 21kb, and has several tandem and dinucleotide repeats.¹⁴ Several observations support the concept that, at higher levels, NO has the ability to impair normal myocardial function and to exert direct toxic effects on the myocardium. NO was found to have direct negative inotropic effects in isolated healthy guinea pig cardiac myocytes through profound inhibition of voltage-dependent calcium channels by high levels of cyclic guanosine monophosphate.¹⁵ The negative inotropic effects of inflammatory cytokines (tumor necrosis factor alpha, interleukin-2, and interleukin-6) are mediated by the generation of NO within the muscle itself.¹⁶ NO donors cause apoptosis of cardiac myocytes in vitro through formation of reactive oxygen and nitrogen species, and finally, NO significantly attenuated the inotropic response to isoproterenol in canine myocytes.^{17,18} There is scarce information about increased NOS III activity in the failing myocardium in ICM, yet NOS II or inducible NOS expression and activity are increased in both ICM and NICM.^{19,20}

It has been shown that the endothelial NOS 4 gene locus is responsible for variations in the genetic control of plasma NO levels. Patients with the endothelial NOS 4a allele have a two-fold higher NO level than those with the 4A allele.²¹ Further studies have revealed that patients with eNOS 4a allele and higher NO levels also have significantly lower extracellular

SOD levels.²² Therefore, NO levels are negatively correlated with extracellular SOD levels, implying that the balance of the above enzyme systems may be important in many pathologic conditions, such as HF. NO and SOD compete for superoxide, and peroxynitrite is generated by the reaction of NO with superoxide anion, with the former being more toxic and long-lived.²³ The fact that plasma NO levels are modified by genetic polymorphisms suggests that the NOS III genotypes may be a useful parameter for studying the role of NO in patients with HF.

A different NOS polymorphism, the Glu298Asp variant of NOS III, localized to exon 7, has been associated with decreased NO levels and increased risk of coronary artery disease. In patients with HF, carriers of the 298Asp variant were found to have a higher mean LVEF, but a decreased functional capacity.²⁴ This may reflect reduced negative inotropic effects of NO in 298Asp homozygotes. It is not clear from this association study whether the Glu298Asp polymorphism is a functional genetic variant or a marker for another functional variant within the gene or an adjacent gene. One possibility is that this variant might influence subcellular targeting or interaction with other regulatory proteins.²⁵ More studies are needed to explore the complex role of NOS III polymorphisms in determining HF phenotype.

Apolipoprotein E (ApoE). ApoE is a plasma protein with known functions in cholesterol transport and metabolism. Metal binding ability (including iron) has been proposed as a mechanism accounting for the antioxidant property of ApoE.²⁶ The *ApoE* gene is located on the long arm of chromosome 19 and a polymorphism exists, with the three most common alleles designated $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$.²⁷ It has been shown that ApoE at physiologic levels has isoform-specific effects in protecting a rat neuronal cell line from oxidative cell death, and that these effects correlated with ApoE's antioxidant activity in *in vitro* assays (ranked $E2 > E3 > E4$).²⁶ In homozygous β -thalassemia, organ damage is mainly attributed to excessive iron deposition through the formation of hydroxyl radicals, with heart involvement being the main cause of death. In a study of Greek thalassemic homozygotes, the ApoE $\epsilon 4$ allele frequency was found to be 7.9% in patients with normal hearts, similar to controls, and 12.8% in patients with cardiomyopathies (OR, 2.11).²⁸ Thus, the decreased antioxidant activity of the ApoE $\epsilon 4$ allele may represent a genetic risk factor for the development of myocardial dysfunction in this well-defined population. Furthermore, a recent European study suggested a gender difference of ApoE genotype on risk of ischemic heart disease; $\epsilon 3$ ver-

