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Although implantable cardioverter defibrillators reduce sudden death in high risk patients with depressed ventricular function, the impact on mortality is blunted by progressive heart failure. Implantable cardioverter defibrillators also provide bradycardia pacing that can vary from continuous to rare back-up pacing. A previous study found that dual-chamber (DDD) pacing increased mortality, likely due to adverse effects of unnecessary right ventricular pacing. Yet DDD pacing systems offer desirable atrial pacing and arrhythmia classification. In this issue of *Circulation*, Olshansky and coworkers conducted a noninferiority trial comparing single-chamber VVI pacing to a DDD pacing algorithm in patients for whom the algorithm minimized right ventricular pacing. DDD pacing did not increase the combined end point of mortality and hospitalizations for heart failure and showed a favorable trend toward benefit. Thus, with attention to pacing programming, DDD pacing can be safely employed in selected implantable cardioverter defibrillator recipients for whom atrial sensing and pacing is desirable. See p 9.

**SILDENAFIL IMPROVES EXERCISE HEMODYNAMICS AND OXYGEN UPTAKE IN PATIENTS WITH SYSTOLIC HEART FAILURE, by Lewis et al.**

The type 5 phosphodiesterase inhibitor sildenafil has been shown to lower pulmonary vascular resistance and pressures in patients with heart failure by augmenting an important determinant of exercise function in heart failure. Lewis and colleagues administered a single oral dose of sildenafil (50 mg) to 13 patients with New York Heart Association class III heart failure. They found that sildenafil lowered pulmonary resistance and pressures and improved exercise capacity in those patients with a pulmonary arterial pressure greater than 25 mm Hg. The Lewis et al study thus provides support for the utility of type 5 phosphodiesterase inhibition in this subset of patients with heart failure. See p 59.

**LOCALIZATION AND QUANTIFICATION OF PLATELET-RICH THROMBI IN LARGE BLOOD VESSELS USING NEAR-INFRARED FLUORESCENCE IMAGING, by Flaumenhaft et al.**

Visualization of clot formation in vivo, particularly real-time visualization, has been limited by the size of blood vessels and the limitations of the currently available technologies. The ability to detect thrombus formation would be important for research as well as clinical events caused by thrombotic occlusion or disruption. In the current issue of *Circulation*, Flaumenhaft and colleagues utilize platelets labeled with a near-infrared fluorophore in animal models and demonstrate the visualization of thrombus in the coronary, carotid, and femoral vessels. The translation of this technology to human patients would have diagnostic implications in a wide variety of clinical scenarios secondary to thrombosis. See p 84.

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**Images in Cardiovascular Medicine**

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Degenerating Heart Valves
Fill Them up With Filamin?

Konstantinos Charitakis, MD; Craig T. Basson, MD, PhD

The intellect is always fooled by the heart.
- La RocheFoucauld

M yxoid valvular heart dystrophies are a frequent cause of valvular diseases. They affect approximately 3% of the population and are the most common cause of isolated mitral regurgitations that require surgical repair.1,2 These valvular diseases are a heterogeneous group of disorders and include isolated nonsyndromic valvular diseases, such as idiopathic mitral valve prolapse and the X-linked myxomatous valvular dystrophy (XMVD), and syndromic entities, such as Marfan syndrome. In this issue of Circulation, Kyndt et al3 report their surprising finding that specific mutations in filamin A (FLNA), a gene previously associated primarily with neurological and skeletal disorders, actually cause XMVD.

XMVD is a rare form of inherited nonsyndromic valvular dystrophy that was identified more than 30 years ago by Monteleone and Fagan.4 Initial reports suggested that only men could be affected, but a subsequent study revealed that the disease has heterogeneous presentations and that women can have milder manifestations.5 Histologically, the valves classically display abnormalities of myxomatous degeneration, with fragmentation of collagen bundles within the valve fibrosa and accumulation of proteoglycan and secondary calcification. The clinical spectrum of XMVD ranges from isolated mild valve defects to severe multivalvular lesions, but XMVD usually affects the mitral and/or aortic valve. The result is mitral valve prolapse and mitral and/or aortic regurgitation. Affected individuals are usually asymptomatic until valvular lesions progress to significant hemodynamic impairment and heart failure. Complications can include endocarditis, spontaneous chordal rupture, and sudden death.5,6

Mutations of fibrillin and collagen genes have been correlated with syndromic cases of myxoid valvar heart dystrophies (eg, as part of Marfan and Ehlers–Danlos syndromes).7,8 but until now, no specific gene had been identified for nonsyndromic valvular dystrophies. Idiopathic mitral valve prolapse exhibits an autosomal dominant inheritance with reduced penetrance and variable expressivity. It has been linked to 3 different loci, at 16p11-p12, 11p15.4, and, recently, 13q31-32.9,10,11 In 1998, Kyndt et al12 mapped XMVD to chromosome Xq28 in a large French family. In this issue of Circulation, Kyndt et al3 identify FLNA mutations in 4 unrelated families with XMVD. Their demonstration that FLNA mutations can cause nonsyndromic myxomatous valvular dystrophy provides novel insight into the origins of cardiovascular defects.

Filamins (A, B, and C) were first described as nonmuscle actin-binding proteins. They are large cytoplasmic proteins consisting of an amino-terminal actin-binding domain and a rodlike domain of 24 repeated antiparallel beta-sheets interrupted by 2 flexible loops that form hinge structures. More than 30 different proteins have been reported to interact with the filamins, suggesting that filamins have a wide role as structural components of the cytoskeleton. Through interactions with both actin and membrane proteins, filamins link the cytoskeleton to the plasma membrane and are believed to be essential in cell motility and membrane stability.

Many of the filamin A interacting proteins are receptors for critical cellular signaling molecules. For instance, the transforming growth factor-β (TGF-β) receptor–activated Smads and the dopamine receptor interact with filamin A. Thus, FLNA is implicated in the regulation of different cellular signaling pathways. FLNA gene defects have previously been associated with developmental disorders of the brain and skeleton.13,14

FLNA mutations were initially identified in patients with an X-linked brain malformation called periventricular heterotopia (PH). In PH, neurons fail to migrate to the correct cortical site during early brain development. The cardinal clinical manifestation is epilepsy presenting during the second decade of life. Perhaps foretelling the new findings of Kyndt et al,3 some women with PH also exhibit congenital cardiovascular abnormalities such as persistent ductus arteriosus and aortic aneurysms. PH is largely diagnosed in women who give birth to few male offspring and who experience miscarriages. This observation suggests X-linked dominant inheritance with prenatal lethality of the hemizygous males. Although FLNA missense mutations can be seen, PH is generally believed to reflect loss of function of one FLNA allele with decreased FLNA dosage.15,16

Interestingly, missense mutations clustered in a unique FLNA domain cause a spectrum of distinct phenotypes that comprise a series of syndromes referred to as otopalatodigital syndrome types 1 and 2, frontometaphyseal dysplasia, and...
Melnick–Needles syndrome. These skeletal dysplasia syndromes are characterized by morphogenetic bone defects and share similar clinical manifestations, including generalized bone dysplasia and facial malformations. They are often distinguished by their associated phenotypes, such as microcephaly and mental retardation in otopalatodigital syndrome type 2. In each syndrome, the expressivity is variable, and more severe clinical manifestations occur in males than females. However, these syndromes do not seem to be associated with PH or with signs of defective neural-cell migration. These genotype–phenotype correlations and the wide range of organ systems affected highlight the critical role of FLNA throughout multiple aspects of human embryonic development.

Kyndt et al\(^3\) widen the spectrum of clinical syndromes caused by FLNA genetic abnormalities with their identification of FLNA mutations in 1 large and 3 smaller families with XMVD. Diagnosis of XMVD was based on transthoracic echocardiography, and family members were considered affected if the thickness of the free edge of either or both mitral valve leaflets was greater than 4 mm, regardless of the presence or the absence of mitral valve prolapse and mitral regurgitation. Given the difficulty of assessing aortic leaflet thickening by transthoracic echocardiography, patients were also considered affected if mild to severe aortic regurgitation was present. Within these families, the disease was inherited with complete penetrance in males and incomplete penetrance in females. However, there was variable expressivity in both genders. Coupling genealogical surveys of the larger family with linkage analyses, Kyndt et al\(^3\) refined the previously mapped locus on chromosome Xq28 to a 2.5-Mb region.\(^{12}\) Screening of candidate genes revealed a P637Q missense mutation in the FLNA gene in the affected members of the larger family. Mutational analyses of the FLNA gene in the other families identified 3 additional FLNA gene mutations: 2 more missense mutations (G288R, V711D) and a 1944 bp in-frame deletion. Male and female carriers were both affected, but the affected females had a less severe phenotype. No signs of PH, otopalatodigital syndrome, frontotemporal dysplasia, Melnick–Needles syndrome, Ehlers–Danlos syndrome, or other extracardiac abnormalities were found.

The mechanism by which these FLNA mutations lead to a cardiac-restricted phenotype remains unknown, and further studies will be required. Helpful tools in these studies will be animal models with mutations in specific domains of the FLNA gene. Hart et al\(^{13}\) recently described a mouse model with loss of filamin A. Absence of the FLNA protein in male mice caused embryonic lethality secondary to incomplete septation of the conotruncus (truncus arteriosus), along with craniofacial and other skeletal defects. A proportion of both male and female mutant mice had other cardiac defects, including dysplasia of the mitral valve, ventricular septal defects, and primum atrial septal defects. These findings highlight the diversity of filamin A contributions to heart development.

In the study by Kyndt et al\(^3\), all 4 XMVD mutations are located within the repeats 1 to 7 of the filamin protein. The 3 substitutions associated with XMVD occur in the same region of the gene, and each replaces a nonpolar amino acid with a polar one within the first, fourth, or fifth repeats. The authors suggest that this change in polarity could cause a significant change in the structural conformation of the beta-pleated sheets of the FLNA protein and could impair binding with other protein partners. On the other hand, the 1944 bp FLNA deletion that Kyndt et al\(^3\) identified produces a truncated protein that lacks the repeats 5 to 7. Although the number of affected individuals in the relevant family is small, it is interesting to note that the newly produced, truncated FLNA protein causes a polyvalvular phenotype in the 2 affected individuals. Further analyses of these patients will highlight the differences in disease severity produced by FLNA-point mutations versus this deletion.

Given that many proteins bind filamin A, it seems reasonable to hypothesize that specific disease phenotypes are driven by the effect of individual FLNA mutations on interactions with different protein partners during embryonic development. To fully appreciate the requirements for filamin A in cardiac valvular morphogenesis, it will be necessary to define the protein(s) that interact with filamin A during cardiogenesis and to dissect the contribution of repeats 1 to 7 in this interactions. During heart valve formation, a subset of endothelial cells overlying the future valve site are programmed to delaminate, differentiate, and migrate into cardiac jelly through a process referred to as endothelial–mesenchymal transformation. Locally expanded swellings of cardiac jelly and mesenchymal cells (referred to as cardiac cushions) undergo extensive remodeling to form the heart valves. Several ligands and signaling pathways are implicated in this process, including vascular endothelial growth factor, NFA/Tc1, Notch, Wnt/beta catenin, BMP, TGF-\(\beta\), ErbB, and NF1/Ras. TGF-\(\beta\) interacts with several of these other pathways to induce endothelial–mesenchymal transformation of epithelial cells during normal embryonic development of the heart.\(^{18}\) Filamin A coordinates localization and activation of TGF-\(\beta\) receptor–activated Smads, particularly Smad2, to act as a positive regulator of TGF-\(\beta\) signaling.\(^{14}\) One potential mechanism underlying cardiac valvular dystrophy could involve dysregulated/disrupted interaction of Smads with filamin A and inhibition of endothelial–mesenchymal transformation via perturbation of the TGF-\(\beta\) signaling.

As previously noted, women with PH can be afflicted with cardiac defects such as persistent ductus arteriosus and aortic aneurysms. Men with PH are rare but can exhibit severe, usually lethal, vascular malformations. Given the identification of TGF-\(\beta\) receptor mutations in some aneurysm syndromes,\(^{19}\) and the association of Smads with filamin A, the TGF-\(\beta\) pathway is an attractive candidate for inducing both valvular and vascular malformations. The dosage of filamin A and TGF-\(\beta\) activity may require precise regulation. Myxomatous mitral valves found in fibrillin-1–deficient mice (which model Marfan syndrome) display excessive TGF-\(\beta\) activation and upregulated expression of FLNA.\(^{20}\) It remains to be seen whether the mutations described by Kyndt et al\(^3\) activate or inactivate filamin A–mediated signaling.

Future research on filamin A and TGF-\(\beta\) signaling holds great promise for deciphering valvular diseases. On the basis of genetic analyses of TGF-\(\beta\) signaling disorders, clinical
trials are now beginning to test new pharmacological interventions for aortic aneurysms. The opportunity to integrate filamin A and events in the cytoskeleton with the TGF-β signaling pathway may present exciting new therapeutic targets for patients with valvular diseases.

Disclosures

None.

References


Key Words: Editorials • valves • mitral valve • heart diseases
Connecting the Missing Link Between Dilated Cardiomyopathy and Viral Myocarditis

Virus, Cytoskeleton, and Innate Immunity

Yuichiro Maekawa, MD, PhD; Maral Ouzounian, MD; M. Anne Opavsky, MD, PhD; Peter P. Liu, MD

Myocarditis classically refers to inflammation of the heart muscle. The mechanisms include host immune dysregulation and viral triggers such as coxsackievirus (CVB), adenovirus, parvovirus, and hepatitis C virus. The pathophysiology is initiated by viral proliferation in a susceptible host, inducing host immune response. The latter, when exuberant, leads to myocyte destruction and dilated cardiomyopathy. Clinically, patients with viral myocarditis will spontaneously recover in one third of cases, persist with the disease in one third, and deteriorate in another third of the cases.

Recent biopsy series in patients with dilated cardiomyopathy have revealed an interesting situation in which patients with symptoms of heart failure may show the presence of the viral genome alone, without any evidence of an overt inflammatory process. This raises the question of whether the virus can be directly responsible for the cardiomyopathy or whether it is merely an incidental bystander.

Viruses are exquisitely designed nanoparticles packaged with just the critical amount of genetic material to allow them to adapt to changing host and environmental conditions and to pass on genetic material to progeny. CVBs are single-stranded RNA viruses that have natural tropism for gut epithelial cells, immune cells, neurons, and cardiomyocytes. All strains of CVBs use the coxsackie–adenoviral receptor molecule to gain entry into host cells (Figure). The coxsackie–adenoviral receptor molecule is a critical tight-junction protein important for cell–cell communication and for maintenance of cell membrane integrity. Interestingly, viruses such as CVB must use host signaling mechanisms, including tyrosine kinases such as Abl, fyn, or p56\(\text{Lck}\), to rearrange host actin and cytoskeleton and thus gain entry into the host cell and release viral RNA into the cytoplasm.

To facilitate entry into cells, it is not surprising that CVBs also produce proteases that can lyse the cell–cell or cell–matrix connections. CVB3 produces protease 2A, a cysteine endopeptidase that can specifically cleave the dystrophin–sarcoglycans complex, which is responsible for the linkage of myocyte cytoskeleton to the extracellular matrix. This cleavage leads to dystrophin dysfunction and to loss of sarcolemmal integrity, likely aiding viral entry. This action resembles that of the naturally occurring human mutations in the dystrophin–glycoprotein complex such as X-linked muscular dystrophies, which are often associated with dilated cardiomyopathy.

Following up on initial in vitro observations, in this issue of the Circulation, Xiong et al have gone on to show that the conditional expression of this protein is alone sufficient to lead to dilated cardiomyopathy in vivo. The presence of protease 2A coincided with the compromise of sarcolemmal integrity and the loss of intact dystrophin by immunostaining. These findings suggest that viral endopeptidases, when present in adequate concentrations or expressed for a sufficient duration, can produce myocyte cytoskeletal and sarcolemmal disruptions, leading to dilated cardiomyopathy. These findings indirectly show that the naturally occurring mutations, as seen in the X-linked dystrophies at the sarcoglycans complex, are critically related to the phenotype of cardiomyopathy. The viral myocarditis model, therefore, is a mimic of the genetically induced cardiomyopathy, disrupting the same cytoskeletal target in an acquired manner.

The advantage of the conditional heart-targeted model used by Xiong et al is that the protein is only expressed in the heart, and the transgene is only produced when the mice are adults. This strategy avoids the complications of having a protease 2A presence in utero, which can lead to unintended effects during development. On the other hand, the transgenic model forces the protease to be produced in relatively high quantities for a prolonged period, which probably does not occur with a natural viral infection. In the latter setting, the protease likely will be produced only for a brief few days when the virus is actively proliferating. Nevertheless, this study provides significant proof that the production of this cytoskeletal-modifying protease is sufficient to produce dilated cardiomyopathy.

What role does the host immune response play in this setting? How does the host deal with the presence of foreign viral particles in the heart and elsewhere and successfully...
clear the viruses from the heart in the majority of the cases? On the other hand, how do we reconcile the presence of the inflammatory cell infiltrates that are seen on many biopsy sections and that can ultimately contribute to the evolution of dilated cardiomyopathy?

The original Dallas criteria for the diagnosis of myocarditis on myocardial biopsies stipulated the presence of lymphocytic infiltrates concurrent with evidence of myocyte necrosis. Most experts would agree that in real practice, the Dallas criteria are overly strict, leading to underdiagnosis of the condition. Nevertheless, these criteria underscore that the host inflammatory response is an integral part of the disease process—but how does the viral infection lead to mobilization of lymphocytes to the myocardium?

The presence of viruses or other foreign particles can trigger the activation of innate immune responses in the host tissue through a family of toll-like receptors. In contrast to the T- and B-cells of acquired immunity, which require perfect matching of specific antigenic peptide sequences for activation, the toll-like receptors require only very general triggers or pathogen-associated molecular patterns. Engagement of the toll-like receptors produces rapid signal transduction in the host, leading to activation of adaptor proteins such as MyD88, IRAK-4, and TRAF-6.10 This activation triggers translocation of interferon regulator factor transcription factors to the nucleus to produce interferons, and this activation also triggers translocation of nuclear factor-κB to produce cytokines. We have shown previously that excessive activation of the MyD-88/nuclear factor-κB axis leads to increased inflammation and higher mortality rates,11 whereas activation of interferon regulator factor-3 and interferons are protective and antiinflammatory for the host, with a reduced mortality rate.12
In addition to the toll-like receptor family, there are other means by which the host cells, and particularly myocytes, can direct innate immunity to enhance host survival, attenuate the virus, or both. In addition to these positive regulators of host-defense responses outlined above, there are also systems of negative modulators that will counteract the cytokine activation. One system is the intracellular suppressors of cytokine signaling (SOCS) system of signaling pathways that negatively regulate innate immune response. The SOCS system particularly downregulates cytokine signals going through the gp130 receptor on the myocytes. The gp130 receptor engages cytokine ligands such as interleukin-6 and cardiotropin-1, which are cardiac myocyte growth and survival factors. Deficiency of gp130 signaling leads to increased apoptosis and susceptibility to other insults and injury such as ischemia or viral infection.

Yajima et al. have previously found that SOCS1 and SOCS3 signaling can downregulate the JAK/STAT signal-transduction pathways and significantly increase the host’s susceptibility to CVB infection, but SOCS2 did not. However, SOCS1 also interferes with interferon signaling, making it difficult to dissociate the interferon-dependent and -independent contributions on host susceptibility. On the other hand, SOCS3 downregulates the JAK/STAT pathway but does not alter interferon signaling. SOCS3 transgenic mice-induced loss of STAT3 led to increased susceptibility to viral injury. This effect is mimicked by ablation of gp130-receptor signaling. The gp130-mediated protective effect seems to be mediated by STAT3 phosphorylation, leading intrinsically to a more robust dystrophin–sarcoglycan complex in the membrane, increasing the sarcolemmal integrity. This is compatible with the overall concept that viral infections engage host growth and replication machinery but also disrupt the membrane apparatus to gain efficient access to the intracellular compartment. If the cytoskeleton is maintained intact and the membrane remains robust, the viral infection is more attenuated, and the host survives better. The converse has been observed in dystrophin-deficient mdx mice, which have increased susceptibility to viral infection.

What are the implications of these findings for the clinician? First, viral infection can directly remodel the myocardium, leading to dilated cardiomyopathy. One example, discussed here, is that the production of viral protease 2A by CVB can directly cleave the dystrophin–sarcoglycan complex, leading to sarcolemmal disruption and dilated cardiomyopathy. On the other hand, the virus can also trigger innate immunity, which can strengthen the host to fight against the infection—for example, through activation of STAT3 and gp130 signaling for survival, and interferon regulator factor-3 triggering interferon production for systemic clearance of the virus. Unfortunately, activation of innate immunity can also have the adverse consequences of production of cytokines and costimulation of T-cells, leading to clonal expansion and inflammatory infiltrate. Excessive inflammation leads to paradoxical destruction of the host myocardium, leaving the patient worse off than he or she would have been with no inflammation at all.

In a perfect world, one would like to see a host capable of recognizing the viral presence at the earliest opportunity and clearing the virus before it could have the chance to proliferate, elaborating proteases, and remodeling the matrix–cytoskeleton connections in the heart. The most effective approaches to date are either vaccination to induce immunologic memory or the administration of type I interferons to enhance viral clearance. Results of ongoing clinical trials evaluating the potential of interferons in rapidly mediating viral clearance and, possibly, improving clinical outcome, are eagerly anticipated. However, other intriguing treatment targets may also be important, including a means to inhibit SOCS3 or promote STAT3 signaling to strengthen the host; such a means could be useful for broad cases of cardiac injury. In addition, the ability to inhibit dystrophin–sarcoglycans breakdown, possibly through blockers of peptidases such as protease 2A, may also prevent cardiomyopathy of diverse origin.

Ultimately, nature may produce the best tools to understand many of our biological enigmas. Indeed, viruses may be the best-designed nanoparticles for unraveling the mysteries of dilated cardiomyopathy, offering novel avenues for treatment of this most challenging condition.

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Disclosures
None.

References


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Is Dual-Chamber Programming Inferior to Single-Chamber Programming in an Implantable Cardioverter-Defibrillator?

Results of the INTRINSIC RV (Inhibition of Unnecessary RV Pacing With AVSH in ICDs) Study

Brian Olshansky, MD; John D. Day, MD; Stephen Moore, DO; Lawrence Gering, MD; Murray Rosenbaum, MD; Maureen McGuire, PhD; Scott Brown, PhD; Darin R. Lerew, PhD

Background—The INTRINSIC RV (Inhibition of Unnecessary RV Pacing with AVSH in ICDs) study tested the hypothesis that dual-chamber rate-responsive (DDDR) with atrioventricular search hysteresis (AVSH) 60-130 programming is not inferior to single-chamber (VVI)–40 programming in an implantable cardioverter defibrillator with respect to all-cause mortality and heart failure hospitalizations using an equivalence margin of 5%.

Methods and Results—At 108 centers, 1530 patients with an implantable cardioverter defibrillator indication received a VITALITY AVT (Guidant Corporation, St. Paul, Minn) implantable cardioverter defibrillator programmed consistently to DDDR AVSH 60-130 for the first week. Of those, 988 patients with <20% right ventricular pacing at 1 week were randomized to DDDR AVSH 60-130 or to VVI-40 programming. Among those randomized, 502 were assigned to DDDR AVSH and 486 to VVI. Groups were similar with regard to coronary disease (68%), gender (21% female), and New York Heart Association functional class >I (79%). A total of 32 patients (6.4%) in the DDDR AVSH arm and 46 patients (9.5%) in the VVI arm died or were hospitalized for heart failure during a mean follow-up of 10.4 months (relative risk = 0.67, \( P = 0.072 \) in favor of DDDR AVSH). DDDR AVSH was not inferior to VVI programming (\( P < 0.001 \)). All-cause mortality was not significantly different between the DDDR AVSH arm (3.6%) and the VVI arm (5.1%; \( P = 0.23 \)). The mean percent right ventricular pacing in the DDDR AVSH arm was 10% (median 4%) versus 3% (median 0%) in the VVI arm.

Conclusions—In the INTRINSIC RV trial, among those randomized, DDDR AVSH was associated with similar outcomes as with VVI backup pacing. (Circulation. 2007;115:9-16.)

Key Words: arrhythmia ■ tachyarrhythmias ■ defibrillation ■ electrophysiology ■ pacing

Data from recent studies have suggested that dual-chamber implantable cardioverter defibrillator (ICD) and pacemaker programming is associated with increased heart failure hospitalizations and total mortality compared with single-chamber (VVI) programming.1–5 These data have led to the belief that dual-chamber pacing in ICDs may worsen or even precipitate heart failure and lead to increased mortality.1 Recently, the Centers for Medicare and Medicaid Services released a decision (CAG-00157R3) stating that providers must justify the medical necessity of implanting any ICD that is not a single-lead/VVI device.1 To date, no controlled clinical trial supports the safety of dual-chamber pacing in an ICD.

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The DAVID (Dual Chamber and VVI Implantable Defibrillator) trial1 showed that 1 specific choice of dual-chamber rate-responsive (DDDR) programming parameters leads to worse outcomes than VVI backup pacing, most likely owing to unnecessary right ventricular (RV) pacing. Recent post hoc analyses support these conclusions and highlight the potential adverse consequences of RV pacing.7 Several randomized trials, however, including those using only pacemakers, suggest potential benefit to dual-chamber programming.2,3,6–9 Nonrandomized and retrospective pacemaker studies10 have shown beneficial...
effects with dual-chamber pacing, including improved hemodynamics, particularly in patients with heart failure. Dual-chamber ICDs have these and other potential benefits, including enhanced arrhythmia detection and treatment options. Rate-responsive pacing has been demonstrated to improve cardiovascular exercise response in pacemaker patients and heart failure patients receiving cardiac resynchronization therapy. A recent study reported a 38% incidence of chronotropic incompetence in ICD recipients, but the utility of rate-adaptive pacing has not been studied in this population.

DDDR programming has potential to cause harm by several mechanisms other than increasing the risk of unnecessary RV pacing. Right atrial pacing may delay left atrial activation, thereby impairing hemodynamics, especially in cardiac resynchronization therapy candidates. Atrioventricular (AV) interval optimization at rest has not been shown to improve overall outcomes in this population. To date, no clinical study of any ICD population supports the use of a device more complex than an ICD programmed to VVI. The outstanding question arising from this apparently conflicting information is whether patients requiring ICDs benefit from DDDR programming without experiencing any of the subsequent potential negative consequences.

One solution may be to attempt to minimize RV pacing with the AV Search Hysteresis (AVSH) algorithm. AVSH allows intrinsic AV conduction beyond the programmed AV delay. The primary objective of the INTRINSIC RV (Inhibition of Unnecessary RV Pacing with AVSH in ICDs) study was to compare the outcomes (all-cause mortality or heart failure hospitalization) of ICD patients randomized to either DDDR AVSH or VVI programming. It was hypothesized that the rate of death or heart failure hospitalization would be noninferior for patients whose ICDs were programmed to DDDR AVSH compared with those who had ICDs programmed to VVI. (A list of investigators and institutions that participated in the INTRINSIC RV study can be found in the online-only Data Supplement.)

Methods

Study Design
Patients had a standard indication for an ICD and had a VITALITY AVT (Guidant Corporation, St. Paul, Minn) ICD implanted. Informed consent was obtained before enrollment in the trial, and the trial was reviewed by the responsible institutional review boards as applicable. After implantation, patients were programmed to DDDR AVSH 60-130 bpm with a 1-touch programming feature to encourage uniform programming. At the 1-week follow-up visit, the rate of RV pacing was assessed with ICD counters (rates of RV pacing are expressed as percentages of paced beats out of all detected beats). Patients who were RV paced <20% of the time at 1 week were randomized to 1 of 2 standardized programming arms (DDDR AVSH 60-130 or VVI-40) in a 1:1 allocation, stratified by clinical site. Because evidence from the DAVID trial suggested that substantial amounts of RV pacing could pose a safety concern, it was deemed inappropriate to randomize patients who had already shown a propensity for RV pacing when programmed to DDDR. For this reason, the conservative cutoff of 20% RV pacing was chosen. VVI-40 parameters were chosen on the basis of programming in DAVID. In comparison, DDDR AVSH 60-130 was deliberately chosen to provide atrial support pacing and to attempt to limit RV pacing in the presence of intrinsic AV conduction. All patients were scheduled to be seen at 3-, 6-, and 12-month postimplantation follow-up visits. The complete study design and rationale have been described previously.

AV Search Hysteresis
AVSH is a proprietary algorithm that actively searches for intrinsic AV conduction every x cycles (where x is a programmable number from 32 to 1024) and extends the AV delay by 10% to 100% to allow for intrinsic conduction, when present, across the programmed lower to upper rate range. The protocol was revised and AVSH programming parameters were optimized after 313 patients were enrolled (100 randomized) because initially specified AVSH settings resulted in fewer randomized patients than expected. Specifically, 2 settings were modified. Rate hysteresis was set at a 20-bpm offset, which allowed the lower rate limit to approach 40 bpm with intrinsic conduction, and AVSH AV increase % was changed from 50% to 100%. Programming parameters have been described previously.

ICD Programming
A 1-touch programming utility was provided to investigators both for convenience and to help standardize complex programming options. Although investigators were permitted to set additional zones, this feature initially provided tachycardia detection in a single-zone configuration starting at 185 bpm.

Inclusion and Exclusion Criteria
Eligible patients met current ICD indications and completed the informed consent process before implantation at approved centers. Complete inclusion/exclusion criteria are shown in Table 1.

Sample Size
The proportion of VVI patients expected to be event-free at the 12-month follow-up was 93.9%, with a similar rate expected for
those whose ICDs were programmed to DDDR AVSH. Sample size was computed with a noninferiority margin of 5%, with desired power of 80%. The significance level for the primary end point was set at 0.05, 1-tailed.

Under these assumptions, an estimated 420 patients per study arm, or a total of 840 patients, were required to be randomized to provide end-point information. After the protocol amendment, total targeted enrollment was increased to 1500 to account for nonrandomized subjects and patient attrition.

Statistical Analysis
The primary end point in INTRINSIC RV was a composite of all-cause mortality and hospitalization for heart failure. The proportions of patients who experienced an event that qualified for the primary end point were compared between the 2 randomized study arms, and the results were analyzed with Blackwelder’s method20 to test noninferiority of DDDR AVSH to VVI. Analyses of study outcomes were performed under the intention-to-treat principle, although for the primary end point, a sensitivity analysis with an as-treated assumption was also performed. Additional tests of continuous variables were performed with $t$ tests, and categorical variables were analyzed with $x^2$ tests. Statistical tests for noninferiority were 1-tailed, whereas all other tests were 2-tailed. All probability values were deemed significant at a level of 0.05 or below. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC).

Data and Safety Monitoring Board
The Data and Safety Monitoring Board, which consisted of 1 electrophysiologist, 1 cardiologist, and 1 statistician independent of the study, and the study sponsor reviewed interim data analysis regularly, including primary and secondary end points. The Data and Safety Monitoring Board was empowered to recommend early termination of the trial; no such cause was found during the conduct of the study.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient Characteristics and Follow-Up
A total of 1530 patients were enrolled over 15 months (ending September 2004) and had an ICD implanted de novo at 1 of the 108 participating clinical sites. Of these, 1461 had their RV pacing assessed at the 1-week visit. At that time, 988 patients were randomized to DDDR AVSH (502 patients) or VVI (486 patients) programming based on RV pacing levels below 20%. Therefore, roughly two thirds of enrolled patients qualified for randomization, a figure that increased to 77% when only patients enrolled after the protocol amendment described above are considered.

Patients’ clinical characteristics at baseline were typical of an undifferentiated ICD population. There were no significant differences between the randomized groups at baseline (Table 2). Mean follow-up for randomized patients was 10.4 months. Figure 1 shows the disposition of patients throughout follow-up. Rates of withdrawal and losses to follow-up were comparable between the randomized arms.

A multiple logistic regression with stepwise selection methods was used to model the likelihood of being randomized at the 1-week visit. Higher body mass index ($P<0.001$), older age ($P<0.001$), male gender ($P=0.002$), lower systolic blood pressure at baseline ($P=0.049$), and history of atrial fibrillation ($P=0.035$) were associated with lower likelihood of randomization, that is, with higher rates of RV pacing at 1 week. Curiously, a history of diabetes mellitus was associated with greater rates of randomization ($P=0.005$).

Primary End Point: Heart Failure Hospitalization and All-Cause Mortality
Compared with patients randomized to VVI, those patients randomized to DDDR AVSH experienced 33% fewer deaths and heart failure hospitalizations. Over the course of the study, 32 patients (6.4%) assigned to DDDR AVSH and 46 (9.5%) assigned to VVI experienced an event that met the definition of the primary end point. Statistical analysis of these results demonstrated noninferiority of outcomes in the DDDR AVSH group ($P<0.001$), meeting the protocol-defined primary end point. In fact, the DDDR AVSH results trended toward superiority ($P=0.072$) compared with VVI. Kaplan-Meier21 curves illustrating time to death or first heart failure hospitalization are shown in Figure 2. An as-treated analysis on 897 patients for whom the programming mode was consistent over follow-up also showed noninferiority of DDDR AVSH ($P<0.001$). There were a total of 91 patients (18%) lost to follow-up in the DDDR AVSH arm and 89 (18%) in the VVI arm. The rates of dropout were similar in both arms, and an examination of causes showed no evidence of differences in the reasons for patients leaving the study. For these reasons, the protocol-defined primary end-point analysis that used proportions of patients experiencing an event would be expected to return statistically valid results. Nevertheless, to account for this censoring, a Cox proportional hazards model22 was also used to analyze the primary end point for both noninferiority and superiority. The noninferiority hypothesis was defined by translating the 5% absolute margin and 9.5% event rate in the control group into a hazard ratio of $0.995+0.05/0.995=1.53$. The Cox model produced a probability value of 0.001 for noninferiority and 0.063 for superiority of DDDR AVSH compared with VVI. These are both similar to the analysis of proportions.

Additionally, all-cause mortality alone favored the DDDR AVSH arm, with 18 deaths (3.6%) occurring in that group versus 25 (5.1%) in the VVI arm, although that result was not statistically significant ($P=0.23$). Kaplan-Meier curves for survival are shown in Figure 3.

Results in the observational arm were less favorable than in either randomized group. Of 473 patients who attended the 1-week visit and were assigned to the observational arm, 59 (12.5%) experienced an event that met the definition of the primary end point, including 26 deaths (5.5%). As previously noted, observational patients tended to be older and more predominantly male than randomized patients, with higher rates of baseline arrhythmias.

Medical Therapy
Medical therapy at 12 months was similar in the randomized groups. Only use of $\beta$-blockers differed significantly between the 2 study arms ($P=0.032$), with patients randomized to VVI pacing receiving the more aggressive treatment (88.2% ver-
sus 82.4% in the DDDR AVSH group). Medical therapies are displayed in Table 3.

**RV Pacing**

RV pacing in patients randomized to DDDR AVSH indicated a mean of 10% and a median of 4%. By comparison, RV pacing in patients randomized to the VVI group showed a mean of 3% and a median of 0%. Regarding atrial pacing, the mean in the DDDR AVSH group was 13%, with a median of 3%.

**Appropriate Versus Inappropriate Shock**

All episodes for which shocks were delivered and a stored electrogram existed were adjudicated by an independent expert. A total of 375 episodes treated with shock occurred in randomized patients, and rates of inappropriate shock were not significantly different between randomized groups. Sixty-three (48.1%) of 131 episodes were deemed inappropriately treated in the DDDR AVSH arm versus 32 (45.7%) of 70 in the VVI arm.

Programming recommendations were the most likely reason for this observation (eg, single-zone detection at 185 bpm). More than half of the INTRINSIC RV population had only a single arrhythmia zone (ventricular fibrillation) programmed, for which heart rate alone was the determinant of therapy. Only 37% had a second (ventricular tachycardia) zone programmed in which detection enhancements were turned on. Of all patients, just 96 (6.3%) of 1530 experienced an inappropriate shock despite

**TABLE 2. Demographic Characteristics by Randomization Group**

<table>
<thead>
<tr>
<th>Demographic Characteristic</th>
<th>DDDR AVSH (n=502)</th>
<th>VVI (n=486)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD (minimum, maximum)</td>
<td>63.8±11.9 (28, 88)</td>
<td>63.4±11.5 (25, 86)</td>
<td>0.65</td>
</tr>
<tr>
<td>Gender, n (% male)</td>
<td>396 (78.9)</td>
<td>381 (78.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>Clinical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular fibrillation</td>
<td>46 (9.2)</td>
<td>39 (8.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>Ventricular tachycardia</td>
<td>216 (43.0)</td>
<td>206 (42.4)</td>
<td>0.85</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>56 (11.2)</td>
<td>38 (7.8)</td>
<td>0.083</td>
</tr>
<tr>
<td>Atrial flutter</td>
<td>14 (2.8)</td>
<td>12 (2.5)</td>
<td>0.84</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>331 (65.9)</td>
<td>311 (64.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>187 (37.3)</td>
<td>180 (37.0)</td>
<td>0.95</td>
</tr>
<tr>
<td>Hypertension</td>
<td>266 (53.0)</td>
<td>245 (50.4)</td>
<td>0.45</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>136 (27.1)</td>
<td>151 (31.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Unexplained syncope</td>
<td>62 (12.4)</td>
<td>60 (12.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>270 (53.8)</td>
<td>273 (56.2)</td>
<td>0.48</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>337 (67.1)</td>
<td>331 (68.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>14 (2.8)</td>
<td>10 (2.1)</td>
<td>0.54</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>357 (71.1)</td>
<td>343 (70.6)</td>
<td>0.89</td>
</tr>
<tr>
<td>Angina</td>
<td>159 (31.7)</td>
<td>136 (28.0)</td>
<td>0.21</td>
</tr>
<tr>
<td>Medications at implantation, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>381 (75.9)</td>
<td>388 (79.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>328 (65.3)</td>
<td>305 (62.8)</td>
<td>0.43</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>60 (12.0)</td>
<td>46 (9.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Diuretics</td>
<td>252 (50.2)</td>
<td>241 (49.6)</td>
<td>0.85</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>67 (13.3)</td>
<td>57 (11.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>45 (9.0)</td>
<td>51 (10.5)</td>
<td>0.45</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>50 (10.0)</td>
<td>34 (7.0)</td>
<td>0.11</td>
</tr>
<tr>
<td>NYHA classification at implantation, n (%)</td>
<td></td>
<td></td>
<td>0.059</td>
</tr>
<tr>
<td>Class I</td>
<td>119 (24.2)</td>
<td>88 (18.5)</td>
<td></td>
</tr>
<tr>
<td>Class II</td>
<td>267 (54.4)</td>
<td>290 (61.1)</td>
<td></td>
</tr>
<tr>
<td>Class III/IV</td>
<td>105 (21.4)</td>
<td>97 (20.4)</td>
<td></td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association.
a mean study follow-up of nearly 1 year. The overall incidence of shock, regardless of appropriateness, across randomized groups was analyzed. Patients in the DDDR AVSH arm did not have a statistically significant difference in shock rates compared with the VVI arm ($P=0.074$).

### Discussion

The DAVID trial raised concerns over the use of DDDR pacing in ICD recipients. That trial indicated that DDDR programming (especially when RV pacing rates were >40%) was associated with increased heart failure hospitalization and all-cause mortality compared with VVI programming. Other trials have found similar results. These studies led to the notion that DDDR ICDs could harm patients. Although high levels of RV pacing (>40%) are associated with problems such as heart failure hospitalization and all-cause mortality, lower levels may have benefit.

The INTRINSIC RV study represents a diverse patient population with standard ICD indications. Those patients with a DDDR ICD programmed to DDDR AVSH fared as well as or better than those patients with ICDs programmed to VVI. The protocol-specified standardized programming with a 1-touch approach simplified the selection process for device settings, with the goal of reducing RV pacing. Typical RV pacing levels among patients randomized to DDDR programming in INTRINSIC RV were drastically lower than in the DAVID trial (mean of 10% versus 59%), which is a likely explanation for the disparity in outcomes of these 2 trials. In fact, DDDR AVSH programming was associated with a trend toward statistical superiority with respect to all-cause mortality and heart failure hospitalization compared with VVI programming.

Dual-chamber ICDs may provide pacing options and increased flexibility in programming compared with VVI devices. For example, they can provide atrial-based pacing support in patients with sinus bradycardia and provide AV synchrony in patients with AV block to potentially ameliorate pacemaker syndrome. Dual-chamber ICDs also

**Figure 1.** Patient flow throughout the study. Patients ($n=1530$) had an ICD implanted, and 1461 of these patients attended the first-week visit; of these, 988 were randomized to receive DDDR AVSH ($n=502$) or VVI ($n=486$) programming of their ICD.

**Figure 2.** Percent of patients free from the primary end point (% event-free [death or heart failure hospitalization]) by randomized group (DDDR AVSH vs VVI programming) over the 1-year follow-up.
offer a second electrogram recording channel that may provide more thorough documentation of the rhythm disturbances that cause ICD activation. Recent data suggest that initial implantation of a dual-chamber ICD can save money compared with implantation of a single-chamber device because there may be need for future upgrade of the single-chamber device to a dual-chamber device. Despite these potential benefits, clinically significant advantages of dual-chamber pacing have not been substantiated. Indeed, in the INTRINSIC RV trial, higher rates of both appropriate and inappropriate shocks were found in the DDDR AVSH arm, and the reasons for this are unknown.

In the INTRINSIC RV study, DDDR AVSH did not reduce inappropriate shock rates. INTRINSIC RV was designed to study the effects of pacing algorithms on the clinical outcomes of heart failure hospitalization and all-cause mortality rather than to study how discrimination algorithms for VVI or DDDR programming affected antitachycardia or defibrillation therapies. Because the wide variation in tachyarrhythmia therapy settings could affect end points, for purposes of the present study, 1-touch programming settings limited complex multizone antitachycardia therapies. Our analysis of programming practices indicated that relatively few physicians deviated from the study protocol in favor of more complex programming. Likewise, a minority of physicians activated detection enhancements. Therefore, although DDDR programming might generally be expected to reduce inappropriate shock rates, programming restrictions likely muted any such effect. Patients receiving inappropriate shocks composed 6.3% of all 1530 subjects.

A greater number of patients in the DDDR AVSH arm received shocks than in the VVI arm; this difference was not statistically significant, however, and DDDR AVSH patients fared as well as if not better than their VVI counterparts. Although 1-touch programming settings limited complex multizone antitachycardia therapies, there were no specific requirements regarding tachycardia therapies (ie, antitachycardia pacing, shocks, or combination schemes). These results pose new challenges for further understanding the risks and benefits of dual-chamber ICD programming.

Closer examination of rate-adaptive programming for ICD recipients has been recommended with specific attention to algorithms that reduce RV pacing in concert with rate-adaptive atrial pacing. The INTRINSIC RV study accomplished this goal. Algorithms other than AVSH exist to reduce ventricular pacing in dual-chamber ICDs; however, no other large randomized, prospective study has shown clinical benefits as were shown in the present study. Carefully controlled clinical trials will be required to show any advantage of a specific programming approach. Indeed, the INTRINSIC RV study is the first to determine whether an algorithm that reduced RV pacing could limit the adverse effects of RV pacing yet preserve the benefits of a dual-chamber ICD.

**Study Limitations**

This study was designed with minimal enrollment restrictions. Because more specific indications were not required, variables such as ejection fraction were not collected. The definition of excessive RV pacing as >20% may have been too conservative, because RV pacing rates as high as 40% may not necessarily incur adverse consequences. Because of this criterion, approximately one third of enrolled patients did not qualify for randomization despite a protocol amendment to adjust programming parameters that ultimately reduced the rate of nonrandomization to less than one quarter.

Investigators and patients were not blinded to randomization assignment. Despite this, examination of follow-up

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**TABLE 3. Medical Therapy at 12 Months**

<table>
<thead>
<tr>
<th>Medication</th>
<th>DDDR AVSH (n=393)</th>
<th>VVI (n=372)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Blockers</td>
<td>324 (82.4)</td>
<td>328 (88.2)</td>
<td>0.032</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>274 (69.7)</td>
<td>243 (65.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>57 (14.5)</td>
<td>45 (12.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Diuretics</td>
<td>209 (53.2)</td>
<td>211 (56.7)</td>
<td>0.35</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>51 (13.0)</td>
<td>49 (13.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>42 (10.7)</td>
<td>39 (10.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>39 (9.9)</td>
<td>26 (7.0)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values are n (%).
data indicated that patients were treated similarly in both randomized groups.

Finally, complex DDDR AVSH programming may appear to be a challenge, but for the convenience of the investigator, this was simplified with use of a 1-touch programming technique.

Conclusion
Among ICD recipients randomized in the INTRINSIC RV trial, DDDR AVSH was not inferior to VVI backup programming with regard to all-cause mortality and heart failure hospitalization. A trend toward lower rates of mortality and heart failure hospitalization in the DDDR AVSH arm was observed but was not statistically significant. The present study is the first in an ICD population to show that DDDR programming can be as good as if not better than VVI programming.

Acknowledgments
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Disclosures
Dr Olshansky serves on the speakers bureau of Boston Scientific CRM, has received honoraria from Boston Scientific CRM, and is a consultant/advisory board member of Boston Scientific CRM. Dr Gering has received honoraria from Boston Scientific CRM, Medtronic, and Biotronik. Dr McGuire has ownership interest in Boston Scientific CRM and is employed by Boston Scientific CRM. Dr Brown is an employee of Integra Clinical Trial Solutions, which is employed as a consultant company to Boston Scientific CRM. Dr Lerew has ownership interest in Boston Scientific CRM and is an employee of Boston Scientific CRM.

References


**CLINICAL PERSPECTIVE**

Dual-chamber (DDDR) implantable cardioverter defibrillators are often the preferred device for patients undergoing implantable cardioverter defibrillator implantation, but some data suggest that compared with single-chamber programming, DDDR programming is associated with an increased risk of heart failure hospitalization and total mortality. We hypothesized that this risk was not due to DDDR programming per se but rather to excessive right ventricular pacing. The INTRINSIC RV (Inhibition of Unnecessary RV Pacing with AVSH in ICDs) study showed in a broad implantable cardioverter defibrillator population that DDDR programming, with atrioventricular search hysteresis to limit excessive right ventricular pacing yet preserve atrioventricular synchrony, resulted in outcomes as good as, if not better than, single-chamber programming. DDDR implantable cardioverter defibrillators programmed to DDDR with atrioventricular search hysteresis should be considered for patients who may benefit from DDDR programming.

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Persistence of Functional Atrioventricular Accessory Pathways in Postseptated Embryonic Avian Hearts

Implications for Morphogenesis and Functional Maturation of the Cardiac Conduction System

Denise P. Kolditz, MSc; Maurits C.E.F. Wijffels, MD, PhD; Nico A. Blom, MD, PhD; Arnoud van der Laarse, MD, PhD; Roger R. Markwald, MD, PhD; Martin J. Schalij, MD, PhD; Adriana C. Gittenberger-de Groot, PhD

Background—During heart development, the ventricular activation sequence changes from a base-to-apex to an apex-to-base pattern. We investigated the possibility of impulse propagation through remnants of atrioventricular (AV) connections in quail hearts.

Methods and Results—In 86 hearts (group A, HH30–34, n=15; group B, HH35–44, n=65; group C, 5 to 6 months, n=6) electrodes were positioned at the left atrium, right ventricular base, left ventricular (LV) base, and LV apex. In group A, LV base activation preceded LV apex activation in the majority of cases (60%; 9 of 15), whereas hearts in group B primarily demonstrated an LV apex-to-base activation pattern (72%; 47 of 65). Interestingly, in group B, the right ventricular base (17%; 11 of 65) or LV base (8%; 5 of 65) exhibited premature activation in 25% (16 of 65) of cases, whereas in 26% (17 of 65), the right ventricular base or LV base was activated simultaneously with the LV apex. Morphological analysis confirmed functional data by showing persistent muscular AV connections in embryonic hearts. Interestingly, all myocardial AV connections stained positive for periostin, a nonmyocardial marker. Longitudinal analysis (HH35–44) demonstrated a decrease in both the number of hearts that exhibited premature base activation (P=0.015) and the number (P=0.004) and width (P=0.179) of accessory AV pathways with developmental stage in a similar time course. In the adult quail hearts, accessory myocardial AV pathways were functionally and morphologically absent.

Conclusion—Thus, impulse propagation through persistent accessory AV connections remains possible at near-hatching stages (HH44) of development, which may provide a substrate for AV reentrant arrhythmias in perinatal life. Periostin positivity and absence of AV pathways in the adult heart suggest that these connections eventually lose their myocardial phenotype, which implicates ongoing AV ring isolation perinatally and postnatally. (Circulation. 2007;115:17-26.)

Key Words: arrhythmia ■ conduction ■ electrophysiology ■ morphogenesis

Atrioventricular (AV) reentrant tachycardias involve the presence of an accessory myocardial AV pathway that bypasses the insulating annulus fibrosis; they are one of the most common arrhythmias in humans.1-3 In children, the first episode of this type of arrhythmia occurs before birth or in the first months of life in ≈60% of cases and appears to resolve spontaneously in two thirds of cases before the age of 1 year.4,5 The natural course in fetuses or neonates is usually benign,5 but a radiofrequency catheter ablation procedure may be necessary to control the arrhythmia.6 Although a causal relationship between abnormal cardiogenesis and arrhythmogenesis has been hypothesized,7 the underlying mechanisms responsible for the development of these accessory pathways (APs) are still not completely understood.

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In the early tubular heart, the atrial myocardium is continuous with the ventricular myocardium, and the blood is driven in a caudal to cranial direction by a slow peristaltic contraction pattern that originates from the primitive pacemaker in the caudal sinus venosus region,8,9 with the endocardial cushions serving as primitive valves.10 Shortly there-
after, the emerging atrium and ventricle in the looped heart start to contract sequentially as a result of the development of alternating slow (sinoatrial region, AV junction, and outflow tract) and fast (atrial and ventricular regions) conducting regions, whereas propagation of the depolarization wave keeps following the direction of the bloodstream. Ultimately, however, ventricular activation shifts from this immature base-to-apex sequence to a mature apex-to-base pattern.12–18

This transition in ventricular activation pattern reflects maturation of the His-Purkinje system (HPS) and coincides with completion of ventricular septation.12,16 Importantly, and almost simultaneously, the existing AV myocardial continuity, which is present on the entire circumference of the AV junction in the looped embryonic heart, disappears because of formation of the fibrous annulus.19 Although remnants of these AV connections, which bypass the insulting AV groove, have been found morphologically in postseptated embryonic and adult chick,20,21 mouse,22 and human23–27 hearts, the electrophysiological properties have not been studied systematically. Recently, a conducting right-side AV myocardial continuity was demonstrated in postseptated CCS-lacZ transgenic mice, which provided a possible explanation for the occurrence of functional atriofascicular bypass tracts via the moderator band, which causes Mahaim tachycardias.28 Nevertheless, the developmental mechanisms that underlie the occurrence, and in many cases underlie early and spontaneous disappearance of APs in fetuses and neonates,4,5 are incompletely understood.

We hypothesized that accessory AV connections that bypass the insulting annulus fibrosis are embryonic remnants of myocardium that retain their conducting properties in postnatal life. By analyzing the ventricular activation sequence in embryonic and adult quail hearts with extracellular electrode recordings and by correlating these electrophysiological data with morphology, we demonstrate that functional remnants of AV connections indeed remain present at late postseptational stages of embryonic heart development.

Methods

Experimental Preparations

Fertilized eggs of the Japanese quail (Coturnix coturnix japonica) were incubated at 37.5°C and 80% humidity. All animal experiments were in accordance with the institutional guidelines of the Leiden University Medical Center. After termination of incubation and staging according to Hamburger-Hamilton (HH) criteria,29 the embryonic hearts (HH30–34, n=15; HH35–44, n=65) were carefully isolated from the embryos after euthanasia by decapitation. Additionally, 6 hearts were harvested from adult quails (5 to 6 months of age) after cervical dislocation.

The hearts were placed in a custom-built, fluid-heated, temperature-controlled tissue bath. Subsequently, the embryonic hearts were superfused (30±0.1°C) and the adult hearts were Langendorff perfused (65 mm Hg, 37±0.1°C) with carboxygenated (95% O2, 5% CO2) Tyrode’s solution (in mmol/L): NaCl 130, KC1 4, KH2PO4 1.2, MgSO4 0.6, NaHCO3 20, CaCl2 1.5, and glucose 10 (pH 7.35).

Technical Features of Electrophysiological Recordings

Unipolar extracellular recordings were performed by consistently positioning 4 tungsten electrodes (tip: 1 to 2 µm; impedance 0.5 to 1.0 MΩ; WPI Inc, Berlin, Germany) on the left atrium, right ventricular base (RVB), left ventricular base (LVB), and left ventricular apex (LVA) (Figure 1). Electrograms were recorded with a high-gain, low-noise, direct-current bioamplifier system (Iso- DAM8; WPI Inc). The signals were band-pass filtered (300 Hz to 1 kHz) and notch filtered (50 Hz) before being digitized at a sampling rate of ≥1 kHz with a computerized recording system (Prucka Engineering Inc, Houston, Tex). Pacing was performed with a stimulator (EP-3, EP MedSystems Inc, West Berlin, NJ), which provided monophasic stimuli (strength 5 to 10 mA, width 1.0 ms).