Table. Candidate Genes of Oxidative Stress in Heart Failure

GENE	LOCUS	POLYMORPHISM	FUNCTION	ASSOCIATION
MnSOD (SOD2)	6q25	Ala16Val	↓ SOD	RF for DCM in Japanese
ecSOD (SOD3)		Arg213Gly	↑ SOD	↓ risk of CM
eNOS (NOS III)	7q35-36	A/a Glu298Asp	↑ NO, ↓ SOD, ↑ peroxynitrite (?) NO	↑ LVEF, ↓ FC
ApoE	19q13.2	$\epsilon 2/3/4$	↓ antioxidant activity of $\epsilon 4$	RF for CM in β -thalassemia RF for ICM
PAF-ah	6p12-12.1	Val279Phe	(?) PAF levels	RF for DCM
β_1 -AR	10q24-26	Ser49Gly Gly389Arg	↓ β_1 -AR stimulation ↑ β_1 -AR-G protein	RF for DCM, ↓ mortality ↑ FC
β_2 -AR	5q31-32	Thr164Ile	↓ inotropy/chronotropy	↓ FC, ↑ mortality
ACE	17q23	I/D	↑ Ang II levels with DD	↑ mortality in DCM
ETA	4	C1363T	(?) ET-1 levels	RF for idiopathic DCM

ACE=angiotensin-converting enzyme; Ang II=angiotensin II; ApoE=apolipoprotein E; β -AR= β -adrenergic receptor; CM=cardiomyopathy; DCM=dilated cardiomyopathy; ecSOD=extracellular SOD; eNOS=endothelial nitric oxide synthase; ET-1=endothelin 1; ET-A=endothelin receptor type A; FC=functional capacity; ICM=ischemic cardiomyopathy; LVEF=left ventricular ejection fraction; MnSOD=manganese-containing SOD; NO=nitric oxide; PAF=platelet activating factor; PAF-ah=PAF acetylhydrolase; RF=risk factor; SOD=superoxide dismutase; I=insertion; D=deletion; ↑=increased; ↓=decreased; (?)=unknown.

sus $\epsilon 33$ is protective in women while $\epsilon 43$ and $\epsilon 44$ versus $\epsilon 33$ ApoE genotypes increase the risk in men.²⁹ Because the ApoE polymorphism is found in white, black, as well as Asian populations, these observations may apply in many parts of the world.

Platelet Activating Factor Acetylhydrolase (PAF-ah). Oxidative stress induces the expression of platelet activating factor (PAF) in endothelial cells and macrophages. PAF may act as a secondary mediator of the effect of ROS in the heart.³⁰ PAF is degraded by PAF-ah, which may protect against PAF-mediated oxidant effects.³¹ Given that PAF-ah constitutes a key defense against increased oxidative stress, alterations in its enzymatic activity may result in predisposition to myocardial damage. The plasma *PAF-ah* gene is located on chromosome 6p12-p21.1 and exhibits a G \rightarrow T polymorphism at position 994 in exon 9, which encodes the catalytic domain.³² This nucleotide change results in Val \rightarrow Phe substitution at position 279 of the protein and a consequent loss of catalytic activity. In Japanese subjects, the G994T polymorphism of the PAF-ah was found to be a risk factor for the development of nonfamilial dilated cardiomyopathy (DCM).³³ Furthermore, LV mass determined by echocardiography in DCM patients differed significantly among *PAF-ah* genotypes. These observations suggest that G994T polymorphism of the plasma *PAF-ah* gene may affect defense mechanisms against oxidative stress and contribute to genetic susceptibility to or progression of nonfamilial DCM.

β -Adrenergic Receptor (β -AR). It is known that the increase of circulating catecholamines and their autooxidation in the failing human heart results in generation of highly cytotoxic free radicals.³⁴ Therefore, ROS may play an important role in catecholamine-induced cardiotoxicity by causing peroxidation of membrane phospholipids, which can result in permeability changes in the membrane, as well as intracellular calcium overload. Furthermore, there is circumstantial evidence that NO produced by myocardial NOS II or inducible NOS attenuates the response of failing hearts to β -adrenergic stimulation.³⁵ A hallmark finding that has been observed with progression of myocardial dysfunction is depressed contractile response to β -agonists.³⁶ Concomitant with this hyporesponsiveness, a decrease in expression and/or function of cardiac β_1 -AR and β_2 -AR would be expected. In fact, it has been shown that uncoupling of β_2 -AR from adenylate cyclase occurs equally in both ICM and NICM and downregulation of β_1 -AR is more pronounced in the former.³⁷

The therapeutic value of β_1 -AR blockade in cardiomyopathy patients with increased LVEF and decreased heart volume has been well delineated in

the Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF).³⁸ The pathophysiology of HF may be influenced by the β_1 -AR genotype, as there is unexplained interindividual variation in the progression of HF in the cardiac response to infused β_1 -agonists used during acute decompensation, and in the clinical response to β -blockers.

The β_1 -AR gene is located on chromosome 10, and studies have shown two different point mutations associated with regulation of cardiac function under pathologic conditions.³⁹ One study showed that the Ser49Gly polymorphism in the N-terminus of the β_1 -AR gene was found more frequently in patients with idiopathic DCM compared to controls.⁴⁰ This may lead to downregulation of β_1 -AR-mediated signal transduction, impairing myocardial response to receptor stimulation. A recent study showed that patients without this mutation have a two-fold increase in risk of death or cardiac transplantation during 5 years of follow-up, compared to controls.⁴¹

In contrast, the Gly389Arg polymorphism, which encodes the G-protein coupling domain of β_1 -AR, causes myocardial protection and a more favorable disease course.

Arg substitution for Gly has been shown to improve exercise capacity in patients with HF.⁴² Based on a previous finding that fibroblasts transfected to express Arg389 receptors have a higher basal and isoproterenol-stimulated adenylyl cyclase activity than cells expressing Gly389 receptors, the human Arg389 variant may enhance receptor-G-protein interaction, increase activation of adenylyl cyclase, and ultimately enhance cardiopulmonary exercise parameters.⁴³

The β_2 -AR is also expressed on atrial and ventricular myocytes and exists in multiple polymorphic forms.⁴⁴ Transgenic mice overexpressing the Thr164Ile polymorphism in the heart exhibit depressed inotropy and chronotropy.⁴⁵ Clinical studies have found that the relative risk for death or cardiac transplantation is 4.81 in patients with the Ile164 polymorphism as compared to carriers of the wild type Thr at this position, with 42% 1-year survival as compared to 76%, respectively.⁴⁶ In a subsequent study of 232 patients with compensated HF, subjects with the Ile164 polymorphism were found to have reduced exercise capacity.⁴⁷

In summary, the experimental and clinical data suggest an association between the disease-modifying β_2 -AR polymorphisms and clinical outcomes in HF caused by ICM or NICM. This could mean, if the data are confirmed in larger populations, that patients with the Ile164 phenotype could be considered for earlier aggressive management with pharmacologic therapies or cardiac transplantation.

Cardiologists who have used β -blockers in the treatment of HF have long known that there are responders and nonresponders to this therapy. This difference may be dictated by the genotype, and β -blockade might be most effective for patients with specific receptor variations. This hypothesis needs to be tested, and further investigations are required to establish the relationship between different β -AR missense mutations, oxidative stress and changes in receptor function.

Angiotensin Converting Enzyme (ACE). The pathophysiologic sequence occurring after myocardial infarction involves activation of the neurohumoral system as an initial adaptation response. The activation of the neurohumoral system is prolonged and excessive in patients with ICM. This prolonged activation is responsible for delayed adaptation or deadaptation in the form of remodeling, dilation of the LV cavity, and clinical HF. An integral part of neurohumoral activation is the renin-angiotensin system, and ACE expression in the perivascular interstitium, developing fibrous tissue, and myocytes after myocardial infarction and cardiomyopathy is well documented.⁴⁸ Benefits of ACE inhibition in HF are attributed to the inhibition in this system; therefore, a possible benefit of therapy with ACE inhibitors is a reduction in fibrosis. Notably, studies have shown that ACE inhibitors also scavenge oxygen free radicals in vitro.⁴⁹ Finally, it has been established by large randomized controlled clinical trials of symptomatic and asymptomatic HF that pharmacologic inhibition of ACE improves clinical symptoms and decreases morbidity and mortality.^{50,51}