The embryonic hearts were stimulated at the high right atrium.

Electrophysiological Recording Protocol

The experimental preparations were allowed to equilibrate for 10 minutes before initiation of the recording protocol. Hearts were categorized into 3 groups: group A (HH30–34, n=15), group B (HH35–44, n=65), and group C (5 to 6 months, n=6). Embryonic hearts in group A, hearts in group B with a stable spontaneous heart rate (HR) of at least 60 beats per minute (bpm; group B1), and hearts in group C were allowed to beat spontaneously, whereas hearts with an HR of <60 bpm (group B2) were stimulated at a fixed cycle length of 500 ms.

After baseline recordings in 15 hearts (HH38–41), 1 mL adenosine (0.3 mg/mL) was superfused on the heart to a final concentration of 0.03 mg/mL (0.11 µmol/L) in the tissue bath to analyze transitions in ventricular activation sequence after conduction through the AV node was slowed.

Definitions and immunohistochemistry are described in detail in the online-only Data Supplement.

Statistical Analysis

HR and AV interval were compared between groups with a 2-tailed Student t test for normally distributed values; otherwise, the Mann-Whitney U test was used (AV interval group B1). The symmetry of the distribution was determined by measurement of the skewness value. For comparison of categorical variables (ventricular activation patterns, AP number, AP width), the P value of <0.05 (2 tailed) was considered statistically significant. All analyses were performed with the Statistical Package for Social Studies version 11.0 (SPSS Inc, Chicago, Ill).

The online-only Data Supplement contains more information about methods used in this study.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Experimental Preparations

In 15 group A hearts (HH30–34), electrograms were recorded during stable heart rhythm of 143±30 bpm (AV interval

Figure 1. A representative HH43 embryonic heart that shows recording electrode placement on the LA, RVB, LVB, and LVA. A bipolar pacing electrode for heart pacing was placed on the high RA. Ao indicates aorta; PT, pulmonary trunk; Bc, brachiocephalic artery; LA, left atrium; and RA, right atrium.
100±20 ms). In 15 group B1 hearts (HH35–44), the HR (91±36 bpm) and the AV interval (80±15 ms) were not significantly different compared with group A (P=0.414 and P=0.415, respectively). During pacing of the right atrium (120 bpm) in the remaining 50 hearts in group B2 (HH35–44), the mean AV interval was 78±28 ms (P=0.758, compared with group B1). The 6 adult quail hearts showed an HR of 199±52 bpm and an AV interval of 80±7 ms. Table 1 summarizes the general (electrophysiological) characteristics of the quail hearts.

**LV Activation Sequence: Base to Apex or Apex to Base?**

Because initial studies mainly reported on LV activation patterns, we first analyzed the relationship between LVA and LVB electrograms. Hearts in group A primarily showed base-to-apex LV activation patterns (9 of 15; 60%), with LVB activation preceding LVA activation by 5±4 ms (Table 2). In contrast, hearts in group B mainly demonstrated apex-to-base LV activation patterns, with LVA activation preceding LVB activation by 4±3 ms in 47 of 65 hearts (72%) (Table 2). In group B, no differences in LV activation patterns were observed between hearts that beat spontaneously and hearts driven by right atrium pacing (P=0.843). Representative examples of electrode recordings in an embryonic heart from group A are shown in Figure 2A and 2B, and recordings in an embryonic heart from group B2 are shown in Figure 2C and 2D.

**Global (LV and RV) Activation Patterns**

Analysis of the more global ventricular activation patterns, which include RVB activation, revealed that quail hearts in group A demonstrated earliest ventricular activation at the LVB in 40% (n=6) of cases, whereas the RVB was the site of earliest ventricular activation in 20% (n=3) of cases (Table 2).

### Table 1. Developmental Stages of the Quail Hearts From Groups A, B, and C With Corresponding HRs and AV Intervals

<table>
<thead>
<tr>
<th>Developmental Stage, HH or months SR/Paced n</th>
<th>HR, bpm, mean±SD (range)</th>
<th>AV Interval, ms, mean±SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (n=15)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 SR</td>
<td>140±33 (100–184)</td>
<td>100±15 (87–125)</td>
</tr>
<tr>
<td>31 SR</td>
<td>142±41 (94–180)</td>
<td>93±10 (82–107)</td>
</tr>
<tr>
<td>32 SR</td>
<td>175</td>
<td>115</td>
</tr>
<tr>
<td>33 SR</td>
<td>140±11 (132–147)</td>
<td>137±12 (129–146)</td>
</tr>
<tr>
<td>34 SR</td>
<td>141±6 (137–145)</td>
<td>76±2 (74–78)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>143±30 (94–184)†</td>
<td>100±20 (74–146)‡</td>
</tr>
<tr>
<td><strong>Group B1 (n=15)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 SR</td>
<td>170±5 (167–174)</td>
<td>91±19 (78–105)</td>
</tr>
<tr>
<td>36 SR</td>
<td>76±21 (61–90)</td>
<td>74±20 (60–89)</td>
</tr>
<tr>
<td>38 SR</td>
<td>76±12 (63–92)</td>
<td>71±9 (62–81)</td>
</tr>
<tr>
<td>39 SR</td>
<td>94±17 (77–112)</td>
<td>72±9 (61–78)</td>
</tr>
<tr>
<td>40 SR</td>
<td>77±13 (63–90)</td>
<td>94±18 (78–114)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>91±36 (41–174)†</td>
<td>80±15 (60–114)*‡</td>
</tr>
<tr>
<td><strong>Group B2 (n=50)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 Paced</td>
<td>120</td>
<td>48</td>
</tr>
<tr>
<td>36 Paced</td>
<td>120</td>
<td>96±40 (62–132)</td>
</tr>
<tr>
<td>37 Paced</td>
<td>120</td>
<td>88±4 (85–91)</td>
</tr>
<tr>
<td>38 Paced</td>
<td>120</td>
<td>93±46 (42–132)</td>
</tr>
<tr>
<td>39 Paced</td>
<td>120</td>
<td>71±24 (47–140)</td>
</tr>
<tr>
<td>40 Paced</td>
<td>120</td>
<td>67±21 (41–89)</td>
</tr>
<tr>
<td>41 Paced</td>
<td>120</td>
<td>96±33 (57–140)</td>
</tr>
<tr>
<td>42 Paced</td>
<td>120</td>
<td>77±28 (47–127)</td>
</tr>
<tr>
<td>43 Paced</td>
<td>120</td>
<td>87±24 (67–120)</td>
</tr>
<tr>
<td>44 Paced</td>
<td>120</td>
<td>58±10 (51–65)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>120</td>
<td>78±28 (41–140)*</td>
</tr>
<tr>
<td><strong>Group C (n=6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 months SR</td>
<td>199±52 (134–251)</td>
<td>80±7 (71–89)</td>
</tr>
</tbody>
</table>

SR indicates sinus rhythm.

*P=0.758 (Student t test); †P=0.414 (Student t test); ‡P=0.415 (Student t test).
TABLE 2. LV Activation Sequences in Groups A, B, and C With Corresponding Locations of Earliest Ventricular Activation

<table>
<thead>
<tr>
<th>LV Activation Sequence</th>
<th>n (%)</th>
<th>LVB First</th>
<th>RVB First</th>
<th>LVA First</th>
<th>LVB or RVB=LVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>9 (60)</td>
<td>6</td>
<td>3</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>5 (33)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>5</td>
</tr>
<tr>
<td>Apex to base</td>
<td>1 (7 )</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td>6 (40)</td>
<td>3 (20)</td>
<td>...</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>7 (11)</td>
<td>5</td>
<td>2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>11 (17)</td>
<td>...</td>
<td>2</td>
<td>...</td>
<td>9</td>
</tr>
<tr>
<td>Apex to base</td>
<td>47 (72)</td>
<td>...</td>
<td>7</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Subtotal</td>
<td>65</td>
<td>5 (8)</td>
<td>11 (17)</td>
<td>32 (49)</td>
<td>17 (26)</td>
</tr>
<tr>
<td>Group B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>1 (7 )</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>3 (20)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>3</td>
</tr>
<tr>
<td>Apex to base</td>
<td>11 (73)</td>
<td>...</td>
<td>...</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td>1 (7)</td>
<td>...</td>
<td>9 (60)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Group B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>6 (12)</td>
<td>4</td>
<td>1</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>Concurrent</td>
<td>8 (16)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>8</td>
</tr>
<tr>
<td>Apex to base</td>
<td>36 (72)</td>
<td>...</td>
<td>7</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Subtotal</td>
<td>50</td>
<td>4 (8)</td>
<td>8 (16)</td>
<td>23 (46)</td>
<td>15 (30)</td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Apex to base</td>
<td>6 (100)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>6 (100)</td>
</tr>
</tbody>
</table>

Values are n (%).

Interestingly, even at late stages of embryonic development (HH35–44) (group B), the LVA was the true site of earliest activation in only 32 of 65 (49%) hearts, whereas the RVB or LVB exhibited earliest ventricular activation in 11 (17%) and 5 (8%) cases, respectively. In the remaining hearts (17 of 65; 26%), concurrent activation of the LVA and RVB or LVB was observed (Table 2). Representative examples of electrogram recordings in embryonic hearts from group B, which displayed early RVB and early LV activation, are shown in Figure 3A/3B and 3C/3D, respectively.

In postseptated hearts (group B) with earliest ventricular activation of the LVB (n=5) or RVB (n=11), the AV intervals were 62±15 ms and 74±31 ms, respectively (P=0.540). Activation of the ventricular base occurred significantly faster in quails with a global base-to-apex pattern (69±26 ms) of ventricular activation than in quails with an apex-to-base pattern (83±22 ms) (P=0.005), which suggests that slow conduction through the AV node was indeed bypassed in these hearts.

Additional longitudinal analysis demonstrated early activation of the ventricular base in 93% (14 of 15) of preseptated HH30–34 hearts, whereas the ventricular base was prematurely activated in 60% (23 of 38) of postseptated HH35–39 and in only 37% (10 of 27) of postseptated HH40–44 hearts (P=0.015).

Ventricular Activation Patterns in the Adult Heart
In all adult quail hearts in group C (n=6; HR 199±52 bpm, AV interval 80±7 ms), the LVA was the location of earliest ventricular activation and was activated 5±4 ms before the LVB or RVB. Surface ECG recordings (n=4) did not reveal ventricular preexcitation: PR intervals were not shortened (69±2 ms, range 66 to 71 ms) and showed an isoelectric segment, and QRS complexes did not show a delta wave (31±2 ms, 29 to 33 ms). A representative example of extracellular and surface ECG recordings in an adult quail heart from group C is shown in Figure 4.

Effect of Adenosine on Ventricular Activation
Adenosine was administered in 15 (HH38–41) hearts from group B, which resulted in a rapid (1- to 2-minute) and marked increase in AV interval from 67±18 ms to 149±9 ms (P<0.001) and concurrent changes in ventricular activation pattern (P=0.022). For instance, in 44% (4 of 9) of hearts with an apex-to-base global ventricular activation pattern (9 of 15, 60%; AV interval 72±18 ms) at baseline, the ventricular activation pattern switched to base to apex (RVB, n=2; LVB, n=1; AV interval 149±12 ms), whereas in 11% (1 of 9) of the cases a concurrent ventricular activation pattern was observed (AV interval 140 ms). The ventricular activation sequence in hearts with a global base-to-apex pattern at baseline (5 of 15, 33%; AV interval 61±15 ms) remained unaltered, whereas the AV interval increased to 154±7 ms. In the remaining heart (AV interval 47 ms) with a concurrent ventricular activation pattern (LVB and LVA activation simultaneously) at baseline, adenosine increased the AV interval to 149 ms, and the RVB was shown to be the location of earliest ventricular activation. Interestingly, in hearts with base-first activation, conduction through the AP also decreased markedly, which indicates intrinsic AV nodal conduction properties.

Immunohistochemical Correlations With Electrophysiological Data
In all 16 sectioned postseptated embryonic quail hearts (HH35–44), an MLC2a-positive myocardial AV continuity was found at the right posteroseptal region. In all hearts, 1 or more mostly right-sided additional AV continuities could be identified until stage HH44. Left-sided continuities were frequently found in HH35–39 hearts (9 of 10; 90%), whereas only 1 of 6 (17%) of HH40–44 hearts showed a left-sided continuity (Table 3). All APs could be followed easily from section to section. Interestingly, all MLC2a-positive myocardial APs found in these embryonic postseptated hearts also stained positive for periostin, a nonmyocardial marker. In Figure 5A through 5H, representative examples of MLC2a and periostin staining in HH36 and HH39 embryonic hearts are given.

Longitudinal analysis showed that with increasing developmental stage, both the number (P=0.004) and width (P=0.179) of APs decreased. Whereas hearts at HH35–39 showed multiple broad APs in various locations, hearts at HH40–44 primarily harbored small AV continuities in the right posteroseptal and right midseptal regions, whereas the adult heart demonstrated complete fibrous annular isolation.
Morphological findings could not be directly correlated with electrophysiological data: Right- or left-sided APs were found both in embryonic hearts that displayed earliest ventricular activation at the RVB or LVB and in hearts with a concurrent or apex-to-base global ventricular activation pattern (Table 3). Morphologically, the APs showed no discriminating features, which could explain these different ventricular activation sequences.

Discussion
We analyzed ventricular activation patterns in embryonic and adult quail hearts with extracellular electrode recording techniques and correlated these activation patterns with the morphology of the insulating AV annulus. A key finding of this study is that although the LV activation pattern in septated hearts changed from an immature base-to-apex to a mature apex-to-base pattern, premature activation of the RVB and LVB remained present in 51% (33 of 65) of postseptated hearts up to stage HH44 (hatching at HH45–46). This premature ventricular base activation can morphologically be explained, as shown in this study, by persistent accessory myocardial continuities between atrium and ventricle.

Transition of the Ventricular Activation Sequence Versus Persistent Early Activation of the Ventricular Base
Whereas hearts at preseptational stages of development (group A) primarily exhibited an immature base-to-apex pattern of LV activation (9 of 15; 60%), hearts at postseptational stages of development (group B) demonstrated a mature apex-to-base pattern in the vast majority of cases (47 of 65; 72%). This transition from an immature base-to-apex to a mature apex-to-base LV activation pattern has been studied previously and is associated with maturation of the HPS. Optical mapping studies showed that this transition marks the emergence of mature “apex-first” epicardial breakthrough near the termini of the bundle branches and demonstrated that right and left bundle branch apical breakthrough sites appear at HH29 and HH35, respectively, which is consistent with the transition of the LV activation sequence that occurs at HH35 in the present study.

Unlike previous studies, we observed in postseptated hearts (HH35–44) that the ventricular base could still be “prematurely” activated in a significant number of cases (33 of 65; 51%). For instance, the RVB was activated before the LVA in 11 (17%) cases and the LVB before the LVA in 5 (8%) cases, whereas in another 17 (26%) hearts the ventricular base and LVA were activated simultaneously. This simultaneous activation can, given the position of our recording electrodes (Figure 1), most likely be explained by simultaneous conduction over 2 different pathways: the AV node/HPS on one hand and an AP on the other hand. Furthermore, in 44% (4 of 9) of hearts with an apex-to-base global ventricular activation pattern at baseline, the ventricular activation pattern switched to base to apex after administration of adenosine, which indicates conduction through an AP.

Thus, in contrast to previous studies, our data show that despite maturation of the HPS and transition of the LV activation sequence from base to apex to apex to base, premature and direct activation of the ventricular base remained present in 51% of postseptated hearts at baseline.

Early Activation of the Ventricular Base in Postseptated Embryonic Hearts Can Be Explained by Persisting AV Continuities
In the present study, continuities between atrial and ventricular myocardium were found in the posteroseptal region of the tricuspid annulus in all 16 postseptated quail hearts that were analyzed. In addition, in several hearts, 1 or more connections were found mostly at the right anteroseptal and
midseptal regions, whereas left-sided pathways were less frequently encountered (Table 3).

The fact that left-sided APs were uncommonly found in late post-septated embryonic HH40–44 hearts (1 of 6; 17%) might reflect a developmental time difference in completion of left and right AV ring isolation, which agrees with a previous description that the left annulus fibrosis in the human adult heart is anatomically usually well formed and nearly always complete, in contrast to the poorly formed and at many sites deficient right annulus fibrosis.23 This is further supported by the demonstrated difference in AV interval between hearts with earliest ventricular activation at the RVB (74±31 ms) versus the LVB (62±15 ms) (P=0.540), and it may be speculated that different developmental mechanisms can be anticipated to cause the appearance of right- and left-sided APs.

Normal Development of the Isolating AV Ring:
Possible Fate of Persisting AV Connections and Periostin Expression
In the looped embryonic heart, the AV junction constitutes one of the slow conducting regions of the heart responsible for the sequential contraction pattern at this developmental stage.11,30 The subsequent separation of the atria and ventricles is thought to be caused by the fusion of the epicardially located AV sulcus with the endocardially situated AV cushions at the ventricular site of the junction.31 The processes that underlie atrial and ventricular myocardium dissociation are, however, still incompletely understood, and the tissues responsible for the formation of the annulus fibrosis yet remain largely unknown. Epicardium-derived cells that migrate through the developing AV-dissociated borderline have been followed in their differentiation and shown to become
conducting AV junctional myocardium in the looped heart. The myocardium of the APs found in the postseptated stages, however, strengthens the hypothesis that APs that cause AV reentrant tachycardias in neonates are remnants of primitive AV myocardium.

Because the sulcus and cushion tissue fuse at the ventricular side of the AV junctional myocardium,31,33,34 we postulate that the myocardium of the APs found in the postseptated quail hearts consists of primitive remnants of the slow-conducting AV junctional myocardium in the looped heart.

This is in ample agreement with the relatively slow conduction through these pathways as found in our present study compared with the higher conduction velocity through the AV node/HPS and the decrease in conduction velocity through the AP after administration of adenosine.

Interestingly in the present study, anatomic AV myocardial continuities were found both in embryonic hearts exhibiting base-first activation and in hearts with a concurrent or apex-first activation pattern. On the basis of morphological data, we were unable to find any discriminating factors that can explain why some of the morphologically demonstrated APs in retrospect gave rise to premature ventricular activation and others did not. We propose that interembryonic variance in conduction properties of the AV connections on one hand and of the AV node/HPS on the other hand can be held responsible for this observation. Poor cellular coupling, a slow upstroke of the action potential, and perhaps "zig-zag conduction" or an unfavorable source–sink relationship at the ventricular insertion side may all contribute to the very slow conduction or even conduction block at the AP, which causes preferential activation via the AV node/HPS.36–38 In précis, the presence of an AP is required to give rise to ventricular preexcitation, but its mere presence does not assure the existence of a faster route for anterograde AV conduction. The high prevalence of functional APs in hearts at late postseptational stages, however, strengthens the hypothesis that APs that cause AV reentrant tachycardias in neonates are remnants of primitive AV myocardium.

Periostin was originally isolated as an osteoblast-specific factor that functions as a cell adhesion molecule involved in osteoblast recruitment, attachment, and spreading. Expression of periostin mRNA was later also found in the embryonic fibroblasts of the fibrous heart skeleton.32 During formation of the annulus fibrosis, the embryonic slow-conducting AV junctional myocardium becomes incorporated in the definitive atrium.31,33,34 With completion of this AV isolation, the primitive AV myocardial connections make way for conduction through the AV node/HPS, which eventually constitutes the only remaining conduction pathway of the adult heart.35 Because the sulcus and cushion tissue fuse at the ventricular side of the AV junctional myocardium,31,33,34 we postulate that the myocardium of the APs in the postseptated quail hearts consists of primitive remnants of the slow-conducting AV junctional myocardium in the looped heart.

Table 3. Locations of AP, Cumulative and Individual Widths, and Corresponding Global Activation Patterns in the 16 Morphologically Analyzed Embryonic Quail Hearts

<table>
<thead>
<tr>
<th>Embryo No.</th>
<th>HH Stage</th>
<th>Location of AP</th>
<th>Cumulative (Individual) Width, µm</th>
<th>Global Activation Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>RAS + RMS + RPS + LAS + LAL</td>
<td>120 (30, 15, 30, 20, 25)</td>
<td>LVA first</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>RAS + RMS + RPS + RML + LPS</td>
<td>100 (25, 15, 30, 15, 15)</td>
<td>LVA first</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>RMS + RPS + RPM + RML</td>
<td>135 (25, 40, 30, 40)</td>
<td>RPS first</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>RMS + RPS + RMP + LMS + LPL</td>
<td>140 (40, 45, 15, 15, 25)</td>
<td>LVA first</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>RMS + RPS + RPS + RPM + LML</td>
<td>120 (20, 25, 30, 20, 25)</td>
<td>LVA first</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>RMS + RPS + RPL + RPM + LPS</td>
<td>140 (35, 30, 40, 20, 15)</td>
<td>RPS + LVA concurrent</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>RMS + RPS + RPS + RPM + LML</td>
<td>115 (20, 35, 25, 20, 15)</td>
<td>LVA first</td>
</tr>
<tr>
<td>8</td>
<td>39</td>
<td>RMS + RPS + RPS + RPM + LAS + LML</td>
<td>80 (15, 30, 15, 10, 10)</td>
<td>RPS + LVA concurrent</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>RMS + RPS + RML + LPS</td>
<td>115 (30, 40, 15, 15, 15)</td>
<td>LVB first</td>
</tr>
<tr>
<td>10</td>
<td>39</td>
<td>RMS + RPS + RPM + LML</td>
<td>85 (25, 35, 15, 10)</td>
<td>LVB first</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>RAS + RPS</td>
<td>45 (15, 30)</td>
<td>LVA first</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>RMS + RPS + RMP</td>
<td>80 (25, 35, 20)</td>
<td>LVA first</td>
</tr>
<tr>
<td>13</td>
<td>41</td>
<td>RMS + RPS</td>
<td>40 (15, 25)</td>
<td>LVA first</td>
</tr>
<tr>
<td>14</td>
<td>41</td>
<td>RMS + RPS</td>
<td>40 (20, 20)</td>
<td>LVA first</td>
</tr>
<tr>
<td>15</td>
<td>42</td>
<td>RMS + RPS + LPS</td>
<td>75 (20, 45, 10)</td>
<td>LVA first</td>
</tr>
<tr>
<td>16</td>
<td>44</td>
<td>RMS + RPS</td>
<td>30 (10, 20)</td>
<td>RVS first</td>
</tr>
</tbody>
</table>

RAS indicates right anteroseptal; RMS, right midseptal; RPS, right posterosetal; RMP, right midposterior; RPL, right posterolateral; LAL, left anterolateral; LAS, left anteroseptal; LAL, left anterolateral; LMS, left midseptal; LPS, left posteroseptal; and LPL, left posterolateral.
mouse and chicken heart in the endocardial cushions that ultimately divide the primitive heart tube into a 4-chambered heart.\textsuperscript{39,40} Periostin is secreted during cushion mesenchym formation\textsuperscript{41} and has been suggested to induce myocardium to transform into mesenchym of a mixed phenotype, which can subsequently transdifferentiate into cells with a fibrous identity, whereas at late stages of development periostin may also serve to maintain the integrity of the fibrous tissues of the heart.\textsuperscript{41,42} At the boundary where myocardial cells directly face endocardial cushion tissue at the AV junction, periostin expression is enhanced and myocardial cells are replaced over time by dense fibrous periostin-positive tissue.\textsuperscript{43} Periostin is also abundantly present in epicardium and epicardium-derived cells.

On the basis of our observations that (1) the functionality, number, and width of persistent APs decreased with developmental stage, (2) the persistent APs all stained positive for periostin, and (3) APs were functionally and structurally absent in the adult quail heart, we assume that periostin expression in persistent myocardial APs perinatally results in inhibition of the myocardial phenotype by transdifferentiation of these myocytes into fibrous tissue. This implicates that these AV connections will disappear within the first weeks to months after birth.

This hypothetically ongoing process of postnatal isolation of the AV ring provides a good etiologic explanation for the clinical observation that AV reentrant tachycardias in human neonates spontaneously disappear before the age of 1 year in the majority of cases,\textsuperscript{4,5} which is further strengthened by the previously reported remarkable morphological transformations of the sinus node, AV node, and bundle of His, which similarly commences about 1 to 2 weeks after birth.\textsuperscript{44–46} Furthermore, local failure or a delay in this remodeling process until adolescence or adulthood may explain the occurrence of reentrant tachycardias later in life.\textsuperscript{46}

Limitations of the Study
The aim of this study was to investigate whether AV conduction remains possible via remnants of AV connections in postseptated hearts despite the well-known maturation of the HPS. Although we indeed showed that early activation of the ventricular base is present in a large number of postseptated hearts, which can be explained by the demonstrated persistent connections between atrial and ventricular myocardium, we did not demonstrate that the strands of tissue found by immunohistochemical staining were indeed the structures responsible for the recorded premature ventricular activation. For this, detailed mapping of impulse propagation via these connections and 1-to-1 correlation with morphology in all hearts will be necessary.

Furthermore, to meet the metabolic demands of the older embryonic hearts, we performed our experiments, similar to others,\textsuperscript{11} at subphysiological temperatures (30°C). Although this might have had an effect on our measurements (eg, slower HRs or longer conduction times), the recorded AV intervals, time differences between apex and base activation, and the developmental stage at which the transition in LV activation sequence occurred were comparable to previous studies.\textsuperscript{12–16}

Figure 5. A, Morphological findings in a representative example of a postseptated HH36 quail heart that demonstrated earliest ventricular activation at the RVB. Histologically, a broad region of AV myocardial continuity was found in the right posteroseptal region of the MLC2a-stained slides. Bar=1000 \( \mu \)m. B, Magnification of boxed area, in which these AV myocardial continuities (arrows) are shown. Bar=100 \( \mu \)m. C, Periostin staining (blue) from adjacent section, superimposed on the MLC2a-stained section, shows periostin expression in the AV myocardial bridges. Bar=1000 \( \mu \)m. D, Magnification of boxed area. Bar=100 \( \mu \)m. E, Morphological findings in a representative example of a postseptated HH39 quail heart, which demonstrated concurrent activation of the RVB and LVA. Histologically, a small right posteroseptal AV myocardial continuity was found in the MLC2a-stained slides. Bar=1000 \( \mu \)m. F, Magnification of boxed area, in which this AP (arrow) is shown to course through the insulating annulus fibrosis. Bar=100 \( \mu \)m. G, Periostin staining (blue) shows marked periostin expression in the AP. Bar=1000 \( \mu \)m. H, Magnification of boxed area. Bar=100 \( \mu \)m.
Conclusions

AV myocardial pathways that bypass the AV node remain present and functional in hearts at late postseptational stages of embryonic development and may provide a physiological substrate for AV reentrant tachycardias in perinatal and postnatal life. However, because (1) the number of embryonic hearts with premature ventricular base activation decreased significantly with developmental stage, (2) a decrease in both AP number and AP width was observed in a similar time course, (3) persistent APs stained positive for periostin, and (4) APs were proven to be structurally and functionally absent in the adult heart, it is likely that these AV connections will disappear within the first weeks to months after birth. Further research should more precisely clarify the processes that cause the disappearance or persistence of these APs.

Disclosures

None.

References

The embryogenesis of the structures involved in atrioventricular (AV) conduction is not fully understood. Nonetheless, knowledge of the anatomic substrates that result in accessory pathway–mediated tachycardia has progressed from being of purely scientific interest to being integral to the management of patients. Within a short time, the primary heart tube transforms into a 4-chambered heart. Whereas sequential activation is initially caused by slow conduction over the circumferential AV continuity, the AV ring becomes isolated in later stages and conduction runs through the AV node/His-Purkinje system. As a result, ventricular activation changes from an immature base-to-apex pattern in preseptated hearts to a mature apex-to-base sequence in postseptated hearts. Abnormal development of the annulus fibrosis that results in accessory pathways may cause AV reentrant arrhythmias. Because these arrhythmias frequently occur in fetuses and neonates, we hypothesized that, during normal development, primitive AV connections that bypass the annulus fibrosis remain present even after development of the His-Purkinje system. We demonstrated that the annulus fibrosis in postseptated prenatal quail hearts is still far from complete, which resulted in functional AV myocardial pathways. We speculate that AV ring isolation continues postnatally, which implicates the disappearance of accessory AV connections within the first weeks after birth and thus provides an etiologic explanation for the clinical observation that AV reentrant tachycardias in human neonates spontaneously obliterate before the age of 1 year in the majority of cases. Local failure or a delay in this remodeling process of the isolating AV ring until adulthood may explain the occurrence of AV reentrant tachycardia later in life.
Statins and the Risk of Lung, Breast, and Colorectal Cancer in the Elderly

Soko Setoguchi, MD, DrPH; Robert J. Glynn, PhD, ScD; Jerry Avorn, MD; Helen Mogun, MS; Sebastian Schneeweiss, MD, ScD

Background—Although most randomized trials and meta-analyses suggest a slight or no increase in the risk of cancer in statin users, results from observational studies have been conflicting, and some have even suggested a large protective effect of statins on certain cancers. Long-term statin users tend to be healthier, less frail, and more adherent to therapy than nonusers, however. This could explain such apparent “protective” effects.

Methods and Results—We conducted the present cohort study by linking data from a large state drug benefit program with cancer registry data and Medicare healthcare utilization data. We identified all initiators of statins; initiators of glaucoma medications, another preventive drug, served as a comparison group. Outcomes included all registry-identified cases of colorectal, lung, and breast cancer. Multivariable Cox proportional models were used to adjust for confounding. Patient characteristics were similar in both groups, but statin initiators (n=24,439) were slightly younger and used some services more frequently than glaucoma drug initiators (n=7,284). The mean follow-up was 2.9 years, with the longest follow-up being 8.4 years. Incidence rates of colorectal, lung, and breast cancers in both groups were very similar to rates in the general population. Adjusted hazard ratios were 0.96 (95% CI, 0.70 to 1.31) for colorectal cancer, 1.11 (95% CI, 0.77 to 1.60) for lung cancer, and 0.99 (95% CI, 0.77 to 1.60) for lung cancer, and 0.99 (95% CI, 0.74 to 1.33) for breast cancer.

Conclusions—These data from a large population of typical older patients who began using statins indicate that it is unlikely that statins confer a clinically important decrease or increase in the risk of colorectal, lung, or breast cancer over the durations studied. (Circulation. 2007;115:27-33.)

Key Words: statins ■ cancer ■ morbidity ■ age

Multiple randomized controlled trials (RCTs) have demonstrated the beneficial effects of statins on cardiovascular morbidity and mortality in a variety of populations.\(^1\)\(^-\)\(^7\) The extensive evidence has led to widespread use of these drugs.\(^8\) Recent epidemiological studies have suggested that statins might reduce the risk of several cancers. As a result, statins are now being studied in clinical trials for cancer prevention.\(^9\)

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However, there is also evidence to suggest an opposite effect of statins on cancer in an RCT of elderly patients.\(^6\) The first concern about a possible association between statins and cancer followed a review of rodent studies that indicated that statins promoted cancer at concentrations equivalent to those commonly prescribed in humans.\(^10\) Subsequently, 4 meta-analyses of RCTs showed no increase or decrease in the risk of cancer.\(^5\)\(^-\)\(^13\) However, these trials have been criticized for having relatively short-term follow-up of highly selected groups of patients.

Although selected populations and short-term follow-up can be addressed in observational studies, the results of earlier observational studies are conflicting.\(^14\)\(^-\)\(^21\) Some observational studies that focused on the elderly population reported a significant protective effect,\(^14\)\(^,\)\(^20\)\(^,\)\(^23\) in contrast to RCT data in the elderly.\(^6\) Because long-term users of statins tend to be healthier, less frail physically and cognitively, and more adherent to therapy and screening than nonusers,\(^23\)\(^-\)\(^26\) these studies may have failed to adjust fully for these factors, possibly leading to residual confounding. With these methodological issues in mind, we conducted a cohort study using glaucoma drug initiators as a comparison group to assess the effect of statins on several specific common cancers in a large elderly population.

Methods

Data Sources and Patients

We conducted a cohort study linking data on drug utilization and the Pharmaceutical Assistance Contract for the Elderly in Pennsylvania between January 1, 1994, and May 31, 2003, with healthcare

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From the Division of Pharmacoepidemiology and Pharmacoconomics, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Mass.

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Reprint requests to Soko Setoguchi, MD, DrPH, Division of Pharmacoepidemiology and Pharmacoconomics, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, 1620 Tremont St, Suite 3030, Boston, MA 02130. E-mail ssetoguchi@partners.org

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of a cancer of interest recorded in the registry (1988 to 2003) before
for any drug during each of 2 consecutive 6-month periods before
required to have had at least 1 clinical encounter and a prescription
from 1994 to 2003. To ensure active system use, subjects were
older who were enrolled in Medicare and the drug benefit programs
protect patients’ privacy.

The study population consisted of all subjects aged 65 years or
older who were enrolled in Medicare and the drug benefit programs
from 1994 to 2003. To ensure active system use, subjects were
required to have had at least 1 clinical encounter and a prescription
for any drug during each of 2 consecutive 6-month periods before
cohort entry. Patients were excluded if they had a previous diagnosis
of a cancer of interest recorded in the registry (1988 to 2003) before
cohort entry.

Cohort Definition
We first identified all patients who filled a prescription for a statin
during the period 1994–2002. To achieve a more homogeneous mix
of users with regard to disease risk, we restricted the cohort to
initiators of statins by ensuring that the patients had not filled a
prescription for a statin for at least 12 months before the first
prescription. We studied statin initiators to reduce the potential for
attrition of susceptible individuals because of side effects or treat-
ment failures, which could introduce bias.31 The design also allowed
us to account for duration of exposure. As a comparison group, we
identified initiators of glaucoma drugs in the same study population.
Subjects of glaucoma drugs were selected as a reference group for the
following reasons. Previous studies suggested that long-term users of
statins tend to be healthier, less frail physically and cognitively, and
more adherent to therapy and screening than nonusers.24,25 Glaucoma
drugs are another type of preventive drug, and their users are likely
to have characteristics similar to those of statin users with regard to
health-seeking behavior and adherence to other preventive proce-
dures. We have previously found that statins and glaucoma drugs
were both prescribed less frequently to subjects at the end of life,23
and there is no evidence that glaucoma drug users pose an increased
risk of cancer compared with the general population.32,33

Exposure Definition
Adherence to statin use declines most rapidly during the first 6
months, and these nonadherent users are likely to have very different characteristics with regard to health-seeking behaviors and/or
preventive procedures.24,34,35 It is also difficult to establish the associ-
ation between statin use and the occurrence of cancer in nonadherent
subjects. Therefore, we required statin initiators to fill 3 or more
prescriptions of any statin (lovastatin, pravastatin, simvastatin, flu-
vasatin, atorvastatin, or cerivastatin) during the first 180 days after
the first prescription of a statin. To ensure the comparability of the
comparison group, the same criteria for adherence were applied to
glaucoma drug users. Statin users who had taken glaucoma drugs
previously or were taking them presently were excluded from the
analyses (and vice versa for glaucoma drug users). We assumed an
induction period of 180 days, so follow-up started 180 days after the
first prescription was filled.

Study Outcomes
Subjects were censored at (1) the occurrence of a defined cancer end
point, (2) initiation of the comparison drug (eg, initiation of
glaucoma drug in statin users), (3) failure to fill ≥2 prescriptions of
the exposure drug every 6 months, (4) death, (5) migration out of the
healthcare system, or (6) end of the study period (May 31, 2003),
whichever came first. The primary study end points were a new
diagnosis of breast, colorectal, or lung cancer according to the cancer
registry data. For colorectal and breast cancer, we excluded in situ
cancer from the primary analyses because detection of in situ cancer
is more likely to be driven by patients’ adherence to screening
procedures. In a secondary analysis, these cases were included. The
event date for each case was defined as the date of diagnosis
recorded in the registry.

Potential Confounding and Its Measurement
We assessed demographic variables, documented risk factors for the
selected cancers (inflammatory bowel disease, benign mammary
dysplasia, arthritis, estrogen use, use of nonsteroidal antiinflamma-
tory drugs, obesity, and tobacco abuse), prevention-related activities
(mammography, gynecologic examination, pap smear, colonoscopy,
and stool occult blood), and healthcare utilization (Charlson comor-
bidity score, number of physician visits, distinct generic medicines
taken, prior hospitalization, and prior nursing home stay) during the
12 months before the index date. These covariates were identified
with International Classification of Diseases, 9th Revision, diagno-
tic codes, current procedural terminology procedure codes, and/or
prescription information.

Statistical Analysis
First, we compared crude incidence rates of the study cancers in the
present cohort to those in the general population. The rates for the
general population were calculated by standardizing the Surveil-
lance, Epidemiology, and End Results cancer rates for age and
gender. In the present study cohort, we excluded the first 6 months
after initiation of the drug from this rate calculation to avoid
immortal person-time bias and to account for 6 months of induction
time.36 We used multivariable Cox proportional hazards regression
to estimate the effects of statin use on incidence of cancer compared
with glaucoma drug use. Statistical significance was assessed with
95% CIs. We tested the proportional hazards assumption by includ-
ing an interaction term between time and exposure in the model. All
statistical analyses were performed with the SAS statistical program
(version 9, SAS, Cary, NC).

Assessment for Unmeasured Confounding With
External Data and Sensitivity Analyses
Using data from the Medicare Current Beneficiary Survey (MCBS),
we assessed the balance of variables not measured in our healthcare
utilization data. The MCBS is conducted in a sample of Medicare
beneficiaries selected each year to be representative of the current
Medicare population, including both aged and disabled beneficiaries
living in the community or in institutions. Previously, the data have
been used to estimate the likelihood of confounding bias.37 We
identified users of statins and glaucoma drugs in MCBS data from
1999 to 2001 and excluded subjects with history of breast, colorectal,
or lung cancers. We estimated the prevalence of smoking status,
body mass index, functional status, education, and aspirin use in
users of statins versus glaucoma drugs.
We further conducted quantitative sensitivity analyses to assess
the impact of important unmeasured confounders, ie, smoking,
aspirin use, and family history of cancer, using the estimated
prevalence of these covariates in glaucoma drug users from MCBS
data.36 We assumed that the rate ratio (RR) of the association
between smoking (ever versus never) and lung cancer was 1.6 from
the estimates for current smoking versus nonsmoking in US data39
and that the effect of aspirin was RR=0.6 for colorectal cancer.40
Because prevalence of family history of cancer was not available in
MCBS data, we assumed the prevalence of family history of lung
and breast cancer was 15%41,42 in glaucoma drug users, with an
effect size of approximately RR=2.0 (RR=1.8 for lung cancer,
with greater risk in younger subjects,43 and RR=1.5 for breast cancer in
elderly women44). We did not perform sensitivity analyses for family
history with regard to colorectal cancer because family history is a strong risk factor in younger populations (especially those aged <45 years) but not in older populations.\textsuperscript{45}

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Patients and Their Characteristics
After the application of exclusion and inclusion criteria, the cohort consisted of 24,439 statin initiators and 7,284 glaucoma drug initiators. The characteristics of the study population measured during the 12-month period before the initiation of either drug class are shown in Table 1. In general, glaucoma users were slightly older than statin users but had comparable characteristics for health service utilization, use of preventive services, and observable risk factors for the cancers of interest.

Incidence Rates of Cancers
Table 2 shows the number of cancers, person-years of follow-up, and incidence of colorectal, lung, and invasive breast cancer in the cohort and in the general population standardized for age and gender with Surveillance, Epidemiology, and End Results data.\textsuperscript{46} The majority of cases in statin users (61% of colorectal cancers and 77% of breast cancers) occurred after 3 years of drug use, whereas 41% of lung cancers occurred after 3 years. The cancer rates we observed in the present study population were comparable to cancer incidence rates in the US general population.

Cancer Risk in Statin Users
Table 3 shows unadjusted, age/gender/race–adjusted, and multivariable adjusted hazard ratios (HRs) of invasive colorectal cancer, lung, and invasive breast cancers. The total numbers of cancers after the exclusion of in situ cancers were 233 for colorectal cancer and 268 for breast cancer. We found no meaningful increase or decrease in the risk of cancers in statin users compared with that in glaucoma drug users. In a secondary analysis, we included in situ cancers in the outcome for colorectal and breast cancers. The multivariable adjusted HRs including in situ cases were unchanged: 0.97 (95% CI, 0.74 to 1.28) for colorectal cancer and 0.93 (95% CI, 0.68 to 1.26) for breast cancer. We also examined the effects of different types of statins. The HR estimates for hydrophobic statins (simvastatin, lovastatin, fluvastatin, and atorvastatin\textsuperscript{77}) and for pravastatin were not meaningfully different from the overall result (point estimates ranged from 0.87 to 1.18).

The test for proportional hazard was statistically significant for breast cancer ($P=0.003$), which indicates that the effect of statin use might be different over time. It was not significant for colorectal cancer ($P=0.39$) or lung cancer ($P=0.65$). Table 4 shows point estimates for short-term effects of statins ($\leq 3$ years) and longer-term effects ($>3$ years). The short-term effect of statins tended to be protective for breast cancer.

Balance in Unmeasured Factors in MCBS Data
Compared with glaucoma drug users, statin users were more educated, less functionally limited, had a slightly higher mean

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>24 439</td>
<td>7284</td>
</tr>
<tr>
<td>Mean follow-up, y</td>
<td>2.9 (2.0)</td>
<td>2.6 (2.0)</td>
</tr>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>76.4 (6.0)</td>
<td>80.1 (6.8)</td>
</tr>
<tr>
<td>Sex, % female</td>
<td>81.8</td>
<td>83.6</td>
</tr>
<tr>
<td>Race, % white</td>
<td>94.2</td>
<td>90.8</td>
</tr>
<tr>
<td>Health service utilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity score</td>
<td>1.9 (1.8)</td>
<td>1.6 (1.7)</td>
</tr>
<tr>
<td>No. of physician visits</td>
<td>9.1 (6.1)</td>
<td>9.6 (6.3)</td>
</tr>
<tr>
<td>No. of medications taken</td>
<td>8.1 (5.0)</td>
<td>7.7 (4.9)</td>
</tr>
<tr>
<td>No. of hospitalizations</td>
<td>0.6 (1.1)</td>
<td>0.4 (0.9)</td>
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<td>Prior nursing home stay</td>
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<td>4.5</td>
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<td>Screening mammogram</td>
<td>12.5</td>
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</tr>
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<td>Mammography</td>
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<td>3.4</td>
</tr>
<tr>
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<td>1.3</td>
</tr>
<tr>
<td>Colonoscopy with removal of polyps</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Colonoscopy without removal</td>
<td>6.0</td>
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</tr>
<tr>
<td>Stool occult blood</td>
<td>11.1</td>
<td>10.6</td>
</tr>
<tr>
<td>Osteoporosis drug use</td>
<td>7.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Other clinical characteristics</td>
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<td></td>
</tr>
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<td>Arthritis</td>
<td>6.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>37.6</td>
<td>31.5</td>
</tr>
<tr>
<td>Inflammatory bowel diseases</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Benign mammary dysplasia</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Estrogen use</td>
<td>5.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Estrogen-progesterone use</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>NSAID use</td>
<td>30.2</td>
<td>31.5</td>
</tr>
<tr>
<td>Use of gastroprotective drugs</td>
<td>32.0</td>
<td>29.8</td>
</tr>
<tr>
<td>Obesity diagnosis</td>
<td>3.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Tobacco abuse diagnosis</td>
<td>3.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

NSAID indicates nonsteroidal antiinflammatory drug. Values are expressed as % for categorical variables and missing categories and mean (SD) for continuous variables.

body mass index, and were more likely to have been smokers and to take aspirin (Table 5).

Sensitivity Analyses on Unmeasured Confounders
The Figure shows the impact of 3 important unmeasured confounders on our observed null effect of statin use on incident cancer. Given the estimated prevalence of smoking and aspirin use in the MCBS survey data, we concluded that the corrected HR would be 0.90 for smoking and 1.02 for aspirin use (see the 2 circled points in the Figure). Because there is no evidence that family history of cancer is associated with statin use versus glaucoma drug use, we can assume that the prevalence in statin users would reasonably be in the
range of 5% to 30% compared with the prevalence of 15% in the general population; then, the corresponding range of corrected HR was 0.87 to 1.07.

**Discussion**

We estimated the risk of common solid cancers among new users of statins compared with new users of glaucoma drugs, and we found no significant increase or decrease in the risk of cancers among statin users. The present study has several strengths compared with earlier studies. First, we restricted the analysis to new users of statins, eliminating the biases that can occur when prevalent drug users are included. Second, we used a comparison group likely to have similar characteristics in health-seeking behaviors and adherence to screening procedures. Third, the present study considered of a very large group of older adults at high risk of cancer. Finally, we used registry-validated cancer diagnosis to measure outcomes.

An overview of RCTs of statins reported no significantly increased risk of cancer (RR, 1.07; 95% CI, 0.90 to 1.26), and 3 meta-analyses found the same result. A nested case-control study using healthcare utilization databases in Quebec, Canada, compared statin users with resin users aged 65 years and older and found no increase but a decrease in cancer incidence with a median follow-up period of 2.7 years. Population-based studies using a health-services database in Saskatchewan, Canada, and the United Kingdom’s General Practice Research Database suggested that statins may be associated with a marginally increased risk of breast cancer or no association with overall cancer risk. Two studies using Danish and Dutch health-service databases found a 20% reduction in overall cancer. Three other studies in younger women in the United States (Case-Control Surveillance Study, Nurses’ Health Study, and a population-based case-control study of 3 Washington counties) showed no significant reduction of the risk for invasive breast cancer (RR ranged from 0.9 to 1.2). Most recently, large cohort studies in the United States found no association between statin use and colorectal and breast cancer. Although the populations in most trials and these observational studies were relatively young compared with the present study population, our findings are similar to the meta-analyses and the most recent studies, and the point estimates from all the other studies are included within the 95% CIs of our estimates.

The results of the present study exclude the strong protective effect of statins found in observational studies in the elderly by Cauley et al and Poynter et al. Cauley et al conducted a prospective cohort study in US elderly women (aged ≥65 years) and reported a 68% reduction in the risk of breast cancer in statin users. A population-based case-control study in northern Israel by Poynter et al reported a 47% reduction in colorectal cancer among long-term statin users (≥5 years) compared with short-term users or nonusers among the elderly (aged ≥60 years). As we have pointed out, however, long-term statin users are likely to be systematically different from nonusers of statins. Health-seeking behavior and the healthier lifestyle of long-term compliant statin users may independently lower the risk of colorectal cancer. In addition, prevention-oriented statin users may be more likely to have precancerous colorectal polyps detected and removed early, which would make statins appear protective. These studies by Poynter et al and Cauley et al may have failed to adjust adequately for these factors, likely leading to residual confounding. The present study attempted to adjust for these possible biases by choosing equally compliant glaucoma drug users as a comparison group. These patients take another kind of preventive drug that, like statins,

**TABLE 2. Unadjusted Incidence Rate of Cancer and Its Comparison With Surveillance, Epidemiology, and End Results Data**

<table>
<thead>
<tr>
<th>Statin Use</th>
<th>Person-Years</th>
<th>Incidence Rate (per 100 000)</th>
<th>SEER Population (Aged ≥65 Years)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All colorectal cancer</td>
<td>190</td>
<td>59640.9</td>
<td>318.6</td>
</tr>
<tr>
<td>All lung cancer</td>
<td>179</td>
<td>59907.7</td>
<td>298.8</td>
</tr>
<tr>
<td>All breast cancer</td>
<td>227</td>
<td>49910.6</td>
<td>454.8</td>
</tr>
<tr>
<td>Invasive colorectal cancer</td>
<td>178</td>
<td>59640.9</td>
<td>298.5</td>
</tr>
<tr>
<td>Invasive breast cancer</td>
<td>203</td>
<td>49910.6</td>
<td>406.7</td>
</tr>
</tbody>
</table>

*SEER indicates Surveillance, Epidemiology, and End Results.

**TABLE 3. Effects of Statin Use on Invasive Colorectal Cancer, Lung Cancer, and Invasive Breast Cancer**

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>Invasive Colorectal Cancer</th>
<th>Lung Cancer</th>
<th>Invasive Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>Lower 95% CI</td>
<td>Upper 95% CI</td>
</tr>
<tr>
<td>Unadjusted*</td>
<td>0.92</td>
<td>0.68</td>
<td>1.24</td>
</tr>
<tr>
<td>Adjusted for sex/age/race†</td>
<td>0.99</td>
<td>0.72</td>
<td>1.36</td>
</tr>
<tr>
<td>Multivariable‡</td>
<td>0.96</td>
<td>0.70</td>
<td>1.31</td>
</tr>
</tbody>
</table>

* Cox proportional hazard regression with study time as a time scale.
† Cox proportional hazard regression with study time as a time scale and age, race, and sex in the model.
‡ Multivariable Cox proportional hazard regression with study time as a time scale and including covariates in Table 1 in the model.
is less frequently prescribed to subjects at the end of life. We found that use of preventive procedures in glaucoma drug users was comparable to that of statin users (Table 1). Long-term statin users have survived and stayed healthy enough to continue to take statins. As a result, in many studies, patients who are vulnerable or susceptible to cancer or other morbid conditions drop out of the long-term statin user cohort, leaving those who are healthier and less susceptible to the risk of cancer (attrition of susceptible individuals). This bias can be avoided by employing a study design that enrolls only initiators of statins or a comparison drug and by comparing their risk at the same point over the course of drug exposure in a Cox model, as we did here. A naïve case-control design (without risk-set sampling) simply comparing long-term users of statins with nonusers has difficulty handling this bias.

The present results raise the possibility that the short-term effect of statins on breast cancer might differ from that of long-term use, with short-term use appearing modestly protective and long-term use seeming to raise the risk slightly, although not significantly. Although we have adjusted for screening behaviors of the study patients, it is possible that statin users had relatively more extensive screening before they reached 65 years of age that was not captured in our data and therefore had fewer events during the first years after their enrolment into Medicare and initiation of statins. The present data do not rule out a possibly increased risk of cancer for long-term statin users beyond the range of our data.

Several limitations of the present study should be noted. A number of possible confounders were not measured in the data (eg, aspirin use and family history of cancer) or were measured incompletely (eg, tobacco use and obesity). These unmeasured risk factors might have biased results if they were differentially associated with statin versus glaucoma drug use. We might have been able to reduce much of this confounding by the choice of comparison group. Using MCBS data, we found that some of the possible confounding factors unmeasured in our data, such as body mass index, smoking, functional status, and aspirin use, were slightly imbalanced; however, sensitivity analyses showed that these differences were not substantial enough to cause significant

### Table 4. Short-Term vs Long-Term Effect of Statin Use on Selected Cancers

<table>
<thead>
<tr>
<th></th>
<th>Short-Term Users (&lt;3 Years)</th>
<th>Long-Term Users (≥3 Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Exposed Cases</td>
<td>HR</td>
</tr>
<tr>
<td>Invasive colorectal cancer*</td>
<td>74</td>
<td>0.93</td>
</tr>
<tr>
<td>Lung cancer*</td>
<td>99</td>
<td>1.18</td>
</tr>
<tr>
<td>Invasive breast cancer*</td>
<td>47</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Multivariable Cox proportional hazard regression with study time as a time scale and including covariates in Table 1 in the model.

### Table 5. Unmeasured Patient Characteristics by Drug Use Categories in Noninstitutionalized Medicare Beneficiaries Aged ≥65 Years (MCBS 1999 and 2001)

<table>
<thead>
<tr>
<th></th>
<th>Statin Users</th>
<th>Glaucoma Drug Users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>3569</td>
<td>894</td>
</tr>
<tr>
<td>Gender, % female</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>75 (6)</td>
<td>80 (7)</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SD)</td>
<td>27 (5)</td>
<td>25 (5)</td>
</tr>
<tr>
<td>Missing</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Bedridden status, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedridden</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Not bedridden</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>Missing</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Functional limitation, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any difficulty in activities of daily living</td>
<td>26</td>
<td>37</td>
</tr>
<tr>
<td>No difficulty in activities of daily living</td>
<td>73</td>
<td>63</td>
</tr>
<tr>
<td>Missing</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>63</td>
<td>57</td>
</tr>
<tr>
<td>Never</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td>Education, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>College or more</td>
<td>69</td>
<td>64</td>
</tr>
<tr>
<td>Missing</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Taking aspirin, %</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

Sensitivity analysis: effect of unmeasured confounding factors. The curves represent how the “true” or corrected RR changes with various values of prevalence of unmeasured confounders in statin users. For glaucoma drug users, the prevalence of aspirin use and smoking is fixed using the estimates for glaucoma users in the MCBS data, and the prevalence of family history of lung and breast cancer is assumed to be 15% based on estimates from the general population. The 2 circled points represent the corrected RR for the values of prevalence of smoking and aspirin use in statin versus glaucoma users in MCBS data. No estimates for family history of cancer were available from MCBS data, but we expect that the prevalence in statins users will be similar to 15%, which is the estimate for the general population.
bias in our estimates. Finally, although we believe that new glaucoma drug users are a more valid comparison group than nonusers of statins, the size of the group was smaller than the statin user group and resulted in less precise estimates.

The mean duration of drug use in the present study population was 2.9 years, slightly longer than that of the RCTs criticized for having a relatively short follow-up.\textsuperscript{6,11–13} Nonetheless, the study population included patients with various durations of follow-up (maximum of 8.4 years), and 40% of the patients had a follow-up of more than 3 years, with 60% of cancers occurring after 3 years of follow-up. Assessment of the possibility of different risk with longer use will require studies with greater exposure durations.

The present data indicate that in the first several years of statin use, it is unlikely that elderly patients have a clinically important decrease or increase in the risk of colorectal, lung, or breast cancer compared with elderly patients using other, unrelated preventive medications. The present data do not rule out a possibly increased risk of long-term statin use beyond the period of exposure studied, however.

**Disclosures**

Dr Glynn reports that he has a contract with AstraZeneca to serve as the independent statistical monitor of its trial of Crestor (rosuvastatin; JUPITER trial). The remaining authors report no conflicts.

**References**


In addition to extensive evidence proving the benefit of statins on cardiovascular morbidity and mortality, recent observational studies have suggested that these drugs might reduce the risk of several cancers; however, long-term statin users tend to be healthier, less frail, and more adherent to therapy and screenings than nonusers. This could explain such apparent “protective” effects of the drug on other outcomes. In contrast, most randomized trials and meta-analyses suggest little or no change in the risk of cancer among patients taking statins. We conducted a cohort study to assess the effect of statins on the incidence of lung, colorectal, and breast cancer in a large population of typical older patients. The present study supports the conclusion that statins are not associated with a clinically important decrease or increase in the risk of cancer in the elderly over the duration studied (mean follow up was 2.9 years, with the longest follow-up being 8.4 years). Until proven otherwise in clinical trials, physicians may not prescribe statins for cancer prevention. These findings suggest that statin use in the elderly should be based solely on the evidence of its cardioprotective effects and previously documented adverse effects rather than on any supposed effect on cancer risk.
Alcohol Consumption and Risk of Heart Failure in the Physicians’ Health Study I

Luc Djoussé, MD, MPH, DSc; J. Michael Gaziano, MD, MPH

Background—Heart failure (HF) is the leading cause of hospitalization among the elderly, and 1 in 5 adults aged 40 years will develop HF in their lifetime. Data on the effects of moderate alcohol consumption on the risk of HF have been sparse and inconsistent. This study sought to evaluate the association between moderate alcohol consumption and incident HF.

Methods and Results—A total of 21,601 participants of the Physicians’ Health Study I who were free of HF and provided data on alcohol intake at baseline were prospectively followed up from 1982 to 2005. Incident HF cases were ascertained through annual follow-up questionnaires and validated with the use of Framingham criteria. During an average follow-up of 18.4 years, 904 incident cases of HF occurred. The crude incidence rates of HF were 25.0, 20.0, 24.3, and 20.6 cases per 10,000 person-years for alcohol categories of 1, 1 to 4, 5 to 7, and >7 drinks per week, respectively. Corresponding hazard ratios (95% CI) were 1.0 (reference), 0.90 (0.76 to 1.07), 0.84 (0.71 to 0.99), and 0.62 (0.41 to 0.96), respectively, with \( P \) for trend = 0.012 adjusted for age, body mass index, smoking, and history of valvular heart disease. There was no evidence for a strong association between moderate alcohol consumption and HF without antecedent coronary artery disease.

Conclusions—Although heavy drinking should be discouraged, our data indicate that moderate drinking may lower the risk of HF. The lack of an association between moderate alcohol intake and HF without antecedent coronary artery disease suggests that possible benefits of moderate drinking on HF may be mediated through beneficial effects of alcohol on coronary artery disease. (Circulation. 2007;115:34-39.)

Key Words: alcohol ■ epidemiology ■ heart failure ■ risk factors

The lifetime risk of heart failure (HF) is estimated at 20% (1 in 5) for both men and women aged 40 years.¹ Although advanced age, hypertension, diabetes mellitus, obesity, valvular heart disease, and myocardial infarction have been recognized as predictors of HF,²,³ limited data are available on the effects of modifiable lifestyle factors on the risk of HF. Specifically, epidemiological data on the role of moderate alcohol consumption on HF risk have been limited. Although heavy drinking has been associated with left ventricular dysfunction⁴–⁶ and dilated cardiomyopathy,⁷–¹⁰ only few studies have examined whether moderate alcohol consumption influences the risk of HF. Mukamal et al¹¹ did not find an association between recent alcohol consumption and HF. In the Survival and Ventricular Enlargement (SAVE) trial,¹² moderate alcohol consumption was associated with a lower incidence of HF in crude analyses but not in multivariable analyses of 2231 post–myocardial infarction patients with left ventricular systolic dysfunction. Contrary to these reports, data from the Framingham Heart Study have shown that moderate alcohol consumption was associated with a lower risk of HF.¹³ In addition, Abramson and colleagues¹⁴ have shown that consumption of 21 to 70 oz of alcohol per month was associated with a 47% reduction in HF risk after 14 years of follow-up among 2235 elderly subjects, and data from the Cardiovascular Health Study showed a lower risk of HF among moderate drinkers.¹⁵ Possible physiological mechanisms by which moderate alcohol consumption might lower the risk of HF include its beneficial effects on coronary artery disease (CAD) and neurohormonal changes that might prevent clinical onset of HF.¹⁶–¹⁸ The present project sought to prospectively assess whether moderate alcohol consumption was associated with a lower risk of HF among US male physicians. In addition, we sought to determine whether moderate alcohol consumption influences the risk of HF without antecedent CAD.

Methods

Study Population
The present study analyzed data from the Physicians’ Health Study (PHS) I, which was a randomized, double-blind, placebo-controlled
Alcohol Consumption

Information about usual alcohol consumption was self-reported on a standard questionnaire. Participants were asked, “How often do you usually consume alcoholic beverages?” Possible response categories included “rarely/never,” “1 to 3/mo,” “1/wk,” “2 to 4/wk,” “5 to 6/wk,” “daily,” and “2+dr.” The response was interpreted as number of alcoholic drinks consumed during the specified period.

Ascertainment of Incident HF

A questionnaire was mailed to each participant every 6 months during the first year and has been mailed annually thereafter to obtain information on compliance with the intervention and the occurrence of new medical diagnoses including HF. In a pilot study, a total of 100 participants who reported a HF diagnosis on a follow-up questionnaire were contacted by mail. The mailing included a HF questionnaire with detailed questions about time and place of HF diagnosis, clinical signs and symptoms, medical treatment, and diagnostic methods (echocardiography, angiography, and radionuclide imaging). Of the 100 physicians, 4 had died, and 8 participants had a routine follow-up method that deviates from the normal mailing procedure (eg, some participants have requested to be contacted by telephone only). After 2 mailings, we obtained a completed questionnaire from 73 of 88 participants (83%). Among respondents, 90% of the HF cases (66 of 73) were confirmed with the use of the Framingham criteria.

Other Variables

Information on age, height, weight, body mass index, cigarette smoking, parental history of myocardial infarction, history of angina, hypertension, atrial fibrillation, valvular disease, diabetes mellitus, and physical activity was collected at baseline. Incident CAD (angina pectoris, myocardial infarction, coronary angioplasty, and coronary bypass surgery) was ascertained through annual follow-up using people consuming 1 to 4 drinks per week as the reference category. Age was a major confounding factor, and because we did not have adequate overlap across categories of alcohol, we conducted stratified analyses by age categories (<50, 50 to 59, 60 to 69, and ≥70 years). In addition, we controlled for age as a continuous variable within age strata when fitting Cox regression models. Smoking was entered in the model as never, past, and current smoker. We also examined whether the effects of alcohol were mediated by myocardial infarction by adjusting additionally for myocardial infarction as a time-dependent covariate. We updated myocardial infarction at the end of 1989, 1994, and 1999. To assess whether alcohol influences the risk of HF without antecedent CAD, we censored noncases at the time of diagnosis of CAD. HF cases that occurred after CAD were also censored at the time of diagnosis of CAD and recoded as noncases. To examine a possible “sick quitter” effect (subjects may have stopped drinking shortly after a diagnosis of a chronic condition), we conducted sensitivity analyses by excluding individuals whose person-times were <2 years and by using people consuming 1 to 4 drinks per week as the reference group. We also verify that alcohol intake was inversely related to myocardial infarction in this cohort. All analyses were completed with the use of SAS, version 9.1 (SAS Institute, NC). The significance level was set at 0.05.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Statistical Analyses

Because the initial analyses conducted with the original 7 alcohol categories showed an inverse association between alcohol consumption and HF (hazard ratio, 1.0, 0.99, 0.82, 0.91, 0.75, 0.81, 0.61 from the lowest to the highest category of alcohol intake, respectively, adjusted for age, smoking, and history of valvular heart disease; P for trend=0.005), we collapsed adjacent categories to obtain stable estimates. Additional adjustment for exercise (categories) did not alter the findings. We calculated person-time of follow-up from baseline until the first occurrence of (1) HF, (2) death, or (3) censoring date (date of receipt of last follow-up questionnaire). Within each alcohol category, incidence rate was computed by dividing the number of HF cases by the corresponding person-time. Actuarial analyses were performed by the Kaplan-Meier method, and the statistical significance was determined with the log-rank test. We used Cox proportional hazard models to compute multivariable adjusted hazard ratios with corresponding 95% CIs using subjects in the alcohol category of “rarely/never” as reference group. We assessed confounding by using 10% change in hazard ratio. Adjustments for the proportional hazard models were tested by including main effects and product terms of covariates and time factor and were met (all P>0.05). We obtained the probability value for linear trend by assigning the midpoint of each alcohol group to a new variable that was modeled as a continuous variable in the Cox regression model. A value of 9 was assigned to the highest open-ended alcohol category. Age was a major confounding factor, and because we did not have adequate overlap across categories of alcohol, we conducted stratified analyses by age categories (<50, 50 to 59, 60 to 69, and ≥70 years). In addition, we controlled for age as a continuous variable within age strata when fitting Cox regression models. Smoking was entered in the model as never, past, and current smoker. We also examined whether the effects of alcohol were mediated by myocardial infarction by adjusting additionally for myocardial infarction as a time-dependent covariate. We updated myocardial infarction at the end of 1989, 1994, and 1999. To assess whether alcohol influences the risk of HF without antecedent CAD, we censored noncases at the time of diagnosis of CAD. HF cases that occurred after CAD were also censored at the time of diagnosis of CAD and recoded as noncases. To examine a possible “sick quitter” effect (subjects may have stopped drinking shortly after a diagnosis of a chronic condition), we conducted sensitivity analyses by excluding individuals whose person-times were <2 years and by using people consuming 1 to 4 drinks per week as the reference group. We also verify that alcohol intake was inversely related to myocardial infarction in this cohort. All analyses were completed with the use of SAS, version 9.1 (SAS Institute, NC). The significance level was set at 0.05.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Among 21,601 participants in the PHS I, the mean age at randomization was 53.8±9.5 years (range, 40 to 86 years). Table I presents baseline characteristics of the study participants. Frequent alcohol consumption was associated with older age; current smoking; higher prevalence of
hypertension, atrial fibrillation, and valvular disease; and lower prevalence of angina pectoris and diabetes mellitus. During an average follow-up of 18.4 years, 904 new cases of HF occurred. The crude incidence rates of HF were 25.0, 20.0, 24.3, and 20.6 cases per 10,000 person-years for usual alcohol consumption of <1, 1 to 4, 5 to 7, and >7 drinks per week, respectively. There was evidence for increased event-free survival from the lowest to the highest category of alcohol consumption ($P = 0.02$, log-rank test).

From the multivariable Cox regression model, hazard ratios (95% CI) for HF were 1.0 (reference), 0.88 (0.75 to 1.05), 0.80 (0.68 to 0.94), and 0.62 (0.40 to 0.95) for alcohol consumption of <1, 1 to 4, 5 to 7, and >7 drinks per week, respectively, after adjustment for age and smoking (3 categories) ($P$ for linear trend$=0.002$; Table 2). Additional adjustment for body mass index (<25, 25 to 29, ≥30 kg/m²), and history of valvular heart disease had only a minimal effect on the results (data not shown). Additional adjustment for myocardial infarction, as a time-dependent covariate, led to a modest attenuation of the main effect of alcohol on HF: Corresponding hazard ratios (95% CI) were 1.0, 0.91 (0.76 to 1.07), 0.87 (0.73 to 1.02), and 0.63 (0.41 to 0.97) from the lowest to the highest alcohol group ($P$ for trend$=0.02$). Exclusion of individuals whose follow-up times were <2 years made the association slightly stronger with fully adjusted relative risks (95% CI): 1.0, 0.90 (0.76 to 1.06), 0.83 (0.70 to 0.99), and 0.58 (0.37 to 0.91) from the lowest to the highest alcohol category, respectively ($P$ for trend$=0.007$). When we used drinkers of 1 to 4 per week as the reference group, our data showed similar results. Multivariable adjusted relative risks (95% CI) were 1.11 (0.94 to 1.32), 1.0 (reference), 0.94 (0.80 to 1.10), and 0.69 (0.45 to 1.06) from the lowest to the highest alcohol group, respectively ($P$ for trend$=0.003$).

Of the total of 904 incident cases of HF, 143 (15.8%) and 346 (38.3%) had antecedent myocardial infarction and CAD, respectively. We examined the association between alcohol intake and HF without antecedent CAD and found no evidence for a strong association. Compared with the lowest alcohol category, hazard ratios (95% CI) for HF without antecedent CAD were 0.93 (0.74 to 1.16), 0.97 (0.79 to 1.21), and 0.84 (0.51 to 1.37) in subjects consuming 1 to 4, 5 to 7, and >7 drinks per week, respectively ($P$ for trend$=0.73$; Table 3). There was evidence for a weaker inverse association between moderate alcohol intake and HF without antecedent myocardial infarction (hazard ratio, 1.0, 0.93 [95% CI, 0.77 to 1.12], 0.91 [95% CI, 0.76 to 1.09], and 0.66 [95% CI, 0.10 to 1.04]) from the lowest to the highest alcohol category, respectively; $P$ for trend$=0.13$). In a multivariable model adjusted for age, body mass index, smoking, and history of hypertension, atrial fibrillation, and valvular heart disease, alcohol consumption was inversely associated with the risk of myocardial infarction in this cohort with corresponding hazard ratio (95% CI) of 1.0, 0.86 (0.76 to 0.98), 0.67 (0.59 to 0.77), and 0.61 (0.43 to 0.86) from the lowest to the highest alcohol group, respectively ($P$ for trend$=0.007$).

### Table 2. Incidence Rate and Hazard Ratios (95% CI) of HF According to Alcohol Consumption

<table>
<thead>
<tr>
<th>Frequency of Alcohol Intake</th>
<th>Cases</th>
<th>Crude Incidence Rate, Cases/10,000 Person-Years</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 drink per week</td>
<td>256</td>
<td>25.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1–4 drinks per week</td>
<td>295</td>
<td>20.0</td>
<td>0.88 (0.75–1.05)</td>
</tr>
<tr>
<td>5–7 drinks per week</td>
<td>330</td>
<td>24.3</td>
<td>0.80 (0.68–0.94)</td>
</tr>
<tr>
<td>&gt;7 drinks per week</td>
<td>23</td>
<td>20.6</td>
<td>0.62 (0.40–0.95)</td>
</tr>
<tr>
<td>$P$ for linear trend</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Adjusted for age (continuous within stratified Cox regression using age strata of <50, 50–59, 60–69, and ≥70), body mass index (<25, 25–29, ≥30 kg/m²), smoking (never, past, and current smokers), and history of valvular heart disease.

### Table 3. Hazard Ratios (95% CI) of HF Without Antecedent Myocardial Infarction and CAD

<table>
<thead>
<tr>
<th>Alcohol, Drinks per Week</th>
<th>HF Without Antecedent Myocardial Infarction</th>
<th>Hazard Ratio (95% CI)</th>
<th>HF Without Antecedent CAD</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>205</td>
<td>1.0</td>
<td>148</td>
<td>1.0</td>
</tr>
<tr>
<td>1–4</td>
<td>246</td>
<td>0.93 (0.77–1.12)</td>
<td>173</td>
<td>0.93 (0.74–1.16)</td>
</tr>
<tr>
<td>5–7</td>
<td>290</td>
<td>0.91 (0.76–1.09)</td>
<td>219</td>
<td>0.97 (0.79–1.21)</td>
</tr>
<tr>
<td>&gt;7</td>
<td>20</td>
<td>0.66 (0.10–1.04)</td>
<td>18</td>
<td>0.84 (0.51–1.37)</td>
</tr>
</tbody>
</table>

$P$ for linear trend... 0.13 0.73

*Adjusted for age (continuous within stratified Cox regression using age strata of <50, 50–59, 60–69, and ≥70), body mass index (<25, 25–29, ≥30 kg/m²), smoking (never, past, and current smokers), and history of valvular heart disease.
highest category of alcohol, respectively (P for trend <0.0001). In secondary analyses, moderate alcohol consumption was associated with a lower risk of HF (although this was not statistically significant) in people with and without diabetes (data not presented). Furthermore, we did not find evidence for an interaction between moderate alcohol consumption and diabetes on the risk of HF (P for interaction=0.53).

Discussion

In observational studies, moderate alcohol consumption has been associated with lower rates of coronary heart disease, a major determinant of HF. Although many researchers have shown that heavy alcohol consumption is associated with cardiomyopathy, limited data are available on the influence of moderate alcohol consumption (up to 2 drinks per day for men and 1 drink per day for women) on the development of HF in a community setting. In the present prospective study, we demonstrated that moderate alcohol consumption was inversely associated with the risk of HF in a dose-response manner and independent of major confounding factors. We did not find a statistically significant association between moderate alcohol consumption and HF without antecedent CAD, however.

Our findings are consistent with many of the previously published data suggesting a reduced risk of HF with moderate alcohol consumption. Abramson et al first reported an inverse association between alcohol consumption and HF among 2235 elderly subjects with a mean age of 74 years; in that cohort, alcohol intake was inversely associated with HF incidence (P for trend=0.02) with a 47% lower risk of HF in subjects consuming 21 to 70 oz of alcohol per month (≈1.5 to 4 drinks per day) compared with abstainers. We have previously reported an inverse association between alcohol consumption and all-cause incident HF in 2796 men and 3493 women of the Framingham Heart Study who reported light to moderate amounts of alcohol consumption; in addition, the Framingham data showed a lower risk of HF without antecedent myocardial infarction among men who consumed 1 to 7 drinks per week (relative risk, 0.41 [95% CI, 0.21 to 0.79]) and suggestive evidence for women consuming 3 to 7 drinks per week (relative risk, 0.55 [95% CI, 0.25 to 1.20]) after adjustment for age, smoking, body mass index, diabetes, valvular disease, and hypertension. Recent data from the Cardiovascular Health Study found a 34% lower risk of HF among elderly people consuming 7 to 13 drinks per week but little effect with heavy alcohol consumption.

In contrast, data from the Kaiser Permanente Medical care Program reported 40% to 60% lower risk of HF hospitalization for drinkers of at least 1 drink per day among 126,235 individuals. This apparent risk reduction for HF hospitalization was restricted to CAD-related HF with only a borderline statistically significant 20% reduction in HF hospitalization risk among individuals consuming <1 drink per day. In contrast, heavy alcohol consumption was associated with a significant increased risk of non–CAD-related HF hospitalization in that cohort (relative risk, 1.7 [95% CI, 1.1 to 2.6]). Specifically, among 55,658 male participants in the Kaiser Permanente Study, consumption of 1 to 2 drinks per day was associated with 40% lower risk of CAD-related HF hospitalization (P=0.001) and a modest 10% increased risk of non–CAD-related HF hospitalization (P=0.7). Our study did not find a statistically significant association between moderate alcohol consumption and HF without antecedent CAD.

On the other hand, other investigators did not find an association between alcohol intake and HF. In a sample of subjects with myocardial infarction, recent alcohol consumption was associated with HF in a crude model, but this association became statistically nonsignificant after controlling for potential confounders. In the SAVE trial, light to moderate alcohol consumption was not related to incident HF after 42 months of follow-up among 2231 patients with left ventricular systolic dysfunction who were randomized to angiotensin-converting enzyme inhibitor or placebo. The discrepancy with our findings merits some comments. Whereas we assessed long-term alcohol consumption, Mukamal et al assessed the immediate effects of alcohol on HF, namely, whether alcohol ingestion can trigger the development of HF. The PHS I included subjects with normal left ventricular function in contrast to the SAVE trial, in which subjects had an ejection fraction <40%. In addition, the shorter mean follow-up in the SAVE trial (3.5 years compared with 19 years in the PHS I) and relative small sample size may have been insufficient for observation of any major effects of alcohol.

There are several biological mechanisms to explain the observed association between alcohol consumption and HF. Previous studies have demonstrated beneficial effects of alcohol on high-density lipoprotein cholesterol, insulin sensitivity, inflammation and endothelial function, coagulation factors, and atrial natriuretic peptide, a cardiac hormone that plays a role in volume homeostasis. Consequently, several studies have reported that moderate alcohol intake may lower the risk of myocardial infarction and fatal coronary events. The attenuation of the hazard ratios on additional adjustment for myocardial infarction and the lack of an association between moderate alcohol intake and HF without antecedent CAD suggest that the observed lower risk of HF among moderate drinkers may be mediated through beneficial effects of alcohol on CAD.

The present study has some limitations. First, we did not collect data to allow separation of former drinkers from lifetime abstainers. The inclusion of former drinkers who stopped drinking because of HF-related events would increase the baseline rate of HF in the reference category and thus inflate the alcohol-HF association. The fact that exclusion of HF cases occurred during the first 2 years of follow-up did not alter our results suggests that we did not have a substantial number of presymptomatic HF cases who reduced or stopped alcohol consumption to be wrongfully classified with lifetime abstainers. Furthermore, the fact that using moderate drinkers as the reference group still showed lower risk of HF for subjects consuming 5 to
7 and >7 drinks per week suggests that our findings are not influenced by sick quitters. Second, we did not collect adequate data to further classify HF on the basis of left ventricular function. Third, there is a possibility of under-reporting of alcohol consumption, especially among heavy drinkers, because these data were self-reported. Such exposure misclassification in the highest alcohol group would lead to attenuation of the effects of moderate alcohol consumption on HF. Fourth, our sample consists of highly educated male physicians who may have different behaviors than the general population. It is thus possible that residual confounding in this cohort by unmeasured factors such as diet may partially explain our findings. The nature of our cohort also limits the generalizability of our findings. Fifth, because only 3% (n=665) of our participants reported consumption of ≥2 drinks per day, we did not have enough data to examine the effects of heavy drinking on HF, and our findings are mainly applicable to moderate drinkers. Finally, we did not have data on beverage types to examine the effects of wine, beer, and spirits consumption on HF. Nevertheless, the large sample size, the longer duration of follow-up, and the fact that participants were physicians who could recognize early signs of HF are strengths of the present study.

In conclusion, our data show an inverse association between moderate alcohol consumption and incident HF. Although individuals always need to be cautioned against the dangers of heavy alcohol drinking, these findings suggest that moderate alcohol consumption may lower the risk of HF, especially CAD-related HF.

Acknowledgements
We are indebted to the participants in the PHS for their outstanding commitment and cooperation and to the entire PHS staff for their expert and unfailing assistance.

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Disclosures
Dr Djoussé received an investigator-initiated research grant (grant-in-aid) from the Alcoholic Beverage Medical Research Foundation (January 2001 to December 2001) to study the effect of alcohol on lung cancer in the Framingham Heart Study. Dr Gaziano has received investigator-initiated research grants from BASF, DSM Pharmaceuticals, Wyeth Pharmaceuticals, McNeil Consumer Products, and Pliva; has received honoraria from Bayer and Pfizer for speaking engagements; and is a consultant for Bayer, McNeil Consumer Products, Wyeth Pharmaceuticals, Merck, Nutraquest, and GlaxoSmithKline.

References
Heart failure (HF) is the leading cause of hospitalization in the elderly population and is associated with higher costs and societal burden. Hypertension, myocardial infarction, obesity, and valvular heart disease are major risk factors for HF, and previous studies have suggested that modifiable lifestyle factors could lower these risk factors and thus prevent HF. Earlier data have reported beneficial effects of moderate alcohol consumption on coronary artery disease and mortality. Available data on the effects of moderate drinking on the risk of HF are limited and inconsistent, however. We examined prospectively the effects of moderate alcohol consumption on the risk of HF among 21,601 US male physicians. Compared with abstainers, the risk of HF was 10%, 16%, and 38% lower among individuals consuming 1 to 4, 5 to 7, and >7 drinks per week, respectively, after adjustment for major confounders. The fact that the relative risks for HF were attenuated on additional adjustment for myocardial infarction and the lack of an association between moderate alcohol intake and HF without antecedent coronary artery disease suggest that the observed lower risk of HF among moderate drinkers may be mediated through beneficial effects of alcohol on coronary artery disease. The most likely biological mechanism appears to be the increase in high-density lipoprotein cholesterol observed with moderate alcohol consumption. Although individuals always need to be cautioned against the dangers of heavy alcohol drinking (because such consumption could increase the risk of hypertension and cardiomyopathy), these findings suggest that moderate alcohol consumption may lower the risk of HF, especially coronary artery disease–related HF.
Mutations in the Gene Encoding Filamin A as a Cause for Familial Cardiac Valvular Dystrophy

Florence Kyndt, PharmD, PhD; Jean-Pierre Gueffet, MD; Vincent Probst, MD, PhD; Philippe Jaafar, MD; Antoine Legendre, MD; Françoise Le Bouffant, PhD; Claire Toquet, MD; Estelle Roy, BS; Lesley McGregor, MD; Sally Ann Lynch, MD; Ruth Newbury-Ecob, MD; Vinh Tran, PhD; Ian Young, MD, PhD; Jean-Noël Trochu, MD, PhD; Hervé Le Marec, MD, PhD; Jean-Jacques Schott, PhD

Background—Myxomatous dystrophy of the cardiac valves affects ≈3% of the population and remains one of the most common indications for valvular surgery. Familial inheritance has been demonstrated with autosomal and X-linked transmission, but no specific molecular abnormalities have been documented in isolated nonsyndromic forms. We have investigated the genetic causes of X-linked myxomatous valvular dystrophy (XMVD) previously mapped to chromosome Xq28.

Methods and Results—A familial and genealogical survey led us to expand the size of a large, previously identified family affected by XMVD and to refine the XMVD locus to a 2.5-Mb region. A standard positional cloning approach identified a P637Q mutation in the filamin A (FLNA) gene in all affected members. Two other missense mutations (G288R and V711D) and a 1944-bp genomic deletion coding for exons 16 to 19 in the FLNA gene were identified in 3 additional, smaller, unrelated families affected by valvular dystrophy, which demonstrates the responsibility of FLNA as a cause of XMVD. Among carriers of FLNA mutation, the penetrance of the disease was complete in men and incomplete in women. Female carriers could be mildly affected, and the severity of the disease was highly variable among mutation carriers.

Conclusions—Our data demonstrate that FLNA is the first gene known to cause isolated nonsyndromic MVD. This is the first step to understanding the pathophysiological mechanisms of the disease and to defining pathways that may lead to valvular dystrophy. Screening for FLNA mutations could be important for families affected by XMVD to provide adequate follow-up and genetic counseling. (Circulation. 2007;115:40-49.)

Key Words: genetics ■ mitral valve ■ regurgitation ■ valves
identified. Autosomal dominant transmission is the usual inheritance, with reduced penetrance and variable expressivity. Three loci have been mapped to chromosomes 16p11–p12, 11p15.4 and 13q31–32, but the underlying genetic defects are not currently known. An X-linked recessive form was originally described by Monteleone and Fagan in 1969 and by Newbury-Ecob et al in 1993.

We previously clinically and genetically characterized a large family (family 1a) affected by X-linked myxomatous valvular dystrophy (XMVD) (OMIM 314400) and used linkage analysis to map the gene to an 8-cM interval on chromosome Xq28. A large familial and genealogical survey led us to refine the locus of the XMVD gene, and a standard positional cloning approach identified a P637Q mutation in the filamin A (FLNA) gene in all affected members of this family. Screening of additional, smaller, unrelated families with severe forms of valvular dystrophy allowed us to find 3 other mutations in this gene. The present study demonstrates that FLNA is the first gene known to be responsible for isolated nonsyndromic valvulopathy.

Methods

Clinical Evaluation

The present study was conducted according to French guidelines for genetic research and approved by the ethics committee of Nantes University Hospital.

Written informed consent was obtained from all participants. Clinical investigation included a review of medical history and a physical examination, with particular attention given to the cardiovascular system and any connective tissue diseases. The phenotypic assignment of family members was based on echocardiographic examination. Family members were followed up with regular echocardiography, and the present data are those recorded during their last examination, with the exception of patients who underwent valvular surgery. Transthoracic echocardiograms were recorded according to the criteria of the American Society of Echocardiography with a Sequoia C256 (Acuson Inc, Mountain View, Calif) equipped with a multifrequency probe (3.5 to 2.0 MHz). Measurements of mitral valves were performed on parasternal, long-axis, 2-dimensional images without second harmonic. The length of each leaflet was determined immediately before valve closure. The thickness of the free edge of the mitral leaflets was measured on a selected diastolic frame that clearly separated the mitral leaflets and chordae. Mitral valve prolapse was considered to exist when 2-dimensional recordings in the parasternal long-axis view showed protrusion of mitral leaflets into the left atrium, crossing the line between the annular hinge points, and when the coaptation point of the leaflets remained at or above the mitral annular plane during systole. Mitral regurgitation was estimated by using standard methods, which include the proximal isovelocity surface area (PISA) analysis. Aortic regurgitation was considered to exist if an abnormal diastolic flow that originated from aortic cusps was identified in the left ventricular outflow tract. Tricuspid valve images were recorded in 4-chamber apical views, and the pulmonary valve was analyzed in the high, left, parasternal, short-axis view.

Patients were defined as affected if the thickness of the free edge of either or both mitral leaflets was >4 mm, with or without mitral valve prolapse and mitral regurgitation on the echocardiograms because of the inconsistency of mitral valve prolapse and mitral regurgitation in affected individuals. Because aortic valve dystrophy is difficult to assess by transthoracic echocardiography, we chose to quantify aortic regurgitation. Patients were also considered as affected in cases of mild to severe aortic regurgitation.

Genetic Analysis

For molecular studies, DNA was isolated from peripheral blood lymphocytes or paraffin-embedded valve tissue with standard methods.

Linkage Analysis, Refined Mapping, and Haplotypic Constructions

Family 1 was already linked to chromosome Xq28. To narrow the candidate region, we selected 8 microsatellite markers (DXS998, DXS8091, DXS8069, DXS8061, DXS15, DXS1073, F8, and DXS1108) from the candidate interval on Xq28 for use in linkage analysis in the expanded family. F8 is a dinucleotide marker located within factor VIII gene intron 13.1 We carried out 2-point linkage analysis with the FASTLINK program in the easyLINKAGE software package (version 4.0; Medical University Clinic at the University of Würzburg, Würzburg, Germany). For linkage calculations, we assumed X-linked inheritance with a disease-allele frequency of 0.0001 and phenocopies at 2%. Penetrance was set at 100% for male family members and 70% for female family members. All family members were included in the analysis. Patients were defined as affected if the thickness of the mitral leaflets was >4 mm and if mild to severe aortic regurgitation was present. Subjects with a mitral leaflet thickness <2 mm without aortic regurgitation were defined as unaffected, and subjects with a 2- to 4-mm thickness of mitral leaflets were considered phenotypically undetermined. To detect more recombination events and refine the locus, 11 microsatellite markers (DXS8103, DXS1684, DXS10052, DXS10053, DXS10054, Afm308yh1, DXS10051, DXS10049, Afn082xa5, GABRA3, and DXS10047) were genotyped in the recombinant individuals to determine the centromeric boundary. Two intragenic microsatellite markers, GAB3 and F8, were used to determine the telomeric boundary.

Analysis of Candidate Genes and FLNA Mutation Analysis

Candidate genes in the Xq28 interval were screened for mutations by sequence analysis in 1 affected patient of family 1a. Coding exons and short flanking intronic sequences were amplified by polymerase chain reaction (PCR), and excess primer was removed from the amplified fragments with the use of exoSAP (Amersham Biosciences, Piscataway, NJ) and sequenced with a dye-terminator cycle-sequencing system (ABI PRISM 377, Perkin-Elmer Applied Biosystems, Foster City, Calif). The coding sequence of FLNA (exons 2 to 48) was amplified from genomic DNA with the use of primers as previously described.

Variants of FLNA identified by sequence analysis were confirmed by restriction-enzyme digestion with HaeIII (P637Q) and Sau96I (G288R): The required exons were amplified by PCR, digested with restriction enzyme, and size fractionated on an 8% acrylamide gel. In the 2 cases, the variant abolished 1 restriction site. The V711D mutation was confirmed by derived cleaved amplified polymorphic sequencing. To assess the frequency of the mutations, we used sequence analysis, restriction-enzyme digestion, or derived cleaved amplified polymorphic sequencing with DNA from family members and from 500 control chromosomes of European, African, or Asian origin.

Model Building

To study the structural consequences of the identified mutations, we constructed a 3-dimensional model of human filamin repeats with the crystal coordinates of Dicyostelium filamin deposited in Protein Data Bank (identification: 1WLH). The automated homology model construction was performed by the protein structure modeling program INSIGHTII (Accelrys, Cambridge, UK).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Clinical Evaluation

Family 1a (Figure 1A) is a previously reported white French family that is affected with XMVD and currently consists
of 91 living members. The proband (patient V-13) underwent aortic replacement for severe regurgitation at age 17. He was of normal size and morphology, and a physical examination found no connective tissue or joint abnormalities. Cardiac auscultation revealed aortic regurgitant murmur, and echocardiography showed severe aortic regurgitation. Aortic root dimensions were normal as confirmed by a nuclear magnetic resonance study of the thoracic aorta, without any aspect of Marfan or Ehlers-Danlos syndrome. Histological examination of the excised valve showed typical features of myxomatous valvular disease, with marked thickening of the free edge of the valve.

The proband’s cousin (patient V-10) underwent valvuloplasty for severe mitral regurgitation due to mitral valve dystrophy at age 20 (Figure 1B). Detection of a mild hemophilia A in these 2 patients and a familial study led to the identification of a very large family of >300 individuals. Among 44 male family members, 10 suffered from progressive mitral valve prolapse (Figure 1B), which was associated in 4 cases with moderate to severe aortic regurgitation, and 4 underwent valvular surgery (Table 1). Among 47 female family members, 10 were considered as affected with mitral or aortic valve abnormalities, although all were asymptomatic. One child (patient V-8), diagnosed at age 10, showed
TABLE 1. Echocardiographic Characteristics of the Affected Male Family Members and Heterozygous Female Family Members of XMVD Family 1a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis</th>
<th>Examination Year (Subject Age, y)</th>
<th>Mitral Prolapse</th>
<th>AML Thickness, mm</th>
<th>PML Thickness, mm</th>
<th>Mitral Regurgitation</th>
<th>Aortic Regurgitation</th>
<th>Tricuspid Regurgitation</th>
<th>Pulmonary Regurgitation</th>
<th>Age and Type of Valve Surgery</th>
<th>Phenotypic Status</th>
<th>Genotypic Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-8</td>
<td>49</td>
<td>1995 (49)</td>
<td>None</td>
<td>7</td>
<td>5</td>
<td>Moderate</td>
<td>Severe</td>
<td>Mild</td>
<td>Mild</td>
<td>Ao replacement at 49</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>II-14</td>
<td>41</td>
<td>2005 (50)</td>
<td>AML/PML</td>
<td>4</td>
<td>3</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Aortic</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>II-17</td>
<td>51</td>
<td>1996 (51)</td>
<td>AML</td>
<td>5</td>
<td>4</td>
<td>Severe</td>
<td>Moderate</td>
<td>Moderate</td>
<td>None</td>
<td>Ao and Mv replacement at 51</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV-42</td>
<td>55</td>
<td>2005 (62)</td>
<td>AML/PML</td>
<td>6</td>
<td>4.5</td>
<td>Moderate</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV-44</td>
<td>29</td>
<td>2005 (38)</td>
<td>AML/PML</td>
<td>4</td>
<td>3</td>
<td>Moderate</td>
<td>None</td>
<td>Mild</td>
<td>ND</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV-46</td>
<td>23</td>
<td>1996 (23)</td>
<td>AML</td>
<td>7</td>
<td>6</td>
<td>Moderate</td>
<td>Mild</td>
<td>Moderate</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>V-10</td>
<td>18</td>
<td>1996 (18)</td>
<td>AML</td>
<td>7</td>
<td>ND</td>
<td>Severe</td>
<td>Mild</td>
<td>None</td>
<td>ND</td>
<td>Mv valvuloplasty at 20</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>V-11</td>
<td>15</td>
<td>2005 (24)</td>
<td>AML</td>
<td>4</td>
<td>5</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>ND</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>V-13</td>
<td>17</td>
<td>1996 (17)</td>
<td>AML/PML</td>
<td>5</td>
<td>5</td>
<td>Moderate</td>
<td>Severe</td>
<td>Mild</td>
<td>Mild</td>
<td>Ao replacement at 17</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>V-15</td>
<td>11</td>
<td>2005 (20)</td>
<td>AML/PML</td>
<td>5.5</td>
<td>4</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-3</td>
<td>82</td>
<td>1996 (82)</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>ND</td>
<td>Unaffected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-5</td>
<td>60</td>
<td>2005 (69)</td>
<td>AML</td>
<td>4</td>
<td>4</td>
<td>Mild</td>
<td>Moderate</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-11</td>
<td>46</td>
<td>2005 (55)</td>
<td>None</td>
<td>3</td>
<td>2</td>
<td>Mild</td>
<td>None</td>
<td>Moderate</td>
<td>ND</td>
<td>None</td>
<td>Undertermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-25</td>
<td>57</td>
<td>2005 (65)</td>
<td>PML</td>
<td>3</td>
<td>3</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-32</td>
<td>56</td>
<td>2005 (65)</td>
<td>None</td>
<td>3</td>
<td>2</td>
<td>Moderate</td>
<td>None</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>Undertermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-2</td>
<td>33</td>
<td>2005 (42)</td>
<td>AML</td>
<td>4</td>
<td>3</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-4</td>
<td>32</td>
<td>1996 (32)</td>
<td>AML</td>
<td>2</td>
<td>2</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Affected</td>
<td>Nonmutated</td>
</tr>
<tr>
<td>IV-10</td>
<td>15</td>
<td>2005 (24)</td>
<td>None</td>
<td>4</td>
<td>3</td>
<td>None</td>
<td>Mild</td>
<td>Mild</td>
<td>ND</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-17</td>
<td>41</td>
<td>2005 (49)</td>
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<td>4</td>
<td>3</td>
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<td>None</td>
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<td>Heterozygous</td>
</tr>
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<td>36</td>
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<td>Mild</td>
<td>None</td>
<td>None</td>
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</tr>
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<td>3</td>
<td>2</td>
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<td>Mild</td>
<td>None</td>
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<td>None</td>
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<td>ND</td>
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<td>Mild</td>
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<td>Undertermined</td>
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</tr>
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<td>24</td>
<td>1996 (24)</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Unaffected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-27</td>
<td>18</td>
<td>2005 (27)</td>
<td>None</td>
<td>3.5</td>
<td>3</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Moderate</td>
<td>None</td>
<td>Undertermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-45</td>
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<td>2005 (35)</td>
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<td>4</td>
<td>2.5</td>
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<td>Mild</td>
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<td>25</td>
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<td>AML/PML</td>
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<td>Undertermined</td>
<td>Heterozygous</td>
</tr>
<tr>
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<td>3</td>
<td>2</td>
<td>Mild</td>
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<td>None</td>
<td>None</td>
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<td>Heterozygous</td>
</tr>
<tr>
<td>V-2</td>
<td>12</td>
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<td>None</td>
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<td>3</td>
<td>None</td>
<td>None</td>
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<td>None</td>
<td>None</td>
<td>Undertermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>V-8</td>
<td>10</td>
<td>2005 (10)</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
<td>None</td>
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</tr>
<tr>
<td>V-9</td>
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<td>2005 (28)</td>
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<td>2</td>
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<td>V-12</td>
<td>8</td>
<td>2005 (6)</td>
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<td>3</td>
<td>ND</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Undertermined</td>
</tr>
</tbody>
</table>

AML indicates anterior mitral leaflet; PML, posterior mitral leaflet; ND, not determined; Ao, aortic valve; and Mv, mitral valve.

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severe aortic regurgitation with aortic stenosis. In all affected family members, the valvular disease was associated with mild hemophilia A (factor VIII activity between 15% and 50%). To increase the size of this family and facilitate the identification of the gene responsible for the disease, we screened our clinical database for patients who both were affected by hemophilia A and had undergone surgery for valvulopathy. Identification of a new proband (patient III-14, family 1b) affected by valvular dystrophy and mild hemophilia A and originating from the same French geographic location, allowed us to identify a new branch of family 1a called family 1b (Figure 1C). Tracing family trees for family 1a and 1b identified a common ancestor born in the 18th century. The new proband (patient III-14, family 1b) underwent aortic valve replacement at the age of 52 for severe aortic regurgitation and was deceased at the time of this study. His mother had moderate mitral and aortic regurgitation. Three other male family members presented with mitral valvular dystrophy (Figure 1B) and variable aortic regurgitation. All of them had mild hemophilia A except patient IV-3, whose factor VIII activity was normal (>50%). Five female family members were considered as affected with variable mitral or aortic regurgitation (Table 2).

Family 2 (Figure 2A) was a British family with XMVD described by Newbury-Ecob et al in 1993. The proband (patient IV-1) was born with severe congenital valvular disease and died at 24 hours of age with severe cardiac failure. Autopsy showed dystrophy of all 4 valves and an atrial septal defect. His grandfather (patient II-2) underwent a triple valve replacement and closure of a persistent foramen ovale at age 41. At surgery, the grandfather’s mitral and aortic valves showed myxomatous dystrophy. The grandfather’s brother (patient II-1) was diagnosed as having mitral and aortic valvular disease at age 30.
TABLE 2. Echocardiographic Characteristics of the Affected Male Family Members and Heterozygous Female Family Members of XMVD Family 1b

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis</th>
<th>Year (Subject)</th>
<th>Mitral Prolapse</th>
<th>Aortic Regurgitation</th>
<th>Tricuspid Regurgitation</th>
<th>Pulmonary Regurgitation</th>
<th>Age and Type of Valve Surgery</th>
<th>Phenotypic Status</th>
<th>Genotypic Status</th>
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<td>Male</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>II-10</td>
<td>49</td>
<td>2005 (55)</td>
<td>AML/PML 5</td>
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<td>Moderate</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
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<td>50</td>
<td>1982 (50)</td>
<td>None ND</td>
<td>Moderate</td>
<td>Severe</td>
<td>None</td>
<td>None Ao replacement at 52</td>
<td>Affected</td>
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</tr>
<tr>
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<td>24</td>
<td>2005 (50)</td>
<td>None 5</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>N-3</td>
<td>21</td>
<td>2005 (27)</td>
<td>AML/PML 5</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>Female</td>
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<td></td>
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<tr>
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<td>1992 (86)</td>
<td>ND ND</td>
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<td>ND</td>
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<td>Affected</td>
<td>Heterozygous</td>
</tr>
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<td>None 3</td>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-1</td>
<td>29</td>
<td>2005 (34)</td>
<td>None 3</td>
<td>Moderate</td>
<td>None</td>
<td>Mild</td>
<td>None</td>
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<td>Heterozygous</td>
</tr>
<tr>
<td>IV-4</td>
<td>18</td>
<td>2005 (24)</td>
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<td>None</td>
<td>None</td>
<td>None</td>
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<td>Heterozygous</td>
</tr>
<tr>
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<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>N-7</td>
<td>26</td>
<td>2005 (32)</td>
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<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>V-2</td>
<td>6</td>
<td>2005 (6)</td>
<td>None ND</td>
<td>None</td>
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<td>None</td>
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</tr>
<tr>
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<td>None</td>
<td>ND</td>
<td>None</td>
<td>Unaffected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>V-5</td>
<td>23</td>
<td>2000 (23)</td>
<td>None ND</td>
<td>Mild</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Unaffected</td>
<td>Heterozygous</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

A male infant was the first child of healthy black African parents (family 3) (Figure 2B). He was diagnosed antenatally with abnormally thick cardiac valves by ultrasound and fetal echocardiography and was born at 38 weeks in good health. Postnatal echocardiography confirmed moderate tricuspid incompetence, trivial mitral and pulmonary incompetence, and mild aortic incompetence. All valves were thickened and dystrophic. At 4 months of age, his growth and developmental assessment were within normal limits and he showed no signs of cardiac failure. An echocardiogram showed excellent ventricular function. The mitral valve remained dystrophic without evidence of regurgitation and with only very mild aortic regurgitation. His mother was examined clinically and showed no evidence of cardiac involvement.

Family 4 (Figure 2C) was of Hong Kong Chinese origin. The 2 boys, 12 and 4 years old, had both mitral and aortic dystrophy. A heart murmur was identified in patient II-1 at age 4 months on a routine check. Subsequent echocardiography revealed he had polyvalvular disease with myxomatous thickening of the mitral tricuspid and aortic valves. He had significant mitral and tricuspid regurgitation with mild aortic regurgitation. Patient II-2 was identified because of his brother’s history. He was shown to have polyvalvular disease with mitral incompetence and stenosis, tricuspid regurgitation, and mild aortic regurgitation. Their mother had an essentially normal echocardiogram at age 38 with mild aortic and pulmonary incompetence.

All 4 families presented no clinically apparent extracardiac abnormalities, no dysmorphic features, and no epileptic seizures.

Linkage Analysis and Refined Mapping to Narrow the Locus

A significant linkage (Zmax = 8.6 at θ = 0 for DXS1108) was obtained for family 1 (Data Supplement Table). To refine the XMVD locus, additional markers were genotyped. Recombination at DXS10049 for unaffected male patient IV-54 of family 1a (Data Supplement Figure) set the centromeric boundary for the locus. Analysis of markers from members of family 1b revealed the same disease haplotype, with the exception of patient IV-3. This person, affected by valvular dystrophy but with normal coagulation factor activity, harbored recombination at microsatellite marker GAB3 (Data Supplement Figure), which set the telomeric boundary for the linked region and showed that valvulopathy and hemophilia were transmitted as independent traits. Therefore, the disease gene was located between DXS10049 and GAB3, spanned 2.5 Mb, and excluded the factor VIII gene.

Mutation Identification

After exclusion of candidate genes in the disease interval, eg, BGN, G4.5, ZNF185, CETN2, DUSP9, STK23, SSR4, RENBP, MGC29729, and several predicted genes, we examined FLNA with a direct sequencing approach. The gene is composed of 48 exons that span ~26 Kb immediately distal to the emerin gene. Mutational analysis of all coding regions of FLNA in affected members of family 1 (families 1a and 1b combined) revealed that all have a C→A transition at nucleotide 1910 in exon 13 (NM_001456: c.1910C>A), which predicts a missense mutation–substitution of a proline (P) for a glutamine (Q) at amino acid 637 (P637Q) (Figure 1D). In family 1, a total of 13 affected male family members and 30 female family members are mutation carriers. One clinically affected woman (patient IV-4) did not inherit the mutation and appeared to be a phenocopy. A G→A transition at nucleotide 1910 in exon 13 (NM_001456: c.1910G>A), which predicts a missense mutation of a valine (V) for an aspartic acid (D) at amino acid 711 (V711D), was found in the affected member of family 3 (Figure 2B). These sequence variations were not found in unaffected relatives of either family or in 500 control
chromosomes of white or African origin. The mutations were confirmed by testing for loss of a HaeIII (P637Q) and Sau96I (G288R) restriction sites and derived cleaved amplified polymorphic sequence (V711D), respectively (data not shown).

Finally, we identified a 1944-bp genomic deletion coding for exons 16 to 19, which corresponds to the 546-bp coding sequence (NT_025965.13:g.942144_944086del1944insTG) that predicts an in-frame deletion of 182 residues (from V761 to Q943) in 2 boys (family 4) diagnosed with plurivalvular dystrophy. The deletion was visualized by PCR analysis from intron 15 to 19 of the FLNA genomic sequence. The father (patient I-1) produced 1 fragment of 3216 bp, the heterozygous mother (patient I-2) produced 2 fragments of 3216 bp and 1272 bp, and the 2 affected boys (patients II-1 and II-2) produced 1 fragment of 1272 bp, which corresponds to the deletion of 1944 bp.

In addition to the mutations identified in FLNA, other known single-nucleotide polymorphisms were identified in the patients. These single-nucleotide polymorphisms occurred at frequencies comparable to those listed in the public National Center for Biotechnology Information (NCBI) database, some of them resulting in amino acid substitutions. No additional disease-associated variants were observed. Screening of 3 other families with potential X-linked valvular dystrophy did not find any mutation in FLNA.

Genotype-to-Phenotype Relations in Family 1

Genotype-to-Phenotype Relations in Male Family Members

In family 1, 13 male subjects were carriers of the P637Q mutation (Table 1). All male carriers had mitral valvulopathy, leading to mitral valve replacement or mitral valvuloplasty in 2 cases. Age for mitral surgery ranged from 20 to 51 years. All but 1 male carrier had mitral valve prolapse, found on the anterior valve in 4 patients (Figure 1B) and on both valves in 8 patients. None had an isolated posterior mitral valve prolapse. The thickness of the mitral valve was 5.3 ± 1.1 mm for the anterior valve and 4.4 ± 0.9 mm for the posterior valve. All but 1 male carrier also had aortic valve regurgitation. This regurgitation was considered mild in 6 cases, moderate in 3 cases, and severe in 3 cases that led to aortic valve replacement. There was no abnormality of the aortic root, and all aortic valves were tricuspid. Age for aortic valve surgery ranged from 17 to 52 years. Mild to moderate tricuspid valve regurgitation was found in 11 male carriers, and mild pulmonary regurgitation was found in 4 male carriers. However, there was no surgery of the tricuspid or pulmonary valves in the family.
Genotype-to-Phenotype Relations in Female Family Members
None of the 30 heterozygous women was symptomatic, and none underwent valvular surgery. Among female carriers of the P637Q mutation, 14 were considered affected, 12 were considered undetermined because of minor valve disease, and 4 were considered unaffected. Mitral valve prolapse was found in 4 cases; these involved the anterior valve in 3 cases and the posterior valve in 1 case. Among heterozygous women, 19 had mild mitral regurgitation and 4 had moderate mitral regurgitation. Mitral valve thickness was 3.3 ± 0.5 mm for the anterior valve and 2.8 ± 0.7 mm for the posterior valve. The anterior mitral valve (P < 0.001) and the posterior mitral valve (P < 0.001) were thicker in male than in female family members. Eight heterozygous women had mild aortic valve regurgitation, 3 had moderate aortic valve regurgitation, and 1 had severe aortic valve regurgitation. Nine heterozygous women had mild tricuspid valve regurgitation, and 1 had moderate tricuspid valve regurgitation. Two heterozygous women had mild pulmonary valve regurgitation, and 1 had moderate pulmonary valve regurgitation.

Structural Aspects of FLNA Mutations in XMVD
Sequence comparisons revealed that all 3 missense mutations modify highly conserved residues and affect the repeat consensus sequence, which includes residues that are common to at least 10 repeats and are highly conserved across a wide range of vertebrate filamins, as compared with consensus sequences derived from the repeating backbones of chicken filamin and Dictyostelium gelation factor (Figure 3A).18–21

The G288R, P637Q, and V711D mutations are located within the first, the fourth, and the fifth repeat, respectively, and the truncated protein corresponds to a smaller protein that lacks repeats 5 to 7 (Figure 3B).

The predicted 3-dimensional model of human filamin repeats shows remarkable similarity to Dictyostelium gelation factor in the overall 3-dimensional fold. The complete structure of filamin repeat consists of 7 antiparallel β-strands arranged in 2 β-sheets of 3 and 4 β-strands (Figure 3C). Modeling the 3-dimensional structure of the filamin repeat showed that the Gly288 residue is located on the external face of the repeat, whereas the Pro637 and Val711 residues are internal. For all mutants, the replacement of a nonpolar residue with a polar residue (basic, uncharged, and acidic for G288R, P637Q, and V711D, respectively) could account for an increase in polarity. It can be expected, therefore, that these mutations would cause a significant change in the structural conformation of the β-strands.

All 3 substitutions, located in the same region and predicted to impact the antiparallel β-strands organization of the protein, could result in impaired partner binding.