Angiotensin II has been shown in vitro to directly stimulate the production of ROS and prostaglandins in vascular and perivascular tissues.^{52,53} Myocardial ischemia and inflammation are known to induce leukocytosis. Furthermore, leukocytes metabolize arachidonic acid to leukotrienes through lipoxigenase; and leukotriene, LTB₄, is known to activate neutrophils, which results in further secretion of ROS.⁵⁴ Thus, chronic HF is a complex condition where increased circulating cytokines and neurohormones, such as angiotensin II, lead to increased oxidative stress, which in turn impacts on the bioavailability and bioactivity of NO.

Insertion (I) or deletion (D) of 287 base pairs in intron 16 of the *ACE* gene, located on chromosome 17q23, leads to variation in ACE activity.⁵⁵ Homozygosity for the presence of the polymorphic segment is designated II; individuals homozygous for the deletion have the DD genotype, and heterozygosity is termed ID. Respective serum ACE levels of II/ID/DD genotypes are 299/393/494 μ g/mL.⁵⁵ The DD genotype is more prevalent in patients with ICM (63%) and NICM

(48%) than in normal controls and is associated with reduced LV systolic performance and with LV hypertrophy (OR, 2.63).⁵⁶⁻⁵⁸ Two studies have shown that the DD genotype is an independent predictor of mortality in idiopathic HF as well as in patients with HF caused by systolic dysfunction.^{59,60} Furthermore, by monitoring heart rate variability, a greater autonomic imbalance was found to be associated with the deletion polymorphism of the ACE, thus contributing to the acceleration of morbidity and mortality in HF.⁶¹ Most recently, Cicoira et al. found that the frequency of aldosterone escape in patients with HF receiving long-term ACE inhibitor treatment was significantly higher in patients with the DD genotype as compared with the other genotypes.⁶²

In summary, the natural history of HF appears to be changed by the *ACE* genotype, as DD subjects have more profound deadaptation (namely remodeling), LV dilation, and clinical symptoms, and consequently a decreased survival rate. Nevertheless, further clarification is needed regarding the genetic, molecular, and pathophysiologic mechanisms related to the *ACE-DD* genotype in relation to generation of ROS. It is important to study the functional significance of *ACE* genotypes in relation to production of ROS in patients with HF.

Endothelin Receptor Type A (ETA). Endothelin-1 (ET-1) is a potent vasoconstrictor that has long-term effects on cellular growth and phenotype.⁶³ It is generated in the myocardium and vasculature by various cell types, including ventricular myocytes, fibroblasts, and vascular endothelial cells. Cardiovascular effects of ET-1 are mediated mainly by the ETA, which is located on several cell types in the myocardium. Plasma ET-1 concentrations are increased in patients with HF and correlate with worsening functional class, LVEF, and exercise capacity.⁶⁴ Physiologic modulation of myocardial function by ET-1 is not well understood. ET-1 may be beneficial during the early stages of HF, decreasing myocardial contractility, yet prolonged stimulation results in maladaptive changes. Recent data have shown that ET-1 induces oxidative stress in human endothelial cells, suggesting that elevated ET-1 levels may mediate development and progression of endothelial dysfunction and cardiovascular diseases.⁶⁵ However, oxidative stress also increases ET-1 promoter activity, preproendothelin-1 mRNA, and big ET-1 protein synthesis.⁶⁶ Thus, it is of critical importance to determine whether increased oxidative stress translates into elevated ET-1 levels.