Discussion
In this study, we describe the identification of FLNA as the first gene responsible for a nonsyndromic valvular dystrophy.

The identification of this genetic defect transmitted with a X-linked recessive pattern was facilitated by a genealogical and geographic approach to the disease. Screening of our hospital files coupled with genealogical analysis of patients affected with myxomatous valvular dystrophy led to the identification of 2 kindreds from the same geographic location who have inherited the same mutation in the FLNA gene from a common ancestor born in the 18th century.

We have several lines of evidence to show that the P637Q mutation in the FLNA gene is the gene responsible for the valvular defect in family 1. Linkage analysis that includes all male and female patients gave a significant positive lod score, and all affected males are carriers of the mutation. Furthermore, screening of additional unrelated families affected by valvular dystrophy potentially transmitted with an X-linked recessive pattern identified 3 other mutations (G288R, V711D, and a 182–amino acid deletion) in the FLNA gene. None of these mutations was found in 500 control chromosomes. All missense mutations are highly conserved across filamin repeats and species.

Within the families with X-linked valvular dystrophy, the disease was inherited with complete penetrance in male family members and incomplete penetrance in female family members, with variable degrees of expression, consistent with different X-inactivation patterns. Among 13 male carriers of the P637Q mutation, 12 had typical mitral valve prolapse characterized by thicker leaflets associated with mild to severe aortic regurgitation, and 11 also had tricuspid regurgitation. Among 30 female carriers, 14 were considered affected with aortic or mitral valve abnormalities. Female mutation carriers had thicker mitral leaflets than normal women but were less severely affected than male patients (no valvular surgery).

Age for valvular surgery ranged from 17 to 52 years in family 1. Age at diagnosis was also highly variable. Within family 1, male family members were diagnosed between age 11 and 55 years. Within family 2, 2 patients (II-1 and II-2) were diagnosed with progressive polyvalvular dystrophy between age 25 and 30 years, and 1 patient (IV-1) was diagnosed at birth. In family 3, polyvalvular disorder was diagnosed antenatally, and it was diagnosed at age 4 and 12 years in family 4.

Filamin A is a ubiquitous phosphoprotein that X-links actin filaments and links the actin cytoskeleton to the plasma membrane by interacting with both actin and membrane proteins such as β-integrins.23,24 Filamin consists of an actin-binding domain at the N-terminus and 24 homologous repeats that correspond to the rod backbone of the protein.16,18 Each repeat consists of 7 antiparallel β-strands that are arranged in 2 β-sheets. Filamins exist in vivo as dimers mediated by interactions between C-terminal sequences.

In addition to its role as a structural component of the cytoskeleton, filamin A has also been implicated in regulating many cellular signaling pathways. Filamin A may contribute to the development of myxomatous changes of the cardiac valves by regulation of transforming growth factor-β (TGF-β) signaling through its interaction with Smads activated by TGF-β receptors.25,26 Defective signaling cascades that involve members of the TGF-β superfamily have been described in impaired remodeling.
of cardiac valves during development. Mice that lack the TGF-β and BMP signaling inhibitor Smad6 show hyperplasia of the cardiac valves.27 On the other hand, BMP6:BMP7 double mutants have hypoplastic valves.28-30 Remodeling of mitral valves might be caused by defects in proteins of the extracellular matrix, similar to cytoskeletal proteins and signaling pathways that mediate transmission between extracellular matrix proteins and the cytoskeleton in syndromic valvular dystrophy. Identification of new genes that cause mitral valve prolapse will allow us to confirm this hypothesis.

Distinct mutations of filamin genes produce different phenotypes. Mutations in FLNB and FLCN genes were identified in skeletal and muscular disorders, respectively.28,29 Mutations in the FLNA gene had previously been described in human periventricular nodular heterotopia (OMIM 300049) and a broad range of congenital malformations: otopalatodigital syndromes (OMIM 311300 and 305620), frontotemporal dysplasia (OMIM 300049), and X-linked myoxomatous valvar dysplasia (XMDV). Numbers above symbols indicate total number of mutations in a given repeat identified in the 5 different FLNA diseases. Most mutations are localized within the ABD and repeat 10. All 4 XMDV mutations are clustered between repeats 1 and 7. Modified from Feng and Walsh23 by permission from Macmillan Publishers Ltd. Copyright 2004. C, Homology model of human filamin repeats WT and mutants based on the crystal structure of Dictyostelium filamin repeat 4 (Protein Data Bank identification, 1WLH), Antiparallel β-strands are shown in yellow. Amino acids are shown in CPK configuration scale to 0.8. In the right panel, Gly288 is present on the surface of the repeat, whereas the other amino acids Pro637 and Val711 are present inside the repeat. In the left panel, the 3 mutants G288R, P637Q, and V711D are shown in orange, green, and red, respectively.

Figure 3. Sequence comparison of filamin repeats and positions of mutations. A. Amino acid sequence alignment of 24 repeats of human FLNA. Human, chicken, and Dictyostelium gelation factor (DGF) consensus sequences that include residues common to at least 10 repeats are listed at the bottom of the figure. Compared showed that the 3 mutations modify highly conserved residues. Mutations are indicated in red, conserved residues are indicated in black, and the deletion is highlighted in red. B, Schematic representation of filamin's actin-binding domain (ABD) and 24 repeats and summary of positions of mutations identified in periventricular heterotopia (PH), otopalatodigital syndromes (OPD), frontotemporal dysplasia (FMD), Melnick-Needles syndrome (MNS), and X-linked myoxomatous valvar dysplasia (XMDV). Numbers above symbols indicate total number of mutations in a given repeat identified in the 5 different FLNA diseases. Most mutations are localized within the ABD and repeat 10. All 4 XMDV mutations are clustered between repeats 1 and 7. Modified from Feng and Walsh23 by permission from Macmillan Publishers Ltd. Copyright 2004.
chromosomes of white or African origin. The mutations were confirmed by testing for loss of a HaeIII (P637Q) and Sau96I (G288R) restriction sites and derived cleaved amplified polymorphic sequence (V711D), respectively (data not shown).

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Genotype-to-Phenotype Relations in Family 1

Genotype-to-Phenotype Relations in Male Family Members

In family 1, 13 male subjects were carriers of the P637Q mutation (Table 1). All male carriers had mitral valvulopathy, leading to mitral valve replacement or mitral valvuloplasty in 2 cases. Age for mitral surgery ranged from 20 to 51 years. All but 1 male carrier had mitral valve prolapse, found on the anterior valve in 4 patients (Figure 1B) and on both valves in 8 patients. None had an isolated posterior mitral valve prolapse. The thickness of the mitral valve was 5.3 ± 1.1 mm for the anterior valve and 4.4 ± 0.9 mm for the posterior valve. All but 1 male carrier also had aortic valve regurgitation. This regurgitation was considered mild in 6 cases, moderate in 3 cases, and severe in 3 cases that led to aortic valve replacement. There was no abnormality of the aortic root, and all aortic valves were tricuspid. Age for aortic valve surgery ranged from 17 to 52 years. Mild to moderate tricuspid valve regurgitation was found in 11 male carriers, and mild pulmonary regurgitation was found in 4 male carriers. However, there was no surgery of the tricuspid or pulmonary valves in the family.


**CLINICAL PERSPECTIVE**

Familial inheritance has been demonstrated for mitral valve prolapse, the most common form of valvular dystrophy, and a familial study is recommended when a case is identified. Autosomal dominant transmission is the usual inheritance, with reduced penetrance and variable expressivity, and 3 loci have been mapped on chromosomes 11, 13, and 16. An X-linked myxomatous valvular dystrophy is a less frequent form of the disease and we have previously mapped it to Xq28, but the underlying genetic defect was not previously known. Clinically affected patients are predominantly male with either mitral or aortic valve defects, whereas female patients present with minor forms of valvular dystrophy. The present study demonstrates that a defect in the filamin A (*FLNA*) gene is responsible for X-linked valvulopathy. These findings could have important clinical implications. For example, the identification of the genetic basis for nonsyndromic valvular dystrophy offers a potential molecular diagnostic tool to detect patients at risk. Early identification of presymptomatic patients might allow a better clinical follow-up to prevent complication of the disease.
Atherosclerotic Renovascular Disease in Older US Patients Starting Dialysis, 1996 to 2001

Haifeng Guo, MS; Philip A. Kalra, MD, FRCP; David T. Gilbertson, PhD; Jiannong Liu, PhD; Shu-Cheng Chen, MS; Allan J. Collins, MD; Robert N. Foley, MB

Background—Temporal trends regarding the epidemiology of atherosclerotic renovascular disease (ARVD) in dialysis populations are poorly defined.

Methods and Results—United States Renal Data System data were used to identify patients aged 67 years or older at dialysis inception between 1996 and 2001 (n = 146,973). Medicare claims in the preceding 2 years were used to identify ARVD and revascularization procedures. Prior ARVD rose from 7.1% to 11.2% between 1996 and 2001 (adjusted odds ratio [AOR], 1.68). Other associations included hypertensive end-stage renal disease (ESRD; AOR, 2.21), ESRD network (AOR, 0.44 in network 17 versus 1.00 in network 1), peripheral vascular disease (AOR, 1.65), black race (AOR, 0.44), urologic cause of ESRD (AOR, 0.57), age >85 years (AOR, 0.58), substance dependency (AOR, 0.62), and inability to ambulate or transfer (AOR, 0.67). The proportion of ARVD patients undergoing revascularization rose from 14.6% to 16.7% between 1996 and 2001 (AOR, 1.27). Other associations included hypertension (AOR, 2.10), ESRD network (AOR, 2.07 for network 13 versus 1.00 in network 1), age >85 years (AOR, 0.53), and black race (AOR, 0.54). The rise in ARVD was not reflected in the proportion of patients with renovascular disease listed as cause of ESRD on the Medical Evidence Report at dialysis inception (5.5% in 1996, 5.0% in 2001).

Conclusions—ARVD diagnoses have become more common in older patients beginning dialysis therapy. The association of demographic factors including age, race, and geographic residence with utilization patterns suggests possible barriers to care. (Circulation. 2007;115:50-58.)

Key Words: atherosclerosis | epidemiology | kidney | revascularization

The number of patients receiving renal replacement therapy for end-stage renal disease (ESRD) is steadily increasing worldwide. Incidence rates almost doubled in the United States between 1991 and 2000 and are projected to increase by an additional 50% by 2015.1,2 The increasing burden of ESRD reflects in part the burgeoning numbers of elderly patients, for whom the risk of chronic kidney disease is several times that of younger patients.3 One would expect the changing age profile of the dialysis population to be accompanied by increases in the proportion of ESRD caused by diseases of aging, and atherosclerotic renovascular disease (ARVD) is a paradigmatic disease of aging. It has been shown to occur as an incidental finding in 6.8% of the elderly population,4 and an incidence rate of ≈ 3.7 cases per 1000 patient-years has been reported in the US Medicare population.5 ARVD is often associated with other vascular pathologies, such as atherosclerotic heart disease,6,7 congestive heart failure,8,9 cerebrovascular disease,10 and peripheral vascular disease.11,12

Clinical Perspective p 58

Few studies have examined the clinical epidemiology of ARVD in latter-day ESRD populations. Over a decade has elapsed since Mailloux and colleagues13 reported that 12.2% of their dialysis population had ARVD, a finding associated with higher than expected mortality rates. The purpose of the present study was to examine the clinical epidemiology of ARVD in dialysis patients at a national level, with emphasis on annual trends in disease burden.

Methods

Objectives The main objective of the present study was to examine annual trends in the proportion of patients starting dialysis therapy with ARVD. Other objectives included enumeration of the following associations of ARVD: annual trends in the use of revascularization, associations of revascularization, and prognostic associations, with or without revascularization.
Patients
We studied US patients with the following characteristics: (1) aged 67 years or older at initiation of maintenance dialysis therapy; (2) initiated dialysis therapy in the years 1996 through 2001; and (3) had Medicare as primary payer for at least 2 years before initiating dialysis therapy. This design was used to determine whether a diagnosis of ARVD, with or without revascularization, was made in the 2 years preceding dialysis initiation. All data were obtained from the United States Renal Data System (USRDS) database, which includes all patients entering the renal replacement therapy program. Patient characteristics at initiation of dialysis were obtained from the USRDS profile and the Centers for Medicare & Medicaid Services Medical Evidence Report (CMS-2728). The Medical Evidence Report was also used to identify patients with renovascular disease (renal artery stenosis, renal artery occlusion, cholesterol emboli, or renal emboli) listed as the primary renal disease at dialysis inception. Note that ARVD and renovascular disease as the primary renal disease are defined differently, with ARVD based on reimbursement claims before dialysis and renovascular disease as the primary renal disease based on physician ratings at the time dialysis began. Estimated glomerular filtration rate at initiation of dialysis was calculated from serum creatinine values from the Medical Evidence Report with the Modification of Diet in Renal Disease study formula14: Glomerular filtration rate = 186×(serum creatinine in mg/dL)\(^{-1.154}\)×(age\(^{-0.203}\))×(1.210 if black race)×0.742 (if female gender).

The USRDS database was linked to back-cast Medicare claims, a database containing all claims before dialysis initiation, including part A claims (inpatient, outpatient, home health agency, hospice, and skilled nursing services) and part B claims (physicians and suppliers). International Classification of Diseases, 9th Revision, Clinical Modification, and Current Procedural Terminology codes, shown in the Appendix (in the online-only Data Supplement), were used to define ARVD and renal revascularization before dialysis initiation and cardiovascular events after dialysis initiation. One or more appropriate codes were required to define ARVD and renal revascularization. Cardiovascular events after initiation of dialysis were defined as the presence of 1 inpatient hospitalization, skilled nursing facility, or home health agency code; 2 outpatient or physician/supplier codes; or 1 outpatient and 1 physician/supplier code <1 year apart. Dates of transplantation and death were obtained from the USRDS database.

Statistical Analysis
A \(\chi^2\) analysis was used for bivariable comparisons and logistic regression for multivariable comparisons of patients with and without ARVD. Comparisons of ARVD patients with and without revascularization were handled similarly. For outcome events after dialysis initiation, potential follow-up extended from dialysis initiation until December 31, 2002, with censoring at renal transplantation. Multivariable Cox proportional hazard models were used to test associations between ARVD and outcome events, adjusted for baseline characteristics. Findings were similar when analyses were replicated with varying lengths of study entry period; similarly, the findings were identical whether geographic network was treated as a fixed effect or a random effect. Hence, only findings from analyses that used a 2-year entry period and the treating geographic network as a fixed effect are reported here. SAS version 8.2 (SAS Institute Inc, Cary, NC) was used for data analysis.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Overall, 9.2% of the 146 973 study patients had a diagnosis of ARVD in the 2-year period before dialysis initiation (Table 1). The proportion of patients with prior ARVD rose during the study period, from 7.1% in 1996 to 11.2% in 2001, with a corresponding adjusted odds ratio (AOR) from multivariable analysis of 1.68 when 2001 was compared with 1996. Regarding the major groupings of primary cause of ESRD, hypertension was associated with an AOR of 2.21 compared with diabetes mellitus as the reference category.

Patients from ESRD network 17 (Northern California and Pacific Islands) were 0.44 times and those from network 13 (Arkansas, Louisiana, and Oklahoma) were 0.56 times as likely to have ARVD as those from network 1 (New England), which served as the reference group. Other main associations (arbitrarily defined as AOR >1.25 or <0.80) included a greater likelihood of ARVD with peripheral vascular disease (AOR, 1.65) and atherosclerotic heart disease (1.26) and a lower likelihood of ARVD with black race (0.44), other race (0.53), other urologic disease as the cause of ESRD (0.57), age >85 years (0.58), drug or alcohol dependency (0.62), Hispanic ethnicity (0.64), cystic kidney disease (0.66), inability to ambulate or transfer (0.67), body mass index >30 kg/m\(^2\) (0.69), and estimated glomerular filtration rate <5 mL·min\(^{-1}\)·1.73 m\(^2\) (0.70).

Of patients with ARVD, 16.2% underwent renal revascularization before beginning dialysis (Table 2). On multivariable analysis, revascularization was more likely later in the study period, especially in 2000 and 2001. Hypertension as the primary cause of ESRD showed the highest AOR for revascularization (2.10). The likelihood of revascularization also varied considerably on a regional basis. With network 1 (New England) as the reference category, revascularization was most likely in network 13 (Arkansas, Louisiana, and Oklahoma; AOR, 2.07), network 14 (Texas; 1.79), and network 6 (Georgia, North Carolina, and South Carolina; 1.72) and least likely in network 3 (New Jersey, Puerto Rico, and US Virgin Islands; 0.96), network 2 (New York; 1.00), and network 1 (New England; AOR 1.00 by definition). Associations with AOR >1.25 included hypertension as the primary cause of renal disease (2.10) and peripheral vascular disease (1.36). Associations with AOR <0.80 were cystic kidney disease (0.31), age >85 years (0.53), black race (0.54), cancer (0.69), inability to transfer or ambulate (0.72), body mass index <18.5 kg/m\(^2\) (0.72), Hispanic ethnicity (0.77), and glomerulonephritis (0.79).

Renovascular disease was listed as the primary cause of ESRD at dialysis initiation for 5.2% of subjects (Table 1), and 38.3% of these had prior ARVD, with a corresponding AOR of 5.38. Of ARVD patients with renovascular disease listed as primary cause of ESRD, 28.8% underwent revascularization (AOR, 2.66; Table 2). The Figure shows the proportions of patients with renovascular disease listed on the Medical Evidence Report as the primary cause of ESRD at initiation and with diagnostic claims indicating ARVD in the 2 years before dialysis. Although the former remained relatively static (5.5% in 1996 and 5.0% in 2001), the latter rose progressively (7.1% in 1996 and 11.2% in 2001).

Table 3 shows the prognostic associations of ARVD, which included greater likelihood of atherosclerotic heart disease (adjusted hazard ratio 1.28), congestive heart failure (adjusted hazard ratio 1.12), cerebrovascular accident or transient ischemic attack (adjusted hazard ratio 1.20), and peripheral vascular disease (adjusted hazard ratio 1.56) but not mortality (adjusted hazard ratio 0.94). Similar findings
**TABLE 1. Baseline Characteristics: Overall Population and Subgroup With ARVD**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (N=146 973), n (%)*</th>
<th>ARVD (N=13 462), %†</th>
<th>P‡</th>
<th>AOR (CI)</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of dialysis initiation</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1996</td>
<td>21 989 (15.0)</td>
<td>7.1</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>1997</td>
<td>23 446 (16.0)</td>
<td>8.5</td>
<td>...</td>
<td>1.18 (1.10–1.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1998</td>
<td>24 120 (16.4)</td>
<td>9.0</td>
<td>...</td>
<td>1.29 (1.20–1.38)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1999</td>
<td>24 963 (17.0)</td>
<td>9.7</td>
<td>...</td>
<td>1.41 (1.32–1.51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2000</td>
<td>25 811 (17.6)</td>
<td>9.1</td>
<td>...</td>
<td>1.33 (1.24–1.43)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2001</td>
<td>26 644 (18.1)</td>
<td>11.2</td>
<td>...</td>
<td>1.68 (1.57–1.80)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mode of dialysis</td>
<td>...</td>
<td>...</td>
<td>0.8</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>5595 (3.8)</td>
<td>10.1</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>141 378 (96.2)</td>
<td>9.1</td>
<td>...</td>
<td>0.99 (0.90–1.08)</td>
<td>0.8</td>
</tr>
<tr>
<td>Age, y</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>67–74</td>
<td>69 401 (47.2)</td>
<td>8.8</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>75–84</td>
<td>65 059 (44.3)</td>
<td>9.9</td>
<td>...</td>
<td>0.91 (0.88–0.95)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥85</td>
<td>12 513 (8.5)</td>
<td>7.3</td>
<td>...</td>
<td>0.58 (0.53–0.62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Male</td>
<td>73 301 (49.9)</td>
<td>9.4</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Female</td>
<td>73 672 (50.1)</td>
<td>8.9</td>
<td>...</td>
<td>1.21 (1.17–1.26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>White</td>
<td>107 267 (73.0)</td>
<td>10.7</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Black</td>
<td>32 789 (22.3)</td>
<td>5.0</td>
<td>...</td>
<td>0.44 (0.41–0.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other</td>
<td>6917 (4.7)</td>
<td>4.6</td>
<td>...</td>
<td>0.53 (0.47–0.60)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hispanic ethnicity</td>
<td>11 762 (8.0)</td>
<td>5.4</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>0.64 (0.59–0.70)</td>
</tr>
<tr>
<td>Primary cause of ESRD</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>60 164 (40.9)</td>
<td>6.5</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Hypertension</td>
<td>52 132 (35.5)</td>
<td>14.2</td>
<td>...</td>
<td>2.21 (2.10–2.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>9550 (6.5)</td>
<td>7.3</td>
<td>...</td>
<td>1.06 (0.97–1.17)</td>
<td>0.2</td>
</tr>
<tr>
<td>Cystic kidney disease</td>
<td>1760 (1.2)</td>
<td>4.8</td>
<td>...</td>
<td>0.66 (0.52–0.82)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Other urologic cause</td>
<td>2959 (2.0)</td>
<td>3.7</td>
<td>...</td>
<td>0.57 (0.46–0.69)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other/unknown/missing</td>
<td>20 408 (13.9)</td>
<td>6.2</td>
<td>...</td>
<td>0.88 (0.82–0.95)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Renovascular disease as primary cause of ESRD</td>
<td>7598 (5.2)</td>
<td>38.3</td>
<td>&lt;0.0001</td>
<td>5.38 (5.10–5.68)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>61 989 (42.2)</td>
<td>9.8</td>
<td>&lt;0.0001</td>
<td>0.96 (0.92–0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Atherosclerotic heart disease</td>
<td>54 601 (37.2)</td>
<td>11.7</td>
<td>&lt;0.0001</td>
<td>1.26 (1.21–1.31)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cardiac arrest or dysrhythmia</td>
<td>14 502 (9.9)</td>
<td>9.9</td>
<td>0.002</td>
<td>0.87 (0.82–0.93)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CVA/TIA</td>
<td>18 004 (12.2)</td>
<td>11.4</td>
<td>&lt;0.0001</td>
<td>1.14 (1.08–1.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>27 803 (18.9)</td>
<td>14.4</td>
<td>&lt;0.0001</td>
<td>1.65 (1.58–1.73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>66 789 (45.4)</td>
<td>7.5</td>
<td>&lt;0.0001</td>
<td>0.92 (0.87–0.96)</td>
<td>0.0008</td>
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<tr>
<td>COPD</td>
<td>15 201 (10.3)</td>
<td>11.9</td>
<td>&lt;0.0001</td>
<td>1.04 (0.98–1.10)</td>
<td>0.2</td>
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<tr>
<td>Current tobacco use</td>
<td>4813 (3.3)</td>
<td>12.5</td>
<td>&lt;0.0001</td>
<td>1.10 (1.00–1.21)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cancer</td>
<td>11 718 (8.0)</td>
<td>7.4</td>
<td>&lt;0.0001</td>
<td>0.80 (0.74–0.86)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alcohol or drug dependency</td>
<td>1113 (0.8)</td>
<td>5.6</td>
<td>0.002</td>
<td>0.62 (0.47–0.80)</td>
<td>0.003</td>
</tr>
<tr>
<td>Inability to ambulate or transfer</td>
<td>7899 (5.4)</td>
<td>6.4</td>
<td>&lt;0.0001</td>
<td>0.67 (0.61–0.74)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ESRD network§</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Network 1</td>
<td>6834 (4.6)</td>
<td>12.8</td>
<td>...</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Network 2</td>
<td>10 798 (7.3)</td>
<td>10.3</td>
<td>...</td>
<td>0.96 (0.87–1.06)</td>
<td>0.5</td>
</tr>
<tr>
<td>Network 3</td>
<td>7381 (5.0)</td>
<td>8.7</td>
<td>...</td>
<td>0.80 (0.71–0.89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 4</td>
<td>8480 (5.8)</td>
<td>11.1</td>
<td>...</td>
<td>0.92 (0.83–1.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>Network 5</td>
<td>9490 (6.5)</td>
<td>10.3</td>
<td>...</td>
<td>0.93 (0.84–1.03)</td>
<td>0.2</td>
</tr>
<tr>
<td>Network 6</td>
<td>12 059 (8.2)</td>
<td>7.2</td>
<td>...</td>
<td>0.68 (0.61–0.76)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
**TABLE 1. Continued**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (N=146 973), n (%)*</th>
<th>ARVD (N=13 462), %†</th>
<th>P‡</th>
<th>AOR (CI)</th>
<th>P§</th>
</tr>
</thead>
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<tr>
<td>Network 7</td>
<td>8668 (5.9)</td>
<td>12.1</td>
<td>...</td>
<td>0.95 (0.86–1.05)</td>
<td>0.3</td>
</tr>
<tr>
<td>Network 8</td>
<td>8285 (5.6)</td>
<td>8.8</td>
<td>...</td>
<td>0.79 (0.71–0.88)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 9</td>
<td>12 810 (8.7)</td>
<td>11.2</td>
<td>...</td>
<td>0.91 (0.83–0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Network 10</td>
<td>7277 (5.0)</td>
<td>8.4</td>
<td>...</td>
<td>0.68 (0.60–0.76)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 11</td>
<td>11 739 (8.0)</td>
<td>10.0</td>
<td>...</td>
<td>0.79 (0.72–0.87)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 12</td>
<td>7140 (4.9)</td>
<td>8.7</td>
<td>...</td>
<td>0.63 (0.57–0.71)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 13</td>
<td>6499 (4.4)</td>
<td>6.6</td>
<td>...</td>
<td>0.56 (0.49–0.63)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 14</td>
<td>9206 (6.3)</td>
<td>6.3</td>
<td>...</td>
<td>0.57 (0.51–0.64)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 15</td>
<td>4748 (3.2)</td>
<td>8.3</td>
<td>...</td>
<td>0.74 (0.65–0.84)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Network 16</td>
<td>3538 (2.4)</td>
<td>8.7</td>
<td>...</td>
<td>0.64 (0.55–0.74)</td>
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<td>Network 17</td>
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<td>Hemoglobin, g/L</td>
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<td>eGFR, mL · min⁻¹ · 1.73 m⁻²</td>
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<td>0.70 (0.66–0.76)</td>
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<td>&gt;10.0</td>
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<td>Body mass index, kg/m²</td>
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<td>18.5–24.9</td>
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<tr>
<td>25–30</td>
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</table>

**CVA/TIA** indicates cerebrovascular accident or transient ischemic attack; **COPD** chronic obstructive pulmonary disease; and **eGFR**, estimated glomerular filtration rate.

*Percentage of 146 973; percentages within column add to 100%.
†Percentage within rows.
‡With the χ² test, with no ARVD as comparison group.
§Multiple logistic regression model including all variables in column 1 except renovascular disease as primary cause of ESRD. Reference groups: incident year 1996, peritoneal modality, age 67 to 74 years, male gender, white race, non-Hispanic ethnicity, diabetes as primary cause of ESRD, no comorbid conditions, network 1, hemoglobin 110–120 g/L, eGFR 5.0–10.0 mL · min⁻¹ · 1.73 m⁻², serum albumin 30–35 g/L, and body mass index 18.5–24.9 kg/m².
¶Multiple logistic regression model including all variables in column 1, except primary cause of ESRD. Reference groups same as for footnote †, other diseases as primary cause of ESRD.

*ESRD networks: 1, Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont; 2, New York; 3, New Jersey, Puerto Rico, US Virgin Islands; 4, Delaware, Pennsylvania; 5, District of Columbia, Maryland, Virginia, West Virginia; 6, Georgia, North Carolina, South Carolina; 7, Florida; 8, Alabama, Mississippi, Tennessee; 9, Indiana, Kentucky, Ohio; 10, Illinois; 11, Michigan, Minnesota, North Dakota, South Dakota, Wisconsin; 12, Iowa, Kansas, Missouri, Nebraska; 13, Arkansas, Louisiana, Oklahoma; 14, Texas; 15, Arizona, Colorado, Nevada, New Mexico, Utah, Wyoming; 16, Alaska, Idaho, Montana, Oregon, Washington; 17, American Samoa, Guam, Hawaii, northern counties of California, Saipan; 18, southern counties of California.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All ARVD (N=13,462), n (%)</th>
<th>Revascularization (N=2,186), %</th>
<th>P‡</th>
<th>AOR (CI)</th>
<th>P§</th>
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<td>67–74</td>
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<td>≥85</td>
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<td>0.01</td>
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<td>Male</td>
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<td>Glomerulonephritis</td>
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<td>0.79 (0.58–1.07)</td>
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<td>0.31 (0.10–1.00)</td>
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<td>Cardiac arrest or dysrhythmia</td>
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<td>0.80 (0.37–1.72)</td>
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<td>Inability to ambulate/transfer</td>
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<td>Revascularization (N=2186), %†</td>
<td>P‡</td>
<td>AOR (CI)</td>
<td>P§</td>
</tr>
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Hemoglobin, g/L

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eGFR mL · min⁻¹ · 1.73 m⁻²

<p>| | | | | | | |</p>
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<td>5.0–10.0</td>
<td>7329 (54.4)</td>
<td>15.5</td>
<td>...</td>
<td>1.01 (0.84–1.03)</td>
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<tr>
<td>&gt;10.0</td>
<td>4939 (36.7)</td>
<td>16.8</td>
<td>...</td>
<td>1.21 (0.82–1.76)</td>
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<tr>
<td>Missing</td>
<td>186 (1.4)</td>
<td>15.1</td>
<td>...</td>
<td>0.87 (0.70–1.01)</td>
<td>0.06</td>
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</tbody>
</table>

Serum albumin, g/L

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<tr>
<td>&lt;30</td>
<td>2255 (16.8)</td>
<td>15.4</td>
<td>...</td>
<td>0.90 (0.74–1.08)</td>
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<tr>
<td>30–35</td>
<td>1283 (9.5)</td>
<td>17.6</td>
<td>...</td>
<td>0.84 (0.70–1.01)</td>
<td>0.06</td>
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<tr>
<td>&gt;35</td>
<td>2994 (22.2)</td>
<td>15.8</td>
<td>...</td>
<td>0.87 (0.70–1.03)</td>
<td>0.1</td>
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<tr>
<td>Missing</td>
<td>6930 (51.5)</td>
<td>16.4</td>
<td>...</td>
<td>0.87 (0.70–1.03)</td>
<td>0.1</td>
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Body mass index, kg/m²

<p>| | | | | | | |</p>
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<thead>
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<tbody>
<tr>
<td>&lt;18.5</td>
<td>1128 (8.4)</td>
<td>13.1</td>
<td>...</td>
<td>0.72 (0.59–0.86)</td>
<td>0.0005</td>
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<tr>
<td>18.5–24.9</td>
<td>6504 (48.3)</td>
<td>17.2</td>
<td>...</td>
<td>1.00 (0.83–1.20)</td>
<td>0.12</td>
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<tr>
<td>25–30</td>
<td>3348 (24.9)</td>
<td>15.9</td>
<td>...</td>
<td>0.95 (0.84–1.06)</td>
<td>0.4</td>
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</tr>
<tr>
<td>&gt;30</td>
<td>1590 (11.8)</td>
<td>14.0</td>
<td>...</td>
<td>0.86 (0.73–1.01)</td>
<td>0.07</td>
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<tr>
<td>Missing</td>
<td>892 (6.6)</td>
<td>18.3</td>
<td>...</td>
<td>1.04 (0.85–1.27)</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

CVA/TIA indicates cerebrovascular accident or transient ischemic attack; COPD, chronic obstructive pulmonary disease; and eGFR, estimated glomerular filtration rate.

*Percentage of 13 462; percentages within column add to 100%.
†Percentages within rows add to 100%.
‡With the χ² test, with no revascularization as comparison group.
§Multiple logistic regression model including all variables in column 1 except renovascular disease as primary cause of ESRD. Reference groups: incident year 1996, peritoneal modality, age 67 to 74 years, male gender, white race, non-Hispanic ethnicity, diabetes as primary cause of ESRD, no comorbid conditions, network 1, hemoglobin 110–120 g/L, eGFR 5.0–10.0 mL · min⁻¹ · 1.73 m⁻², serum albumin 30–35 g/L, and body mass index 18.5–24.9 kg/m².
¶ESRD networks: 1, Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont; 2, New York; 3, New Jersey, Puerto Rico, US Virgin Islands; 4, Delaware, Pennsylvania; 5, District of Columbia, Maryland, Virginia, West Virginia; 6, Georgia, North Carolina, South Carolina; 7, Florida; 8, Alabama, Mississippi, Tennessee; 9, Indiana, Kentucky, Ohio; 10, Illinois; 11, Michigan, Minnesota, North Dakota, South Dakota, Wisconsin; 12, Iowa, Kansas, Missouri, Nebraska; 13, Arkansas, Louisiana, Oklahoma; 14, Texas; 15, Arizona, Colorado, Nevada, New Mexico, Utah, Wyoming; 16, Alaska, Idaho, Montana, Oregon, Washington; 17, American Samoa, Guam, Hawaii, northern counties of California, Saipan; 18, southern counties of California.
were seen in an analysis comparing patients with and without renovascular disease listed on the Medical Evidence Report as the primary cause of ESRD. Table 3 also shows an analysis in which ARVD with and without revascularization was compared with the reference category of patients without ARVD. These analyses suggested that ARVD patients who had undergone revascularization before initiation of dialysis had a lower likelihood of death and a greater likelihood of atherosclerotic heart disease, cerebrovascular accident or transient ischemic attack, and peripheral vascular disease than those who had not undergone revascularization.

**Discussion**

The proportion of elderly US patients commencing dialysis with a prior diagnosis of ARVD rose steadily during the 5-year study period. Hypertensive ESRD was the most frequent primary disease category associated with ARVD, whereas the main comorbid factors associated with a greater likelihood of ARVD were peripheral vascular disease, and atherosclerotic heart disease. The variation of ARVD prevalence across the dialysis networks was striking. Overall, 16.2% of ARVD patients had renal revascularization before beginning dialysis, and as with ARVD prevalence, the proportion undergoing revascularization rose throughout the study period. The disparity between claims diagnoses and the listed cause of ESRD at inception was notable. Although ARVD was associated with higher rates of cardiovascular disease on dialysis therapy, mortality rates were marginally lower, a finding that is difficult to explain. Among ARVD patients, revascularization exhibited a similar pattern: association with lower death rates but higher rates of cardiovascular events.

We found that the proportion of patients with diagnostic claims indicating prior ARVD was almost twice the proportion with renovascular disease listed as the primary cause of ESRD. Many cases of ARVD may in fact be incidental and causally unrelated to any accompanying renal failure. Also, accurate attribution of the cause of ESRD is often difficult, especially in patients with vascular diseases, who often have increased burdens of risk factors for parenchymal renal

Proportions of patients with renovascular disease listed as primary cause of ESRD at dialysis initiation (——) and with diagnostic claims indicating atherosclerotic renovascular disease in the 2 years before dialysis initiation (—-).

---

**TABLE 3. Prognostic Associations of ARVD, Revascularization After ARVD, and Renovascular Disease as Primary Cause of ESRD (Follow-Up to December 31, 2002)**

<table>
<thead>
<tr>
<th>ARVD</th>
<th>Death</th>
<th>Atherosclerotic Heart Disease</th>
<th>Congestive Heart Failure</th>
<th>Cerebrovascular Accident or Transient Ischemic Attack</th>
<th>Peripheral Vascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (ref)</td>
<td>AHR (CI)*</td>
<td><em>P</em></td>
<td>AHR (CI)*</td>
<td><em>P</em></td>
<td>AHR (CI)*</td>
</tr>
<tr>
<td>102,145</td>
<td></td>
<td>0.92 (0.89–0.95)</td>
<td>&lt;0.0001</td>
<td>1.24 (1.21–1.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Event rate per 1000 patient-years</td>
<td>348</td>
<td>0.98 (0.95–0.99)</td>
<td>&lt;0.0001</td>
<td>1.26 (1.24–1.29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ARVD</td>
<td>No (ref)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes†</td>
<td>0.94 (0.92–0.96)</td>
<td>&lt;0.0001</td>
<td>1.12 (1.10–1.14)</td>
<td>&lt;0.0001</td>
<td>1.20 (1.16–1.23)</td>
</tr>
<tr>
<td>Revascularization</td>
<td>No‡</td>
<td>0.95 (0.93–0.98)</td>
<td>&lt;0.0001</td>
<td>1.11 (1.09–1.14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Yes‡</td>
<td>0.88 (0.83–0.93)</td>
<td>&lt;0.0001</td>
<td>1.17 (1.13–1.23)</td>
<td>&lt;0.0001</td>
<td>1.30 (1.23–1.38)</td>
</tr>
<tr>
<td>Renovascular disease as primary cause of ESRD</td>
<td>No (ref)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>0.90 (0.89–0.95)</td>
<td>&lt;0.0001</td>
<td>1.07 (1.04–1.10)</td>
<td>&lt;0.0001</td>
<td>1.21 (1.17–1.26)</td>
</tr>
</tbody>
</table>

AHR indicates adjusted hazard ratio; and ref, reference group.

*Proportional hazards model including all variables in column 1 of Table 1 except renovascular disease as primary cause of ESRD.
†ARVD as 2 categories: no (reference group) and yes (ARVD patients with and without revascularization as a single group).
‡ARVD as 3 categories: no (reference group), ARVD with no revascularization, and ARVD with revascularization.
in the nondialyzed general Medicare population.5

marginally lower death rates than their respective compara-
however, ARVD and revascularization were associated with
were compared with those who had not. Paradoxically,
when ARVD patients who had undergone revascularization
of cardiovascular events, an association pattern replicated
the proportion of ARVD patients undergoing revascularization.

Paralleling the increase in ARVD prevalence recorded
in the 5-year study period was a modest stepwise increase in
the use of renal revascularization among ARVD
patients who commenced dialysis (14% greater likelihood in
2001 than in 1996). Although case series have demonstrated
that a small proportion of patients with critical ARVD can be
rescued from dialysis by renal revascularization procedures,18–20 to date, there is no randomized controlled trial
evidence that revascularization can improve renal functional
outcome, and especially prevent dialysis need, in ARVD
patients.21–24 As also noted in the elderly nondialysis Medi-
care population, revascularization was performed in ≈16% of
all patients diagnosed with ARVD,4 and selection for revas-
cularization appeared to be a nonrandom process.

Several associations suggested that the utilization of diag-
nostic and therapeutic resources may have been influenced by
nonbiological factors. For example, age >75 years, especially
age >85 years, and black race were associated with substan-
tially lower likelihoods of ARVD and substantially lower
likelihoods of revascularization when ARVD was diagnosed.

Evidence that revascularization can improve renal functional
outcome, and especially prevent dialysis need, in ARVD
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nostic and therapeutic resources may have been influenced by
nonbiological factors. For example, age >75 years, especially
age >85 years, and black race were associated with substan-
tially lower likelihoods of ARVD and substantially lower
likelihoods of revascularization when ARVD was diagnosed.
Similarly, ESRD networks showed substantial variability in
the proportion of patients diagnosed with ARVD and in the
proportion of ARVD patients undergoing revascularization.

We found that ARVD was associated with increased rates
of cardiovascular events, an association pattern replicated
when ARVD patients who had undergone revascularization
were compared with those who had not. Paradoxically,
however, ARVD and revascularization were associated with
marginally lower death rates than their respective comparators.
Similar findings were seen among ARVD patients
identified in the nondialyzed general Medicare population.5
Although a clear explanation cannot be provided, survivor
bias may explain the paradox of higher cardiovascular disease
event rates and lower death rates while undergoing dialysis.
Thus, patients with ARVD who survive to ESRD may be
resistant to death from cardiovascular disease in ways yet to
be determined. Similarly, selection of intrinsically healthier
patients could explain the lower mortality seen in ARVD
patients who underwent revascularization before dialysis
inception. Clearly, careful prospective studies are needed to unravel this paradox. In this regard, Mailoux and col-
leagues13 found 5-year survival of ARVD patients as low as
18%, lower than all other disease categories except diabetes mellitus.

The limitations of the present study should be mentioned.
It was retrospective, and many of the major study outcomes
were based on administrative claims data. The validity of
using ARVD claims to establish ARVD could not be ascer-
tained. Other studies, however, suggest that diagnoses based
on administrative claims tend to be specific but insensitive in
patients with chronic kidney disease.25 Conceivably, ARVD
may often be an incidental finding during the diagnostic
workup in patients with disease in nonrenal vascular beds.
Medicare coverage is available to the older US patient
population, and one should exercise caution in generalizing
the present findings to younger populations or other coun-
tries. Definitions of comorbidity were based on the Medical
Evidence Report. Although this has been shown to underes-
timate disease burdens,26 the error is unlikely to differ
dramatically from year to year or between populations with
and without ARVD.

Despite its limitations, we believe that the present study
provides useful information. It suggests that ARVD is a
common entity in older dialysis patients in the United States.
It also suggests that although ARVD may be among the most
rapidly rising causes of ESRD, current data systems are
poorly designed to capture this disturbing trend. Most strik-
ingly, it suggests considerable variation in the frequency of
ARVD diagnosis and the use of renal revascularization for
ARVD treatment among the geographic ESRD networks and
in certain subgroups of the US population.

Acknowledgments

The authors thank Beth Forrest and Nan Booth, MSW, MPH, of the
USRDS Coordinating Center for manuscript preparation and editing,
respectively.

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was performed as a deliverable under contract No. N01-DK-9-2343
(National Institute of Diabetes and Digestive and Kidney Diseases,
National Institutes of Health, Bethesda, Md).

Disclosures

None.

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Collins AJ, Foley RN. Atherosclerotic renovascular disease in United
States patients aged 67 years or older: risk factors, revascularization, and


**CLINICAL PERSPECTIVE**

Shared risk factors and societal aging lead us to hypothesize that atherosclerotic renovascular disease (ARVD) might account for a growing proportion of end-stage renal disease that requires dialysis therapy in the United States. We looked for a diagnosis of ARVD in the preceding 2 years in 146,973 older patients who started dialysis in the United States between 1996 and 2001. Prior ARVD was more common in successive years, rising from 7.1% in 1996 to 11.2% in 2001. Diagnostic and therapeutic management of ARVD varied substantially by geographic location. Although mortality rates in ARVD patients were similar to those without ARVD, ARVD patients who underwent revascularization had lower mortality rates than those who did not. The present study suggests that ARVD may be a rapidly emerging cause of end-stage renal disease in the United States. Viewed in the context of an aging society, the rising burden of disease suggests that ARVD may become a principal cause of end-stage renal disease in the future.
Sildenafil Improves Exercise Hemodynamics and Oxygen Uptake in Patients With Systolic Heart Failure

Gregory D. Lewis, MD; Justine Lachmann, MD; Janice Camuso, RN; John J. Lepore, MD; Jordan Shin, MD, PhD; Maryann E. Martinovic, BS; David M. Systrom, MD; Kenneth D. Bloch, MD; Marc J. Semigran, MD

Background—Heart failure (HF) is frequently associated with dysregulation of nitric oxide–mediated pulmonary vascular tone. Sildenafil, a type 5 phosphodiesterase inhibitor, lowers pulmonary vascular resistance in pulmonary hypertension by augmenting intracellular levels of the nitric oxide second messenger, cyclic GMP. We tested the hypothesis that a single oral dose of sildenafil (50 mg) would improve exercise capacity and exercise hemodynamics in patients with chronic systolic HF through pulmonary vasodilation.

Methods and Results—Thirteen patients with New York Heart Association class III HF underwent assessment of right heart hemodynamics, gas exchange, and first-pass radionuclide ventriculography at rest and with cycle ergometry before and 60 minutes after administration of 50 mg of oral sildenafil. Sildenafil reduced resting pulmonary arterial pressure, systemic vascular resistance, and pulmonary vascular resistance, and increased resting and exercise cardiac index (P<0.05 for all) without altering mean arterial pressure, heart rate, or pulmonary capillary wedge pressure. Sildenafil reduced exercise pulmonary arterial pressure, pulmonary vascular resistance, and pulmonary vascular resistance/systemic vascular resistance ratio, which indicates a selective pulmonary vasodilator effect with exercise. Peak VO₂ increased (15±9%) and ventilatory response to CO₂ output (Ve/VCO₂ slope) decreased (16±5%) after sildenafil treatment. Improvements in right heart hemodynamics and exercise capacity were confined to patients with secondary pulmonary hypertension (rest pulmonary arterial pressure >25 mm Hg).

Conclusions—The present study shows that in patients with systolic HF, type 5 phosphodiesterase inhibition with sildenafil improves peak VO₂, reduces Ve/VCO₂ slope, and acts as a selective pulmonary vasodilator during rest and exercise in patients with HF and pulmonary hypertension. (Circulation. 2007;115:59-66.)

Key Words: cyclic GMP ■ heart failure ■ pulmonary hypertension ■ sildenafil ■ type 5 phosphodiesterase
congenital systemic-to-pulmonary shunts. In patients with PH secondary to chronic HF, sildenafil lowers resting PVR and PCWP and increases CI without causing systemic hypotension.

Abnormal systemic vascular tone is also a hallmark of HF, which contributes to diminished skeletal muscle perfusion and heightened systemic vascular resistance (SVR). Sildenafil has been shown to improve endothelium-dependent, flow-mediated brachial artery dilation in patients with HF. Furthermore, sildenafil may improve nonuniformity in skeletal muscle unit perfusion that predisposes HF patients to early anaerobic metabolism during exercise. Hence, multiple mechanisms exist by which PDE5 inhibition may augment exercise capacity and ameliorate symptoms in patients with HF.

Sildenafil has previously been shown to increase peak VO₂ and increase the efficiency of ventilatory response to CO₂ output (Ve/VCO₂, slope) in patients with predominantly New York Heart Association class II HF. However, no studies to date have investigated the effects of sildenafil on exercise hemodynamic measurements and peripheral oxygen extraction, variables which may provide insight into the mechanisms by which sildenafil improves exercise capacity in HF. We carried out simultaneous measurement of exercise capacity and hemodynamics in response to sildenafil to evaluate the short-term effects of this agent on RV and LV performance and pulmonary and systemic vascular tone. We tested the hypothesis that administration of the pulmonary vasodilator sildenafil can augment exercise capacity by improving RV function in patients with class III HF.

Methods

The study population included patients with LVSD (LVEF <0.35) and stable New York Heart Association class III HF for at least 3 months despite maximal medical therapy who were referred to the Massachusetts General Hospital Heart Failure Service. Patients with provokable ischemia, chronic obstructive pulmonary disease, or ongoing nitrate therapy were excluded. Data compiled for each patient included clinical history and physical examination, exercise test results, radionuclide ventriculography, and spirometry. The study protocol was approved by the Subcommittee of the Massachusetts General Hospital on Human Studies, and informed consent was obtained from all patients.

Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) with upright cycle ergometry and respiratory gas exchange was performed with previously reported methods. In brief, exercise testing was carried out after an overnight fast with a 6.25 to 12.5 W/min incremental ramp protocol. Breath-to-breath respiratory gas exchange was continuously measured with a metabolic cart interfaced to the ergometer (Medical Graphics Corp., St. Paul, Minn). Minute ventilation (Ve), oxygen uptake (VO₂), CO₂ output (VCO₂), and respiratory exchange ratio were calculated. Peak VO₂ was defined as the highest VO₂ measured during the last minute of symptom-limited exercise. The ventilatory anaerobic threshold (AT) was determined by the V-slope method. Ventilatory efficiency was assessed by calculation of the slope of the increase in ventilation with respect to CO₂ output (Ve/VCO₂) with values measured between rest and AT.

First-Pass Radionuclide Ventriculography and Hemodynamic Measurements

Rest and exercise first-pass radionuclide ventriculography of both ventricles were performed at the time of cycle ergometry as previously described. In brief, a multicrystal camera (System 77; Baird Corp., Bedford, Mass) was used to detect technetium-labeled red blood cells in a region of interest placed over either the left or right ventricle. Volumetric measurements were taken as the average of values from 6 to 8 consecutive heartbeats and were indexed to body size by dividing by the calculated body surface area. The EF of each ventricle was calculated as (end-diastolic counts – end-systolic counts)/end-diastolic counts.

Right heart catheterization was performed by insertion of a 7F balloon-tipped triple-lumen pulmonary artery catheter via the right internal jugular vein before the second CPET. Right atrial, RV, pulmonary arterial, and pulmonary capillary wedge pressures were measured, and cardiac output was determined with the Fick oxygen technique. RV stroke work index (gm-m/m²) was calculated as the stroke volume index (ml/m²) × [mean PAP – RAP] mm Hg × 0.0136. A 22-gauge radial arterial catheter was placed for continuous measurement of mean arterial pressure, and lactic acid and blood gas assessments were performed at 1-minute intervals during CPET. SVR, PVR, and CI were calculated with standard formulas. PH was defined by resting PAP >25 mm Hg.

Measurement of Serum Lactate and Sildenafil Levels

Systemic arterial blood samples were deproteinated and enzymatically assayed for lactate concentration using an Analox Instruments LM3 analyzer (London, England). Serum sildenafil levels were measured by liquid chromatography-tandem mass spectrometry (Covance Laboratories, Indianapolis, Ind) as previously described.

Study Protocol

All patients underwent a baseline maximal CPET (CPET #1). Two days (±2 days) after CPET #1, patients underwent placement of a pulmonary artery catheter and arterial line, followed by repeat CPET with continuous hemodynamic monitoring (CPET #2; Figure 1). During CPET #2, patients were permitted to exercise only until they reached 90% of their VO₂ at which their AT occurred in CPET #1. This threshold was chosen to permit complete recovery and repeat exercise testing with the pulmonary artery catheter in place for a limited period of time, so as to minimize potential risks to patients.

Patients underwent a 120-minute rest period in between their submaximal exercise test (CPET #2) and their final maximal CPET (CPET #3). All patients received a single oral dose of sildenafil (50 mg) 60 minutes before the start of exercise in CPET #3. Blood
TABLE 1. Baseline Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>No.</td>
<td>13</td>
</tr>
<tr>
<td>Age, mean y ± SEM</td>
<td>47±9 (33 to 59)</td>
</tr>
<tr>
<td>Cause</td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>6</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>7</td>
</tr>
<tr>
<td>Gender, M:F</td>
<td>11:2</td>
</tr>
<tr>
<td>Heart failure pharmacotherapy, n/N</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor or angiotensin receptor blocker</td>
<td>11/13</td>
</tr>
<tr>
<td>β-Adrenergic receptor antagonist</td>
<td>9/13</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>6/13</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>6/13</td>
</tr>
<tr>
<td>Weight, mean kg ± SEM</td>
<td>87.3±3.3 (70.0 to 102.2)</td>
</tr>
<tr>
<td>LVEF, mean±SEM</td>
<td>0.32±0.02 (20 to 38)</td>
</tr>
<tr>
<td>RVEF, mean±SEM</td>
<td>0.33±0.03 (16 to 50)</td>
</tr>
<tr>
<td>FVC, mean % predicted ± SEM</td>
<td>79±5.9 (52 to 114)</td>
</tr>
<tr>
<td>FEV1, mean % predicted ± SEM</td>
<td>74±5.4 (51 to 116)</td>
</tr>
<tr>
<td>DLco mean % predicted ± SEM</td>
<td>79.8±4.2 (56 to 110)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second; and DLco pulmonary diffusing capacity. Numbers in parentheses indicate the range of values.

All measurements are presented as means±SEM. Measurements of gas exchange variables were compared between CPETs #1 and #3 before and after the administration of sildenafil. Measurements of hemodynamic variables during CPET #3 before sildenafil administration were compared with hemodynamic measurements during the submaximal portion of CPET #3 at a matched workload. Paired Student t tests were used to test hypotheses that compared before and after sildenafil administration outcomes within subjects for continuous variables that were demonstrated to be normally distributed with the Wilk-Shapiro test. Unpaired Student t tests were used for between-group comparisons of normally distributed continuous variables. The relationship between changes in exercise variables after sildenafil treatment and hemodynamic measurements were assessed with a Pearson correlation coefficient. A probability value ≤0.05 was accepted as statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Population

Thirteen patients with New York Heart Association class III HF participated in the present study. Baseline patient characteristics, medications, ventriculographic measurements, and pulmonary function tests are shown in Table 1. All patients had undergone optimization of their HF pharmacotherapy in the 30 days before the study. None of the patients had evidence of a significant underlying pulmonary disease. Mean resting PAP and PCWP before sildenafil administration were elevated at 28±4 mm Hg and 14±4 mm Hg, respectively, and CI was depressed at 1.8 L/min per m². PVR was 290±36 dyne-sec/cm⁵ (Table 2) and 7 of the patients had resting PH.

As assessed in CPET #1, exercise capacity was depressed as indicated by peak VO₂ of 1015±84 mL/min and a peak workload of 83±7 W.