A recent study⁶⁷ found that exon 8 C/T polymorphism in the 3'-untranslated region of the *ETA* gene appears to be a genetic risk factor for IDC in a Euro-

pean population. Individuals who were homozygous for the T allele of ETA were at significantly increased risk for IDC (OR, 1.9). The functional consequences of this polymorphism are unknown, but because of its location in the 3'-untranslated region, it might be related to regulatory sequences of the transcription of the gene and thus be associated with differences in the level of gene expression leading to alterations of plasma ET-1 levels. The identification of a genetic risk factor for idiopathic forms of HF strengthens the interest of clinical studies using ETA antagonists, and might help in defining subgroups of responders to such treatment.

Summary and Conclusions

There is a growing body of evidence to support the role of various genetic polymorphisms in susceptibility to and progression of HF. The influence of these genetic factors is mainly through modulating plasma and tissue levels of their biologically active products. This review summarizes the role of various genetic variants of key signaling molecules involved in oxidative stress, which may have pathophysiological consequences within the context of HF. Patients with specific adverse polymorphisms and HF, such as the ETA and β -AR polymorphisms, may be candidates for earlier aggressive intervention or cardiac transplantation.

Notably, some drugs that have been found to be effective in the treatment of HF, such as carvedilol, have important antioxidant properties, which are discussed in a separate review in this issue. Furthermore, there is evidence that carvedilol, metoprolol, and ACE inhibitors can reduce oxidative stress.⁶⁸ Physicians who use β blockers in the treatment of HF are aware that there are responders and nonresponders to this therapy. This difference may be dictated by the genotype, i.e. ACE DD genotype, and β blockade might be most effective for patients with specific receptor variations.⁶⁰

Genetic association studies need to be carefully performed and the functional significance of the polymorphisms has to be elucidated. Assuming that there is some genetic importance to a certain polymorphism, the question remains whether this specific genotype is merely a marker for the disease and in linkage disequilibrium with a neighboring mutation of functional importance, or whether it is in fact causative. Although most designs provide a powerful tool for detecting the effects of disease-modifying genes, such studies are sensitive to the effects of undetected confounding or bias that may arise during the selection of either case or control subjects.

The demand for molecular genetic testing will certainly increase as physicians and patients be-

come better educated as to how these data can influence patient care, family-planning decisions, and pharmacologic treatment.

Obviously, the genomic approach to HF has just started, and the results provide a new approach to the pathology of the disease, which could lead to better identification of individuals at risk and application of pharmacogenomics in the treatment of patients with HF.

REFERENCES

- 1 American Heart Association. 2001 Heart and Stroke Statistical Update. Dallas, TX: American Heart Association; 2000.
- 2 Przyklenk K, Whittaker P, Kloner RA. In vivo infusion of oxygen free radical substrates causes myocardial systolic, but not diastolic dysfunction. *Am Heart J*. 1990;119:807-815.
- 3 McMurray J, Chopra M, Abdullah I, et al. Evidence of oxidative stress in chronic heart failure in humans. *Eur Heart J*. 1993;14:1493-1498.
- 4 Diaz-Velez CR, Garcia-Castineiras S, Mendoza-Ramos E, et al. Increased malondialdehyde in peripheral blood of patients with congestive heart failure. *Am Heart J*. 1996;131:146-152.
- 5 Keith M, Geranmayegan A, Sole MJ, et al. Increased oxidative stress in patients with congestive heart failure. *J Am Coll Cardiol*. 1998;31:1352-1356.
- 6 Prasad K, Gupta JB, Kalra J, et al. Oxygen free radicals in volume overload heart failure. *Mol Cell Biochem*. 1992;111:55-59.
- 7 Dieterich S, Bieligg U, Beulich K, et al. Gene expression of antioxidative enzymes in the human heart: increased expression of catalase in the end-stage failing heart. *Circulation*. 2000;101:33-40.
- 8 Yang J, Moravec CS, Sussman MA, et al. Decreased SLIM1 expression and increased gelsolin expression in failing human hearts measured by high-density oligonucleotide arrays. *Circulation*. 2000;102:3046-3052.
- 9 Abdellatif M. Leading the way using microarray: a more comprehensive approach for discovery of gene expression patterns. *Circ Res*. 2000;86:919-920.
- 10 Wan XS, Develaraja MN, St Clair DK. Molecular structure and organization of the human manganese superoxide dismutase gene. *DNA Cell Biol*. 1994;13:1127-1136.
- 11 Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, et al. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem Biophys Res Commun*. 1996;226:561-565.
- 12 Hiroi S, Harada H, Nishi H, et al. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. *Biochem Biophys Res Commun*. 1999;261:332-339.
- 13 Sandstrom J, Nilsson P, Karlsson K, et al. 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J Biol Chem*. 1994;269:19163-19166.
- 14 Marsden PA, Heng HHQ, Scherer SW, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem*. 1993;268:17478-17488.
- 15 Brady AJB, Warren JB, Poole-Wilson PA, et al. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol*. 1993;265:H176-H182.
- 16 Finkel MS, Oddis CV, Jacob TD, et al. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science*. 1992;257:387-389.
- 17 Olivetti G, Abbi R, Quaini F, et al. Apoptosis in the failing human heart. *N Engl J Med*. 1997;336:1131-1141.

- 18 Yamamoto S, Tsutsui H, Tagawa H, et al. Role of myocyte nitric oxide in beta-adrenergic hyporesponsiveness in heart failure. *Circulation*. 1997;95:1111-1114.
- 19 Bauersachs J, Bouloumie A, Fraccarollo D, et al. Endothelial dysfunction in chronic myocardial infarction despite increased vascular endothelial nitric oxide synthase and soluble guanylate cyclase expression: role of enhanced vascular superoxide production. *Circulation*. 1999;100:292-298.
- 20 Haywood GA, Tsao PS, von der Leyen HE, et al. Expression of inducible nitric oxide synthase in human heart failure. *Circulation*. 1996;93:1087-1094.
- 21 Wang XL, Mahaney MC, Sim AS, et al. Genetic contribution of the endothelial constitutive nitric oxide synthase gene to plasma nitric oxide levels. *Arterioscler Thromb Vasc Biol*. 1997;17:3147-3153.
- 22 Adachi T, Wang XL. Association of extracellular-superoxide dismutase phenotype with the endothelial constitutive nitric oxide synthase polymorphism. *FEBS Letters*. 1998;433:166-168.
- 23 Radi R, Beckman J, Bush KM, et al. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem*. 1991;266:4244-4250.
- 24 Ramani RN, Tokarczyk TR, Palmer AD, et al. Asp298 variant of endothelial nitric oxide synthase (eNOS) and functional capacity in patients with heart failure [abstract]. *Circulation*. 2000;102(suppl II):378.
- 25 Feron O, Saldana F, Michel JB, et al. The endothelial nitric-oxide synthase-caveolin regulatory cycle. *J Biol Chem*. 1998;273:3125-3128.
- 26 Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet*. 1996;14:55-61.
- 27 Zannis VI, Breslow JL, Utermann G, et al. Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes. *J Lipid Res*. 1982;23:911-914.
- 28 Economou-Petersen E, Aessopos A, Kladi A, et al. Apolipoprotein E epsilon4 allele as a genetic risk factor for left ventricular failure in homozygous beta-thalassemia. *Blood*. 1998;92:3455-3459.
- 29 Frikke-Schmidt R, Tybjaerg-Hansen A, Steffensen R, et al. Apolipoprotein E genotype: epsilon32 women are protected while epsilon43 and epsilon44 men are susceptible to ischemic heart disease. *J Am Coll Cardiol*. 2000;35:1192-1199.
- 30 Alloati G, Montrucchio G, Camussi G. Role of platelet activating factor (PAF) in oxygen radical-induced cardiac dysfunction. *J Pharmacol Exp Ther*. 1994;269:766-771.
- 31 Stremel KE, Stafforini DM, Prescott SM, et al. Human plasma platelet-activating factor acetylhydrolase: oxidatively fragmented phospholipids as substrates. *J Biol Chem*. 1991;266:11095-11103.
- 32 Stafforini DM, Satoh K, Atkinson DL, et al. Platelet-activating factor acetylhydrolase deficiency: a missense mutation near the active site of an anti-inflammatory phospholipase. *J Clin Invest* 1996;97:2784-2791.
- 33 Ichihara S, Yamada Y, Yokota M. Association of a G994->T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with genetic susceptibility to nonfamilial dilated cardiomyopathy in Japanese. *Circulation*. 1998;98:1881-1885.
- 34 Graham DG, Tiffany SM, Bell WR, et al. Autooxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine and related compound toward C1300 neuroblastoma cells in vitro. *Mol Pharmacol*. 1978;14:644-653.
- 35 Hare JM, Loh E, Creager MA, et al. Nitric oxide inhibits the positive inotropic response to beta-adrenergic stimulation in humans with left ventricular dysfunction. *Circulation*. 1995;92:2198-2203.
- 36 Fowler MB, Laser JA, Hopkins GL, et al. Assessment of the beta-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation*. 1986;74:1290-1302.
- 37 Bristow MR, Anderson FL, Port D, et al. Differences in beta-adrenergic neuroeffector mechanisms in ischemic versus dilated cardiomyopathy. *Circulation*. 1991;84:1024-1039.