Effects of Sildenafil on Hemodynamic Measurements

Lactate levels immediately before CPET #2 and #3 were similar (1.8±0.2 versus 1.7±0.2 mmol/L), which suggests that metabolic recovery occurred before CPET #3. The mean sildenafil level immediately before the start of CPET #3 was 237±23 ng/mL, which is similar to levels reported in pharmacokinetic studies in normal individuals.20,21 Sildenafil was well tolerated by all patients; specifically there was no symptomatic hypotension, facial flushing, or vision changes.

TABLE 2. Hemodynamic Values at Rest and During Exercise Before and After Sildenafil Administration

<table>
<thead>
<tr>
<th></th>
<th>CPET #2 Rest</th>
<th>Sildenafil Rest</th>
<th>%Δ</th>
<th>t × (df)</th>
<th>df=12</th>
<th>P</th>
<th>CPET #2 Exercise</th>
<th>Sildenafil Exercise</th>
<th>%Δ</th>
<th>t × (df)</th>
<th>df=12</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>76±3</td>
<td>73±3</td>
<td>±3</td>
<td>1.32</td>
<td>0.21</td>
<td>80±3</td>
<td>78±3</td>
<td>0±3</td>
<td>0.55</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats × min⁻¹</td>
<td>76±4</td>
<td>77±5</td>
<td>±2</td>
<td>0.27</td>
<td>0.66</td>
<td>91±6</td>
<td>91±6</td>
<td>0±3</td>
<td>0.03</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>4±1</td>
<td>5±1</td>
<td>±2</td>
<td>0.59</td>
<td>0.56</td>
<td>9±2</td>
<td>7±1</td>
<td>13±7</td>
<td>-1.73</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PAP, mm Hg</td>
<td>28±4</td>
<td>22±2</td>
<td>±3</td>
<td>-2.25</td>
<td>0.03</td>
<td>36±5</td>
<td>31±4</td>
<td>-10±3</td>
<td>-2.55</td>
<td>0.03</td>
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<td></td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>14±4</td>
<td>12±2</td>
<td>±5</td>
<td>-0.80</td>
<td>0.19</td>
<td>19±3</td>
<td>18±3</td>
<td>-6±5</td>
<td>-1.13</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVR, dyne · s · cm²</td>
<td>290±36</td>
<td>215±25</td>
<td>±7</td>
<td>-2.98</td>
<td>0.007</td>
<td>280±62</td>
<td>192±30</td>
<td>-21±5</td>
<td>4.24</td>
<td>0.04</td>
<td></td>
<td></td>
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<tr>
<td>SVR, dyne · s · cm²</td>
<td>1700±133</td>
<td>1374±176</td>
<td>±4</td>
<td>-4.70</td>
<td>0.0005</td>
<td>1102±91</td>
<td>990±78</td>
<td>-8±5</td>
<td>-1.42</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVR/SVR</td>
<td>0.17±0.27</td>
<td>0.16±0.2</td>
<td>±6</td>
<td>-0.96</td>
<td>0.35</td>
<td>0.24±0.06</td>
<td>0.19±0.05</td>
<td>-11±8</td>
<td>-2.16</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI, L/min per m²</td>
<td>1.8±0.1</td>
<td>2.1±0.2</td>
<td>±6</td>
<td>3.70</td>
<td>0.003</td>
<td>2.7±0.2</td>
<td>3.0±0.2</td>
<td>12±4</td>
<td>2.85</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV stroke work index</td>
<td>7.7±1.6</td>
<td>6.7±1.1</td>
<td>±1</td>
<td>-1.21</td>
<td>0.26</td>
<td>8.2±1.0</td>
<td>8.3±1.2</td>
<td>2±6</td>
<td>0.23</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(a–v)O₂, ml O₂/dL blood</td>
<td>8.3±0.4</td>
<td>7.6±0.3</td>
<td>±3</td>
<td>-2.48</td>
<td>0.028</td>
<td>11.9±0.6</td>
<td>11.1±1.2</td>
<td>-4±2</td>
<td>2.14</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM unless otherwise indicated. %Δ indicates mean±SEM percent change after sildenafil administration; MAP, mean arterial pressure; and C(a–v)O₂, difference in oxygen content between arterial and venous blood.
The administration of sildenafil 60 minutes before CPET #3 reduced resting mean PAP (14±5%), PVR (21±7%), and SVR (19±4%), and increased CI (20±2%) (all P<0.05) but did not alter resting heart rate, mean arterial pressure, PCWP, RAP, RV stroke work index, C(a-v)O2, or PVR/SVR ratio (Table 2). Exercise hemodynamic variables were measured at 90% AT before and after sildenafil administration (CPET #2 and CPET #3, respectively). Similar lactic acid levels were observed at submaximal exercise before and after sildenafil (2.3±0.2 versus 2.4±0.2 mmol/L). Sildenafil decreased mean exercise PAP and PVR (11±3% and 21±5%, respectively, both P<0.05) and increased CI (12±4%, P<0.05) (Table 2). Sildenafil did not change exercise SVR or RV stroke work index, and PVR/SVR with exercise fell by 11±8% (P=0.05). The magnitude of reduction in exercise PVR/SVR after sildenafil administration correlated directly with resting PAP (r=0.73, P<0.01). Sildenafil did not significantly change mean arterial pressure, HR, or PCWP with exercise.

Sildenafil was associated with a significant increase in RVEF both at rest (0.34±0.03 to 0.4±0.03, P=0.001) and with exercise (0.34±0.03 to 0.44±0.04, P<0.001), whereas LVEF was unchanged.

**Effects of Sildenafil on Parameters of Exercise Capacity**

Measurements of exercise capacity were compared between CPET #1 and CPET #3 (after sildenafil administration). Peak VO2 increased from 1.01±0.08 L/min to 1.10±0.05 L/min, and VO2/VO2 slope decreased from 39±3 to 34±2 (both P<0.05) after sildenafil treatment. Augmentation of peak VO2 with sildenafil correlated with mean resting PAP before sildenafil administration (r=0.75, P<0.01). Sildenafil administration was associated with a modest reduction in oxygen extraction C(a-v)O2 at 90% AT (Table 2) but no change in peak workload (83±7 versus 88±6 W [P=0.17]).

**Effects of Sildenafil on Patients With and Without Pulmonary Hypertension**

As sildenafil appeared to show a pulmonary vasodilator effect proportionate to the degree of baseline PH, patients were separated into 2 groups. Patients with PH (Group +PH, n=7) had a mean resting PAP of 37±6 mm Hg, and those without PH (Group -PH, n=6) had a mean resting PAP of 17±1 mm Hg. The 2 groups did not differ in age, sex, cause of HF, or sildenafil levels (data not shown).

Sildenafil administration in Group +PH reduced mean resting PAP (27±7%), PVR (34±6%), and SVR (22±4%, all P<0.05) without altering PCWP (19±6 mm Hg) or cardiac output (Figure 2). Sildenafil had no effect on hemodynamic parameters in Group -PH patients at rest. In Group +PH, sildenafil decreased mean exercise PAP and PVR (15±5% and 40±6%, respectively, P<0.05) (Figure 2) without altering PCWP (25±4 mm Hg). SVR tended to decrease but not significantly (8±6%, P=0.08). Sildenafil increased exercise CI (14±6%) and decreased PVR/SVR ratio (26±6%) in Group +PH (both P<0.05, Figure 2 and 3, respectively). The reduction in PVR and PVR/SVR ratio was greater in the Group +PH than in the Group -PH, in which no changes in any of the exercise hemodynamic parameters were observed.

Between-group (+PH versus -PH) comparisons of hemodynamic changes associated with sildenafil administration demonstrated that exercise PVR and PVR/SVR ratio were reduced to a greater extent in +PH compared with -PH patients, whereas other hemodynamic variables did not differ significantly at rest or with exercise (Figure 2).

Resting RVEF increased from 0.34±0.04 to 0.42±0.04 (P<0.01), and exercise RVEF increased from 0.35±0.03 to 0.5±0.05 (P<0.001) after sildenafil treatment in Group +PH (Figure 4). No differences in RVEF were observed in Group -PH patients after treatment with sildenafil. A modest increase in resting LVEF was observed in the +PH group but...
Figure 3. Effect of sildenafil on the ratio of PVR to SVR. Rest and exercise measurements were performed before (baseline) and 60 minutes after sildenafil administration. Patients were divided into 2 groups based on whether pulmonary hypertension (PAP >25 mm Hg) was present (Group +PH, n=7) or absent (Group -PH, n=6). *P<0.05 for comparisons between baseline and sildenafil. #P<0.05 for between-group comparison of patients in the +PH and -PH groups.

Figure 4. Effect of sildenafil on RV ejection fraction (A) and LV ejection fraction (B), as determined by radionuclide ventriculography. Rest and exercise measurements were performed in CPET #1 (baseline) and 60 minutes after sildenafil administration (CPET #3). Patients were divided into 2 groups based on whether pulmonary hypertension (PAP >25 mm Hg) was present (Group +PH, n=7) or absent (Group -PH, n=6). *P<0.05 for comparisons between baseline and sildenafil. #P<0.05 for between-group comparison of patients in the +PH and -PH groups.

Discussion

In this study, we evaluated the hypothesis that administration of a single oral dose of the PDE5 inhibitor sildenafil (50 mg) would improve, through pulmonary vasodilation, exercise capacity and exercise hemodynamics in patients with chronic systolic HF. We found that sildenafil, at plasma levels similar to those reported in pharmacokinetic studies in normal individuals at 60 minutes,20,21 (1) improves exercise capacity, (2) reduces PAP, PVR, and PVR/SVR ratio and augments CI during exercise, (3) increases RVEF both at rest and with exercise, and (4) improves ventilatory efficiency (Ve/VCO2 slope). Sildenafil did not alter HR, PCWP, or mean arterial pressure and did not cause hypotension in any patients. Post hoc stratification of patients by presence or absence of PH demonstrates that improvements in hemodynamics and exercise capacity were predominantly seen in patients with secondary PH.

Effects on Hemodynamics

Our group has previously reported that sildenafil produces pulmonary and systemic vasodilation, decreases LV filling pressure, and increases CI without causing systemic hypotension in resting patients with PH secondary to HF.12 In this study, we extended these findings by demonstrating that sildenafil acts predominantly as a pulmonary vasodilator during exercise. In patients with HF and PH, sildenafil reduces PAP and PVR, which leads to an improved RV performance (higher RVEF) and increased CI.

PDE5 is the principle enzyme responsible for cGMP catabolism in the lungs and has been shown in animal models to be upregulated in pulmonary vascular smooth muscle cells under conditions associated with PH.22,23 We observed that the relative selectivity of sildenafil for the pulmonary vasculature during exercise correlated with resting PAP in the present study. Hence, our observation that sildenafil is a relatively selective pulmonary vasodilator in HF may be attributable to a higher relative abundance of PDE5 in the pulmonary vasculature compared with the systemic vasculature in the presence of PH. In the NO-deficient state of HF with secondary PH, putative regional differences in PDE5 activity may only become apparent with a stimulus that increases NO availability, such as exercise-mediated increase in blood flow.

Our observation that sildenafil lowers PAP and PVR at rest is consistent with that of Guazzi et al12; however, unlike Guazzi and colleagues, in this study we observed that sildenafil increased CI at rest. Our patient population differed from that studied by Guazzi et al in that mean baseline CI was...
lower in our patients, and PVR was >2 times greater. Hence, the beneficial effects on RV afterload reduction may have been more apparent in our patient population at rest. Moreover, in patients with PH without LV systolic HF studied by Michelakis and colleagues, sildenafil increased CI by 17% at rest.10 Finally, a 12-week study of sildenafil administration in patients with PH showed a dose-dependent increase in resting CI.11 Thus, patients with HF who develop the phenotype of secondary PH may derive clinical benefit from treatment with sildenafil in a manner that is proportionate to the severity of PH.

**Effects of Sildenafil on Exercise Performance**

The improvement in maximal exercise capacity observed after sildenafil treatment may be attributable to several mechanisms. First, sildenafil may have improved CI through a NO-cGMP–mediated reduction in PAP and PVR, a hypothesis which is supported by the improvement in RVEF observed with sildenafil. The correlation between the change in peak \(\dot{V}O_2\) with sildenafil treatment and baseline PAP also suggests that reduction in RV afterload may have mediated the improvement in exercise capacity. Consistent with this finding, our group previously demonstrated that inhaled NO augments exercise capacity in the subset of HF patients with impaired RVEF but not in HF patients with preserved RVEF.6

Sildenafil has also been shown to improve flow-mediated vasodilatation in HF13,14 and therefore may augment skeletal muscle perfusion. We did not directly measure flow-mediated vasodilatation in this study, but we did observe an augmentation in CI after sildenafil administration that likely resulted in increased skeletal muscle perfusion. Patients with HF have intrinsic skeletal muscle changes, including atrophy,24 and reduced oxidative enzyme capacity25 that predispose them to impaired oxygen extraction. However, the peak exercise C(a-v)\(O_2\) of 14.3±0.6 mL/dL in our study population with a mean hemoglobin of 14.2±0.5 g/dL indicates normal maximal oxygen extraction,26 and we observed no improvement in oxygen extraction at rest or with exercise after sildenafil administration. At rest, C(a-v)\(O_2\) was inversely correlated with resting cardiac output \((r=0.7, P<0.05)\). This relationship persisted with exercise \((r=-0.83, P<0.005)\), which indicates a preserved compensatory ability to extract oxygen to a greater extent when cardiac output is limited during exercise. If abnormal extraction was the limiting factor to exercise capacity, this tight inverse relationship would not be expected. Hence, sildenafil likely improved peak \(\dot{V}O_2\) by augmenting the contribution of cardiac output to \(\dot{V}O_2\), via pulmonary vasodilation–mediated improvement in RV performance.

An exaggerated ventilatory response \((V_E)\) to \(CO_2\) output \((V_{E}O_2)\) occurs in HF patients and inversely correlates with prognosis.27 Possible explanations for this inefficient ventilation include either an augmentation of the central drive to ventilation or abnormal ventilation/perfusion \((V/Q)\) matching.28 It is unlikely that sildenafil improved \(V/Q\) matching, as we did not observe a decrease in dead space ventilation during exercise after its administration. On the other hand, the reduction in PAP with exercise observed after sildenafil administration may have led to a decrease in J receptor activation in the pulmonary interstitium,29 and thus a decrease in the central ventilatory drive. Furthermore, sildenafil has been reported to improve perfusion of hypoxemic skeletal muscle in HF patients.13 This effect may have lead to attenuation of an exaggerated chemoreflex-mediated ventilatory drive during exercise, and an improvement in ventilatory efficiency.

Several vasodilators have been shown to reduce PVR and SVR and thereby improve cardiac performance in HF.30–32 However, only inhaled NO has been shown to be a selective pulmonary vasodilator in HF, and it can lead to an increase in PCWP in HF patients. Sildenafil is a relatively selective pulmonary vasodilator with exercise that does not increase PCWP, likely because of concomitant reduction in LV afterload. In a previous study from our laboratory, sildenafil was observed to decrease resting LV end-systolic pressure by 11%.12 Therefore, compared with inhaled NO, the hemodynamic effects of sildenafil make it a potential candidate for the treatment of HF patients with elevated PVR. Further investigation of the long-term clinical effects of sildenafil are indicated to determine whether the observed hemodynamic benefits of this agent translate to improved HF outcomes.

**Limitations**

This study should be considered as a preliminary pilot investigation because of its small sample size and lack of adjustment for multiple comparisons. Our findings in this
pilot study have prompted a larger confirmatory study that is currently underway.

The patients in the present study underwent 2 exercise tests on the same day to limit risk of repeated pulmonary arterial catheter separation on separate days. The study was not placebo-controlled; hence, we are unable to determine whether antecedent exercise influenced hemodynamic or gas exchange parameters and therefore confounded interpretation of sildenafil treatment. However, in similar studies of HF patients given placebo, even repeating maximum CPET on the same day resulted in no differences in peak workload achieved, peak VO₂, or other gas exchange parameters. In addition, at the start of the CPET #3 on day 2 (±2 days) of our protocol, lactate levels had returned to baseline, which suggested metabolic recovery from initial exercise. Furthermore, in similar patients undergoing CPET with hemodynamic monitoring, we observed that the hemodynamic parameters return to baseline values within 10 minutes of cessation of exercise (data not shown). In our study, 120 minutes elapsed between exercise studies; therefore it is unlikely that hemodynamic changes in CPET #3 were attributable to initial exercise.

**Conclusions**

The data presented in this study indicate that the oral PDE5 inhibitor sildenafil can be safely administered to patients with chronic severe HF in whom it reduces RV afterload and increases CI both at rest and with exercise. In addition, administration of a single dose of sildenafil increases RV EF and peak VO₂ and improves ventilatory efficiency, all of which have been shown to predict prognosis in HF. The observed benefits in exercise capacity and hemodynamic parameters were apparent only in patients with HF and secondary PH. If proven safe and effective for longer periods of administration, PDE5 inhibition may be useful in the treatment of HF, particularly of HF with secondary PH.

**Acknowledgments**

We thank the staff of the cardiopulmonary exercise laboratory for helping with data collection.

**Sources of Funding**

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**Disclosures**

Dr Semigran has a sponsored research agreement with Pfizer Inc.

**References**


In chronic systolic heart failure (HF), dysregulation of vascular smooth muscle tone commonly leads to an increase in pulmonary vascular resistance. Sildenafil, a type 5 phosphodiesterase inhibitor, lowers pulmonary vascular resistance in pulmonary arterial hypertension by augmentation of intracellular levels of the nitric oxide second messenger, cyclic GMP. In the present study, a single oral dose of sildenafil (50 mg) was safely administered to 13 patients with chronic severe HF undergoing right heart catheterization and exercise testing. Sildenafil reduced pulmonary vascular resistance and increased cardiac index both at rest and with exercise. In addition, administration of sildenafil increased exercise capacity and improved ventilatory efficiency, both of which have been shown to predict prognosis in HF. The observed benefits in exercise capacity and hemodynamic parameters were apparent only in patients with HF and secondary pulmonary hypertension. If proven safe and effective for longer periods of administration, type 5 phosphodiesterase inhibition may be useful in the treatment of HF, particularly of HF with secondary pulmonary hypertension.
Hemodynamic Modulation of Endocardial Thromboresistance

Navin K. Kapur, MD; Clayton B. Deming, MS; Sunil Kapur, BS; Ce Bian, MD; Hunter C. Champion, MD, PhD; J. Kevin Donahue, MD; David A. Kass, MD; Jeffrey J. Rade, MD

**Background**—Patients with heart failure are at increased risk for thromboembolic events, including stroke. Historically attributed to blood stasis, little is known about the adverse effects of elevated chamber filling pressure on endocardial function, which could predispose to intracardiac thrombus formation.

**Methods and Results**—We investigated changes in the expression of thrombomodulin, a key component of the anticoagulant protein C pathway, in rats subjected to acute atrial pressure overload caused by aortic banding. Acute elevation of left atrial filling pressure, without an associated decline in ventricular systolic function, caused a 70% inhibition of atrial endocardial thrombomodulin expression and resulted in increased local thrombin generation. Targeted restoration of atrial thrombomodulin expression with adenovirus-mediated gene transfer successfully reduced thrombin generation to baseline levels. In vitro co-culture studies revealed that thrombomodulin downregulation is caused by the paracrine release of transforming growth factor-β from cardiac connective tissue in response to mechanical stretch. This was confirmed in vivo by administration of a neutralizing transforming growth factor-β antibody, which effectively prevented thrombomodulin downregulation during acute pressure overload.

**Conclusions**—These findings suggest that increased hemodynamic load adversely affects endocardial function and is a potentially important contributor to thromboembolus formation in heart failure. (Circulation. 2007;115:67-75.)

**Key Words:** endocardium ■ heart failure ■ thrombosis ■ thrombomodulin

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Binds thrombin and renders it incapable of enzymatically cleaving fibrinogen or activating cellular thrombin receptors, but enables the activation of circulating protein C. Activated protein C (APC) degrades factors Va and XIIIa of the coagulation cascade, potently inhibiting further thrombin generation. Deletion of the thrombomodulin gene causes lethal thrombosis in mice, and the acquired loss of thrombomodulin in humans is thought to contribute to the thrombotic manifestations of bacterial sepsis, radiation enteropathy, and coronary atherosclerosis.

We recently identified pressure-induced vascular stretch as a novel and potent inhibitory stimulus for endothelial thrombomodulin expression. This was first observed in rabbit vein segments implanted into the arterial circulation; thrombomodulin protein expression decreased by 95% and resulted in increased local thrombin generation and microthrombus formation. On the basis of these studies, we hypothesize that pressure-induced chamber stretch might also negatively regulate endocardial thrombomodulin expression and contribute to intracardiac thrombus formation during heart failure. To explore this, we characterized endocardial thrombomodulin expression and function in a rat model of acute pressure...
overload. We then used adenovirus-mediated gene transfer to determine the relationship between thrombomodulin function and endocardial thrombogenesis and identified an important role of paracrine effects of transforming growth factor-β (TGF-β) in regulation of endocardial thrombomodulin expression.

Methods

Rat Model of Pressure Overload

Animal protocols were approved by the Johns Hopkins Animal Care and Use Committee. A well-characterized rat model was used with modification.14 Six-week-old male Wistar rats that weighed ~100 g (Charles River Laboratories, Wilmington, Mass) were anesthetized with 1% to 2% isoflurane and mechanically ventilated. Through a right thoracotomy, a 23-gauge needle was placed adjacent to the ascending aorta and a 4-0 silk suture ligature was applied around both the needle was then removed to create an immediate >70% luminal stenosis. Sham control animals underwent thoracotomy without suture placement. To investigate the effects of TGF-β, rats were administered 1 mg/kg of either a neutralizing anti–TGF-β antibody (MAB240; R&D Systems, Minneapolis, Minn) or an IgG1 isotype control antibody (MAB005; R&D Systems) via peritoneal injection 24 hours before surgery, with a second dose of 0.5 mg/kg administered 48 hours after surgery. The antibody dose was based on previous studies in the rat that demonstrated effective neutralization of TGF-β.15

At euthanization, echocardiographic measurements were obtained with a portable Sonos 550 Ultrasound System (Hewlett-Packard, Andover, Mass) equipped with a 15-MHz probe (Acuson, Siemens, Malvern, Pa) with validated techniques in rodents.16 Ventricular pressures were measured directly via cardiac puncture with a 21-gauge needle attached to a pressure monitor (SpaceLabs, Redmond, Wash).

Atrial Gene Transfer

The construction of adenovirus vectors that express human thrombomodulin (AdTMh5) and no transgene (AdNull) have been previously described.13 The left atrium was exposed via a left thoracotomy, a 23-gauge needle was placed adjacent to the ascending aorta with 1% to 2% isoflurane and mechanically ventilated. Through a right thoracotomy, a 23-gauge needle was placed adjacent to the ascending aorta and a 4-0 silk suture ligature was applied around both the needle was then removed to create an immediate >70% luminal stenosis. Sham control animals underwent thoracotomy without suture placement. To investigate the effects of TGF-β, rats were administered 1 mg/kg of either a neutralizing anti–TGF-β antibody (MAB240; R&D Systems, Minneapolis, Minn) or an IgG1 isotype control antibody (MAB005; R&D Systems) via peritoneal injection 24 hours before surgery, with a second dose of 0.5 mg/kg administered 48 hours after surgery. The antibody dose was based on previous studies in the rat that demonstrated effective neutralization of TGF-β.15

At euthanization, echocardiographic measurements were obtained with a portable Sonos 550 Ultrasound System (Hewlett-Packard, Andover, Mass) equipped with a 15-MHz probe (Acuson, Siemens, Malvern, Pa) with validated techniques in rodents.16 Ventricular pressures were measured directly via cardiac puncture with a 21-gauge needle attached to a pressure monitor (SpaceLabs, Redmond, Wash).

Assessment of Neurohormonal Activation

At the time of euthanization, levels of angiotensin II, atrial natriuretic peptide, and brain natriuretic peptide were determined in extracted serum with EIA kits that recognize rat peptides (Phoenix Pharmaceuicals, Belmont, Calif) in accordance with the manufacturer’s instructions. Serum tumor necrosis factor-α concentrations were measured by rat tumor necrosis factor-α ELISA kit (BioSource, Camarillo, Calif) in accordance with the manufacturer’s instructions.

Real-Time Quantitative Polymerase Chain Reaction

RNA from freshly harvested tissue or cultured cells was extracted using TRIzol Reagent (Invitrogen, Carlsbad, Calif). Real-time quantitative polymerase chain reaction (PCR) was performed with a 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif). Triplicate samples were subjected to reverse transcription and real-time PCR with TaqMan One-Step RT-PCR Master Mix Reagents with gene-specific primers and probes designed by Primer Express software (Applied Biosystems) based on published nucleotide sequences (see Data Supplement). Thrombomodulin gene expression was normalized to endothelial-specific RNA content by quantification of rat CD31 gene expression. Thrombomodulin gene expression in cultured cells and TGF-β gene expression in both tissue samples and cultured cells were normalized to 18S ribosomal RNA (TaqMan Ribosomal RNA Reagents with VIC-labeled probe; Applied Biosystems).

Western Blot and Immunohistochemical Analyses

Western blot analysis was performed with anti-thrombomodulin antibodies (#3381 and #2380; American Diagnostica) and an anti-CD31 antibody (M0823; Dako, Carpinteria, Calif) as described.13 Bands were detected by autoradiography and quantified densitometrically with UN-SCAN-IT software (Silk Scientific, Orem, Utah). Immunohistochemical analysis was performed on formalin-fixed and paraffin-embedded sections of rat left atria with antibodies against rat thrombomodulin (#3381; American Diagnostica), human TGF-β latent associated peptide (EF01; R&D), and rat prolyl 4-hydroxylase (6-9H6; Acris Antibodies, Hiddenhausen, Germany) as described.8

In Situ Protein C and Thrombin Activity Assays

Capacity to generate APC was measured on freshly-harvested whole left atria with a previously described protocol with modification.13 The atria were washed in Hank’s buffered salt solution and then incubated in 250 μL Hank’s buffered salt solution that contained 40 mmol/L human α-thrombin (Sigma-Aldrich, St Louis, Mo) and 1 mmol/L human protein C (American Diagnostica) at 37°C. After 60 minutes, thrombin was neutralized by excess lepirudin (Bolger Laboratories, Montville, NJ). Aliquots measuring 100 μL were incubated at room temperature with a 3-mmol/L solution of the chromogenic substrate S-2366 (DiaPharma, West Chester, Ohio). The rate of substrate conversion was determined spectrophotometrically with a Vmax Kinetic Microplate Reader ( Molecular Devices, Sunnyvale, Calif) and the amount of protein C activation calculated by comparison with a human APC (American Diagnostica) standard curve. Bound thrombin activity on the atrial endocardial surface was measured as previously described with some modification.13 Freshly excised whole atria were washed in Hank’s buffered salt solution, then incubated in 250 μL of substrate buffer (30 mmol/L Tris-HCl, 175 mmol/L NaCl, and 2 mmol/L CaCl2 [ pH 7.8] that contained 333 μmol/L of the chromogenic substrate, S-2238 (DiaPharma) at 37°C for 30 minutes. The change in absorbance at 405 nm before and after lepirudin treatment represented thrombin-specific substrate conversion. Bound thrombin activity was then calculated by comparison with a human α-thrombin standard curve.

In Vitro Stretch Experiments

Human atrial endocardial cells isolated in our laboratory (see Data Supplement) and human cardiac fibroblasts purchased from Cell Applications (San Diego, Calif) of passage 2 to 3 were plated onto type I collagen-coated 6-well Bioflex plates (Flexcell International, Hillsborough, NC) and grown in either EGM-2 (endocardial cells) or FGM-2 (fibroblasts) culture media (BioWhittaker). When nearly confluent, cells were refreshed in basal media and subjected to 0% to 10% cyclic strain delivered at 1 Hz for 24 hours at 37°C and 5% CO2 with a FX-4000T Tension Plus System (Flexcell International). For co-culture experiments, atrial endocardial cells were grown on 24-mm diameter Transwell inserts with a 0.4-μm pore size (Corning, Corning, NY) in EGM-2 medium. When confluent, the inserts were placed into the individual wells of the Bioflex plates with cardiac fibroblasts in basal medium (BioWhittaker). The cardiac fibroblasts were then subjected to 0% to 10% cyclic strain delivered at 1 Hz for 24 hours. To investigate the effects of TGF-β, 0.5 μg/mL of either a neutralizing anti–TGF-β antibody (MAB240; R&D) or IgG1 isotype control antibody (MAB005; R&D) were added to the medium before stretching the fibroblasts at 10% cyclic strain delivered at 1 Hz for 24 hours.

Statistical Analysis

All data are presented as mean±SEM. Comparison between 2 groups is by 2-tailed t tests and between multiple groups is by 1-way ANOVA with a Bonferroni correction for intergroup comparisons.
Results

Effects of Acute Pressure Overload on Endocardial Thrombomodulin Expression

The ascending aortae of 6-week-old rats were suture-banded to induce a >70% luminal constriction. Because this degree of acute increase in afterload results in pulmonary edema and death within 7 days of surgery, all hemodynamic and echocardiographic measurements were obtained 96 hours after banding. Compared with sham-operated controls, left ventricular systolic pressure increased 40% in banded animals, whereas left ventricular diastolic pressure, equivalent to mean left atrial pressure, increased by nearly 400% \((P<0.0001\) for both; Figure 1A). Ventricular wall thickness and diastolic diameter were unchanged, whereas left atrial diameter increased slightly (Figure 1B). Systolic function was not depressed in this acute period, with slight increases in ejection fractions \((77\pm9\%\) banded versus \(65\pm5\%\) sham controls, \(P<0.01\)), which likely reflect acute compensation to high afterload.\(^{17}\) Serum atrial natriuretic peptide and brain natriuretic peptide levels did not change from controls (Figure 1C).

To determine the impact of acute hemodynamic changes on endocardial thrombomodulin expression, tissue from the left atrium and ventricle of banded rats was subjected to quantitative PCR analysis and compared with sham controls (Figure 2A). Aortic tissue, distal to the suture ligation, was analyzed as an additional control. Data were normalized to CD31, an endothelial-specific adhesion molecule whose expression does not change with pressure-induced cell stretch.\(^{18}\) Thrombomodulin expression in the left atrium of banded rats declined by 60% compared with sham controls \((P=0.01\)), whereas thrombomodulin expression in the left ventricle or in the distal aorta was unchanged. Western blot analysis of left atrial tissue confirmed a 70% decline in thrombomodulin expression \((P<0.002\); Figure 2B). Left atrial tissue stained with an anti-mouse thrombomodulin antibody verified decreased thrombomodulin expression by the atrial endocardial endothelium (Figure 2C). These data suggest that endocardial thrombomodulin expression is directly modulated by changes in chamber loading rather than by systemic factors induced by acute heart failure.

We also determined whether acute pressure overload altered the atrial expression of other molecules known to modulate endocardial thromboresistance (Figure 3). Aortic banding did not alter the expression of endothelial nitric oxide synthase but was associated with increased tissue factor pathway inhibitor and decreased tissue factor expression. The net effect of these changes would be expected to reduce local thrombin generation at the endocardial surface. Interestingly, banding also increased the expressions of both tissue plasminogen activator and plasminogen activator inhibitor-1.

Consequences of Thrombomodulin Downregulation on Endocardial Thromboresistance

Thrombomodulin exerts its anticoagulant effect via activation of circulating protein C. Endocardial capacity to generate APC was measured in resected whole left atria 96 hours after surgery. Aortic banding reduced the capacity to generate APC in the left atria of banded rats by >35% compared with sham controls \((P=0.01\); Figure 4A). To determine the effect on in situ thrombin generation, the activity of thrombin bound to the atrial endocardial surface was then quantified. Thrombin generated at sites of vascular injury binds to fibrin strands within a developing clot and is protected from inactivation by circulating inhibitors. Bound thrombin activity is therefore proportional to the degree of thrombus that is present and is capable of detecting the presence of microscopic amounts of fibrin clot.\(^{12}\) Bound thrombin activity in the atria of banded rats was significantly higher than in atria from sham-operated controls and approached that observed in atria subjected to direct mechanical forceps injury (Figure 4B).
We then determined the effects of thrombomodulin expression restoration on capacity to generate APC and bound thrombin activity. This was accomplished with a “gene painting” technique, in which solutions that contain adenovirus vectors that express either human thrombomodulin (AdTM5) or no transgene (AdNull) were applied to the epicardial surfaces of the rat left atria. Previous studies in the pig revealed that this technique results in efficient transmural transduction of atrial cells, including the endocardial endothelium. Figure 1 illustrates the degree of gene transfer that can be attained with this method in the rat. Three days after transduction, protein lysates of left atrial tissue subjected to ELISA that detects only the human form of thrombomodulin revealed an average of $76.5 \pm 24.9$ ng of human thrombomodulin per milligram of atrial tissue in AdTMh5-transduced atria ($n=4$), whereas no human thrombomodulin was detected in AdNull-transduced atria ($n=3$; $P<0.004$). These results were confirmed by Western blot analysis (Figure 4C). To verify that acute pressure overload does not attenuate transgene expression, transduced atria from suture-banded rats were assayed for the expression of both human and native rat thrombomodulin by quantitative PCR 96 hours after surgery. Figure 4D demonstrates that human thrombomodulin gene expression remains robust throughout the experimental period in AdTMh5-transduced atria, whereas no human thrombomodulin gene expression could be detected in AdNull-transduced atria. Restoration of thrombomodulin expression with adenovirus-mediated gene transfer effectively prevented the loss of capacity to generate APC induced by aortic banding (Figure 4A) and reduced the levels of bound thrombin activity to baseline values (Figure 4B). Taken together, these data support the concept that downregulation of endocardial thrombomodulin contributes to increased local thrombin generation.

**Effects of Stretch on Thrombomodulin Expression in Isolated Endocardial Endothelial Cells**

We have previously shown that thrombomodulin downregulation in vein segments exposed to arterial pressure results from pressure-induced vascular stretch and not from the direct effects of pressure per se. To determine whether stretch can directly inhibit thrombomodulin gene expression, endocardial endothelial cells isolated from human right atrial appendages were grown on collagen-coated silastic membranes and subjected to increasing

![Figure 2. Effect of acute pressure overload on endocardial thrombomodulin (TM) expression. A, Thrombomodulin gene expression, normalized to CD31, was determined by quantitative PCR of tissue extracts obtained from the left atrium ($n=10$ per group), left ventricle ($n=10$ per group), and the distal aorta ($n=8$ per group) of banded and sham rats 96 hours after surgery. B, Thrombomodulin protein expression, normalized to CD31, was determined by densitometric analysis of Western blots of left atrial tissue extracts obtained from banded and sham rats ($n=4$ to 5 per group) 96 hours after surgery. The right panel depicts a representative Western blot. C, Histological sections of left atrial tissue obtained from sham and banded rats 96 hours after surgery and stained with an anti-mouse thrombomodulin antibody that shows decreased endocardial thrombomodulin protein expression.](image)

![Figure 3. Effect of acute pressure overload on the expression of molecules that modulate endocardial thromboresistance. Gene expression, normalized to CD31, was determined by quantitative PCR of tissue extracts obtained from the left atrium of banded and sham rats 96 hours after surgery ($n=10$ per group). eNOS indicates endothelial nitric oxide synthase; TF, tissue factor; TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator; and PAI-1, plasminogen activator inhibitor-1.](image)
amounts of cyclic stretch for 24 hours. Surprisingly, cyclic stretch resulted in a trend toward increased thrombomodulin gene expression (Figure 5A). Similar results were observed in stretched endothelial cells isolated from both rabbit jugular veins and human umbilical veins (data not shown). Because endocardial endothelial cells in situ lie adjacent to cardiac connective tissue, we next tested whether this interaction might influence thrombomodulin expression by stretch. Human atrial endocardial cells were cultured on stationary filters suspended in the media of wells that contain human cardiac fibroblasts subjected to increasing amounts of cyclic stretch. Stretching of cardiac fibroblasts resulted in a dose-dependent decrease in thrombomodulin expression in the stationary endocardial cells (Figure 5B). Experiments that substituted human aortic smooth cells for cardiac fibroblasts yielded identical results (data not shown). These results indicate that endocardial thrombomodulin expression is modulated in paracrine fashion by a soluble factor released by cardiac connective tissue in response to stretch.

Modulation of Thrombomodulin Expression by Paracrine Release of TGF-β

TGF-β is a multifunctional stretch-induced growth factor known to be intimately involved in cardiac remodeling induced by heart failure and has been reported to inhibit thrombomodulin protein expression in human umbilical vein endothelial cells. TGF-β gene expression in cardiac fibroblasts exhibited a dose-dependent increase in response to cyclic stretch (Figure 6A). In addition, thrombomodulin gene expression in human atrial endocardial cells was inhibited in a dose-dependent manner after 24-hour exposure to recombinant TGF-β (Figure 6B). To determine whether TGF-β is the paracrine factor responsible for thrombomodulin downregulation, human endocardial cells were plated on stationary filters submerged in the media of cardiac fibroblasts subjected to 10% cyclic stretch for 24 hours in the presence of a neutralizing anti-TGF-β antibody or an isotype control antibody. Figure 6C shows that neutralization of TGF-β effectively
prevented the downregulation of endocardial thrombomodulin gene expression.

To determine whether paracrine release of TGF-β is responsible for in vivo modulation of thrombomodulin, we first assessed TGF-β expression in the cardiovascular tissue of rats subjected to aortic banding. Compared with sham-operated controls, TGF-β gene expression in the left atrium of banded rats increased by >200% (P<0.001), whereas there was no significant change in TGF-β expression in the left ventricle or in the distal aorta (Figure 7A). Banding was also associated with increased TGF-β activation, as evidenced by positive immunostaining for latency-associated peptide, the cleaved propeptide of the TGF-β precursor molecule that remains noncovalently attached to active TGF-β. Latency-associated peptide staining localized predominantly to fibroblast-appearing cells in the subendocardial space that expressed an abundance of prolyl 4-hydroxylase, an enzyme involved in collagen synthesis (Figure 7B). To determine whether TGF-β inhibition could prevent thrombomodulin downregulation, rats were administered either a neutralizing anti–TGF-β antibody or an isotype control antibody in the perioperative period. Neutralization of TGF-β effectively prevented thrombomodulin downregulation in the left atrium 96 hours after aortic banding (Figure 7C). Importantly, there was no meaningful difference in the hemodynamic response to banding between rats administered the anti–TGF-β antibody versus the isotype control antibody (left ventricular end-diastolic pressure 17.7±0.6 versus 15.8±0.2 mm Hg, respectively; P=0.02). These data confirm that the effects of pressure overload on endocardial thrombomodulin expression and thromboresistance are mediated via paracrine release of TGF-β.

Discussion

The major findings of this study are (1) acute elevations in filling pressure adversely affect atrial endocardial thrombomodulin expression; (2) downregulation of endocardial thrombomodulin expression impairs local protein C activation and contributes to local thrombin generation; and
(3) endocardial thrombomodulin expression is negatively regulated during acute pressure overload by the paracrine effects of TGF-β secreted by cardiac connective tissue in response to pressure-induced chamber stretch.

It has long been recognized that patients with heart failure develop intracardiac thrombi that cause cerebral, pulmonary, and peripheral arterial thromboembolization. Thrombus formation has traditionally been viewed as a consequence of blood stasis that results from impaired ventricular function. If the presence of atrial fibrillation is excluded, however, the degree of left ventricular dysfunction only weakly correlates with thromboembolic risk. This suggests that factors other than stasis contribute to intracardiac thrombus formation in patients with heart failure, of which the overwhelming majority remain in sinus rhythm. We believe that ours is the first study to convincingly demonstrate that elevated cardiac filling pressures can adversely affect endocardial function and predispose to intracardiac thrombus formation independent of blood stasis.

Thrombomodulin is but 1 of several anticoagulant molecules expressed by endothelial cells that protect against pathological thrombosis. The level of thrombomodulin expression varies among endothelial cell types and therefore its relative contribution to vascular thromboreistance may differ between vascular beds. Several elements of the present study suggest that thrombomodulin is a critical contributor to endocardial thromboreistance. The first is that thrombomodulin is expressed in abundance by the endocardial endothelium. In addition to the rat, we have also found that in situ endocardial thrombomodulin expression is also robust in the rabbit and in humans (Figure II). Second, increased local thrombin generation in our model was proportional to the downregulation of thrombomodulin and occurred despite changes in the expression of other anticoagulant molecules that would be expected to inhibit thrombus formation. Third, and most important, targeted restoration of thrombomodulin expression with adenovirus-mediated gene transfer effectively reduced local thrombin generation.

In prior studies with rabbit vein grafts, we identified pressure-induced vascular stretch as a novel and potent negative regulator of in vivo thrombomodulin expression. The present study extends these findings by providing evidence of a second, and uniquely important, vascular bed in which stretch modulates thrombomodulin expression and by identifying paracrine release of TGF-β as the critical molecular mediator. TGF-β is a multifunctional dimeric polypeptide growth factor involved in a diverse array of biological processes that include embryogenesis, tumor growth, wound healing, and tissue remodeling. TGF-β is intimately involved in the adaptive response of cardiac and vascular tissue to pressure overload. In systolic heart failure, for example, TGF-β expression is known to be markedly increased in cardiac myocytes and fibroblasts, where it is a recognized autocrine and paracrine mediator of hypertrophy and fibrosis.

In the model of acute pressure overload used for this study, a marked increase in local TGF-β expression was observed in the left atrium but not in the left ventricle and appeared to originate predominantly from cardiac fibroblasts. This was likely the result of the relatively greater hemodynamic load imposed on the atrium and explains why endocardial thrombomodulin expression in the ventricle did not change. A recognized limitation of this model is the absence of ventricular remodeling and systolic dysfunction common to most chronic heart failure models. Although the model mostly replicates features of diastolic heart failure, it was precisely the absence of systolic dysfunction that obviated potential confounding effects of blood stasis on intracardiac thrombin generation. In models of chronic systolic dysfunction, TGF-β expression is increased in ventricular tissue, and it is reasonable to predict that thrombomodulin expression in the overlying endocardium would also be reduced. Adaptive responses, however, may complicate this issue, as there is evidence that myocardial TGF-β expression may vary according to the cause of heart failure and may change over time as heart failure progresses. This raises the possibility that the risk and chamber origin of thrombus formation may change over time, and the risk may be greater for different types of heart failure. With the observed association between TGF-β expression and endocardial dysfunction as a conceptual framework, future studies that use chronic models of systolic heart failure can be used to investigate these possibilities.

Extremely little is known about the molecular mechanism by which TGF-β regulates thrombomodulin expression in endothelial cells. TGF-β signal transduction is initiated via binding to specific serine/threonine type I and type II receptor complexes located on the endothelial cell surface. Receptor engagement causes the phosphorylation of several intracellular effector molecules known as Smads. Whereas most of the Smad proteins positively regulate TGF-β signal transduction, Smad6 and Smad7 are known to exert inhibitory effects. In the only published study to date that investigates the mechanism of thrombomodulin modulation by TGF-β, antisense inhibition of Smad7 potentiated the effects of TGF-β, whereas antisense inhibition of a splice variant of Smad6, known as Smad6.30, unexpectedly blunted the downregulation of thrombomodulin in human umbilical endothelial cells by TGF-β. As Smad6 and Smad7 are differentially expressed in the endothelium of normal and atherosclerotic arteries, they may be able to differentially modulate the paracrine effects of TGF-β on thrombomodulin expression in various vascular beds and disease states. What remains completely unknown at the present time is the nature of the signaling pathway distal to Smad activation that may regulate thrombomodulin gene transcription.

In addition to heart failure, our findings may have implications for understanding the thrombogenic potential of other forms of cardiovascular disease, such as atrial fibrillation. Thrombus formation in atrial fibrillation is also primarily ascribed to blood stasis in a left atrial appendage that is not contracting. However, it is well known that atrial fibrillation in patients with structurally normal hearts, ie, "lone atrial fibrillation," is associated
with a relatively small risk (<0.5% per year) of thromboembolic stroke compared with atrial fibrillation that occurs in the setting of hypertension, heart failure, or valvular heart disease (5% to 12% per year). As these latter conditions are all characterized by pressure-induced atrial distension, it is possible that endocardial dysfunction and altered thrombomodulin expression may contribute substantially to the thromboembolic risk in these patients. This concept is supported by data from a rat model of atrial fibrillation in which atrial thrombomodulin expression decreased by 35% after 8 hours of rapid atrial pacing. Interestingly, the left atrial pressure in this model triples during pacing, which raises the possibility that the effects on thrombomodulin expression were primarily the result of hemodynamic rather than electrical influences.

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**Disclosures**

None.

**References**


CLINICAL PERSPECTIVE

Patients with congestive heart failure, atrial fibrillation, and valvular heart disease are at risk for thromboembolic events, including stroke. Thromboembolus formation in these patients has historically been attributable to blood stasis within the cardiac chambers. The present study reveals that elevated cardiac chamber filling pressure, which is common to each of these conditions, can adversely affect endocardial function and contribute to intracardiac thrombus formation. This may help explain why patients with heart failure and preserved ventricular function remain at risk for thromboembolic stroke, whereas patients with atrial fibrillation and structurally normal hearts are at relatively low risk. The present study also identifies transforming growth factor-β, released in paracrine fashion by pressure-induced chamber stretch, as a potent negative regulator of endocardial thromboresistance. Targeted inhibition of the transforming growth factor-β signaling pathway could represent a novel therapeutic alternative for prevention of thromboembolus formation in selected cardiac patients currently being treated with systemic anticoagulation.
Prospective Familial Assessment in Dilated Cardiomyopathy
Cardiac Autoantibodies Predict Disease Development in Asymptomatic Relatives

Alida L.P. Caforio, MD, PhD; Niall G. Mahon, MD; M. Kamran Baig, MD; Francesco Tona, MD, PhD; Ross T. Murphy, MD; Perry M. Elliott, MD; William J. McKenna, MD

Background—In autoimmune disorders, circulating autoantibodies identify healthy relatives at risk years before clinical presentation. Healthy relatives of patients with dilated cardiomyopathy (DCM) who have echocardiographic changes, including left ventricular enlargement or depressed fractional shortening at baseline, have increased medium-term risk for DCM development. Approximately one third of relatives have serum anti-heart autoantibodies (AHAs) at baseline; we intended to assess their potential role in predicting DCM development.

Methods and Results—Baseline evaluation, including electrocardiography, echocardiography, and AHA, was performed in 592 asymptomatic relatives of 169 consecutive DCM patients (291 males and 301 females; mean age 36±16 years). Relatives were classified in accordance with published echocardiographic criteria; those who did not have DCM were followed up (median of 58 months). DCM among relatives was diagnosed by echocardiography at follow-up. Of the 592 individuals evaluated, 77% were assessed as normal, 4.4% as having DCM, and 19% as possibly affected on the basis of depressed fractional shortening without ventricular dilatation in 17 and left ventricular enlargement without systolic dysfunction in 94. Five-year follow-up of 311 relatives revealed that 26 had progressed (13 to DCM, 11 to left ventricular enlargement, and 2 to depressed fractional shortening). Relatives who developed DCM were more frequently AHA-positive than those who did not (69% versus 37%, P=0.02). Five-year probability of progression to DCM, among normal or possibly affected relatives, was higher in AHA-positive cases (P=0.03). By Cox regression, positive AHAs at baseline were independent predictors of progression (RR 2.26, CI 1 to 5.1, P=0.03).

Conclusions—Among healthy relatives of DCM patients, AHAs are independent predictors of disease development within 5 years. (Circulation. 2007;115:76-83.)

Key Words: cardiomyopathy | antibodies | immunology | inflammation

In autoimmune disorders, circulating organ-specific autoantibodies are detected in asymptomatic relatives of affected index patients years before disease presentation and are useful serological markers for subjects at risk. Dilated cardiomyopathy (DCM) is a genetically heterogeneous disease with multifactorial pathogenesis. It may be familial, genetic, viral, and/or immune. Autoimmunity is recognized to play a pivotal role in the pathogenesis of a substantial proportion of cases, possibly triggered by various causes of cardiac injury in genetically predisposed individuals. Circulating autoantibodies to distinct cardiac autoantigens, including α- and β-myosin heavy chain, are found in animal models and in humans, which represent autoimmune markers in a subset of patients. With indirect immunofluorescence, organ- and disease-specific anti-heart autoantibodies (AHAs) are found in 30% of DCM patients at clinical presentation and in 20% to 30% of their symptom-free relatives. We have reported that symptom-free relatives of DCM patients with subtle echocardiographic changes, in particular, left ventricular enlargement (LVE) or depressed fractional shortening (dFS) at baseline, have increased medium-term risk for DCM development. Healthy relatives with or without LVE or dFS have serum AHAs at baseline. These relatives have other features of immune activation, including cytokine activation in peripheral blood and intramyocardial inflammation and reduced peak oxygen consumption.

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We hypothesized that similar to other autoimmune disorders, AHAs, found in asymptomatic relatives at initial family evaluation, might identify at a preclinical stage those
at risk of DCM. In the present study, we assessed the potential role of AHAs, obtained as part of the baseline clinical and immunologic characterization of healthy relatives, in predicting DCM development.

Methods

Probands and Relatives
Study subjects were 592 asymptomatic first- or second-degree relatives (291 males; age 36±16 years; 248 from 54 familial pedigrees and 344 from the 115 nonfamilial pedigrees) of 169 consecutive DCM probands (127 males; age 43±13 years). The study protocol was approved by the human research committee at St George’s Hospital, London, United Kingdom; informed consent was obtained from patients and their relatives. Sera for AHA testing were taken at control evaluation as part of the routine immunologic assessment for possible DCM. The diagnosis of DCM was based on World Health Organization criteria. Probands were evaluated by noninvasive clinical and invasive examination, including coronary angiography and endomyocardial biopsy where indicated. We classified DCM as familial if at least 1 relative (in addition to the proband) had DCM during life or at postmortem examination or if there was a history of unexplained sudden cardiac death before the age of 30 years.

Family screening was offered irrespective of the presence or absence of an overt family history, and inclusion was based on willingness to participate. Assessment of asymptomatic relatives included medical history, clinical examination, 12-lead ECG, 2D echocardiogram, and AHA testing. Relatives assessed as having DCM proceeded to the same full evaluation as for probands.

Echocardiographic Criteria
2D echocardiograms were performed by trained operators working in a dedicated DCM clinic who were unaware of the clinical data. Measurements of chamber dimensions and wall thickness were obtained at the mitral valve–tip level from 2D-guided M-mode or short-axis-view recordings. Predicted normal values for the left ventricular end-diastolic cavity dimension (LVDD) were calculated from Henry’s formula and corrected for age and body surface area (BSA). The percent predicted LVDD (%LVDD) was calculated as measured LVDD/predicted LVDD×100. LVDD was defined as %LVDD 25.28 Using these cutoff levels, asymptomatic relatives with noninflammatory heart disease (1%) or ischemic heart disease (1%) were classified as having DCM (%LVDD 112 and %FS 0.03(age)−7.2±12.2). The percent predicted LVDD (%LVDD) was calculated as (measured LVDD/predicted LVDD)×100. LVDD was defined as %LVDD 25.112 of predicted normal values; dFS was defined as percent fractional shortening (%FS) 25.28 Using these cutoff levels, asymptomatic relatives were classified as having DCM (%LVDD 112 and %FS 0.03(age)−7.2±12.2). The percent predicted LVDD (%LVDD) was calculated as measured LVDD/predicted LVDD×100. LVDD was defined as %LVDD 25.28 Using these cutoff levels, asymptomatic relatives were classified as having DCM (%LVDD 112 and %FS 0.03(age)−7.2±12.2).

Follow-Up of Asymptomatic Relatives
We offered follow-up evaluation, including clinical examination, 12-lead ECG, and 2D echocardiogram, to all relatives regardless of outcome of initial assessment, and we based inclusion on their willingness to participate and their geographic availability. Relatives initially classified as having DCM were offered annual follow-up, and those found to be normal with positive AHAs had 2-year follow-up. No treatment during follow-up was initiated unless criteria for DCM were fulfilled. AHA-negative individuals who were assessed as normal were reevaluated at a median of 4 years. As part of a larger follow-up program, 132 AHA-negative normal individuals were studied. Clinical and echocardiographic follow-up was available in 311 relatives initially classified as normal or as having DCM.

AHA Testing by Standard Indirect Immunofluorescence
For AHA detection, sera were tested by standard indirect immunofluorescence. The frequency of AHAs in healthy relatives of DCM patients was compared with that observed in our established control groups of noninflammatory heart disease (n=160, 80 males, age 37±17 years, of whom 55 had rheumatic heart disease, 67 had hypertrophic cardiomyopathy, and 38 had congenital defects), ischemic heart disease (n=141, 131 males, age 44±14 years), and normal subjects (n=270, 123 males, age 35±11; 14.20 Forty-one of the 141 ischemic patients (age 47±12 years, 28 males; 31 in New York Heart Association class III and 10 in class IV) had suffered a documented myocardial infarction 6 months to 10 years (median 2 years) previously; ejection fraction ranged from 16% to 44% (mean 30±7%).