- 38 The MERIT-HF Study Group. Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomised intervention trial in congestive heart failure (MERIT-HF). *Lancet*. 1999;353:2001-2007.
- 39 Frielle T, Collins S, Daniel KW, et al. Cloning of the cDNA for the human beta 1-adrenergic receptor. *Proc Natl Acad Sci U S A*. 1987;85:9494-9498.
- 40 Podlowski S, Wenzel K, Luther HP, et al. beta1-adrenoceptor gene variations: a role in idiopathic dilated cardiomyopathy? *J Mol Med*. 2000;78:87-93.
- 41 Borjesson M, Magnusson Y, Hjalmarson A, et al. A novel polymorphism in the gene coding for the beta1-adrenergic receptor associated with survival in patients with heart failure. *Eur Heart J*. 2000;21:1853-1858.
- 42 Wagoner LE, Lamba S, Craft LL, et al. Polymorphic Gly389 (I1 adrenergic receptors depress exercise capacity in heart failure. *Circulation* 2000;102:suppl II-378 (abstract 1843).
- 43 Mason DA, Moore JD, Green SA, et al. A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. *J Biol Chem*. 274;18:12670-12674.
- 44 Reihnsaus E, Innis M, MacIntyre N, et al. Mutations in the gene encoding for the beta2-adrenergic receptor in normal and asthmatic subjects. *Am J Resp Cell Mol Biol*. 1993;8:334-339.
- 45 Turki J, Lorenz JN, Green SA, et al. Myocardial signaling defects and impaired cardiac function of a human beta2-adrenergic receptor polymorphism expressed in transgenic mice. *Proc Natl Acad Sci U S A*. 1996;93:10483-10488.
- 46 Liggett SB, Wagoner LE, Craft LL, et al. The Ile164 beta2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. *J Clin Invest*. 1998;102:1534-1539.
- 47 Wagoner LE, Craft LL, Singh B, et al. Polymorphisms of the [beta]2-adrenergic receptor determine exercise capacity in patients with heart failure. *Circ Res*. 2000;86:834-840.
- 48 Hirsch AT, Talsness CE, Schunkert H, et al. Tissue-specific activation of cardiac angiotensin-converting enzyme in experimental heart failure. *Circ Res*. 1991;69:475-482.
- 49 Suzuki S, Sato H, Shimada H, et al. Comparative free radical scavenging action of angiotensin-converting enzyme inhibitors with and without the sulfhydryl radical. *Pharmacology*. 1993;47:61-65.
- 50 Pfeffer MA, Braunwald E, Moye LA, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after acute myocardial infarction. Result of the survival and enlargement trial. The SAVE Investigators. *N Engl J Med*. 1992;327:669-677.
- 51 The SOLVD investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med*. 1992;327:685-691.
- 52 Zafari AM, Ushio-Fukai M, Akers M, et al. Role of NADH/NADPH oxidase-derived H2O2 in angiotensin II-induced vascular hypertrophy. *Hypertension*. 1998;32:488-495.
- 53 Nasjletti A, Malik KU. Interrelationships among prostaglandins and vasoactive substances. *Med Clin North Am*. 1981;65:881-889.
- 54 Fantone JC, Ward PA. Role of oxygen derived free radicals and metabolites in leukocyte dependent inflammatory reaction. *Am J Pathol*. 1982;107:397-418.
- 55 Rigat B, Hubert C, Alhenc-Gelas F, et al. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half of the variance of serum enzyme levels. *J Clin Invest*. 1990;86:1343-1346.
- 56 Reynolds MV, Bristow MR, Bush EW, et al. Angiotensin-converting enzyme DD genotype in patients with ischaemic or dilated cardiomyopathy. *Lancet*. 1993;342:1073-1075.
- 57 Candy GP, Skudicky D, Mueller UK, et al. Association of left ventricular systolic performance and cavity size with angiotensin-converting enzyme genotype in idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1999;83:740-744.
- 58 Schunkert H, Hense HW, Holmer SR, et al. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med*. 1994;330:1634-1638.
- 59 Andersson B, Sylven C. The DD genotype of the angiotensin-converting enzyme gene is associated with increased mortality in idiopathic heart failure. *J Am Coll Cardiol*. 1996;28:162-167.