Statistical Analysis
Results for quantitative features are given as mean±SD. Student t test, 1-way ANOVA, χ2, or Fisher exact test was used as appropriate. The Kaplan-Meier method was used to construct life tables of the probability of survival free from progression to DCM or from any progression (from normal, LVE, or dFS to DCM and from normal to LVE or dFS). The equality of the survival distributions was tested by log-rank test. Multivariable analysis of potential risk factors for progression was performed by the stepwise proportional hazard method of Cox. Results are expressed with the relative risk (RR) ratios and their associated 95% CIs. Variables identified as significant by univariate analysis were included in multivariable analysis. All probability values were 2-tailed; probability values below 0.05 were considered to indicate statistical significance. All statistical analyses were performed with the SPSS statistical software package for Windows, version 13.0 (SPSS Inc, Chicago, Ill).

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Baseline Features and AHA Status of Relatives at Initial Evaluation
At baseline evaluation of the 592 asymptomatic relatives, 455 (77%) were assessed as normal, 26 (4.4%) as having DCM, and 111 (19%) as possibly affected, of whom 17 (3%) had dFS and 94 (16%) had LVE. AHAs of IgG class were detected in 188 relatives (32%); the frequency of AHAs was higher (P<0.0001) in healthy relatives (32%) than in controls with noninflammatory heart disease (1%) or ischemic heart disease (1%) or normal blood donors (2.5%). AHAs were more common in those relatives classified as having asymptomatic DCM (13/26, 50%) than in the other relatives (175/566, 31%; P=0.04). AHA titers were as follows: 1/10 in 113 positive sera (60%), 1/20 in 54 (28%), and 1/40 or higher in 21 (11%). The proportion of first-degree relatives was higher in nonfamilial pedigrees; relatives from familial DCM cases had higher LVDD, higher LVDD%, and lower %FS (Table 1). High-titer AHAs (1/20 or higher) were more frequent in familial pedigrees (P=0.0001; Table 1). In 112 (66%) of the pedigrees, AHAs were found in the proband and/or in at least 1 family member and were more common among relatives with AHA-positive probands (41%) than among those with negative probands (34%, P=0.001; Table 2). AHAs were also more common in familial than in nonfamilial cases (98/248, 39.5% versus 90/344, 26%; P=0.0001). When features were compared after exclusion of the 26 relatives assessed as having asymptomatic DCM at baseline, relatives from familial DCM cases still had lower %FS and higher %LVDD (34±6 versus 36±6, P=0.001, and 106±9 versus 104±9, P=0.03, respectively) and a higher
frequency of AHA (85/222 versus 90/344, \( P = 0.002 \)), but the proportions of relatives with LVE or dFS were similar.

Baseline features did not differ in asymptomatic relatives with and without AHA, except for a higher proportion of pedigrees with positive AHAs in familial than in nonfamilial DCM (45/54, 83% versus 67/115, 58%; \( P = 0.001 \)). Similar proportions of relatives with and without AHAs were classified as possibly affected (LVE or dFS). This also applied when features were compared after exclusion of the 26 asymptomatic relatives who were found to have DCM at baseline.

**Univariate and Multivariable Risk Factor Analysis for Disease Development According to Antibody Status**

When baseline features were compared in relatives with \( (n=311) \) or without \( (n=255) \) follow-up, those who were followed up were younger (32±14 versus 40±18 years, \( P = 0.0001 \)), were more frequently AHA-positive (40% versus 22%, \( P = 0.0001 \)), were from familial pedigrees 44% versus 33% of the time (\( P = 0.009 \)), and had a higher prevalence of LVE or dFS (30% versus 6%, \( P = 0.0001 \)), but a substantial number (42%) were assessed as normal and AHA-negative.

Median follow-up time was 58 months (range 1 to 132 months) in all relatives and 54 months (range 1 to 109 months) when those relatives with DCM at family screening were excluded, and follow-up time was shorter in those with LVE or dFS (with or without positive AHAs) than in normal and AHA-negative relatives (54±26 versus 64±26 months, \( P = 0.001 \)). At follow-up, 26 (8%) of the 311 normal or possibly affected asymptomatic relatives progressed (13 to DCM, 11 to LVE, and 2 to dFS). Table 3 shows baseline features of relatives with or without progression to DCM. Relatives who developed DCM had higher mean LVDD, had lower %FS, had higher %LVDD, were more frequently AHA-positive, and were more commonly classified as having LVE or dFS than those who did not develop DCM. The same

**TABLE 1. Baseline Features of the 592 Asymptomatic Relatives of DCM Patients in Relation to Familial or Nonfamilial Disease**

<table>
<thead>
<tr>
<th></th>
<th>Familial Disease ( (n=248) )</th>
<th>Nonfamilial Disease ( (n=344) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at study, y</td>
<td>35±16</td>
<td>36±16</td>
<td>0.41</td>
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<tr>
<td>No. (%) of males</td>
<td>121 (49)</td>
<td>170 (49)</td>
<td>0.90</td>
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<td>First-degree/second-degree relative</td>
<td>164/84</td>
<td>262/82</td>
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<td>2D echocardiogram</td>
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<tr>
<td>LVDD, mm</td>
<td>51±7</td>
<td>49±5</td>
<td>0.0001</td>
</tr>
<tr>
<td>%FS</td>
<td>32±7</td>
<td>36±6</td>
<td>0.0001</td>
</tr>
<tr>
<td>%LVDD</td>
<td>108±12</td>
<td>104±9</td>
<td>0.0001</td>
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<tr>
<td>No. (%) of relatives with</td>
<td></td>
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<tr>
<td>DCM</td>
<td>26 (10)</td>
<td>0 (0)</td>
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<td>LVE</td>
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<tr>
<td>dFS</td>
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<td></td>
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<tr>
<td>None</td>
<td>169 (68)</td>
<td>286 (83)</td>
<td></td>
</tr>
<tr>
<td>No. (%) of AHA-positive relatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>98 (40)</td>
<td>90 (26)</td>
<td>0.001</td>
</tr>
<tr>
<td>LVE</td>
<td>47 (19)</td>
<td>28 (8)</td>
<td>0.0001</td>
</tr>
<tr>
<td>dFS</td>
<td>21 (8)</td>
<td>20 (6)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* \( P \) value refers to 2 \( \times \) 4 contingency table.

**TABLE 2. Association of AHA Status of Relatives and of Proband**

<table>
<thead>
<tr>
<th>Proband AHA Status</th>
<th>AHA-Positive Relatives ( (n=188) )</th>
<th>AHA-Negative Relatives ( (n=404) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not assessed</td>
<td>49 (26)</td>
<td>61 (15)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>78 (41)</td>
<td>136 (34)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>61 (32)</td>
<td>207 (51)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are n (%). This table shows that AHA-positive status in probands is associated with AHA positivity among their relatives.

**TABLE 3. Baseline Features in Asymptomatic Relatives in Relation to Progression to DCM**

<table>
<thead>
<tr>
<th></th>
<th>Progression ( (n=13) )</th>
<th>No Progression ( (n=298) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at study, y</td>
<td>36±14</td>
<td>32±14</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex ratio (M/F), n</td>
<td>6/7</td>
<td>146/152</td>
<td>0.92</td>
</tr>
<tr>
<td>First-degree/second-degree relative</td>
<td>9/4</td>
<td>211/87</td>
<td>0.90</td>
</tr>
<tr>
<td>Familial/nonfamilial disease</td>
<td>8/5</td>
<td>142/156</td>
<td>0.30</td>
</tr>
<tr>
<td>2D echocardiogram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVDD, mm</td>
<td>54±5</td>
<td>50±5</td>
<td>0.02</td>
</tr>
<tr>
<td>%FS</td>
<td>28±7</td>
<td>34±6</td>
<td>0.01</td>
</tr>
<tr>
<td>%LVDD</td>
<td>115±11</td>
<td>106±9</td>
<td>0.02</td>
</tr>
<tr>
<td>No. (%) of AHA-positive relatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>6 (46)</td>
<td>78 (26)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>LVE</td>
<td>4 (31)</td>
<td>9 (3)</td>
<td></td>
</tr>
<tr>
<td>dFS</td>
<td>3 (23)</td>
<td>211 (71)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>9 (69)</td>
<td>112 (37)</td>
<td>0.02</td>
</tr>
<tr>
<td>No. (%) of high-titer (1/20 or higher) AHA-positive sera</td>
<td>3 (23)</td>
<td>43 (14)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

M indicates males; F, females.

* \( P \) value refers to 2 \( \times \) 3 contingency table.
Positive AHAs were weakly but not significantly associated with progression among relatives of AHA-negative probands; 15% of AHA-positive relatives progressed versus 6% of AHA-negative relatives (\(P=0.08\); Table 5). Similar results were obtained when relatives from familial and nonfamilial DCM probands were analyzed separately.

By Kaplan-Meier analysis, 5-year probability of survival free from progression to DCM (Figure 1), as well as to DCM, LVE, or dFS (Figure 2), among relatives initially classified as normal or as having LVE or dFS at baseline. AHA+ indicates AHA-positive; AHA−, AHA-negative. status did not attain statistical significance (\(P=0.3\), RR=1.97, 95% CI 0.75 to 3.19; Table 6). The sensitivities and specificities of AHAs alone or in conjunction with an abnormal echocardiogram as predictors of progression are shown in Table 7.

### Table 4. Baseline Features in Asymptomatic Relatives in Relation to Progression to DCM (n=13), LVE (n=11), or dFS (n=2)

<table>
<thead>
<tr>
<th>Progression</th>
<th>No Progression</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=26)</td>
<td>(n=285)</td>
<td></td>
</tr>
<tr>
<td>Mean age at study, y</td>
<td>33±12</td>
<td>31±14</td>
</tr>
<tr>
<td>Sex ratio (M/F), n</td>
<td>14/12</td>
<td>138/147</td>
</tr>
<tr>
<td>First-degree/second-degree relatives</td>
<td>17/9</td>
<td>203/82</td>
</tr>
<tr>
<td>Familial/nonfamilial disease</td>
<td>15/11</td>
<td>135/150</td>
</tr>
<tr>
<td>2D echocardiogram</td>
<td>(\text{LVDD, mm})</td>
<td>53±4</td>
</tr>
<tr>
<td></td>
<td>(%\text{FS})</td>
<td>30±6</td>
</tr>
<tr>
<td></td>
<td>(%\text{LVDD})</td>
<td>112±9</td>
</tr>
<tr>
<td>No. (%) of relatives with</td>
<td>LVE</td>
<td>6 (23)</td>
</tr>
<tr>
<td></td>
<td>dFS</td>
<td>4 (15)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>16 (61)†</td>
</tr>
<tr>
<td>No. (%) of AHA-positive relatives</td>
<td>16 (61)†</td>
<td>105 (37)</td>
</tr>
<tr>
<td>No. (%) of high-titer (1/20 or higher)</td>
<td>6 (23)</td>
<td>40 (14)</td>
</tr>
</tbody>
</table>

M indicates males; F, females.

*\(P\) value refers to 2×3 contingency table.

†Of the 16 normal subjects who progressed, 3 progressed to DCM, 11 to LVE, and 2 to dFS.

### Table 5. Progression to DCM, LVE, or dFS by AHA Status if Proband Is AHA-Negative

<table>
<thead>
<tr>
<th>Progression (n=12)</th>
<th>No Progression (n=131)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHA-positive relatives, n (%)</td>
<td>6 (15)</td>
<td>35 (85)</td>
</tr>
<tr>
<td>AHA-negative relatives, n (%)</td>
<td>6 (6)</td>
<td>96 (94)</td>
</tr>
</tbody>
</table>

This table shows that AHA-positive status was weakly but not significantly associated with progression among relatives of AHA-negative probands.

**Figure 1.** Probability of remaining free of DCM during follow-up (months) according to AHA status at initial evaluation for asymptomatic relatives classified as normal or as having LVE or dFS at baseline. AHA+ indicates AHA-positive; AHA−, AHA-negative.

**Figure 2.** Probability of remaining free of any progression (to DCM, LVE, or dFS) during follow-up (months) according to AHA status at initial evaluation for asymptomatic relatives classified as normal or as having LVE or dFS at baseline. AHA+ indicates AHA-positive; AHA−, AHA-negative.
Target-organ function. The present data indicate that AHAs on the onset of clinical symptoms or detectable abnormalities of heart dysfunction. In keeping with this, positive AHA status alone had higher sensitivity (61%) than its combination with abnormal echocardiogram (sensitivity 27%) as a predictor of progression to DCM, LVE, or dFS. In other words, positive AHAs with a normal echocardiogram identified a proportion of relatives at risk of progression to DCM, or of progression from normal to preclinical DCM (eg, LVE or dFS), that would not have been identified by echocardiography alone. In addition, the present data suggest that both techniques are necessary in DCM family screening and counseling. In fact, the positive predictive value (PPV) for progression to DCM was higher (18%) for both abnormal echocardiogram and positive AHAs than for AHAs alone (7%) or echocardiogram alone (10%). Similarly, the PPV for any progression (eg, DCM, LVE, or dFS) was higher (18%) for both an abnormal echocardiogram and positive AHAs than for AHAs alone (13%) or echocardiogram alone (10%). The combination of abnormal echocardiography and positive AHAs appears to identify relatives at a more advanced stage of preclinical DCM, who need closer follow-up and could potentially benefit from therapeutic intervention to attenuate or prevent disease development. Finally, negative AHAs alone or in combination with a normal echocardiogram had a good negative predictive value (98%) and allowed the identification of the majority of subjects at low risk of progression at least up to 5 years. An important issue that needs further study is the long-term outcome of these AHA-negative relatives with normal echocardiograms. In type 1 diabetes mellitus, a staging of preclinical disease has been proposed for siblings of affected children based on a combination of the initial number of antibodies and FPIR to intravenous glucose: no prediabetes (no antibodies), early prediabetes (1 antibody specificity, normal FPIR), advanced prediabetes (2 or more antibodies, normal FPIR), and late prediabetes (at least 1 antibody, reduced FPIR). By analogy, if the same applies to DCM, the staging could be as follows: no pre-DCM (negative AHAs, normal echocardiogram), early (positive AHAs, normal echocardiogram), advanced (AHA-positive and positivity for 1 or more of the other antibodies described in DCM13–22), and late pre-DCM (at least 1 antibody marker and LVE or dFS). Although 98% of relatives with negative AHAs and precede echocardiographic abnormalities; AHA-positive relatives, classified as normal at first screening, progressed to DCM, LVE, or dFS. Thus, there is, in our opinion, good justification for equating the AHA-positive relatives in the present study with others diagnosed with noncardiac autoimmune disorders. The prospective data presented here are in keeping with earlier observations that revealed that these relatives have other features of immune activation.24,25

Relative Utility of Echocardiography and AHAs in DCM Family Studies

Prospective family studies indicate that echocardiographic abnormalities, defined as LVE and dFS, represent early, preclinical DCM or asymptomatic left ventricular dysfunction in symptom-free relatives, similar to the first-phase insulin response (FPIR) to intravenous glucose in prediabetes. Conversely, AHAs, similar to the multiple antibody markers in preclinical diabetes, precede other diagnostic abnormalities of heart dysfunction. In keeping with this, positive AHA status alone had higher sensitivity (61%) than its combination with abnormal echocardiogram (sensitivity 27%) as a predictor of progression to DCM, LVE, or dFS. In other words, positive AHAs with a normal echocardiogram identified a proportion of relatives at risk of progression to DCM, or of progression from normal to preclinical DCM (eg, LVE or dFS), that would not have been identified by echocardiography alone. In addition, the present data suggest that both techniques are necessary in DCM family screening and counseling. In fact, the positive predictive value (PPV) for progression to DCM was higher (18%) for both abnormal echocardiogram and positive AHAs than for AHAs alone (7%) or echocardiogram alone (10%). Similarly, the PPV for any progression (eg, DCM, LVE, or dFS) was higher (18%) for both an abnormal echocardiogram and positive AHAs than for AHAs alone (13%) or echocardiogram alone (10%). The combination of abnormal echocardiography and positive AHAs appears to identify relatives at a more advanced stage of preclinical DCM, who need closer follow-up and could potentially benefit from therapeutic intervention to attenuate or prevent disease development. Finally, negative AHAs alone or in combination with a normal echocardiogram had a good negative predictive value (98%) and allowed the identification of the majority of subjects at low risk of progression at least up to 5 years. An important issue that needs further study is the long-term outcome of these AHA-negative relatives with normal echocardiograms. In type 1 diabetes mellitus, a staging of preclinical disease has been proposed for siblings of affected children based on a combination of the initial number of antibodies and FPIR to intravenous glucose: no prediabetes (no antibodies), early prediabetes (1 antibody specificity, normal FPIR), advanced prediabetes (2 or more antibodies, normal FPIR), and late prediabetes (at least 1 antibody, reduced FPIR). By analogy, if the same applies to DCM, the staging could be as follows: no pre-DCM (negative AHAs, normal echocardiogram), early (positive AHAs, normal echocardiogram), advanced (AHA-positive and positivity for 1 or more of the other antibodies described in DCM13–22), and late pre-DCM (at least 1 antibody marker and LVE or dFS). Although 98% of relatives with negative AHAs and
normal echocardiograms did not progress up to 5 years, a long latency period and slow progression are features of organ-specific autoimmune disease,1–4 and therefore, a proportion of them may develop AHAs in the future, thus becoming at risk. Therefore, longer follow-up is needed to completely reassure these subjects, and it may be appropriate to provide echocardiographic and immunologic testing, although less frequently than for those with AHAs and/or abnormal echocardiography.

### Limitations of Cardiac Autoantibody Tests for Risk Assessment in DCM Family Studies

Several autoimmune features seen in DCM resemble those found in type 1A diabetes mellitus: male preponderance, human leukocyte antigen (HLA)-DR4 association,1,4,30 familial aggregation,1–6 familial clustering of other autoimmune diseases,1,31 and presence of multiple cardiac autoantibody specificities among patients and asymptomatic relatives.1–4,7,15–22,32 The detection of multiple autoantibodies or of a single high-titer antibody increases the PPV of autoimmune serology in siblings of type 1A diabetes mellitus.4,29,32 In addition, some of these markers appear early and are closely associated with the initial pathogenetic events, whereas others are detected later, in relation to epitope spreading; consequently, each antibody or antibody combination has distinct predictive value.33

Three of the autoantigens recognized by the AHA detected by indirect immunofluorescence in DCM were identified by our group as α- and β-myosin heavy chain and myosin light chain-1v by Western blotting.14 AHAs were not directed against tropomyosin, actin, or troponin, but other unknown antigens were present.14 The finding of disease-specific anti-myosin antibodies in myocarditis/DCM has been confirmed by others.15,16 Although myosin is one of the relevant antigens responsible for the AHAs detected by indirect immunofluorescence, Western blotting, or ELISA in DCM, it is unknown whether subjects classified as seronegative for 1 antibody are positive for another, which is the temporal sequence of appearance of the various antibodies (antimyosin, troponin, β-adrenoceptor, mitochondrial, and other antigens) and whether single or multiple antigen-specific antibody tests will be superior to a non–antigen-specific technique such as indirect immunofluorescence as screening tools.13–22 Collaborative work among laboratories testing the individual antibodies is warranted. Further work on IgG subclass20 and epitope mapping21 for the individual antibodies may lead to an improvement in the predictability of the various tests.13–22

In type 1A diabetes mellitus, standardization and quantification of islet cell antibody has been advantageous to refine its PPV, particularly in relation to high-titer antibody conferring additional risk.3 To date, this is the first study showing that AHAs identify symptom-free relatives at risk of DCM; the follow-up is relatively short, and the study was not initially planned to prospectively test AHAs as disease predictors. The low incidence of primary and secondary end points (n =13 and 26, respectively) may result in low precision to estimate effect size and low power to detect effects. Finally, there is no equivalent of Juvenile Diabetes Foundation (JDF) units3 for AHAs. Although high AHA titers were not associated with progression, the present study was not powered to perform quantitative analysis on end-point–titrated positive sera. Further quantitative work should be performed as soon as the number of progressors increases, to test the hypothesis that high-titer AHAs may confer higher risk.

Will genetic markers combined with AHAs increase prediction among relatives at risk of DCM? In type 1A diabetes mellitus, the predisposing HLA markers identify relatives at higher risk when used in conjunction with antibody testing.4,29 This strategy is useful to assess the risk at the

### TABLE 7. AHA and Echocardiography Status as Predictors of Disease Progression at 100 Months

<table>
<thead>
<tr>
<th>Progression to DCM</th>
<th>AHA Alone, Abnormal/Normal</th>
<th>Echo Alone, Abnormal/Normal</th>
<th>Both Echo and AHA, Abnormal/Normal</th>
<th>Either Echo or AHA, Abnormal/Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>9/4</td>
<td>10/3</td>
<td>7/6</td>
<td>12/1</td>
</tr>
<tr>
<td>No</td>
<td>112/186</td>
<td>87/211</td>
<td>32/266</td>
<td>167/131</td>
</tr>
<tr>
<td>P</td>
<td>0.02</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>69</td>
<td>77</td>
<td>54</td>
<td>92</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>62</td>
<td>71</td>
<td>89</td>
<td>44</td>
</tr>
<tr>
<td>PPV, %</td>
<td>7</td>
<td>10</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>NPV, %</td>
<td>98</td>
<td>98.5</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Progression to DCM, LVE, or dFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16/10</td>
<td>10/16</td>
<td>7/19</td>
<td>19/7</td>
</tr>
<tr>
<td>No</td>
<td>105/180</td>
<td>87/198</td>
<td>32/253</td>
<td>160/125</td>
</tr>
<tr>
<td>P</td>
<td>0.01</td>
<td>0.40</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>61.5</td>
<td>38</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>63</td>
<td>69.5</td>
<td>89</td>
<td>44</td>
</tr>
<tr>
<td>PPV, %</td>
<td>13</td>
<td>10</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>NPV, %</td>
<td>95</td>
<td>92.5</td>
<td>93</td>
<td>95</td>
</tr>
</tbody>
</table>

PPV indicates positive predictive value; NPV, negative predictive value; AHA+/AHA−, AHA-positive/AHA-negative; and echo, 2D echocardiogram.
individual level or to recruit high-risk subjects for intervention trials; conversely, because it usually leads to reduced sensitivity, autoantibodies alone are recommended as the first-line screening in siblings. A weak HLA-DR4 association has been reported in nonfamilial DCM, but there have been negative studies. Because DCM is genetically and etiologically heterogeneous, further studies are needed to evaluate the potential contribution of HLA to genetic susceptibility and risk prediction. We had shown, and confirmed here, that in \( \approx 30\% \) of pedigrees, AHAs are not found, whereas positive AHAs in the proband were associated with AHAs among relatives. This reinforces the issue of heterogeneity in DCM, although long-term follow-up might reveal that some relatives from non-AHA families will develop these markers. AHA was also weakly but not significantly associated with progression among relatives of AHA-negative probands. This may relate to the lower number of progressors in this subset analysis; negative AHAs in the proband may also reflect reduced titers with disease progression. Thus, extended follow-up is needed to clarify whether there is an AHA form and a non-AHA form of DCM.

The lack of a greater association of AHAs with disease progression in first- versus second-degree relatives may reflect failure to reach statistical significance due to the low number of progressors, but it may also relate to the fact that autoimmune diseases are not entirely genetically determined, because they are thought to result from a polygenic HLA and non-HLA-linked susceptibility and its interaction with the environment. However, single gene defects might account for DCM in some families, eg, as for type 1A diabetes mellitus in autoimmune polyendocrinopathy-candidiasis–ectodermal dystrophy families.

In conclusion, the presence of organ-specific AHAs in asymptomatic relatives predicts development of DCM at 5 years and provides a noninvasive immune marker in 60% of familial and nonfamilial cases of DCM. PPV of AHA was low. PPV of autoantibodies in autoimmune disease is a function of many variables, in particular, prevalence of the disease in the population, type of autoimmune disease, at-risk population screened (eg, for type 1A diabetes mellitus, twins versus first-degree relatives versus schoolchildren), autoantibody specificity, titer and persistence or fluctuation during follow-up, genetic risk (HLA predisposing or protective haplotypes), and follow-up length, owing to the long latency period. Thus, an accurate comparison of AHA with other organ-specific autoantibodies is not feasible, at least at this stage. However, the PPV of AHA is similar to that of islet cell antibody alone in some populations at risk of type 1A diabetes mellitus. So far, evidence for a direct pathogenic role of AHA is lacking. The relatively high proportion of AHA relatives who did not progress in the mid term is likely to reflect the long latency period and slow progression of disease. Long-term prospective clinical and immunologic follow-ups are warranted.

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**Disclosures**

None.

**References**


In autoimmune disorders, circulating autoantibodies identify healthy relatives at risk years before clinical presentation. Dilated cardiomyopathy (DCM) is a genetically heterogeneous disease with multifactorial pathogenesis. It may be familial/genetic, viral, and/or immune. Autoimmunity is recognized to play a pivotal role in the pathogenesis of a substantial proportion of cases, possibly triggered by various causes of cardiac injury in genetically predisposed individuals. Using indirect immunofluorescence, organ- and disease-specific anti-heart autoantibodies (AHAs) are found in ~30% of DCM patients at clinical presentation, in 20% to 30% of their symptom-free relatives, and in 60% of familial and nonfamilial pedigrees. Symptom-free relatives of DCM patients with subtle echocardiographic changes, in particular, left ventricular enlargement or depressed fractional shortening at baseline, have increased medium-term risk for DCM development. In this prospective article, the authors demonstrate for the first time that similar to other autoimmune disorders, serum AHAs at initial family evaluation identify at a preclinical stage asymptomatic relatives at risk of DCM development. In this prospective article, the authors demonstrate for the first time that similar to other autoimmune disorders, serum AHAs at initial family evaluation identify at a preclinical stage asymptomatic relatives at risk of DCM development.
Localization and Quantification of Platelet-Rich Thrombi in Large Blood Vessels With Near-Infrared Fluorescence Imaging

Robert Flaumenhaft, MD, PhD; Eiichi Tanaka, MD, PhD; Gwenda J. Graham, PhD; Alec M. De Grand, BS; Rita G. Laurence, BS; Kozo Hoshino, MD; Roger J. Hajjar, MD; John V. Frangioni, MD, PhD

Background—Imaging of thrombus formation in vivo has been limited by the inability to directly visualize and measure thrombi in large blood vessels in real time. Near-infrared light, with its superior tissue penetration and reduced scatter, could potentially solve this problem.

Methods and Results—Platelets were labeled with the near-infrared fluorophore IR-786. Optimal total fluorescence yield occurred at 6 attomoles of IR-786 per platelet. IR-786–labeled platelets were tested for their ability to detect thrombus formation in large animal model systems relevant to common human vascular procedures. Invisible near-infrared light did not distort the surgical field in any way, and even after optimization of per-platelet fluorescent yield, platelets remained fully functional. Intravenous infusion of just $3.6 \times 10^{10}$ labeled platelets into a 35-kg Yorkshire pig permitted thrombus visualization, with a signal-to-background ratio $\geq 2$, for at least 2 hours in coronary, carotid, and femoral vessels. Platelet-rich, actively growing clots were monitored in real time and quantified with respect to size and kinetics after injury to vessels, cutaneous incisions, intravascular stent insertion, or introduction of embolic coils. Similarly, formed clots were monitored in real time during thrombolysis with streptokinase and heparin. Vessel patency was assessed independently with a second near-infrared fluorescent blood pool agent.

Conclusions—IR-786–labeled platelets provide sensitive, specific, and real-time visualization of thrombi in thick-walled blood vessels. In addition to immediate application in cardiac, transplant, and vascular surgery, the mechanisms that underlie thrombus formation in large blood vessels can now be investigated. (Circulation. 2007;115:84-93.)

Key Words: imaging ■ platelets ■ thrombosis ■ fluorescence

Among the emerging imaging techniques in cardiovascular medicine is the use of near-infrared (NIR) light (700 to 900 nm) to monitor the circulatory system.1–5 The fundamental advantage of imaging in the NIR range is that photon penetration into living tissue is higher because of lower photon absorption and scatter.6 An additional advantage is that tissue emits limited intrinsic fluorescence (ie, autofluorescence) in the 700- to 900-nm range. Therefore, fluorescence contrast agents that emit in the NIR range demonstrate a favorable signal-to-background (SBR) ratio when used in animal models or for patient care.

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Real-time in vivo visualization of thrombus formation is an important goal in both research and clinical settings but is problematic because the vasculature is a closed system. In small animal models, thrombi have been visualized with probes such as fluorophore-conjugated antibodies or platelets labeled with fluorescent dyes.7–10 The fluorophores used in these approaches emit light in the visible spectrum. Because of high absorption and scatter, visible wavelengths cannot penetrate large vessels and, thus, this approach has been largely limited to small vessels such as those within the mesenteric and cremaster microvasculatures of rodents.7–10 Dyes that emit NIR light have been used to assess perfusion of cardiac vessels intraoperatively.2,4,11–13 Although these dyes are useful for detecting stenosis and obstruction, they have only modest sensitivity for thrombus detection and cannot distinguish evolving clots from stabilized clots. New approaches that can detect thrombus formation in vessels that approximate the size of human coronary, carotid, and femoral vessels are required to study thrombus formation in large animal models and to detect thrombi during vascular procedures.

We hypothesized that platelets loaded with a dye that emits NIR light could be used to image thrombus formation after injury of large muscular vessels. To evaluate this possibility, we labeled platelets with IR-786, a lipophilic, cationic,
heptamethine indocyanine-type NIR fluorophore. We now show that IR-786–labeled platelets used in conjunction with an integrated NIR fluorescence imaging system are able to quantitatively monitor thrombus formation and dissolution in real time. This method enables visualization and measurement of thrombus formation in major vessels of large animals. Use of IR-786–labeled platelets in conjunction with an NIR fluorescence imaging system demonstrates several favorable features compared with current approaches of thrombus detection.

Methods

Preparation of IR-786–Labeled Washed Platelets
Washed human platelets were prepared by differential centrifugation of fresh blood obtained from aspirin-free donors as described previously. Washed pig platelets were isolated from fresh blood obtained from anesthetized Yorkshire pigs. Platelet-rich plasma was prepared by centrifugation at 200g for 20 minutes. Platelets were then isolated from platelet-rich plasma by centrifugation at 1400g for 10 minutes in the presence of 50 ng/mL prostaglandin E1 and 10% v/v acid citrate/dextrose, pH 4.6, and resuspended at a concentration of 4×10^11 cells/mL in Tyrode’s-HEPES buffer. The perchlorate salt of IR-786 (CAS #102185-03-5) was purchased from Sigma-Aldrich (St. Louis, Mo).

Quantification of IR-786 Uptake Into Platelets
Platelet counts were measured with the HEMAVET Multispecies Hematology Analyzer (Drew Scientific, Oxford, Conn). For platelet-loading experiments, 1-mL samples of washed platelets (~4×10^10 total) were incubated for 0, 15, 30, 60, 90, or 120 minutes at room temperature with gentle rocking in Tyrode’s-HEPES supplemented with 5, 2.5, 1.25, 0.625, or 0 μmol/L IR-786. For measurements, platelets were pelleted for 5 minutes at 2000g in the presence of prostaglandin E1. Pellets were lysed with 500 μL absolute methanol by repeated pipetting and sonication for 1 minute at a 50% duty cycle. Sample fluorescence was measured by comparison to IR-786 calibration standards in methanol (pellets) or Tyrode’s-HEPES (supernatant).

Spectral Measurements and NIR Fluorescence Microscopy
Absorbance and fluorescence measurements were performed as described previously. For measurement of relative fluorescence yield, IR-786 samples in Tyrode’s-HEPES, methanol, or concentrated in washed platelets were matched for absorbance (0.1 A units) and area under the fluorescence emission curve calculated after excitation with a 5-mW 655-nm laser diode. NIR fluorescence microscopy was performed as previously described.

Platelet Aggregation Studies
Platelets resuspended at a density of 4×10^11 cells/mL in modified Tyrode’s-HEPES buffer were stimulated with agonists in siliconized glass tubes in an optical aggregometer (Chronolog, Haverton, Pa). Assays were performed at 37°C and with constant stirring. Aggregation was monitored by measurement of optical density of the platelet suspension.

Animal studies were performed in accordance with an approved institutional protocol. Yorkshire pigs (E.M. Parsons and Sons, Hadley, Mass) that weighed 35 kg were anesthetized with 4.4 mg/kg IM tiletamine/zolazopam (Telazol, Fort Dodge Labs, Fort Dodge, Iowa). Animals were intubated with a 7-mm cuffed endotracheal tube and anesthesia was maintained with oxygen and isoflurane 0.5 to 5.0% to effect. Animals were prepped and draped in the usual sterile fashion, and the indicated vessels were exposed with standard surgical techniques.

Vessel Injury Models
Platelets (3.6×10^10 total) in Tyrode’s-HEPES were labeled with 2 μmol/L IR-786 for 30 minutes at room temperature and infused intravenously before induction of thrombi with FeCl3, embolic coil, intravascular stent, or cutaneous incision. For induction of thrombus formation with FeCl3, a 0.5×1-cm2 swatch of grade 413 Whatman filter paper was saturated with a 50% solution of FeCl3 (Sigma-Aldrich) and applied beneath the vessel so as not to impede visualization of IR-786–labeled platelet accumulation. For induction of thrombi with either embolic coils or stents, a 5F Pinnacle sheath introducer (Terumo Medical, Elkton, Md) was inserted into the vessel and used to deploy devices. Thrombus formation was then imaged continuously until fluorescence signal stabilized. Streptokinase was from Sigma-Aldrich and heparin was from American Pharmaceutical Partners (Schauflburg, Ill).

Intraoperative NIR Fluorescence Imaging System
The imaging system has been described in detail previously, with the following modifications. Three wavelength-isolated excitation sources were utilized: one that generates 400- to 680-nm “white” light (0.5 mW/cm^2); a second that generates 680- to 700-nm low-NIR fluorescence excitation light (1 mW/cm^2) with model number L-660-66-60-550 high-power light-emitting diodes (Marubeni Epitek, New York, NY) and custom excitation filters; and a third that generates 725- to 775-nm NIR fluorescence-excitation light (5 mW/cm^2). All sources operated over a 15-cm diameter field of view. Photon collection is achieved with custom-designed optics that maintain the separation of the white light and NIR fluorescence emission (ie, 700 to 725 nm versus >795 nm) channels. With custom LabVIEW software (National Instruments, Austin, Tex), anatomic (white light) and functional (NIR fluorescence light) images can be displayed separately and merged. To create a single image that displays both anatomy (color video) and function (NIR fluorescence), the NIR fluorescence image was pseudocolored (eg, in lime green) and overlaid with 100% transparency on top of the color video image of the same surgical field. All images are refreshed up to 15 times per second. The entire apparatus is suspended on an articulated arm over the surgical field, which thus permits unobtrusive imaging.

Vessel patency was assessed by intravenous injection of 1 mL of 1% (10 mg total) methylene blue (Mayne Pharma, Paramus, NJ) with continuous imaging of NIR fluorescence (700 to 725 nm) emission (E.T., unpublished data, 2006).

Quantification of In Vivo Thrombi
NIR fluorescence excitation light, and field of view were held constant for all quantitative comparisons. Regions of interest of a defined shape and pixel number could be moved anywhere within the field of view to quantify NIR fluorescence emission signal intensity. SBR was assessed by quantification of fluorescence signal from a region of interest that encompasses the thrombus compared with an intravascular region of interest of the same size proximal to the thrombus.

Statistics
A Wilcoxon rank-sum test was used for statistical comparison of SBR of thrombi. The number of thrombi used for each comparison is indicated in the text. Two femoral thrombi (right and left) were formed in each animal.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Incorporation of IR-786 Into Human Platelets
To develop a contrast agent with the capability to detect thrombi in major vessels of large animals, we labeled platelets with IR-786. IR-786 is a highly hydrophobic nonsulfonated heptamethine that emits NIR light (Figure 1A). Initial ex vivo studies were designed to determine the optimal fluores-
cence yield after platelet loading. Time course studies demonstrated maximal incorporation of IR-786 into platelets after 30 minutes of incubation (Figure 1B). A dose-response curve showed that platelet loading occurred in a linear manner until 2.5 μmol/L (Figure 1C). At concentrations >2.5 μmol/L, the linearity of dose-dependency was lost, which indicates self-quenching and/or dye aggregation. All subsequent experiments were therefore performed with 2 μmol/L IR-786. At
this concentration, platelets incorporated ≈6 attomoles (=3 600 000 molecules) of IR-786 per platelet.

Spectral analysis demonstrated that platelet incorporation of IR-786 resulted in a characteristic red shift. The excitation and emission maxima of IR-786 in aqueous buffer were 769.3 and 788.8 nm, respectively (Table 1). In contrast, its excitation and emission maxima after incorporation into platelets were 788 and 804 nm, even redder than emission found in neat methanol (Table 1).

We next characterized the subcellular location of IR-786 in labeled platelets. Although there was a small degree of homogeneous staining of lamellipodia and pseudopodia consistent with plasma membrane staining, the majority of fluorescence was punctate and localized in the central granulomere (Figure 1D). This pattern of fluorescence suggests incorporation of dye into intracellular structures and is consistent with staining patterns observed in other cell types.16

To ensure that platelets labeled with 2 μmol/L IR-786 retained function, we assessed the ability of IR-786–labeled platelets to aggregate in response to agonists. IR-786–labeled platelets demonstrated normal aggregation in response to either thrombin or collagen-related protein (Figure 1E). Evaluation of resting platelets labeled with 2 μmol/L IR-786 showed no significant P-selectin surface expression, which demonstrates that incubation with IR-786 does not activate platelets (data not shown). These data indicate that platelets remain functionally intact after labeling.

Clearance of IR-786–Labeled Platelets In Vivo
A porcine model was used to test the clearance of platelets labeled with IR-786. Washed pig platelets (3.6 × 10^10) were labeled with 2 μmol/L IR-786 for 30 minutes. IR-786–labeled platelets were then infused through a cannula in the internal jugular vein of the pig and the line was extensively flushed with saline. Blood samples were obtained at the indicated times after infusion, and platelet-rich plasma was analyzed (Figure 2A). Evaluation of platelet-associated fluorescence demonstrated a rapid increase in fluorescence after infusion of the IR-786–labeled platelets. After this increase, there was a period of sharp decline in fluorescence until ≈20 minutes. A slow decline in fluorescence then followed. Clearance of IR-786–labeled platelets was also analyzed by manually counting labeled and unlabeled platelets in platelet-rich plasma. Microscopic analysis

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showed that 2.0±0.4% of platelets were labeled at 15 minutes after infusion and that 2.6±0.6% (n=3, P=0.4) were labeled at 150 minutes after infusion. Based on these data, we conclude that a majority of IR-786–labeled platelets remain in circulation 150 minutes after infusion into pigs. The decline in fluorescence observed in Figure 2A may not be primarily the result of platelet clearance. We suspect that free IR-786 is rapidly cleared by the liver after infusion16 and that the fluorescent signal in the platelets slowly declines over time as demonstrated in Figure 1B. Overall, these data indicate that the majority of IR-786–labeled pig platelets are not rapidly cleared from the circulation.

We also tested the ability of pig platelets to aggregate after incubation with IR-786. Washed pig platelets incubated for 30 minutes with 2 μmol/L IR-786 aggregated normally in response to thrombin or collagen-related protein (Figure 2B). Evaluation of aggregometry of pig platelets obtained 2 hours after infusion of IR-786–labeled platelets demonstrated that labeling with 2 μmol/L IR-786 did not affect platelet aggregation. Furthermore, NIR microscopy of platelets after aggregation studies demonstrated that IR-786–labeled platelets incorporate into aggregates (data not shown). Evaluation of erythrocyte and platelet counts after infusion of IR-786–labeled platelets demonstrated that infusion of this contrast media had no significant effects on circulating numbers of these blood cells (data not shown).

Figure 3. Real-time detection and quantification of thrombus formation after FeCl₃-induced injury. A, One hour after treatment of the femoral artery with FeCl₃. Shown are color video (left), NIR fluorescence (middle; 67 ms exposure time), and a pseudocolored (lime green) merge of the color video and NIR image (right). Arrow indicates location of intravascular thrombus. A representative image from 8 independent femoral artery thrombi is shown. B, One hour after treatment of the femoral vein with FeCl₃, with imaging as described in 3A. C, Hemotoxylin and eosin (H&E) histology and NIR fluorescence from the same tissue section of a FeCl₃-induced thrombus in the femoral artery. Note characteristic changes to the vessel wall exposed to FeCl₃. A representative image from 8 independent femoral artery thrombi is shown. D, IR-786–labeled platelets were infused 30 minutes after application of FeCl₃ to femoral arteries. The image shows H&E histology and NIR fluorescence from a longitudinal section of an injured femoral artery. The image represents 1 of 4 independent femoral artery thrombi that were evaluated under these conditions.
IR-786–Labeled Platelets Detect Thrombus Formation In Vivo

We next determined whether we could detect thrombi in live pigs and in real time with IR-786–labeled platelets. Thrombus formation after oxidant injury induced by exposure of vessels to filter paper saturated with FeCl₃ is a widely used and reliable method for in vivo induction of thrombus formation.⁷,¹⁹,²⁰ We hypothesized that the high fluorescence yield of IR-786 in platelets and the enhanced tissue penetration of NIR light would enable us to visualize thrombi in large, thick-walled vessels. FeCl₃-induced injuries to the femoral arteries were studied first. Imaging demonstrated the accumulation of platelets at the site of injury as represented by increased fluorescence signal (Figure 3A). Development of platelet-rich thrombi could also be visualized after FeCl₃-induced injury of the femoral vein (Figure 3B). Quantification of images demonstrated that platelet accumulation began 25 to 35 minutes after application of FeCl₃ (see Applications of IR-786–Labeled Platelets). This delay may represent the time required for diffusion of the FeCl₃ through the vessel wall or the time required for oxidative denudation of the endothelium. Thrombus formation began after this delay and continued to increase over the 150-minute experiment. These studies demonstrated that IR-786–labeled platelets accumulate at sites of thrombus formation, which thereby provides precise localization of thrombi within large, thick-walled vasculature.

Hematoxylin and eosin staining of these vessels demonstrated large thrombi oriented toward the portion of the vessel exposed to FeCl₃, (Figure 3C). NIR microscopy showed that IR-786–labeled platelets are diffusely incorporated throughout the body of the thrombus (Figure 3C). There is no evidence for incorporation of IR-786 into the underlying vasculature, which indicates that IR-786 remains platelet-associated.

To determine whether IR-786–labeled platelets can accumulate at sites of preexisting thrombi, IR-786–labeled platelets were infused 30 minutes after exposure of femoral arteries to FeCl₃. Under these conditions, fluorescence accumulated at the injury site within minutes of infusion of IR-786–labeled platelets. Histology of the injured artery demonstrated a component of the thrombus devoid of fluo-
rescence directly apposed to the inferior surface of the vessel, adjacent to the site of FeCl₃ application. Only the more luminal component of the thrombus contained IR-786–labeled platelets (Figure 3D). These results indicate that IR-786–labeled platelets adhered to and accumulated at the site of a preexisting thrombus.

Applications of IR-786–Labeled Platelets

Although the FeCl₃-induced injury is widely used to model thrombus formation in vivo, we sought to determine whether IR-786–labeled platelets could detect thrombi formed under circumstances encountered during surgical or vascular procedures. We observed that, after cutaneous incision and wound irrigation, a rim of thrombus formation could be visualized at the edge of wounds (Figure 4A). Occasionally, a thrombus would form at the site of electrocautery, as is shown in Figure 4A for the carotid artery. Reproducible thrombus formation occurred after insertion of intravascular devices into major vessels. Placement of an embolic coil into the iliac artery in an unheparinized animal resulted in rapid thrombus formation (Figure 4A). Similarly, a thrombus developed in the iliac artery after placement of a stent in an unheparinized animal (Figure 4A). The onset of thrombus formation after surgical manipulation of vessels or placement of intravascular devices was significantly more rapid than that after FeCl₃ exposure (Figure 4B). The average maximal SBR after FeCl₃ exposure was 4.4±1.7 (n=4) compared with an average maximal SBR of 3.3±1.9 (n=4) after embolic coil placement. These examples demonstrate that IR-786–labeled platelets constitute a versatile and quantitative contrast medium for detection of thrombi formed after a variety of vascular manipulations.

Monitoring Thrombolysis With IR-786–Labeled Platelets

We next asked whether IR-786–labeled platelets could be used to monitor the dynamics of thrombus growth and dissolution. As shown in Figure 5A, placement of an embolic coil in the femoral artery resulted in rapid formation of an intravascular thrombus, the extent of which could be quantified with NIR fluorescence. By 40 minutes, the thrombus had stabilized in size, at which point streptokinase and heparin were infused and dissolution of the thrombus was monitored in real time. Thrombolysis resulted in a 22.5% (P=0.0312, n=6) decrease in fluorescence signal. A second pattern of thrombus behavior after streptokinase and heparin infusion is shown in Figure 5B for a femoral artery treated with FeCl₃. In this case, thrombolytics triggered embolization of the thrombus, which then reformed slowly in the vessel. These data demonstrate that IR-786–labeled platelets can be used to monitor the efficacy of thrombolytic therapy in vivo and in real time.

Assessment of Vascular Patency During Thrombus Formation

An intravascular thrombus, even a large one, does not necessarily result in cessation of blood flow. One of the many advantages of NIR light is that the “NIR window” (wavelength range 700 to 900 nm) is 200 nm wide. This permits >1 NIR fluorophore to be used simultaneously. In recent work (E.T., unpublished data, 2006), we have characterized the NIR fluorescent properties of methylene blue, an agent already approved by the US Food and Drug Administration as a blue dye for surgery. Because methylene blue fluorescence peaks at ~700 nm, its fluorescence is well separated from that of IR-786. As shown in Figure 6, methylene blue can thus be used to assess vessel patency simultaneously with IR-786–labeled platelets used to monitor thrombus size and location. In the example shown, a thrombus is seen growing in the vessel until vascular occlusion occurs, at which point the vessel is supplied only by back-fill through a small collateral vessel.
The present study demonstrates that IR-786–labeled platelets are a sensitive reagent for the optical detection of thrombi in the major vessels of large animals. Previously described molecular probes designed to detect thrombi in vivo with NIR fluorescence have typically been engineered with a single fluorophore molecule per probe. The intracellular probe described in the present study concentrated to 10^6 molecules of fluorophore per platelet, which corresponds to an intracellular concentration of 700 μmol/L. This enormous concentration of probe compensates for the fact that the fluorescence yield from IR-786 incorporated into platelets is significantly lower than that of free IR-786 in methanol or aqueous buffer (Table 1) as a result of quenching and internal absorption. In addition to producing a concentrated dye, the platelet is uniquely adapted as a probe to detect blood clots because of its ability to adhere to sites of vascular injury and incorporate into thrombi. This biological signal amplification enables thrombi to be imaged while circulating labeled platelets remain undetectable.

IR-786–labeled platelets demonstrate several benefits compared with previously described fluorescent contrast agents and probes used to detect thrombi in vivo. This reagent can detect thrombus formation in femoral, carotid, and coronary vessels of large animals. Thrombi formed in these thick-walled arteries and veins cause clinically important occlusion. There is considerable interest in both research and clinical settings to develop reagents capable of real-time imaging of thrombus formation in this aspect of the vasculature. Sensitivity is a second advantage of this reagent. Typical cardiac contrast agents produce only a negative image of the thrombus. IR-786–labeled platelets incorporate directly into thrombi, and thus produce a positive signal. These platelets appear to be a more sensitive indicator of thrombus formation than blood pool agents, as thrombi that fail to cause a filling defect are detected by IR-786–labeled platelets (Figure 6). In addition, IR-786–labeled platelets provide precise localization of the thrombus. Localization of thrombi with standard contrast agents can be confounded if, eg, an occlusion results in altered blood flow to tributaries and misrepresents the thrombus location (Figure 6). The fact that NIR fluorescent platelets actively incorporate into growing thrombi enables evaluation of the kinetics of thrombus growth. In this manner, an actively evolving thrombus can be distinguished from a stable thrombus because only the former will actively incorporate platelets. Because IR-786 remains associated with platelets and does not transfer to underlying structures (Figure 5), thrombolysis can also be followed with this reagent. These characteristics represent meaningful advantages over presently available probes and contrast agents used in cardiovascular imaging.

Some limitations of IR786–labeled platelets are evident from the present study. Although the tissue penetration of NIR light is considerably improved over that of visible light, NIR light emitted from within a vessel cannot penetrate the dermis. Thus, an invasive procedure, such as surgical exposure or angioscopy, is required to visualize IR-786–labeled platelets in vivo. IR-786–labeled platelets are not as sensitive as blood pool agents for evaluation of vessel patency. For this reason, we have developed a method that uses methylene blue
in conjunction with IR-786–labeled platelets to monitor both thrombus formation (IR-786–labeled platelets emitting at 800 nm) and vessel patency (methylene blue as a blood pool agent emitting at 700 nm). This method provides direct visual assessment of the relationship between thrombus formation and vessel patency.

We envision several applications for IR-786–labeled platelets. Presently, there are no large animal models in which the kinetics of platelet accumulation into thrombi can be imaged in real time. This model will offer new opportunities for study of thrombus formation in vessels that approximate the size of human vessels affected in myocardial infarction, stroke, and peripheral vascular disease. The model will be useful for testing stents and other vascular devices in which thrombus formation is a known complication. In addition, testing antiplatelet drugs and other antithrombotic pharmaceuticals with real-time imaging of vessels with blood flow velocities and shear rates similar to those of critical human vessels will now be possible. In clinical medicine, IR-786–labeled platelets could enable improved visualization of thrombus formation during vascular surgery. For example, intraoperative graft occlusion occurs as a complication of cardiac bypass surgery during ~1.5 to 5.0% of procedures. Enhanced detection of intraoperative thrombi with IR-786–labeled platelets along with an integrated NIR fluorescence imaging system could provide useful information that can direct required interventions during surgeries.

Another potential application of IR-786–labeled platelets is in the characterization of atherosclerotic plaque in conjunction with NIR fluorescence angioscopy. There is increasing evidence that platelets function at multiple stages in atherogenesis. Platelets incorporate and deposit cytokines into early atherosclerotic lesions, and initiate thrombus formation at sites of ruptured plaque. Techniques to distinguish vulnerable plaque from quiescent plaque with near-infrared angiography are currently being developed. Interaction of platelets with plaque may provide further prognostic information regarding plaque stability. Future studies will evaluate potential uses of IR786–labeled platelets to study the interaction of platelets with atherosclerotic plaque in animal models and in the clinical setting.

Acknowledgments
We thank Barbara L. Clough for editing and Grisel Vazquez for administrative assistance.

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This work was funded by National Institutes of Health grants R01-CA-115296 and R21-CA-110185 to Dr Frangioni; R01-HL-078691 to Dr Hajjar; and R01-HL-63250 to Dr Flaumenhaft; a Center for Integration of Medicine and Innovative Technology Application Development Award to Dr Frangioni; an American Society of Hematology Junior Faculty Scholar Award to Dr Flaumenhaft; a Grant-In-Aid from the American Heart Association to Dr Flaumenhaft; and a Special Programs Award from Bayer Healthcare to Dr Flaumenhaft.

Disclosures
All intellectual property for the intraoperative NIR fluorescence imaging system used in the present study is owned by the Beth Israel Deaconess Medical Center, although, as inventor, Dr Frangioni may someday receive royalties if the system is commercialized. Dr Frangioni also received a sponsored research grant from GE on the intraoperative imaging system, and has a collaborative research agreement with GE funding. The other authors report no conflicts.

References


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**CLINICAL PERSPECTIVE**

The intact vasculature is opaque to the human eye. Hence, the formation of intravascular thrombi cannot currently be detected in real time, especially in the intraoperative setting, where occult thrombi can lead to significant patient morbidity. Invisible near-infrared (NIR) light, in the wavelength range of 700 to 900 nm, is capable of penetrating deeply into living tissue, and NIR fluorescence can provide highly sensitive detection of labeled targets. In the present report, we describe highly NIR fluorescent autologous platelets that retain full bioactivity. When injected intravenously at low doses, they circulate for hours, yet concentrate and become detectable in the setting of intravascular thrombi. When used in conjunction with an intraoperative NIR fluorescence imaging system, NIR fluorescent platelets permit sensitive detection of thrombi in muscular arteries and veins such as coronary, carotid, and femoral vessels. This technology has immediate application for detection of thrombus formation during common surgical procedures such as coronary artery bypass surgery, endarterectomy, and peripheral vascular surgery. NIR fluorescent platelets could also be used in conjunction with NIR fluorescence angioscopy for characterization of atherosclerotic plaque and may ultimately be used to distinguish quiescent from vulnerable plaque.
Inducible Cardiac-Restricted Expression of Enteroviral Protease 2A Is Sufficient to Induce Dilated Cardiomyopathy

Dingding Xiong, MD, PhD; Toshitaka Yajima, MD, PhD; Byung-Kwan Lim, PhD; Antine Stenbit, MD, PhD; Andrew Dublin, MD; Nancy D. Dalton, RDCTS; Daphne Summers-Torres, BS; Jeffery D. Molkentin, MD; Herve Duplain, MD; Rainer Wessely, MD; Ju Chen, PhD; Kirk U. Knowlton, MD

**Background**—Enterovirus infection is a cause of cardiomyopathy. We previously demonstrated that enteroviral protease 2A directly cleaves the cytoskeletal protein dystrophin. However, the direct effect of protease 2A in enteroviral cardiomyopathy is less clear because other viral proteins are also expressed with viral infection.

**Methods and Results**—A transgenic mouse with inducible cardiac-restricted expression of enteroviral protease 2A was generated. In the transgenic mouse, a tamoxifen-regulated Cre-loxP system, MerCreMer (MCM), was used to induce genetic recombination in cardiac myocytes, which led to protease 2A expression. Protease 2A and MCM double transgenic (2AxMCM) mice were treated with tamoxifen; the controls included 2AxMCM mice treated with diluents for tamoxifen and tamoxifen-treated MCM littermates. Protease 2A activity was significantly induced after tamoxifen in the 2AxMCM mice compared with controls. Echocardiographic analysis demonstrated an increase in left ventricular end-diastolic and end-systolic chamber size, with decreased fractional shortening in tamoxifen-treated 2AxMCM mice.

There was an increase in heart weight-to-body weight ratio in 2AxMCM mice treated with tamoxifen. Only a small increase in interstitial fibrosis and inflammation was found in tamoxifen-treated 2AxMCM mice; however, ultrastructural analysis demonstrated myofibrillar collapse with abnormalities of intercalated discs and sarcolemmal membranes. Evans blue dye–positive myocytes with disruption of dystrophin were present in 2AxMCM mice treated with tamoxifen. Disruption of dystrophin was also found in cultured myocytes isolated from 2AxMCM mice with Cre in the nucleus.

**Conclusions**—Protease 2A has a significant role in enteroviral cardiomyopathy and alone is sufficient to induce dilated cardiomyopathy, which is associated with disruption of the sarcolemmal membrane and cleavage of dystrophin with protease 2A expression. (*Circulation. 2007;115:94-102.)*

**Key Words:** cardiomyopathy ■ enterovirus ■ heart failure ■ myocarditis ■ protease
mediated cardiomyopathy by infection of dystrophin-deficient mice and showed that dystrophin deficiency increases enterovirus-induced cardiomyopathy with an increase in enteroviral replication and propagation in the heart. In sum, these findings suggest that 2APro-mediated cleavage of dystrophin during CVB3 infection contributes to a cascade of viral-mediated events that leads to dilated cardiomyopathy. However, it is not known whether 2APro alone in the absence of other viral proteins and activation of the viral-mediated immune response is sufficient to induce cardiomyopathy in the adult heart.

Therefore, to address this hypothesis, we generated a transgenic mouse with inducible cardiac-restricted expression of 2APro. Our results indicate that inducible expression of enteroviral 2APro in the adult cardiac myocyte at levels comparable to those observed with enteroviral infection is sufficient to cause dilated cardiomyopathy. In addition, the present study also demonstrated that 2APro expression increased disruption of the sarcolemmal membrane with disruption of sarcolemmal localization of dystrophin in a subset of cells. These results add significantly to the evidence that an interaction between enteroviral 2APro and dystrophin contributes to the development of cardiomyopathy after enteroviral infection and suggests that strategies directed at inhibition of 2APro are likely to have a significant, beneficial effect on enteroviral-mediated cardiomyopathy.

Methods

Construction of Transgene

The transgenic construct (α-MHC-IREs-FloxedLacZ-2APro) was created as follows: (1) an encephalomyocarditis virus internal ribosome entry site (IRES)11 from pCITE4b (Novagen) was inserted downstream from a cardiac-specific murine α-actin mouse heavy chain (α-MHC) promoter12,13 to allow expression of the transgene in the presence of 2APro; (2) consensus translation initiation sequences were positioned upstream from a loxP site followed by a β-galactosidase (LacZ) cDNA with a stop codon; and (3) another loxP site followed by a 2APro cleavage site and the 2APro cDNA were inserted downstream from the loxP sites and followed by a polyadenylation signal (pa). α-MHC-IREs-FloxedLacZ-2APro is likely to have a significant, beneficial effect on enteroviral-mediated cardiomyopathy.

Generation of Transgenic Mice

The 10-kb expression cassette of α-MHC-IREs-FloxedLacZ-2APro construct was isolated after cleavage with BamH1 and microinjected into the male pronuclei of fertilized eggs from superovulated C57BL/6 × Balb/c mice. Injected eggs were transferred into the oviduct of pseudopregnant CD1 mice. Founder mice were bred with Balb/c mice and maintained in a pathogen-free environment. The α-MHC-IREs-FloxedLacZ-2APro mice were bred with α-MHC-MerCreMer (MCM)13 to create double transgenic mice (2AProMCM).

4-Hydroxytamoxifen Treatment and Recombination Analysis

We dissolved 4-hydroxytamoxifen (4OHTAM, Sigma, St Louis, Mo) in peanut oil (Sigma) at 5 mg/mL. Adult transgenic mice (6 to 8 weeks of age) were treated with 4OHTAM by intraperitoneal injection once daily for 5 days at a dose of 20 mg/kg per day. In separate experiments, adult cardiac myocytes were isolated from 2AProMCM mice with the protocol described in the Appendix. The cells were then treated with 10 μmol/L 4OHTAM for 36 hours.

Polymerase chain reaction (PCR) primers were designed to assess transgene recombination at the loxP sites in the DNA extracted from the hearts of transgenic mice. In the first PCR, the sense primer was located within the IRES sequence (P1: 5′-CAATGGCTACCTCAAGCC-3′). The antisense primer was in the 2APro cDNA (P2: 5′-CGTTGTACACCGGCTG-3′). After excision of the LacZ sequence, PCR with these primers yielded a 561-bp band. The theoretical 4-kb product was not detected. The second set of primers annealed to the IRES and floxed LacZ sequence; sense primer is P1 and reverse primer is within LacZ (P3: 5′-AGGGAGATCGCACTCCAGCC-3′). The intact nonrecombined transgene yields a PCR fragment of 496 bp with the second set of primers, and no amplification occurs after Cre-mediated excision.

Assay for 2APro Activity

Heart protein extracts from either transgenic mice or CVB3-infected CSH/HeJ mice were analyzed for 2APro activity. The CSH/HeJ mice were inoculated with intraperitoneal injection with 5 × 106 plaque-forming units of CVB3 at 4 weeks of age. The infected mice were euthanized 6 days after infection; the tamoxifen-stimulated mice were euthanized 12 days after initiation of tamoxifen and their hearts were harvested. Total proteins were extracted from the hearts of tamoxifen-treated transgenic mice (n = 3), control mice (n = 3), and CVB3-infected CSH/HeJ mice (n = 3). Five μg total protein from each heart were incubated with 50 μmol/L Ac-LSTT-AFC (LSTT-AFC), polymerase chain reaction (PCR) primers were designed to assess transgene recombination at the loxP sites in the DNA extracted from the hearts of transgenic mice. In the first PCR, the sense primer was located within the IRES sequence (P1: 5′-CAATGGCTACCTCAAGCC-3′). The antisense primer was in the 2APro cDNA (P2: 5′-CGTTGTACACCGGCTG-3′). After excision of the LacZ sequence, PCR with these primers yielded a 561-bp band. The theoretical 4-kb product was not detected. The second set of primers annealed to the IRES and floxed LacZ sequence; sense primer is P1 and reverse primer is within LacZ (P3: 5′-AGGGAGATCGCACTCCAGCC-3′). The intact nonrecombined transgene yields a PCR fragment of 496 bp with the second set of primers, and no amplification occurs after Cre-mediated excision.
Histochemistry
Heart tissue was embedded in OCT Tissue Tek (Sakura Finetechnical, Torrance, Calif) and snap-frozen in isopentane chilled in dry ice, and 6-μm sections were cut by cryosection. LacZ was detected with 5-bromo-4-chloro-3-indolyl-D-galactopyranoside (X-Gal) staining. In addition, the number of cells that expressed X-Gal was quantified with adult myocytes isolated from tamoxifen- or peanut oil–treated 2AxMCM and expressed as the percentage of the total number of cells.

Fluorescent staining with Evans blue dye (EBD) was used to assess the impairment of sarcolemmal membrane integrity as previously described.10 Dystrophin in the hearts of transgenic mice was detected by immunofluorescence with monoclonal antibodies that recognize epitopes that map toward the N-terminus of dystrophin (sc-135776) and at the C-terminus (sc-7461) (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif). Dystrophin levels of cultured cardiomyocytes were detected by immunofluorescence with a monoclonal antibody that recognizes the mid-rod domain of dystrophin (Dy4/6D3; NovoCasra, Newcastle, UK). Cre protein in the hearts of transgenic mice and cultured cardiac myocytes was detected by immunofluorescence with a rabbit polyclonal antibody against Cre (BABCO). Cell membrane glycoproteins were visualized with FITC-labeled wheat germ agglutinin.14 The specimens used for conventional electronmicroscope were fixed and embedded as described previously.15

Echocardiography
M-mode echocardiograms were performed as described previously.16

Statistical Analysis
Data are expressed as mean±SE unless otherwise noted. Statistical significance was evaluated with the unpaired Student t test for comparisons between 2 means. For multiple comparisons, a 1-way ANOVA with Tukey-Kramer post hoc test was used. To test mean changes in left ventricular end-diastolic and end-systolic dimension as well as percent fractional shortening among 3 groups from baseline to 22 days after 4HHTAM administration, repeated-measure ANOVA was used. Probability values <0.05 were considered significantly different.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Generation of Transgenic Mice With Cardiac Restricted Expression of 2APro Regulated by a Cre-LoxP-Inducible System
To assess the effect of enteroviral 2APro in the adult heart, a tamoxifen-inducible Cre-LoxP system was used to regulate 2APro expression. To accomplish this, we generated transgenic mice that harbored a floxed LacZ-2APro cDNA expression vector. This construct contained a cardiac-specific murine α-MHC promoter followed by a picornaviral IRES. The viral IRES was inserted to facilitate translation in the presence of 2APro expression.17–19 A translation initiation start site was inserted downstream of the IRES. Downstream of the LacZ expression cassette was a cDNA that contained loxP DNA sequence, a 2APro cleavage site, and the 2APro coding sequence followed by a stop codon. The construct terminates with an SV40 polyA signal sequence (Figure 1A, a). Of a total of 6 genotype-positive founder transgenics, 2 were identified that expressed LacZ in the hearts at baseline. Both were healthy, bred normally, and had phenotypically normal hearts (data not shown). The 2APro transgenic mice were bred with α-MHC MCM transgenic mice15 to establish double-transgenic mice with 2APro and MCM transgenes (referred to as 2AxMCM mice). MCM transgene expresses a Cre-recombinase fusion protein with mutant estrogen-receptor domains (Mer) under the control of the α-MHC promoter (Figure 1A, b).

In the absence of tamoxifen, the MCM fusion protein is sequestered in the cytoplasm of cardiomyocytes, and in the unstimulated 2AxMCM mice, LacZ is expressed without expression of 2APro (Figure 1B). Conversely, stimulation of the mice with tamoxifen causes translocation of MCM fusion protein to the nucleus, where the LacZ cDNA is excised by Cre-mediated recombination (Figure 1C, a) and 2APro is expressed (Figure 1C, b). The 2APro then cleaves the amino terminus of the protein at the cleavage site to generate an exact copy of the enteroviral 2APro (Figure 1C, c).

To determine the extent of translocation of MCM after tamoxifen administration, immunofluorescence analysis was performed on ventricular sections from 2AxMCM mice 12 days after initiation of tamoxifen administration. Antibody to Cre protein and DAPI were used to visualize the MCM fusion protein and cell nuclei, respectively. In the absence of tamoxifen, the MCM fusion protein was seen diffusely throughout the cytoplasm of ventricular myocytes. In contrast, tamoxifen induced detectable nuclear localization of MCM in 38±4% of total nuclei (Figure 2A). Cre-mediated recombination was analyzed by PCR at the same time point. Recombination of the transgene was confirmed in 2AxMCM mice treated with tamoxifen (Figure 2B). The efficiency of tamoxifen-inducible recombination was also analyzed by quantitative assessment of LacZ-positive cells with X-gal staining at 12 days after initiation of tamoxifen administration. There was very little or no LacZ expressed after stimulation with tamoxifen when compared with LacZ expression in the absence of tamoxifen in either tissue section (Figure 2C) or in isolated adult myocytes (Figure 2D). These data clearly demonstrate that tamoxifen induced Cre-recombinase-mediated DNA recombination in this transgenic mouse model.

Expression of 2APro was determined 12 days after initiation of tamoxifen administration with a substrate-based fluorescent cleavage assay as described in Methods. No 2APro activity was detected in the hearts of the MCM mice treated with tamoxifen. Minimal 2APro activity was detected in the hearts of the 2AxMCM mice in the absence of tamoxifen, whereas administration of tamoxifen increased 2APro activity. Activity of 2APro in the tamoxifen-treated 2AxMCM mice was compared with 2APro activity of CVB3-infected heart extracts 6 days after infection (Figure 2E). These results demonstrated that the presence of tamoxifen can significantly induce 2APro expression in
the hearts of 2AxMCM mice, with minimal expression of 2APro in the hearts of 2AxMCM mice in the absence of tamoxifen. The level of 2APro expression in the whole heart was comparable to that observed during the acute phase of CVB3 infection.

Induction of 2APro Expression in Adult Heart Leads to Dilated Cardiomyopathy

To determine whether 2APro expression is sufficient to induce dilated cardiomyopathy, male double-transgenic 2AxMCM mice 6 to 8 weeks of age were treated with tamoxifen. Two types of control mice were evaluated; ie, 2AxMCM mice treated with the diluent peanut oil, and MCM mice that lacked the 2APro transgene were treated with tamoxifen. All the mice, 5 in each group, underwent echocardiography at days 0, 9, and 22 after initiation of tamoxifen administration. Their hearts were harvested for analysis as described below at 22 days after initiation of treatment.

The 2AxMCM mice treated with tamoxifen had a time-dependent increase in left ventricular end-diastolic dimension and end-systolic dimension (Figure 3A and 3B). Abnormal systolic performance was observed because of a corresponding decrease in the fractional shortening of the left ventricle (Figure 3B); however, there was no significant left ventricular dilation or decrease in the fractional shortening of the left ventricle in control mice. There were no significant differences in average body weight and heart rate between 2AxMCM mice treated with tamoxifen and the controls (data not shown). These results indicate that cardiac-restricted enteroviral 2APro expression is sufficient to induce dilated cardiomyopathy.

Dilated cardiomyopathy was also demonstrated in the 2AxMCM mice treated with tamoxifen in terms of heart weight-to-body weight ratio and heart morphology. The heart weight-to-body weight ratio significantly increased in the 2AxMCM mice 22 days after administration of tamoxifen as compared with controls (Figure 4A). There were no significant differences in the body weight of the 2AxMCM mice treated with peanut oil when compared with the tamoxifen-treated MCM mice. In addition to severe ventricular enlargement, there were bialtrial enlargement, pleural effusions, and ascites in the 2AxMCM mice treated with tamoxifen (data not shown). Transverse sections of hearts were stained with hematoxylin and eosin and Masson trichrome (Figure 4B). Minimal cellular infiltrate and a small amount of interstitial fibrosis were noted in the tamoxifen-treated 2AxMCM mice. The controls were normal. Ultrastructural analysis with electron microscopy revealed striking structural alterations. Compared with controls, the 2AxMCM mice treated with tamoxifen displayed “fuzzy” Z-lines and shortened sarcomeres (Figure 4C). In addition, cardiac cells also demonstrated severe cytoskeletal changes, such as myofibrillar collapse with a haphazard arrangement, concomitant with a decrease in myofibril number; the increased presence of swollen mitochondria were often observed. Although it is difficult to assess sarcolemmal membrane integrity with electron microscopy, the 2AxMCM mice treated with tamoxifen demonstrated consider-
Cardiac 2A<sup>Pro</sup> Expression Induces Sarcolemmal Disruption and Loss of Sarcolemmal Localization of Dystrophin

Because we have previously shown that coxsackieviral 2A<sup>Pro</sup> cleaves dystrophin in vitro and that there is cleavage of dystrophin and disruption of the sarcolemma in CVB3-infected cultured myocytes and infected mouse hearts, we hypothesized that the diluted cardiomyopathy induced by 2A<sup>Pro</sup> expression would be associated with sarcolemmal disruption and loss of dystrophin localization to the sarcolemma.

To determine whether disruption of the sarcolemmal membrane occurred in the intact heart with enteroviral 2A<sup>Pro</sup> expression, we evaluated sarcolemmal membrane integrity in vivo by injecting EBD, a large tracer molecule that can only enter into the myocytes with a disrupted sarcolemma. There was uptake of EBD in scattered myocytes of the 2AxMCM mice treated with tamoxifen. Disruption of the sarcolemmal membrane, however, was completely absent in the MCM mice treated with tamoxifen and 2AxMCM mice treated with peanut oil (Figure 5A). To compare this with a mouse model of cardiomyopathy from a different cause, muscle lim protein knockout mice with cardiomyopathy and heart failure were also injected with EBD. In contrast to tamoxifen-treated 2AxMCM mice, there was only very weak, diffuse EBD staining that was clearly distinct from the bright staining that was detected in the 2AxMCM mice (Figure 5A). It is also notable that the pattern of EBD staining in the 2AxMCM mice was distinct from that seen in CVB3 infected mice in that the staining occurred in scattered individual myocytes as opposed to the positive staining of foci of adjacent myocytes observed after CVB3 infection. This finding indicates that sarcolemmal disruption in the 2AxMCM mice treated with tamoxifen was associated with protease 2A expression and not an indirect effect of cardiomyopathy alone.

To determine whether the disruption of the sarcolemma in mice with 2A<sup>Pro</sup> expression was associated with 2A<sup>Pro</sup>-mediated disruption of dystrophin at the sarcolemma as seen in virally infected hearts, mice were injected with EBD and sections were immunostained for the presence of dystrophin using antibodies directed against the C-terminus and toward the N-terminus of dystrophin. This demonstrated that EBD uptake occurred in the myocytes with a disrupted C-terminal dystrophin-staining pattern (Figure 5B). The disruption of dystrophin was similar, with the antibody directed nearer the N-terminus (data not shown). Double staining with wheat germ agglutinin, which stains membrane glycoproteins in general, and EBD demonstrated that loss of the dystrophin in the sarcolemma of the myocytes with EBD-positive staining was not the result of general disintegration of plasma membrane, as evidenced by preserved wheat germ agglutinin stain in the cells with sarcolemmal disruption (Figure 5C). However, there was partial disruption of caveolin-3 stain at the sarcolemma in EBD-positive cells (data not shown). These data indicate that impairment of sarcolemmal membrane in the mice with 2A<sup>Pro</sup> expression is associated with disruption of dystrophin.

In addition, to further demonstrate that 2A<sup>Pro</sup> expression in cardiomyocytes is sufficient to disrupt dystrophin, adult myocytes from 2AxMCM mice were isolated and treated with tamoxifen for 36 hours. Co-immunostaining of the myocytes from 2AxMCM mice were isolated and treated with tamoxifen (4OH TAM + MCM, left panels), a 2AxMCM mouse treated with peanut oil (peanut oil + 2AxMCM, center panels), and a 2AxMCM mouse with tamoxifen (4OH TAM + 2AxMCM, right panels). The double-headed vertical arrows indicate the left ventricular chamber dimension at end diastole (longer arrow) and at end systole (shorter arrow). Images in the upper panels were obtained before treatment; images in the lower panels were obtained 22 days after initiation of treatment with tamoxifen or diluent (post-treatment). The left ventricle was dilated in the 2AxMCM mice after administration of tamoxifen and there was reduced wall motion (right, lower panel) compared with pretreatment (right, upper panel), which demonstrated the decrease in ventricular systolic function. However, no difference in left ventricular dimension was found in the tamoxifen-treated MCM mouse (left, lower panel) and the peanut oil-treated 2AxMCM mouse (center, lower panel) compared with pretreatment (corresponding upper panels). There was no significant difference in the average heart rates between pre and posttreatment in any of the groups. B, Transthoracic M-mode echocardiographic measurements of end-diastolic dimension, end-systolic dimension, and fractional shortening. There was a significant increase in end-diastolic and end-systolic dimensions in 2AxMCM mice treated with tamoxifen (n=5) compared with tamoxifen-treated MCM mice (n=5) and 2AxMCM mice treated with peanut oil (n=5, *P<0.05). No significant difference was found between tamoxifen-treated MCM mice and 2AxMCM treated with peanut oil. The fractional shortening was lower in the 2AxMCM mice treated with tamoxifen compared with controls (*P<0.05). There was no significant difference in fractional shortening between tamoxifen-treated MCM mice and 2AxMCM mice treated with peanut oil.

Cardiac 2A<sup>Pro</sup> Expression Induces Sarcolemmal Disruption and Loss of Sarcolemmal Localization of Dystrophin

Because we have previously shown that coxsackieviral 2A<sup>Pro</sup> cleaves dystrophin in vitro and that there is cleavage of dystrophin and disruption of the sarcolemma in CVB3-infected cultured myocytes and infected mouse hearts,
curred in myocytes in which MCM was localized to the nucleus (Figure 5D). The result indicated that expression of enteroviral 2APro in isolated cardiomyocytes is sufficient to induce disruption of the sarcolemmal localization of dystrophin.

Discussion

It has been clearly demonstrated that coxsackieviral 2APro can directly cleave dystrophin, that CVB3 infection of the heart is associated with disruption of the dystrophin glycoprotein complex, and that dystrophin deficiency can have a role in determination of susceptibility to enteroviral-mediated cardiomyopathy.9,10 Because enteroviral infection is accompanied by expression of all viral proteins7 and activation of a potent immune response,2 it is not known whether expression of 2APro alone is sufficient to induce dilated cardiomyopathy or whether it would be associated with disruption of sarcolemmal membrane integrity. Therefore, the present study demonstrates for the first time that the presence of coxsackieviral 2APro in the cardiac myocyte is sufficient to induce cardiomyopathy with disruption of the sarcolemmal membrane and loss of localization of dystrophin in the intact heart. In addition, the present study also demonstrates that expression of 2APro in isolated adult myocytes is able to disrupt dystrophin localization to the cell membrane, which suggests that 2APro-mediated cleavage of dystrophin plays an important role in 2APro-induced dilated cardiomyopathy. These findings contribute to our understanding of the pathogenesis of viral-mediated dilated cardiomyopathy.

Hallmarks of dilated cardiomyopathy in humans include a decrease in systolic ventricular function and an increase in ventricular chamber size. Microscopic features include variable degrees of interstitial fibrosis, degeneration of myocytes, and occasional clusters of lymphocytes.24 Because the major objective of this study was to determine whether cardiac restricted expression of enteroviral 2APro could induce cardiomyopathy, we sought to determine whether some or all the characteristics of dilated cardiomyopathy were present in the transgenic mice with 2APro.
expression. The manifestations of dilated cardiomyopathy and heart failure found in the 2AxMCM mice treated with tamoxifen included an increase in left ventricular end-diastolic and end-systolic chamber size in tamoxifen-treated transgenic mice, which was associated with decreased fractional shortening, a marker of systolic dysfunction. Other findings consistent with cardiomyopathy included atrial enlargement and an increased ratio of heart weight to body weight, as well as pleural effusions and ascites. Histopathological analysis of transgenic mice demonstrated mild fibrosis and degeneration of myocytes in the absence of a significant cellular immune response. Ultrastructural analysis revealed disruption of myofibril structure and irregularities in the sarcolemmal membrane and intercalated discs. These results clearly indicate that cardiac-restricted expression of enteroviral 2APro is sufficient to induce dilated cardiomyopathy in the absence of other viral proteins required for viral replication.

In the present study, we used a tamoxifen-regulated Cre-loxP system to induce enteroviral 2APro expression in adult mouse hearts. In the absence of tamoxifen in 2AxMCM mice or in the presence of tamoxifen in MCM mice without the 2APro transgene, there was no evidence of echocardiographic or histologic abnormality with exception that <10% of 2AxMCM mice had evidence of cardiomyopathy, presumably secondary to occasional “leaky” expression of 2APro in the absence of tamoxifen. Stimulation of the mice with tamoxifen resulted in a significant induction of 2APro activity and development of cardiomyopathy in all treated mice. These results demonstrate that the dilated cardiomyopathy observed in tamoxifen-treated 2AxMCM mice results from expression of 2APro induced by a tamoxifen-regulated Cre-LoxP system rather than tamoxifen or MCM fusion protein.

To establish a reference for the level of 2APro activity in the transgenic hearts, we compared them to the activity of 2APro in CVB3-infected hearts. The level of 2APro activity in the whole heart was similar to that observed in coxsackieviral-infected hearts 6 days after infection. Despite the similarity in 2APro expression, it should be noted that are significant differences in the development of the disease process between the transgenic mice and mice infected with CVB3. These differences include the absence of expression of all viral proteins and lack of a potent cellular immune response in the transgenic mice. In addition, there is a difference in the time course and localization of 2APro expression between the transgenic mice and mice infected with CVB3. Activity of 2APro is transient in acute viral infection secondary to host immune responses against viral infection. After the virus is completely cleared by host immune responses, 2APro expression will be decreased. However, the transgenic mice represent
a more chronic pattern of 2A\textsuperscript{Pro} expression because it is driven by the \(\alpha\)-MHC promoter. This is more reminiscent of persistent viral infection of the heart. In addition, although we were not able to stain directly for 2A\textsuperscript{Pro} in the tissue, the EBD staining was dispersed throughout the myocardium rather than in focal areas of adjacent myocytes as occurs with CVB infection. This finding, in combination with the known pattern of expression of genes driven by the \(\alpha\)-MHC promoter,\textsuperscript{12,13} indicates that transgenic expression of 2A\textsuperscript{Pro} is more diffuse than that observed after CVB infection. Because the total 2A\textsuperscript{Pro} activity is similar in the transgenic and CVB-infected hearts, it is likely that the level of 2A\textsuperscript{Pro} activity in each myocyte in the transgenic mice is lower than that of CVB3-infected myocytes.

We have previously shown that expression of a replication-defective enteroviral genome in the heart can also lead to cardiomyopathy. Although there are similarities between the previously reported model and that reported in the present article, there are also significant differences. Similarities include deterioration in ventricles between the previously reported model and that replication-defective enteroviral genome in the heart can cause cardiomyopathy and the role for acquired disruption of the cytoskeleton.

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Disclosures
None.

References

**CLINICAL PERSPECTIVE**

Coxsackievirus infection is one of the common causes of viral myocarditis. Activation of the cellular immune system has a role in the pathogenesis of viral heart disease. However, it has recently been shown that viral proteases may be important in the pathogenesis of viral-mediated heart disease by cleaving proteins that stabilize the cell membrane. This article demonstrates that when the coxsackieviral protease 2A alone is expressed in the adult cardiac myocyte, it is sufficient to induce severe cardiomyopathy. This indicates that viral proteases are potentially important therapeutic targets for the treatment of viral-mediated heart disease and that protease inhibitors would likely limit the viral-mediated damage caused by proteases in the host cell.
Pulmonary vein stenosis is a fascinating yet frustrating and difficult to manage condition with an exceptionally high mortality rate. Until recently, the disease was seen almost exclusively in young children with or without various forms of congenital heart disease. Pulmonary vein stenosis is a relatively rare condition. In most published series from large centers, there has been an average of \( \approx2 \) or 3 cases per year that require treatment. Pulmonary vein stenosis in the adult population is even more rare, and the small number of reported cases has often been associated with mediastinal processes such as neoplasms or fibrosing mediastinitis. However, with the advent of aggressive treatment strategies for atrial fibrillation, we have seen a new group of pulmonary vein stenosis patients. The stenosis appears as a complication of radiofrequency ablation procedures around the pulmonary veins. Small series of new surgical and interventional catheterization procedures for treatment of both the pediatric and adult forms of pulmonary vein stenosis suggest an improving prognosis in centers with specialized expertise. However, the prognosis of patients affected with pulmonary vein stenosis remains guarded and requires diligent follow-up and often repeated procedures. The purpose of this article is to review concepts of causation and possible treatments for this rare but serious condition as they evolve.

**Embryology and Anatomy of the Pulmonary Veins**

The left atrium and pulmonary veins initially develop separately in the 3- to 5-mm embryo (25 to 27 days gestation).\(^1\) The primordial pulmonary venous system is part of the splanchnic plexus, which initially connects to the cardinal and umbilicovitelline veins. At 27 to 29 days gestation, a small endothelial outgrowth from the posterior superior wall of the primordial left atrium develops just to the left of the developing septum primum. At 28 to 30 days gestation, this common pulmonary venous out-pouching engages the pulmonary venous portion of the splanchnic plexus and begins to drain blood from the pulmonary system. In normal development, the connections to the cardinal and umbilicovitelline systems atrophy, which results in complete separation between the pulmonary and systemic venous systems.

The sequence of connection of the out-pouching of the left atrium to the pulmonary venous plexus, followed by incorporation of the confluence of the common pulmonary venous system into the left atrium, results in the typical anatomic appearance of the normal heart. In most hearts, approximately half of the left atrium is comprised of the common pulmonary vein and the other half, which includes the left atrial appendage, forms from the primitive left atrium.\(^2\) In most hearts, the embryological confluence of structures leads to the formation of 2 right-sided and 2 left-sided pulmonary veins that enter the smooth portion of the posterior left atrium.

Failure of the out-pouching of the left atrium to connect with the pulmonary venous plexus may result in persistence of the connections of the pulmonary veins to portions of the systemic venous system,\(^3\) which leads to the various forms of partial or total anomalous pulmonary venous return. If the connection between the left atrium and the pulmonary veins fails to occur at a time in development after connections of the pulmonary venous system to the systemic venous system have become obliterated, the result is the very rare condition of complete pulmonary vein agenesis.

The syndrome of “primary” endoluminal pulmonary vein stenosis with no preceding surgery or catheter intervention has been postulated to result from abnormal incorporation of the common pulmonary vein into the left atrium in the later stages of cardiac development.\(^3\) Affected patients most often become symptomatic in the first few months to years of life, frequently have 1 or more additional cardiac anomalies, and have no active inflammation in or around the involved segments of vein. Estimates of the incidence of associated cardiac defects have ranged from 30% to 80%\(^4,5,6\) The most commonly associated congenital heart defects are septal defects, but pulmonary vein stenosis has been seen in conjunction with all major types of congenital cardiac malformations. Stenosis of the pulmonary veins may appear as a relatively discrete shelf, as a longer segment of narrowing at the junction of the pulmonary vein to the left atrium that extends slightly into the pulmonary vein, or as diffuse hypoplasia of the pulmonary veins.\(^4,7\) Pulmonary vein stenosis in children and even adults with no apparent preceding or concomitant cause of stenosis has been termed “congenital”. However, except in the small group of patients with diffusely hypoplastic pulmonary veins, we prefer the term “primary” pulmonary vein stenosis as the designation. The reason for this difference in terminology is that it is becoming more apparent that the disease is often progressive and may not even be evident at birth. Some feel that the rapidity of
progression with no evidence of inflammation in many patients suggests a neoproliferative process. Sadr et al found apparently proliferative “myofibroblastic” cells in a small number of autopsy specimens. Staining and electron microscopic characteristics of these cells showed simultaneous features of myocytes and fibroblasts, which is consistent with a myofibroblast cell type. In other areas of the body, these types of cells retain the ability to differentiate into either myocytes or fibroblasts. It is unknown whether antiproliferation therapy such as radiation or chemotherapy might alter growth of these cells in patients with pulmonary vein stenosis. It is also not clear whether these types of cells may be particularly widespread in the pulmonary veins of some patients and involved with the apparently overly exuberant growth after a traumatic insult such as surgery or radiofrequency ablation.

**Clinical Picture of Pulmonary Vein Stenosis in Childhood**

The timing and severity of symptoms in pediatric patients with pulmonary vein stenosis appears to depend largely on the number of pulmonary veins involved and the severity of obstruction to individual pulmonary veins. Most patients present in the first months to years of life with a history of significant respiratory symptoms. Patients are often tachypneic and have recurrent pneumonias. As the disease progresses, signs of pulmonary hypertension become increasingly prominent. Patients may have diffuse or more localized evidence of pulmonary edema, based on whether 1 or more pulmonary veins are involved. Hemothysis may become a prominent symptom, especially in older patients.

Approximately one half of patients with primary pulmonary vein stenosis have some type of associated cardiac defect. It is therefore imperative that echocardiographic evaluations of patients with all forms of congenital heart disease specifically include evaluation of the pulmonary veins. Recent studies have documented progression from normal pulmonary venous flow patterns in a significant number of patients who later developed progressive pulmonary vein stenosis. Evaluation for stenotic pulmonary veins is indicated in any young patient with severe pulmonary hypertension.

Pulmonary vein stenosis may also be secondary in pediatric patients and occurs most often after anomalous pulmonary vein surgery. Clinically significant stenosis occurs postoperatively in ≈10% of patients after repair of total anomalous pulmonary venous return in most series. The site of obstruction may be at the anastomotic site of the pulmonary veins and pulmonary arteries. In our experience, the primary limitations of magnetic resonance imaging relate to relatively long acquisition times, sensitivity to motion artifacts and arrhythmias, and somewhat limited spatial resolution. Sensitivity to artifacts from metallic objects in the chest and contraindications in patients with a pacemaker can also be a problem in a significant portion of patients.

We have found multidetector CT angiography to be an excellent technique for detailed analysis of the pulmonary vein stenosis.
veins in patients with known or suspected pulmonary vein stenosis (Figure 2). The primary concern with this technique, especially with small children, is the ionizing radiation should repeated studies be needed. Excellent images can be obtained rapidly and with good spatial resolution. We have found, however, that the resolution may still be inadequate to differentiate between completely occluded pulmonary veins and those with a tiny residual opening that may still be adequate for treatment by catheter techniques.26

Angiography provides the most selective and detailed views of the pulmonary veins. A pulmonary arterial catheter can be manipulated selectively to arterial segments that drain to each of the pulmonary veins. In regions with severe pulmonary vein stenosis, there may be little or no prograde flow under normal conditions. Contrast dye may actually flow “backwards” into arteries that drain into less stenotic veins. For optimal visualization, we therefore occlude a small segmental pulmonary artery with a balloon wedge catheter and inject nonionic contrast media followed by saline flush under careful fluoroscopic visualization (Figure 3). With this technique, we have been able to demonstrate even very small openings in some patients with presumed complete occlusion by noninvasive imaging. Direct visualization of nonoccluded pulmonary veins can be performed by transseptal catheterization and manipulation of the catheter through the obstructed area. Small injections of nonionic contrast are generally well tolerated and provide the most detailed pictures of the involved area of stenosis (Figure 4).

Asymmetrical pulmonary venous stenosis results in redistribution of flow between and throughout the 2 lungs. We have found that radionuclide quantitative pulmonary flow imaging provides the best evaluation of flow distribution (Figure 5). We strongly recommend this technique for any patients with pulmonary vein stenosis both before any type of intervention and as an excellent test for following patients over time.

Treatment and Prognosis of Pediatric Pulmonary Vein Stenosis

Patients with the pediatric form of pulmonary vein stenosis, either primary or secondary, have a very guarded prognosis. Without treatment, patients with involvement of most or all of
the pulmonary veins nearly always have relentless progression, and long-term survival is rare. The mode of demise is usually a pulmonary hypertensive crisis, intercurrent pulmonary infection, or hemoptysis. Patients with single-ventricle physiology may have progressively severe cyanosis or the clinical picture of a failing Fontan. Patients with only 1 or 2 pulmonary veins involved have a significantly more benign course. Breinholt et al found a mortality rate of 83% in patients with 3 or 4 stenosed pulmonary veins versus 0% in patients with 1 or 2 stenosed pulmonary veins. More cases of mild forms of pulmonary vein stenosis are undoubtedly being diagnosed in relatively asymptomatic patients as a result of increased awareness and improvements in noninvasive imaging modalities. The precise natural history of milder forms of pulmonary vein stenosis is therefore not entirely clear.

Repair of primary and secondary forms of pulmonary vein stenosis has been attempted with similar techniques and with similar outcomes. Pulmonary vein stenosis after repair of anomalous pulmonary venous return occurs in $\approx 10\%$ of patients. This can be a particularly devastating complication in patients with associated single-ventricle physiology. Advances in the technique of surgical repair of pulmonary vein stenosis have been based on the concept of reducing trauma to the veins in hopes of reducing any stimulus for regrowth of obstructive tissue. A technique by which the pericardium around the pulmonary veins is attached to the left atrium avoids any stitches in the cut edges of the pulmonary veins and is now considered the best approach. Limited experience suggests that this sutureless marsupialization may be superior to previous approaches that used direct anastomosis after resection of stenotic segments or patching of the stenotic veins. Overall, freedom from reoperation or death at 5 years, however, is still only $\approx 50\%$. Patients with milder degrees of stenosis and stenosis of only 1 or 2 pulmonary veins clearly have a better prognosis. Progressive pulmonary vein stenosis isolated to 1 lung may be survivable even though flow studies demonstrate little or no flow to the involved lung. Pneumonectomy may be necessary for hemoptysis. In a small number of patients with unrelenting progression and development of severe pulmonary hypertension, lung transplantation has been successful. Short-term results in patients who survived long enough to undergo bilateral sequential lung transplants have been good, but the long-term prognosis is guarded at best.

Single-catheter interventions for treatment of pediatric pulmonary vein stenosis have also met with limited success. Immediate improvement is usually seen angiographically, but recurrent stenosis occurs in a large majority of patients. We have found that these lesions may be very
Pulmonary vein stenosis is a rare condition with a bimodal age distribution. In pediatric patients the primary (ie, not associated with any preceding surgery) form of the disease may be related to inadequate embryological connections between the intrapulmonary venous system, the common pulmonary vein, and the left atrium. However, the stenosis is usually not static, and postnatal worsening of stenosis may be caused by abnormal proliferation of unusual myofibroblastic cells. It is not clear whether the same cell type may be involved in secondary pulmonary vein stenosis after surgical procedures that involve the pulmonary veins, such as in patients with anomalous pulmonary venous return. Recurrence of stenosis in both primary and secondary forms of pediatric pulmonary vein stenosis occurs in the majority of patients. Our current approach to most of these patients is to attempt surgical repair with the sutureless marsupialization procedure. Patients must then be followed carefully, and noninvasive imaging is usually adequate for screening. Catheter intervention is performed in veins with evidence of increasing stenosis. We generally perform high-pressure balloon dilation or cutting balloon dilation initially. Repeat procedures are frequently needed, but we have found that these repeat procedures may eventually slow the progress of restenosis. Placement of stents in the pulmonary veins of pediatric patients is usually considered only as a final mode of therapy before lung transplantation.

Pulmonary vein stenosis in adult patients is now most commonly associated with prior radiofrequency ablation procedures for atrial fibrillation. Balloon angioplasty and stenting are reasonably successful in treating these patients. Repeat procedures are commonly needed, but aggressive intervention to prevent complete occlusion has resulted in good long-term clinical results.

Disclosures

None.

References


**KEY WORDS:** veins, anomalous pulmonary, heart diseases, congenital pulmonary heart disease, catheter ablation, pulmonary veins, surgery, pulmonary veins, pathology.
This article gives an overview of survival methods in medical studies. We briefly describe survival data and discuss the methods used for analysis of such data. We apply these methods to data from a clinical trial and discuss the results. Survival methods are applicable when the measure of interest is time to an event such as mortality or occurrence of disease. The concept of censoring makes survival methods unique. If a patient goes through the study without having the event, his time to the event is (right) censored, in the sense that we only know that the event happened after the last time we observed the patient. Thus, for each patient we have 2 pieces of data: the first is a time that is either the patient’s event time or the time that the patient was last followed up, and the second is an indicator that denotes whether the time is an event time or a follow-up time. Another way of thinking of censoring is to assume that each patient has an event time and a censoring time after which the patient would no longer be observed. Whenever the censoring time is less than the event time, the event time is missing. Survival methods also assume that the censoring time is unknown when it is greater than the event time.

Survival distributions are usually described in terms of 2 functions: the survival function, \( S(t) \), defined as the probability that a person survives past a specified time \( t \); and the hazard function, \( h(t) \), which is the instantaneous failure rate and is defined as:

\[
h(t) = \frac{dS(t)}{d(t)} / S(t).
\]

Suppose a patient has survived to time \( t \); then the hazard function is the probability that the patient will have an event in the next instant. The hazard function is conceptually useful in describing survival distributions but is rarely published. The greater the hazard function, the shorter is the survival time.

The survival methods that we describe require that the censoring time is independent of the event time. This is called noninformative censoring. An example that illustrates when this assumption would always be met is a clinical trial in which patients enter the study over a period of time and there are no dropouts. If the patient does not have an event before the end of the study, the patient’s event time will be censored. The distribution of the potential censoring time will only depend on when the patient entered the study. This time will be independent of the patient’s time to event as long as there are no secular trends in the survival distribution. An extreme example of an instance when these assumptions would not be met is a study of time to death, where patients are no longer followed up after they recover from a disease. Patients who are lost to follow-up in a clinical trial or drop out of a clinical trial are problematic because the time to their last observation may or may not be related to their unobserved event time. For instance, if patients who feel better drop out of the study, the censoring may be informative. Approaches to this problem have been the focus of an extensive literature.

### Estimating the Survival Function

The most commonly used descriptive statistics for survival data are based on an estimate of the survival function. Often the median is reported, which is the value of \( t \) where the survival function, \( S(t) \), equals 0.5 (ie, 50% of the cohort is event free). Sometimes the value of \( S(t) \) is reported at \( t=1,5, \) or 10. The mean survival time is rarely reported because, as we shall see, it cannot be estimated reliably. Each of these descriptive statistics starts with the estimation of the survival function. Often the estimate of the entire function is included in a report of a study. The advantage of this is that the behavior of the function over various time periods may be of interest. The curve may drop steeply at first because of early events or may level off if patients who survive past a certain point without an event are unlikely to have one in the future. As we see in the next section, the curve cannot be estimated past the longest follow-up time.

The Kaplan-Meier method is frequently used to estimate the survival function when there are censored data. The best way to understand this method is to break up the time scale into intervals that end at each event time. Let \( t_1, t_2, \ldots \) be ordered event times. Then, because no event occurs before time \( t_1 \), the value of \( S(t) \) is 1 from \( t=0 \) to just before \( t=t_1 \). Suppose that \( n_i \) is the number of patients that are being observed at time \( t_i \) (by convention, if a patient’s censoring time is \( t_i \), the patient is considered to be observed at \( t_i \)), and \( m_i \) is the number of events at \( t_i \). Then, \( S(t) \) is

\[
\frac{(n_i-m_i)}{n_i}
\]
for \( t=t_1 \) up until just before \( t_2 \). Note that this is simply the proportion of patients that survive past \( t_1 \) among those who survived until \( t_1 \). At \( t_2 \), one estimates the probability that a patient survives past \( t_2 \) given that the patient lives up to \( t_2 \) by

\[
\frac{(n_1 - m_1)}{n_2}.
\]

The estimate of \( S(t) \) is then

\[
\left(\frac{n_1 - m_1}{n_1}\right) \times \left(\frac{n_2 - m_2}{n_2}\right)
\]

from \( t=t_2 \) up to just before \( t=t_3 \). Most computer programs that compute the Kaplan-Meier survival curve start with 2 columns of data; the first is the survival or censoring time, and the second is a censoring indicator that is 0 if the time is a censoring time and 1 if the time is an event time.

The plots with survival time on the horizontal axis and the proportion surviving, \( S(t) \), on the vertical axis when there is censoring are called the KM curves. These start at 1 (because probability of survival beyond time 0 is 1) and step down toward zero. If there are subjects who survive beyond the study time, then the survival curve does not go to zero but stays horizontal. Because the survival curve does not change in intervals in which no events occur, one can calculate the curve at event times only. The mean time to an event is estimated by the area under the survival function. If the largest event is an event time, then the survival function goes to zero at that time, and this estimate is finite. Otherwise, the mean time cannot be estimated. This is why median survival is used more often than mean survival. The median survival time is estimable only if the survival curve drops to or below 0.5. The median survival time is estimated by the value on the horizontal axis at the intersection of a horizontal line drawn from the vertical axis to the survival line where \( S(t)=0.5 \). If the KM curve drops to or below 0.5 but does not equal 0.5, then the first event time when the curve falls below 0.5 is used.

Survival curves can be compared to assess differences in treatment effects. If \( k \geq 2 \) groups are being compared, survival curves are plotted for each group. If the survival curves are parallel to each other, then the group that consistently has a higher survival curve than the other has longer survival, and the treatment given to this group is concluded to be the better of the treatments being studied. Although visually the survival curves for the 2 groups might seem different from each other, we need to test whether the “true” survival curves are statistically different by using a formal test.

**Hypothesis Testing**

In most studies, one is interested in evaluating the effect of 1 or more treatments or exposures (eg, aspirin/placebo, smoking) on an outcome of interest (eg, first myocardial infarction) either by itself or adjusted for other covariates. In survival analysis, the outcome of interest is the survival time, and one is interested in comparing the survival times between groups or assessing the relationship of exposure/covariates to the survival time. Standard methods of data analysis (eg, t tests, linear or logistic regressions) cannot be applied to survival data because they do not account for censoring. If censored observations are excluded from the analysis, the results will be biased.

**Proportional Hazards or Log-Rank Test**

The log-rank test can be used to test the hypothesis of no difference in survival between the 2 groups. This test makes the assumptions that the observations are independent and that the censoring distribution is independent of the survival distribution; notably, the censoring distribution can be different in each group, so that the test can be used to compare a current treatment group with a historical control.

This tests the null hypothesis that there is no statistical difference between the survival curves in the 2 groups. The basic idea behind the test is that at each event time \( t \) there will be \( n_i \) patients in group 1 and \( n_2 \) patients in group 2. Under the null hypothesis of no treatment effect, the probability that the treatment group of the patient who had the event will be in group 1 is

\[
\frac{n_{i1}}{(n_{i1} + n_{i2})}.
\]

Thus, if we define an indicator variable \( \delta_i \) to equal 1 if the patient is from group 1 and zero if the patient is from group 2, then

\[
\sum \delta_i \left(\frac{n_{i1}}{(n_{i1} + n_{i2})}\right)
\]

has a mean equal to zero. This is the numerator of the log-rank test. The denominator is the standard deviation of this quantity:

\[
\sqrt{\sum \frac{n_{i1}n_{i2}(m_{i1} + m_{i2})(n_{i1} + n_{i2} - m_{i1} - m_{i2})^2}{(n_{i1} + n_{i2})^2(n_{i1} + n_{i2} - 1)^{1/2}}}
\]

The log-rank test can be used to compare \( k \geq 2 \) survival curves. It is preferable to use the multivariable regression method to assess the relationship of many risk factors to survival.

**Cox Proportional Hazards Model**

This is the most popular method to evaluate the relationship between covariates and survival with the use of a mathematical model. This is called a semiparametric model because it does not assume any distribution for the baseline hazard. The model is defined as

\[
h(t; x_1, x_2, \ldots, x_k) = \lambda_0(t)e^{\lambda_1 x_1 + \lambda_2 x_2 + \ldots + \lambda_k x_k}
\]

where \( \lambda_0(t) \) is the baseline hazard at time \( t \) and \( x_1, x_2, \ldots, x_k \) are \( k \) independent covariates. No assumptions are made regarding the baseline hazard function.

We can test the association of each of the independent variables with survival time adjusted for other covariates. It is important to understand the meaning of the parameters in a proportional hazards model. Suppose first that the covariate, say \( x_i \), has 2 values, 0 and 1. Then, \( e^{\lambda_i} \) is the hazard ratio for patients with \( x_i = 1 \) versus those with \( x_i = 0 \). That is the instantaneous probability of an event in one group divided by that probability in the other. Notice that we are modeling the hazard so that if patients for whom \( x_i = 1 \) have longer survival times, then \( \lambda_i \) will be negative. The model specifies that the
hazard ratio is constant over time and for the values of all the other covariates. When the covariate is continuous, then \( \exp(\lambda_i) \) is the hazard ratio for a unit change in the value of \( x_i \). It is often helpful to divide continuous covariates by their standard deviation so that the units for each covariate are comparable, and \( \lambda_1, \lambda_2, \ldots \) have the same scale, which would be 1 standard deviation. Hazard ratios are approximately equal to the relative risk (ratio of risk in the exposed group to the risk in the unexposed group) and are used interchangeably.

### Parametric Methods

Parametric methods assume that the survival times follow a specified distribution. Exponential, Weibull, Gompertz, Gamma, and log-normal distribution are often used for survival times. In the exponential model the hazard function is constant, which means a person’s probability of an event in the future is independent of how long the person has gone without an event. Weibull, Gompertz, or Gamma can be used when the hazard function is monotonically increasing or decreasing. The log-normal distribution is assumed when the hazard increases in the beginning and then decreases.

Probably the most commonly used parametric survival model is the exponential model, which is defined as:

\[
h(t; x_1, x_2, \ldots, x_k) = \exp(\lambda_0 + \lambda_1 x_1 + \lambda_2 x_2 + \ldots + \lambda_k x_k) .
\]

This model has some very useful properties. Without covariates, the survival function is \( S(t) = \exp(-t \exp(\lambda_0)) \). The hazard function is constant \( \exp(\lambda_0) \), the mean survival is \( \exp(-\lambda_0) \), and the median survival is \( -\exp(-\lambda_0) \times \log(0.5) \).

To estimate \( \lambda_0 \), let \( n \) be the total number of events and let \( f \) be the total amount of follow-up; then \( \lambda_0 \) is estimated by \( \log(n/f) \), which has a standard error of \( 1/\sqrt{n} \). The estimates from the exponential model are the same as those from the proportional hazards model when the data are exponential, which is why the exponential distribution is often used for sample size calculations even when the data are to be analyzed with a proportional hazards model or a log-rank test.

Kalbflesisch and Prentice provide more details on parametric methods.

A brief discussion of other issues related to survival analysis is presented in the next section.

### Additional Topics

#### Competing Risks

The concept of competing risks is another important issue to consider when survival over time is studied. For example, subjects in a cohort might be at risk of cardiovascular (other than myocardial infarction) mortality or dying because of myocardial infarction. The analysis approaches involve either computing the all-cause hazards, in which all events are taken into account, eg, cardiovascular mortality or death due to myocardial infarction (whichever occurs first is the outcome), and the cause-specific hazards, in which only the time to the event of interest is observed, and the times to other events are censored. For example, if cardiovascular mortality (other than a myocardial infarction) is of interest, then only time to this event is observed, and subjects who die due to myocardial infarction are censored. Plots of cumulative hazard functions are generally preferred when cause-specific hazards are used, and tests based on cumulative incidence have been developed because the Kaplan-Meier curves and log-rank tests may give biased results. For more information, see References by Gail through Allison.

#### Interval Censoring

It is important to identify the time origin in a survival analysis. The usual time origin is the entry into the study. In that case, at time 0 the value of \( n_0 \) is the total number of patients. However, many survival techniques will also work without this restriction. For instance, one can analyze age at death among nursing home patients using survival theory. In that case, the time variable is age, and \( n_0 \) will not decrease but will vary as people of different ages enter the nursing home. This is known as interval censoring. For those interested in this type of survival analysis, see Hyde and Turnbull. There are also a wealth of techniques for the situation in which the event time is only known up to an interval; for instance, if the event were the development of a condition that could only be diagnosed by an ultrasound or a laboratory test, all that one would know was that it occurred after the last negative test and before the next positive one. This is known as interval censoring.

#### Time-Varying Covariates

The assumptions of proportional hazards may not hold for a given data set. The proportional hazards model can be made more general because one can add time-varying covariates to handle situations in which the hazard ratio is not constant over time or add interaction and quadratic terms when the hazard ratio is not constant over other covariates. For in-
stance, the covariate $t \times Z$ would model a situation in which the hazard ratio increases linearly when $Z = 1$ compared with $Z = 0$. Thus, issues of whether the model “fits” the data are actually issues about whether the model is correctly specified. Tests of fit are described by Schoenfeld,3,14 and Wei,15 and the consequences of misspecification on testing are described by Lagakos and Schoenfeld16 and Gail et al.17

If the model is used for testing, the conclusion is that misspecification is not a great problem.16 However, estimates are biased and represent a weighted average of the hazard over the duration of the study.

One of the advantages of covariate adjustment is that it can help to ameliorate the effects of informative censoring. In an analysis with covariates, the censoring distribution can depend on the covariates. Informative censoring in this case would be a dependency between the event time and the censoring time for patients with the same value of all covariates included in the model. Thus, for instance, if a covariate affected both the censoring time and the event time, then the censoring would be informative in a model without the covariate but noninformative in a model with the covariate.