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- 60 McNamara DM, Holubkov R, Janosko K, et al. Pharmacogenetic interactions between beta-blocker therapy and the angiotensin-converting enzyme deletion polymorphism in patients with congestive heart failure. *Circulation*. 2001;103:1644-1648.
- 61 Binkley PF, Louis-Stratton Y, Hatton PS, et al. The deletion polymorphism of the ACE gene is a determinant of accelerated autonomic imbalance in congestive heart failure [abstract]. *Circulation*. 2000;102(suppl II):379.
- 62 Ciccoira M, Zanolla L, Rossi A, et al. Failure of aldosterone suppression despite angiotensin-converting enzyme (ACE) inhibitor administration in chronic heart failure is associated with ACE DD genotype. *J Am Coll Cardiol*. 2001;37:1808-1812.
- 63 Colucci WS. Myocardial endothelin: does it play a role in myocardial failure? *Circulation*. 1996;93:1069-1072.
- 64 Ponick K, Vogelsang M, Heinroth M, et al. Endothelin receptors in the failing and nonfailing human heart. *Circulation*. 1998;97:744-751.
- 65 Saito T, Itoh H, Chun T, et al. Oxidative stress suppresses the endothelial secretion of endothelin. *J Cardiovasc Pharmacol*. 1998;31S345-S347.
- 66 Kahler J, Mendel S, Weckmuller J, et al. Oxidative stress increases synthesis of big endothelin-1 by activation of the endothelin-1 promoter. *J Mol Cell Cardiol*. 2000;32:1429-1437.
- 67 Charron P, Tesson F, Poirier O, et al. Identification of a genetic risk factor for idiopathic dilated cardiomyopathy: involvement of a polymorphism in the endothelin receptor type A gene. CARDIGENE group. *Eur Heart J*. 1999;20:1587-1591.
- 68 Kukin ML, Kalman J, Charney RH, et al. Antioxidant effect of carvedilol and metoprolol in congestive heart failure. *J Am Coll Cardiol*. 1998;31:189A.