**Power Calculations**

Power calculations are useful for designing studies. To calculate the power for survival analysis, one needs to know the total number of subjects in the trial, accrual time (time during which subjects are recruited in the study), failure time (time at which an event or death occurs), median survival ratio (or hazard ratio) or the minimum detectable hazard, and the significance level.3,4,13,18 Standard software packages like SAS, SPSS, S-PLUS, and STATA have procedures that are simple to use for all methods described in this article.

**Example**

As an example of how these methods are used in practice, consider the report “Prevention of Fatal Arrhythmias in High-Risk Subjects by Fish Oil n-3 Fatty Acid Intake,” which recently appeared in this journal.19 We briefly summarize the uses of survival methods in that report.

The aim of the study was to evaluate whether n-3 fatty acids prevent potentially fatal ventricular arrhythmias in high-risk patients. A total of 402 patients with implantable cardioverter-defibrillators (ICDs) were randomly assigned to double-blind treatment with either a fish oil or olive oil daily supplement for 12 months. The primary end point was time to first ICD event for ventricular tachycardia or fibrillation confirmed by stored electrograms or death from any cause. Analyses were performed both according to the intention to treat and according to actual treatment. All randomized

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**Comparison of Baseline Characteristics of All Enrollees for the Placebo and Fish Oil Treatment Arms in the Fatty Acid Antiarrhythmia Trial: Analysis of Time to First Event**

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intention-to-treat analysis (n=402)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Confirmed events</td>
<td>0.72</td>
<td>0.51–1.01</td>
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<tr>
<td>Including probable events</td>
<td>0.69</td>
<td>0.49–0.97</td>
<td>0.033</td>
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<td>Multivariable analysis*</td>
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<td></td>
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<tr>
<td>Confirmed events</td>
<td>0.67</td>
<td>0.47–0.95</td>
<td>0.024</td>
</tr>
<tr>
<td>Including probable events</td>
<td>0.66</td>
<td>0.46–0.92</td>
<td>0.016</td>
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<tr>
<td><strong>On-treatment analysis for all on treatment† (n=402)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Controlling for baseline left ventricular ejection fraction</td>
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<tr>
<td>Confirmed events</td>
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<tr>
<td>Confirmed events</td>
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<td>0.46–0.98</td>
<td>0.037</td>
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<tr>
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<td>0.65</td>
<td>0.45–0.95</td>
<td>0.026</td>
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<tr>
<td><strong>On-treatment analysis for at least 11 months‡ (n=236)</strong></td>
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<td>Confirmed events</td>
<td>0.62</td>
<td>0.39–0.97</td>
<td>0.034</td>
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<tr>
<td>Including probable events</td>
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<td>Confirmed events</td>
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<td>Including probable events</td>
<td>0.53</td>
<td>0.34–0.84</td>
<td>0.0070</td>
</tr>
</tbody>
</table>

*Multivariable model controlled for gender, left ventricular ejection fraction (continuous), New York Heart Association class III congestive heart failure, history of myocardial infarction, history of prior defibrillator therapies for ventricular tachycardia/ventricular fibrillation, time from ICD implant (continuous), and sustained ventricular tachycardia as the indication for the ICD (all measured at baseline).

†On-treatment analysis for all subjects who had taken any of their prescribed oil supplements; the follow-up was censored at 2 months after medication was stopped.

‡On-treatment analysis only for those subjects who were on treatment at least 11 months.
subjects in this study were included in the intention-to-treat analysis. The primary analysis, based on confirmed events, was an intention-to-treat analysis of the survival free of appropriate ICD events for ventricular tachycardia/ventricular fibrillation and/or death from any cause, which included all ICD events that occurred during the 12-month period after the first dose of the study drug, irrespective of the duration of treatment. An “on-treatment” analysis was done that included all ICD events that occurred no later than 2 months after treatment was stopped. In this analysis, the date of cessation of treatment plus 2 months was used as the censoring variable. To ensure that the time to event was independent of time to noncompliance, conditional on covariates in the model, the authors tested for associations between baseline variables and time to noncompliance and used any that were significant as covariates in this analysis.20

Time to first event analysis was calculated by the Kaplan-Meier method, and survival time across the 2 groups was compared with log-rank tests. Cox proportional hazards models were also performed to calculate hazard ratios and to adjust for clinical covariates that were associated with non-compliance in the on-treatment analysis and with the primary end point in the multivariable analysis.

The survival plots displayed in the Figure and the results of the analysis displayed in the Table were published previously in Circulation.19

Time to First Event Analyses

In the primary analysis, according to the intention-to-treat principle, there was a trend toward a longer time to first ICD event for ventricular tachycardia/ventricular fibrillation confirmed by electrograms or death from any cause among patients randomized to fish oil compared with those randomized to the olive oil placebo ($P=0.057$). According to the KM estimates (Figure), 28% of patients in the fish oil arm ($n=57$) and 39% of patients in the olive oil arm ($n=78$) had reached the primary end point at 12 months. This difference corresponds to a hazard ratio of 0.72. The multivariable analysis that controlled for baseline clinical characteristics resulted in a hazard ratio of 0.67, 95% confidence limits of 0.47 to 0.95, and significance of $P=0.024$ (Table).

In the “on-treatment” analysis of “confirmed” events, which included all who had taken any prescribed oil supplements during the 12-month period, the hazard ratio was 0.73, which was not significant ($P=0.11$). This analysis controlled for baseline left ventricular ejection fraction, which was the only variable affecting time to noncompliance. After the investigators controlled for more baseline variables, the reduction in risk associated with use of fish oil became significant (hazard ratio, 0.67; $P=0.037$) (multivariable analysis, Table).

It is interesting that the $P$ value decreases in the multivariable analysis compared with the analysis that only considers treatment. This is not necessarily due to confounding, which was not large in this study. The effect is rather due to the fact that a multivariable model that controls for factors that affect the time to event increases the power to see a treatment effect when the covariates are equally distributed between the treatment groups, which is usually the case in clinical trials.

This effect is quantified in Schoenfeld et al.21 When a study is designed, it is often a conundrum to decide whether the covariate analysis should be a primary or secondary analysis, but the decision regarding the primary analysis and the covariates to be used in the analysis should be made before data collection for a confirmatory type of study. It has more power at the cost of complexity.

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Disclosures

None.

References


Key Words: proportional hazards models ■ Kaplan-Meier estimate ■ survival
Primary Prevention of Cardiovascular Diseases in People With Diabetes Mellitus

A Scientific Statement From the American Heart Association and the American Diabetes Association

John B. Buse, MD, PhD, Co-chair; Henry N. Ginsberg, MD, FAHA, Co-chair; George L. Bakris, MD, FAHA; Nathaniel G. Clark, MD, MS, RD; Fernando Costa, MD, FAHA; Robert Eckel, MD, FAHA; Vivian Fonseca, MD; Hertzel C. Gerstein, MD, MSc, FRCP; Scott Grundy, MD, FAHA; Richard W. Nesto, MD, FAHA; Michael P. Pignone, MD, MPH; Jorge Plutzky, MD; Daniel Porte, MD; Rita Redberg, MD, FAHA; Kimberly F. Stitzel, MS, RD; Neil J. Stone, MD, FAHA

Abstract—The American Heart Association (AHA) and the American Diabetes Association (ADA) have each published guidelines for cardiovascular disease prevention: The ADA has issued separate recommendations for each of the cardiovascular risk factors in patients with diabetes, and the AHA has shaped primary and secondary guidelines that extend to patients with diabetes. This statement will attempt to harmonize the recommendations of both organizations where possible but will recognize areas in which AHA and ADA recommendations differ. (Circulation. 2007;115:114-126.)

Key Words: AHA Scientific Statement ■ cardiovascular diseases ■ diabetes mellitus ■ primary prevention

Diabetes mellitus is a disease defined by abnormalities of fasting or postprandial glucose and frequently is associated with disorders of the eyes, kidneys, nerves, and circulatory system. Circulatory disorders associated with diabetes include coronary heart disease (CHD), stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure. Diabetes generally results in early death from cardiovascular diseases (CVDs). In 1999, the American Diabetes Association (ADA) and the American Heart Association (AHA) published a joint statement with the National Heart, Lung, and Blood Institute (NHLBI), the National Institute of Diabetes and Digestive and Kidney Diseases, and the Juvenile Diabetes Foundation International indicating the need for multorganizational cooperation for prevention of CVD in patients with diabetes.1 The present statement represents a joint response of the ADA and AHA to this challenge.

The ADA and AHA each have published guidelines for CVD prevention that overlap with the present statement: The ADA has issued separate recommendations for each of the cardiovascular risk factors in patients with diabetes, and the AHA has shaped primary and secondary guidelines that extend to patients with diabetes. The present document will attempt to harmonize the recommendations of both organizations where possible but will recognize areas in which ADA and AHA recommendations differ.

Clear clinical trial evidence published over the past decade suggests that broad-based treatment of dyslipidemia, hypertension, and hypercoagulability (as well as interventional cardiology and cardiovascular surgery during the acute coronary syndrome2) can improve the event-free survival rate in people with diabetes who already have clinical CVD. However, a much smaller body of clinical trial data addresses the issue of primary prevention of CVD in patients with diabetes and no known CVD. This is a critical issue because patients with diabetes have twice the risk of incident myocardial infarction and stroke as that of the general population. Furthermore, large numbers of people with diabetes do not survive their first event, and if they do survive, their mortality...
rate over the subsequent months to years is generally greater than that of the general population. As many as 80% of patients with type 2 diabetes mellitus will develop and possibly die of macrovascular disease. This represents a great societal cost, with major loss of life expectancy and quality of life.\(^{3,4}\) Although the incidence of CVD events in patients with diabetes seems to have declined over the past decade,\(^5\) implementation of preventive strategies is often inadequate.\(^6\)

To facilitate clinical practice, the present statement is condensed into essential recommendations. No endeavor is made to recapitulate all of the clinical trial evidence that is thoroughly documented in the ADA and AHA reports on management of individual risk factors. For each of the risk factors, a sampling of relevant studies is discussed and referenced. Recommendations are made on the totality of evidence in the field, including studies of several types, such as controlled clinical trials (Table 1). When possible, studies under way that will further address these issues are also noted. With the exception of recommendations related to control of hyperglycemia, the recommendations provided in this document are appropriate for people both with and without diabetes; however, because of their higher risk for CVD, people with diabetes should derive even more benefit from these recommendations.

**Comprehensive Risk Assessment**

Recent guidelines for CVD management in diabetes are based on the premise that most patients with diabetes are at high risk for future CVD events. When diabetes exists in patients with established CVD, absolute risk for future events is very high. Even in the absence of CVD, both the ADA and the AHA identify diabetes as a high-risk condition for macrovascular CVD.\(^7,8\) This conclusion was based on several factors, including a relatively high 10-year risk for CVD events, increased morbidity after the onset of CVD, and a high long-term risk for developing CVD.\(^9\) For these reasons and to simplify the assessment of risk, the NHLBI Adult Treatment Panel III (ATP III) designated diabetes as a “CVD risk equivalent” for setting treatment goals for low-density lipoprotein cholesterol (LDL-C).\(^10\) The same general strategy for LDL lowering is recommended by the ADA\(^8\) and the British Hypertension Society guidelines.\(^11\) This approach has also been applied to treatment of hypertension by both the ADA and the NHLBI.\(^12\)

Nonetheless, it is widely recognized that absolute risk for macrovascular CVD varies among individuals with diabetes, and an accurate assessment of risk clearly depends on the individuals’ characteristics.\(^13\)\(^{-18}\) Indeed, it seems self-evident that some patients, such as children and young adults with recent-onset diabetes, are at relatively low risk of CVD over an intermediate time frame (eg, 10 years). For this reason, some investigators favor individualizing risk assessment on the basis of risk-prediction algorithms to provide more appropriate risk factor interventions than those recommended by general guidelines that are geared toward middle-aged and older individuals with type 2 diabetes mellitus. Three such risk calculators are the Framingham risk calculator (available at http://hin.nhlbi.nih.gov/atp3ii/calculator.asp?usertype=prof),\(^19\) the UK Prospective Diabetes Study (UKPDS) risk engine (available for download at http://www.dtu.ox.ac.uk/riskengine),\(^20\) and the ADA’s Diabetes PHD (Personal Health Decisions; available at http://diabetes.org/diabetesPHD), which has been extensively validated against clinical trials.\(^21\)

It is important to realize that unresolved issues still exist relating to the assessment of risk in many people with diabetes mellitus. For example, the AHA and the NHLBI have issued a statement on management of the metabolic syndrome and maintain that with regard to risk for CVD, the metabolic syndrome and type 2 diabetes mellitus can coexist in one person.\(^22\) The ADA, in contrast, contends that once type 2 diabetes mellitus is present, the metabolic syndrome no longer pertains because CVD risk factors characteristic of the metabolic syndrome are largely subsumed in the type 2 diabetes mellitus syndrome.\(^23\)

**Lifestyle Management**

Lifestyle measures such as medical nutrition therapy and aerobic exercise have been demonstrated to modify lipids and reduce blood pressure and are integral to the management of glycemia and weight control.\(^24,25\) Numerous epidemiological analyses suggest that nutrition and physical activity are predictors of age-specific mortality and cardiovascular event rates. Although lifestyle intervention in patients with type 2 diabetes mellitus has traditionally focused almost exclusively on weight loss, most experts in the field today believe the major focus of lifestyle intervention should be on improving glycemic control and controlling other major CVD risk factors. Weight control remains an important component of lifestyle management. Reeducation of the patient about food selection and the importance of regular physical activity, combined with regular reevaluation and behavioral interventions to maintain adherence, may be the most successful approach to improve long-term outcomes.\(^22,24\) To date, short-term studies of medical nutrition therapy,\(^7,24\) physical activity, and comprehensive lifestyle approaches have been shown to improve the control of risk factors and intermediate markers of CVD risk.

**Weight**

Weight reduction in obese persons will reduce all of the CVD risk factors associated with type 2 diabetes mellitus and will improve hyperglycemia. Moderate weight loss (eg, 7% to 10% of body weight in 1 year) is often attainable, whereas efforts to achieve ideal body weight in short periods of time usually fail. Even if no weight reduction can be achieved, weight maintenance is certainly preferable to weight gain. Diets low in carbohydrate (and therefore high in fat) may be associated with greater weight loss in the short term but have not been demonstrated to result in greater weight loss after 1 year than diets with more balanced proportions of fats and carbohydrates.\(^26,27\)

No long-term, large-scale study of lifestyle intervention or intentional weight loss has been adequately powered to examine CVD end points in individuals with diabetes mellitus. In the Look AHEAD (Action for Health in Diabetess) study, patients with type 2 diabetes mellitus with a body mass index \(\leq 25\) kg/m\(^2\) have been randomized to an intensive weight loss program (calorie restriction and physical activity) or to diabetes support and education and are being followed
TABLE 1. Recommendations for Primary Prevention of CVD in People With Diabetes

### Lifestyle Management

#### Weight

Structured programs that emphasize lifestyle changes such as reduced fat (<30% of daily energy) and total energy intake and increased regular physical activity, along with regular participant contact, can produce long-term weight loss on the order of 5% to 7% of starting weight, with improvement in blood pressure.

For individuals with elevated plasma triglycerides and reduced HDL-C, improved glycemic control, moderate weight loss (5% to 7% of starting weight), dietary saturated fat restriction, increased physical activity, and modest replacement of dietary carbohydrate (5% to 7%) by either monounsaturated or polyunsaturated fats may be beneficial.

#### Medical Nutrition Therapy

To achieve reductions in LDL-C:

- Saturated fats should be <7% of energy intake
- Dietary cholesterol intake should be <200 mg/d.
- Intake of trans-unsaturated fatty acids should be <1% of energy intake.

Total energy intake should be adjusted to achieve body-weight goals.

Total dietary fat intake should be moderated (25% to 35% of total calories) and should consist mainly of monounsaturated or polyunsaturated fat.

Ample intake of dietary fiber (≥14 g per 1000 calories consumed) may be of benefit.

If individuals choose to drink alcohol, daily intake should be limited to 1 drink for adult women and 2 drinks for adult men. One drink is defined as a 12-oz beer, a 4-oz glass of wine, or a 1.5-oz glass of distilled spirits. Alcohol ingestion increases caloric intake and should be minimized when weight loss is the goal. Individuals with elevated plasma triglyceride levels should limit alcohol intake, because intake may exacerbate hypertriglyceridemia.

In both normotensive and hypertensive individuals, a reduction in sodium intake may lower blood pressure. The goal should be to reduce sodium intake to 1200 to 2300 mg/d (50 to 100 mmol/d), equivalent to 3000 to 6000 mg/d of sodium chloride.

#### Physical Activity

To improve glycemic control, assist with weight loss or maintenance, and reduce risk of CVD, at least 150 minutes of moderate-intensity aerobic physical activity or at least 90 minutes of vigorous aerobic exercise per week is recommended. The physical activity should be distributed over at least 3 days per week, with no more than 2 consecutive days without physical activity.

For long-term maintenance of major weight loss, a larger amount of exercise (7 hours of moderate or vigorous aerobic physical activity per week) may be helpful.

#### Blood Pressure

Blood pressure should be measured at every routine diabetes visit. Patients found to have systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥80 mm Hg should have blood pressure confirmed on a separate day.

Patients with diabetes should be treated to a systolic blood pressure <130 mm Hg and a diastolic blood pressure <80 mm Hg.

Patients with a systolic blood pressure of 130 to 139 mm Hg or a diastolic blood pressure of 80 to 89 mm Hg should initiate lifestyle modification alone (weight control, increased physical activity, alcohol moderation, sodium reduction, and emphasis on increased consumption of fresh fruits, vegetables, and low-fat dairy products) for a maximum of 3 months. If, after these efforts, targets are not achieved, treatment with pharmacological agents should be initiated.

Patients with hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) should receive drug therapy in addition to lifestyle and behavioral therapy.

All patients with diabetes and hypertension should be treated with a regimen that includes either an ACE inhibitor or an ARB. If one class is not tolerated, the other should be substituted. Other drug classes demonstrated to reduce CVD events in patients with diabetes (β-blockers, thiazide diuretics, and calcium channel blockers) should be added as needed to achieve blood pressure targets.

If ACE inhibitors, ARBs, or diuretics are used, renal function and serum potassium levels should be monitored within the first 3 months. If stable, follow-up could occur every 6 months thereafter.

Multiple-drug therapy is generally required to achieve blood pressure targets.

In elderly hypertensive patients, blood pressure should be lowered gradually to avoid complications.

Orthostatic measurement of blood pressure should be performed in people with diabetes and hypertension when clinically indicated.

Patients not achieving target blood pressure despite multiple-drug therapy should be referred to a physician specializing in the care of patients with hypertension.

#### Lipids

In adult patients, lipid levels should be measured at least annually and more often if needed to achieve goals. In adults under the age of 40 years with low-risk lipid values (LDL-C <100 mg/dL, HDL-C >50 mg/dL, and triglycerides <150 mg/dL), lipid assessments may be repeated every 2 years.

Lifestyle modification deserves primary emphasis in all diabetic individuals. Patients should focus on the reduction of saturated fat and cholesterol intake, weight loss (if indicated), and increases in dietary fiber and physical activity. These lifestyle changes have been shown to improve the lipid profile in patients with diabetes.

In individuals with diabetes who are over the age of 40 years, without overt CVD, but with 1 or more major CVD risk factors, the primary goal is an LDL-C level <100 mg/dL (2.6 mmol/L). If LDL-lowering drugs are used, a reduction of at least 30% to 40% in LDL-C levels should be obtained. If baseline LDL-C is <100 mg/dL, statin therapy should be initiated based on risk factor assessment and clinical judgment. Major risk factors in this category include cigarette smoking, hypertension (blood pressure >140/90 mm Hg or use of antihypertensive medication), low HDL cholesterol (<40 mg/dL), and family history of premature CHD (CHD in male first-degree relative <55 years of age; CHD in female first-degree relative <65 years of age).

In individuals with diabetes who are under the age of 40 years, without overt CVD, but who are estimated to be at increased risk of CVD either by clinical judgment or by risk calculator, the LDL-C goal is <100 mg/dL, and LDL-lowering drugs should be considered if lifestyle changes do not achieve the goal.

The ADA and AHA suggest different approaches to the management of HDL- and triglyceride-associated CVD risk.
up to determine the effect of these interventions on CVD events.28

**Medical Nutrition Therapy**

Although numerous studies have attempted to identify the optimal combination of macronutrients to prevent CVD, it is unlikely that any one such combination of macronutrients exists. The best mix of carbohydrate, protein, and fat seems to vary according to individual circumstances. The cardiovascular efficacy and safety of low- or moderately low-carbohydrate diets in diabetes have not been well studied. Very-low-carbohydrate diets (eg, those that restrict carbohydrate intake to <130 g/d) are not recommended for patients with diabetes because ample intake of fruits, vegetables, grains, legumes, and low-fat dairy products provides vitamins, minerals, fiber, and protein. In the general population, studies of a variety of medical nutrition therapy techniques to reduce blood pressure have focused on weight loss, sodium restriction, reduction of alcohol intake, and an increase in the intake of potassium and calcium. For example, the Dietary Approaches to Stop Hypertension (DASH) diet, which encourages the intake of fruits, vegetables, and low-fat dairy products, particularly when those foods are combined with sodium restriction, was associated with substantial improvements in blood pressure.29 The restriction of saturated fats, dietary cholesterol, and trans-unsaturated fats and the incorporation of increased dietary fiber and monounsaturated and polyunsaturated fats into the diet are recommended dietary strategies to improve lipids.7 Overall, the AHA diet and lifestyle recommendations,30 the therapeutic lifestyle changes suggested by the National Cholesterol Education Program’s ATP III,7 and the ADA nutrition guidelines8 address all of these issues.

Supplementation of a healthy diet with antioxidant vitamins, B vitamins to lower homocysteine, or specific fatty acids (such as omega-3 fatty acids) is not recommended by either the AHA30 or the ADA at this time for healthy persons.8 Although each of these has been demonstrated to be associated with lower CVD risk in published epidemiological analyses, no consistent findings have emerged from large-scale, randomized trials in people with diabetes.30–33 Of all the supplements, the strongest data for benefit are with omega-3 fatty acids in individuals with established CHD. For this reason, the AHA currently recommends 1 g/d eicosapentaenoic acid + docosahexaenoic acid for individuals with established disease.34,35 On the other hand, randomized trials of vitamin E, folate, and B vitamins, as well as other antioxidants such as beta-carotene or antioxidant cocktails, have not shown benefit.36,37

**Physical Activity**

To improve glycemic control, assist with weight maintenance, and reduce the risk of CVD (on the basis of epidemiological studies), at least 150 minutes of moderate-intensity aerobic physical activity per week or at least 90 minutes of vigorous aerobic exercise per week is recommended. Thus, patients with diabetes should be encouraged to perform 30 to 60 minutes of moderate-intensity aerobic activity such as brisk walking on most (preferably all) days of the week, supplemented by an increase in daily lifestyle activities (eg,
walking breaks during the workday, gardening, and household work). For long-term maintenance of major weight loss, a larger amount of exercise (a minimum of 7 hours of moderate or vigorous aerobic physical activity per week) is helpful.

Before beginning a program of physical activity that is more vigorous than brisk walking, people with diabetes should be assessed for conditions that might contraindicate certain types of exercise or predispose to injury (eg, severe autonomic neuropathy, severe peripheral neuropathy, proliferative or proliferative retinopathy). One potential area of controversy is the circumstance under which a graded exercise electrocardiogram stress test is indicated. Unfortunately, no randomized trials or large cohort studies have evaluated the utility of exercise stress testing specifically in people with diabetes. Moreover, if cardiac stress imaging is performed, it is difficult to identify which individuals with diabetes are at low risk. The low predictive value of a negative stress test in those with diabetes confirms the need to treat risk factors for atherosclerosis intensively regardless of the results of exercise testing and indicates that patients with diabetes require close follow-up, with a lower threshold for proceeding to angiography than patients without diabetes. Indeed, those patients with diabetes who are unable to exercise are at the greatest risk of CHD events, and in some analyses, the most important prognostic variables for CVD and all-cause death were not exercise ECG changes but fitness-related variables such as exercise duration and heart rate recovery. Because of these uncertainties, the decision to perform stress testing for patients beginning a vigorous exercise program must be made on an individual basis.

**Recommendations for Lifestyle Intervention for Primary Prevention of CVD**

**Weight Management**

- Structured programs that emphasize lifestyle changes such as reduced fat (<30% of daily energy) and total energy intake and increased regular physical activity, along with regular participant contact, can produce long-term weight loss on the order of 5% to 7% of starting weight, with an improvement in blood pressure.
- For individuals with elevated plasma triglycerides and reduced high-density lipoprotein cholesterol (HDL-C), improved glycemic control, moderate weight loss (5% to 7% of starting weight), dietary saturated fat restriction, increased physical activity, and modest replacement of dietary carbohydrate (5% to 7%) by either monounsaturated or polyunsaturated fats may be beneficial.

**Medical Nutrition Therapy**

- To achieve reductions in LDL-C, saturated fats should be <7% of energy intake, dietary cholesterol intake should be <200 mg/d, and intake of trans-unsaturated fatty acids should be <1% of energy intake.
- Total energy intake should be adjusted to achieve body-weight goals.
- Total dietary fat intake should be moderated (25% to 35% of total calories) and should consist mainly of monounsaturated or polyunsaturated fat.
- Ample intake of dietary fiber (≥14 g per 1000 calories consumed) may be of benefit.
- If individuals choose to drink alcohol, daily intake should be limited to 1 drink for adult women and 2 drinks for adult men. One drink is defined as a 12-oz beer, a 4-oz glass of wine, or a 1.5-oz glass of distilled spirits. Alcohol ingestion increases caloric intake and should be minimized when weight loss is the goal. Individuals with elevated plasma triglyceride levels should limit alcohol intake because intake may exacerbate hypertriglyceridemia. Alcohol ingestion can also increase blood pressure.
- In both normotensive and hypertensive individuals, a reduction in sodium intake may lower blood pressure. The goal should be to reduce sodium intake to 1200 to 2300 mg/d (50 to 100 mmol/d), equivalent to 3000 to 6000 mg/d of sodium chloride.

**Physical Activity**

- To improve glycemic control, assist with weight loss or maintenance, and reduce risk of CVD, at least 150 minutes of moderate-intensity aerobic physical activity or at least 90 minutes of vigorous aerobic exercise per week is recommended. The physical activity should be distributed over at least 3 days per week, with no more than 2 consecutive days without physical activity.
- For long-term maintenance of major weight loss, a larger amount of exercise (7 hours of moderate or vigorous aerobic physical activity per week) may be helpful.

**Blood Pressure**

Epidemiological analyses and randomized clinical trials have demonstrated the impact of elevated blood pressure as a risk factor for both microvascular and macrovascular disease in diabetes. As a result, many have argued that blood pressure management is the most critical aspect of the care of the patient with diabetes. Epidemiological analyses show that higher risk for cardiovascular events and mortality starts at a blood pressure >115/75 mm Hg in the general population and doubles for every 20-mm Hg systolic or 10-mm Hg diastolic increase. However, the question of what systolic and diastolic blood pressure goals should be targeted is not completely answered by currently available outcome trials.

The Hypertension Optimal Treatment trial randomized patients with diastolic blood pressure of 100 to 115 mm Hg to diastolic blood pressure targets of ≤90, ≤85, and ≤80 mm Hg. Although the overall study did not demonstrate a benefit from lower diastolic blood pressure targets, a post hoc analysis of subjects with diabetes did demonstrate a significant decline in the rate of major cardiovascular events with lower diastolic blood pressure targets. In the group randomized to a diastolic target of ≤80 mm Hg, the risk of major cardiovascular events was halved compared with the group with a target of ≤90 mm Hg. For patients with diabetes, it generally is agreed that the appropriate diastolic blood pressure target is ≤80 mm Hg.

Although studies similar to the Hypertension Optimal Treatment trial have not been conducted to examine specific systolic blood pressure targets, placebo-controlled studies demonstrate robustly that systolic blood pressure levels <140 mm Hg are associated with improved outcomes compared with higher levels. In the ABCD trial (Appropriate
Blood Pressure Control in Diabetes), a mean systolic blood pressure of 132 mm Hg was achieved in the more intensively treated group; however, no significant reduction in CVD end points occurred, although total mortality rate was reduced.\textsuperscript{43} Thus, although it is unclear exactly how much the systolic blood pressure should be lowered below 140 mm Hg, various groups have recommended systolic blood pressure targets of $<135 \text{ mm Hg}$\textsuperscript{42} and $<130 \text{ mm Hg}$\textsuperscript{8,44}. The ACCORD (Action to Control Cardiovascular Risk in Diabetes) blood pressure study\textsuperscript{45} will explicitly test the cardiovascular efficacy of lowering the systolic blood pressure below 140 mm Hg. It has randomized participants to 2 levels of systolic blood pressure control to determine whether a therapeutic strategy that targets a systolic blood pressure of $<120 \text{ mm Hg}$ reduces the rate of CVD events more than a strategy that targets a systolic blood pressure of $<140 \text{ mm Hg}$\textsuperscript{45}.

Multiple studies that have used thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), $\beta$-blockers, and calcium channel blockers have demonstrated benefits on microvascular end points and combined cardiovascular end points.\textsuperscript{46} In general, more consistent clinical trial evidence supports the hypothesis that blood pressure should be lowered to the safest minimal level to reduce adverse CVD outcomes than the notion that there is a clear rank ordering in the effectiveness of various antihypertensive agents. However, several relatively small trials suggest that ACE inhibitors may be associated with better CVD outcomes than are dihydropyridine calcium channel blockers. Furthermore, many\textsuperscript{46–48} but not all\textsuperscript{49} recent studies with ACE inhibitors and ARBs suggest benefits that cannot be fully attributed to blood pressure lowering in preventing and delaying the progression of advanced diabetic kidney disease.\textsuperscript{46,47} For these reasons, current guidelines\textsuperscript{8} suggest that ACE inhibitors are the drugs of choice in the initial management of hypertension in people with diabetes or kidney disease.

Regardless of the initial therapy, most patients will require multiple-drug therapy for hypertension in the setting of diabetes. Thiazide diuretics, $\beta$-blockers, ACE inhibitors, ARBs, and calcium channel blockers are beneficial in reducing CVD incidence in patients with diabetes. Although ACE inhibitors and ARBs may be the preferred agents for the initial therapy of hypertension in diabetes, a low-dose thiazide diuretic generally should be one of the first 2 drugs used for management of hypertension in these patients. Calcium channel blockers and $\beta$-blockers are effective blood pressure–lowering agents and certainly should be considered as additional therapy in patients treated with ACE inhibitors or ARBs.\textsuperscript{50}

**Recommendations for Blood Pressure Control**

- Blood pressure should be measured at every routine diabetes visit. Patients found to have systolic blood pressure $\geq 130 \text{ mm Hg}$ or diastolic blood pressure $\geq 80 \text{ mm Hg}$ should have blood pressure confirmed on a separate day.
- Patients with diabetes should be treated to a systolic blood pressure $<130 \text{ mm Hg}$ and a diastolic blood pressure $<80 \text{ mm Hg}$.
- Patients with a systolic blood pressure of 130 to 139 mm Hg or a diastolic blood pressure of 80 to 89 mm Hg should initiate lifestyle modification alone (weight control, increased physical activity, alcohol moderation, sodium reduction, and emphasis on increased consumption of fresh fruits, vegetables, and low-fat dairy products) for a maximum of 3 months. If, after these efforts, targets are not achieved, treatment with pharmacological agents should be initiated.
- Patients with hypertension (systolic blood pressure $\geq 140 \text{ mm Hg}$ or diastolic blood pressure $\geq 90 \text{ mm Hg}$) should receive drug therapy in addition to lifestyle and behavioral therapy.
- All patients with diabetes and hypertension should be treated with a regimen that includes either an ACE inhibitor or an ARB. If one class is not tolerated, the other should be substituted. Other drug classes demonstrated to reduce CVD events in patients with diabetes ($\beta$-blockers, thiazide diuretics, and calcium channel blockers) should be added as needed to achieve blood pressure targets.
- If ACE inhibitors, ARBs, or diuretics are used, renal function and serum potassium levels should be monitored within the first 3 months. If levels are stable, follow-up could occur every 6 months thereafter.
- Multiple-drug therapy generally is required to achieve blood pressure targets.
- In elderly hypertensive patients, blood pressure should be lowered gradually to avoid complications.
- Orthostatic measurement of blood pressure should be performed in people with diabetes and hypertension when clinically indicated.
- Patients who do not achieve target blood pressure despite multiple-drug therapy should be referred to a physician specializing in the care of patients with hypertension.

**Lipids**

In patients with type 2 diabetes mellitus, triglycerides are often elevated, HDL-C is generally decreased, and LDL-C may be elevated, borderline, or normal. LDL particles are small and dense, carrying less cholesterol per particle. Thus, the LDL-C concentration may be misleading: There will be more LDL particles for any cholesterol concentration if the LDL particles are small and dense. Additionally, these small, dense LDL particles may be more atherogenic than would be suspected by their concentration alone, because in vitro and cell culture studies suggest they may be more readily oxidized and glycated.\textsuperscript{10,51} Although an elevated LDL-C level generally is not recognized as the major lipid abnormality in patients with type 2 diabetes mellitus, clinical trials amply demonstrate that LDL-C lowering with drugs will reduce risk for major coronary events regardless of diabetes status.\textsuperscript{52}

Elevated LDL-C is identified as the primary target of lipid-lowering therapy by both the ADA and the AHA. The focus on LDL-C is supported by results of controlled clinical trials that have shown that LDL-C lowering with statins will reduce the risk of major CVD events in patients with diabetes. For example, the Heart Protection Study and the Collaborative Atorvastatin Diabetes Study both included large numbers of patients with diabetes who were $>40$ years of age and had
no known vascular disease but had at least 1 major cardiovascular risk factor or evidence of retinopathy or microalbuminuria. Subjects were randomized in a double-masked, placebo-controlled fashion to simvastatin 40 mg/d in the Heart Protection Study and atorvastatin 10 mg/d in the Collaborative Atorvastatin Diabetes Study, which produced, respectively, a 33% and 40% reduction in LDL-C associated with a 31% and 37% reduction in combined cardiovascular end points. Although these trials showed an increased absolute CHD risk associated with higher LDL-C values at baseline, the observed benefits (relative risk reduction) were independent of baseline LDL-C and other lipid values. Indeed, these results supported the epidemiological observations that the relationship between CHD risk and blood LDL-C is approximately linear when CHD is plotted on a logarithmic scale. This explains the uniform relative reduction in CHD risk seen with LDL-C reductions of 30% to 40% over a wide range of LDL-C values.

Triglyceride-rich lipoproteins, especially very-low-density lipoproteins, are often elevated in patients with diabetes, appear to be atherogenic, and represent a secondary target of lipid-lowering therapy (after the goal for LDL-C is attained). The ADA recognizes serum triglycerides as a surrogate for atherogenic triglyceride-rich lipoproteins and suggests a target of <150 mg/dL. The AHA suggests an alternative approach—namely, for patients with diabetes and no clinical CVD whose triglyceride level is >200 mg/dL, the AHA recommends a non-HDL target of <130 mg/dL.

The “fibrate” class of lipid-lowering drugs is useful for lowering elevated triglyceride or non–HDL-C levels; however, clinical trials of these drugs have reported mixed results. In the Helsinki Heart Study, 135 patients with diabetes and no known vascular disease were randomized to gemfibrozil 600 mg twice daily or placebo. In association with a 10% reduction of LDL, a 6% increase in HDL, and a 26% reduction in triglycerides, there was a 68% relative risk reduction in coronary death and nonfatal myocardial infarction; this result did not reach statistical significance, however, because of the small number of patients. The FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) trial randomized 9795 people with type 2 diabetes mellitus with average total cholesterol levels (116 to 251 mg/dL) and an elevated total cholesterol–to–HDL-C ratio (>4) or triglycerides >89 mg/dL to fenofibrate or placebo. In the overall population, fenofibrate treatment did not reduce the primary end point of first myocardial infarction or CHD death. Almost 80% of the FIELD population was free of clinical CVD at the start of the study, and in this prespecified subgroup, there was a 19% reduction in total cardiovascular events (CVD death, nonfatal myocardial infarction, stroke, and coronary revascularization; $P=0.004$) in the fenofibrate-treated group. The effect of fenofibrate on the primary end point in subjects without prior CVD was not provided. A concern in the FIELD trial was a rise in creatinine of ≈15% overall in the group treated with fenofibrate; this was completely reversible at 6 weeks after the end of the study and the cessation of fenofibrate therapy. It is not known whether the temporary rise in creatinine over the course of the study had any adverse consequences. Additionally, when fibrates are used in combination with statins, attention must be paid to the risk for myositis and rhabdomyolysis. The ACCORD study will examine whether a fibrate combined with a statin is safe and whether together they provide CVD benefits beyond those of statin therapy alone.

Although both the ADA and the AHA support efforts to raise HDL-C in high-risk patients when these levels are reduced, there is one difference in the organizations’ recommendations. The ADA specifies therapeutic goals for HDL-C (>40 mg/dL, with consideration of a higher target of >50 mg/dL in women), whereas the AHA advocates efforts to raise HDL-C without specifically designating goals of therapy. The most effective available drug for raising HDL-C levels is nicotinic acid. Clinical trials suggest CVD risk reduction with nicotinic acid, although no trials of this drug that specifically target patients with diabetes have been performed. Furthermore, at higher doses, nicotinic acid can worsen hyperglycemia.

**Recommendations for Lipid Management**

- In adult patients, lipid levels should be measured at least annually and more often if needed to achieve goals. In adults under the age of 40 years with low-risk lipid values (LDL-C <100 mg/dL, HDL-C >50 mg/dL, and triglycerides <150 mg/dL), lipid assessments may be repeated every 2 years.
- Lifestyle modification deserves primary emphasis in all diabetic individuals. Patients should focus on the reduction of saturated fat and cholesterol intake, weight loss (if indicated), and increases in dietary fiber and physical activity. These lifestyle changes have been shown to improve the lipid profile in patients with diabetes.
- In individuals with diabetes who are over the age of 40 years, without overt CVD, but with 1 or more major CVD risk factors, the primary goal is an LDL-C level <100 mg/dL (2.6 mmol/L). If LDL-lowering drugs are used, a reduction of at least 30% to 40% in LDL-C levels should be obtained. If baseline LDL-C is <100 mg/dL, statin therapy should be initiated on the basis of risk factor assessment and clinical judgment. Major risk factors in this category include cigarette smoking, hypertension (blood pressure ≥140/90 mm Hg or use of antihypertensive medication), low HDL-C (<40 mg/dL), and family history of premature CHD (CHD in male first-degree relative ≤55 years of age; CHD in female first-degree relative ≤65 years of age).
- In individuals with diabetes who are under the age of 40 years, without overt CVD, but who are estimated to be at increased risk of CVD either by clinical judgment or by risk calculator, the LDL-C goal is <100 mg/dL, and LDL-lowering drugs should be considered if lifestyle changes do not achieve the goal.
- The ADA and AHA suggest different approaches to the management of HDL-C and triglyceride-associated CVD risk. The AHA suggests that in patients with triglyceride levels of 200 to 499 mg/dL, a non–HDL-C (total cholesterol minus HDL-C) goal of ≤130 mg/dL is a secondary target. If triglycerides are ≥500 mg/dL, therapeutic options include fibrate or niacin before...
LDL-lowering therapy and treatment of LDL-C to goal after triglyceride-lowering therapy. A non–HDLC level ≤130 mg/dL should be achieved if possible. The ADA suggests lowering triglycerides to <150 mg/dL (1.7 mmol/L) and raising HDL-C to >40 mg/dL (1.15 mmol/L); in women, an HDL-C goal 10 mg/dL higher (>50 mg/dL) should be considered.

- Combination therapy of LDL-lowering drugs (eg, statins) with fibrates or niacin may be necessary to achieve lipid targets, but this has not been evaluated in outcomes studies for either CVD event reduction or safety.

Tobacco
Cigarette smoking is a strong and modifiable risk factor for macrovascular disease both in the general population and for patients with diabetes. Recently, a randomized, prospective trial of smoking cessation with long-term follow-up to assess effects on cardiovascular outcomes demonstrated a reduction in mortality rate with a trend toward reduction of CVD deaths. These data have not been reported for individuals with diabetes, nor have rates for nonfatal CVD events been reported.

Smoking history must be ascertained and reviewed regularly. All patients with diabetes should be counseled not to start smoking or to quit if they are smoking. In patients willing to consider stopping smoking, it is appropriate to refer them to a formal smoking cessation program and to consider prescribing nicotine substitutes and/or bupropion hydrochloride.

Recommendations for Tobacco Use Cessation
- All patients with diabetes should be asked about tobacco use status at every visit.
- Every tobacco user should be advised to quit.
- The tobacco user’s willingness to quit should be assessed.
- The patient can be assisted by counseling and by developing a cessation plan.
- Follow-up, referral to special programs, or pharmacotherapy (including nicotine replacement and bupropion) should be incorporated as needed.

Antiplatelet Agents
Aspirin is widely regarded as the most cost-effective intervention to reduce CVD in the general population and in patients with diabetes. The Early Treatment of Diabetic Retinopathy Study is the only large randomized, controlled trial of aspirin in people with diabetes (n=3711), but it included people with and without CVD; for the overall population in this study, the relative risk among aspirin-treated patients was 0.91 for death and 0.83 for fatal and nonfatal myocardial infarction. Numerous epidemiological studies support these findings. It is commonly recognized that aspirin is associated with an increased risk of gastrointestinal bleeding; to minimize the potential that the risk might exceed the benefits, it is generally recommended that aspirin therapy not be used for CVD prevention in populations with annual CVD risks substantially <1% and that aspirin be limited to doses of 75 to 162 mg/d.

Recommendations for Antiplatelet Therapy
- Aspirin therapy (75 to 162 mg/d) should be recommended as a primary prevention strategy in those with diabetes at increased cardiovascular risk, including those who are >40 years of age or who have additional risk factors (family history of CVD, hypertension, smoking, dyslipidemia, or albuminuria).
- People with aspirin allergy, bleeding tendency, existing anticoagulant therapy, recent gastrointestinal bleeding, and clinically active hepatic disease are not candidates for aspirin therapy. Other antiplatelet agents may be a reasonable alternative for patients at high risk.
- Aspirin therapy should not be recommended for patients under the age of 21 years because of the increased risk of Reye’s syndrome associated with aspirin use in this population. People under the age of 30 years have not been studied.

Glucose Management
Glycemic control clearly reduces microvascular complications in patients with diabetes; however, one of the most hotly debated clinical questions in diabetes is whether better glycemic control is associated with a reduction in CVD outcomes and how low we should go in pursuing glycemic targets. The ADA recommends a glycosylated hemoglobin A1c (A1c) target of <7.0% in general but suggests targeting an A1c as close to normal (<6%) as possible without causing significant hypoglycemia in individual patients. Other guidelines are generally consistent with this recommendation, although the specific numbers recommended are different. These recommendations are largely based on epidemiological studies that suggest that each 1% increase in A1c is associated with a 15% and 18% increase in the relative risk of CVD for patients with type 1 and type 2 diabetes mellitus, respectively. In support of these observational studies, both the UKPDS and the Diabetes Control and Complications Trial reported a nonsignificant trend toward a lower risk of CVD with lower A1c levels. A recent long-term follow-up of the Diabetes Control and Complications Trial suggested that 6 years of intensified insulin therapy has long-term CVD benefits. Nevertheless, no clinical trials of a glycemic intervention have provided clear-cut evidence that glucose lowering reduces the risk of CVD. Moreover, as lower targets are achieved, the risk of severe hypoglycemia increases. Thus, there is certainly a floor below which benefits will be counterbalanced by risk. In the ACCORD trial, 10 000 subjects with type 2 diabetes mellitus have been randomized to either a standard treatment group, with an A1c goal of ≤7.5%, or an intensive treatment group, with an A1c goal of <6.0%. There are also 2 other ongoing clinical trials that directly test the hypothesis that more intensive glucose lowering in the setting of type 2 diabetes mellitus will be associated with a reduction in CVD events. Among patients with diabetes, glycemic control to reduce microvascular complications is clearly of benefit.
Recommendations for Glycemic Control

- The A1c goal for patients in general is <7%.
- The A1c goal for the individual patient is as close to normal (<6%) as possible without causing significant hypoglycemia.

Type 1 Diabetes Mellitus

The absolute CVD risk in patients with type 1 diabetes mellitus is lower than in patients with type 2 diabetes mellitus, in part because of their younger age and the lower prevalence of CVD risk factors. However, the relative risk of CVD in people with type 1 diabetes mellitus compared with that of nondiabetics of similar age is dramatically increased in men and women and is associated with classic cardiovascular risk factors and nephropathy but not glycermic control. 77–80 No data suggest that the interventions documented to be of benefit in reducing CVD are less effective in patients with type 1 diabetes mellitus than in type 2 diabetes mellitus. This is particularly true of lipid lowering with a statin, 53 aspirin therapy, 64 and glucose management. 72

Recommendations for Patients With Type 1 Diabetes Mellitus

At the present time, all of the recommendations listed above for patients with type 2 diabetes mellitus appear appropriate for those with type 1 diabetes mellitus as well.

Summary

People with either type 1 or type 2 diabetes mellitus are at increased risk for CVD and have worse outcomes after surviving a CVD event. In this joint statement, we have attempted to summarize the evidence supporting lifestyle and medical interventions that will prevent the development of CVD in people with diabetes. The aggressive use of lifestyle modifications can reduce or delay the need for medical intervention. Appropriate lifestyle and medical interventions will reduce the occurrence of CVD and allow people with diabetes to live healthier and longer lives.
## Writing Group Disclosures

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<th>Consultant/Advisory Board</th>
<th>Other</th>
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<td>University of North Carolina</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
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<tr>
<td>Henry N. Ginsberg, MD, FAHA</td>
<td>Columbia University</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
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<tr>
<td>George L. Bakris, MD, FAHA</td>
<td>St. Luke’s Medical Center</td>
<td>AstraZeneca, Abbott (modest)</td>
<td>None</td>
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<td>Novartis, Merck, Abbott, Biovail, AstraZeneca</td>
<td>None</td>
</tr>
<tr>
<td>Nathaniel G. Clark, MD, MS, RD</td>
<td>American Diabetes Association</td>
<td>Pending</td>
<td>Pending</td>
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<td>Pending</td>
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<tr>
<td>Fernando Costa, MD, FAHA</td>
<td>American Heart Association</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<td>Robert Eckel, MD, FAHA</td>
<td>University of Colorado</td>
<td>Merck (significant)</td>
<td>None</td>
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<td>None</td>
<td>FDA (significant), Schering, Dowden Health Media, Medical Decision Point (modest)</td>
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<tr>
<td>Vivian Fonseca, MD</td>
<td>Tulane University Medical Center</td>
<td>Pfizer, GSK, Takeda, Aventis, Novartis (significant), AstraZeneca (modest)</td>
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<tr>
<td>Hertzel C. Gerstein, MD, MSc, FRCPC</td>
<td>McMaster University Medical Center</td>
<td>Sanofi-Aventis, GSK, King, Wyeth-Ayerst (significant)</td>
<td>Aventis (significant)</td>
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<tr>
<td>Richard W. Nesto, MD, FAHA</td>
<td>Lahey Clinic</td>
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<td>Michael P. Pignone, MD, MPH</td>
<td>University of North Carolina</td>
<td>Bayer, Inc, Pfizer (modest)</td>
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<tr>
<td>Jorge Plutzky, MD</td>
<td>Brigham &amp; Women’s Hospital</td>
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<td>AstraZeneca (modest)</td>
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<tr>
<td>Daniel Porte, MD</td>
<td>VA San Diego Health Care Systems</td>
<td>None</td>
<td>None</td>
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<td>None</td>
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<tr>
<td>Rita Redberg, MD, FAHA</td>
<td>University of California, San Francisco</td>
<td>None</td>
<td>None</td>
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<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Kimberly F. Stitzel, MS, RD</td>
<td>American Heart Association</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Neil J. Stone, MD, FAHA</td>
<td>Northwestern Feinberg School of Medicine</td>
<td>None</td>
<td>None</td>
<td>AstraZeneca, Merck, Pfizer, Reliant, Sanofi, SonoSite (modest)</td>
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FDA indicates US Food and Drug Administration; GSK, GlaxoSmithKline; and VA, Veterans Affairs.

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<tr>
<td>Alice H. Lichtenstein</td>
<td>Tufts University</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Joyce Green Pastors</td>
<td>University of Virginia</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>James R. Sowers</td>
<td>University of Missouri-Columbia</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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References


AHA Scientific Statement

Essential Features of a Surveillance System to Support the Prevention and Management of Heart Disease and Stroke

A Scientific Statement From the American Heart Association Councils on Epidemiology and Prevention, Stroke, and Cardiovascular Nursing and the Interdisciplinary Working Groups on Quality of Care and Outcomes Research and Atherosclerotic Peripheral Vascular Disease

David C. Goff, Jr, MD, PhD; Lawrence Brass, MD†; Lynne T. Braun, PhD, RN, CNP; Janet B. Croft, PhD; Judd D. Flesch; Francis G.R. Fowkes, MD, PhD; Yuling Hong, MD, PhD; Virginia Howard, MSPH; Sara Huston, PhD; Stephen F. Jencks, MD, MPH; Russell Luepker, MD, MS; Teri Manolio, MD, PhD; Christopher O’Donnell, MD, MPH; Rose Marie Robertson, MD; Wayne Rosamond, PhD; John Rumsfeld, MD, PhD; Stephen Sidney, MD, MPH; Zhi Jie Zheng, MD, PhD

Executive Summary

A strategic goal of the American Heart Association (AHA) is to reduce heart disease, stroke, and risk for both by 25%, and Healthy People 2010 (HP2010) established 4 national goals for heart disease and stroke prevention and management. However, the current health tracking systems (surveillance) in the United States cannot track progress toward these goals in a comprehensive and systematic manner. This article provides a brief overview of these goals, prevention and management strategies, and the role of surveillance in monitoring the impact of prevention and treatment efforts. It also provides a review of the existing surveillance system for monitoring progress toward preventing heart disease and stroke in the United States and recommendations for filling important gaps in that system. This information will serve as an important basis for advocacy to guide the development of a comprehensive surveillance system to support the current HP2010 and AHA goals and the likely future goal of eliminating the epidemic burden of heart disease and stroke. Recommendations are categorized as overarching (fundamental recommendations that cut across goal areas) or as goal-specific. They are further classified according to priority (P) (I for high priority and II for intermediate priority. No low-priority recommendations were made), staging (S) (I for early staging [1–2 years], II for intermediate staging [2–4 years], and III for later staging), and cost (C) ($ for items estimated to cost less than $10 million per year, $$ for estimates of $10 to $100 million, and $$$ for estimates exceeding $100 million). In addition, potential barriers to action are addressed.

Overarching Recommendations

1. A National Heart Disease and Stroke Surveillance unit should be established to produce annual reports on key indicators of progress in the prevention and management of heart disease and stroke. P I, S I, C $.
2. Cardiovascular disease (CVD), including cardiac arrests, acute coronary syndromes (heart attack and unstable angina), stroke, chronic heart failure (CHF), and related interventional procedures, should be classified as reportable conditions. P I, S III (although developmental work should begin earlier), C $$$.
3. Data collection about patients’ encounters with the healthcare system should be revised to include collection of data on lipoprotein cholesterol concentrations, blood sugar, and glycohemoglobin values. P I, S I, C $.
4. Data elements should be standardized across surveys, and unnecessary duplication in data sources should be avoided. P I, S I, C $ (potentially cost saving).

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

†Deceased.

This statement was approved by the American Heart Association Science Advisory and Coordinating Committee on September 22, 2006. A single reprint is available by calling 800-242-8721 (US only) or writing the American Heart Association, Public Information, 7272 Greenville Ave, Dallas, TX 75231-4596. Ask for reprint No. 71-0386. To purchase additional reprints: up to 999 copies, call 800-611-6883 (US only) or fax 413-665-2671; 1000 or more copies, call 410-528-4121, fax 410-528-4264, or e-mail kelle.ramsay@wolterskluwer.com.

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5. The design and conduct of nationally representative surveillance programs should be revised to facilitate oversampling by states, territories, and tribal organizations and to provide meaningful estimates on ethnic subgroups in the populations. Sampling within states, territories, and tribal organizations should be designed to facilitate oversampling by counties. P I, S II, C $$. to $$$ (depending on extent of oversampling achieved).

6. Mechanisms should be developed to enable linkage between healthcare data systems, including the national surveillance programs (eg, National Ambulatory Medical Care Survey [NAMCS], National Hospital Discharge Survey [NHDS], and National Death Index), and electronic health records. P I, S II, C $$$. (startup) and $$ (maintenance).


**Recommendations for HP2010 Goals 1 (Risk Factor Prevention) and 2 (Risk Factor Detection, Treatment, and Control)**

Data collection in national surveys should be expanded to include important measures that are currently missing from the data collection process, such as information on awareness, detection, treatment, and control of physical inactivity, unhealthy diet, cigarette smoking, and obesity. P I, S I, C $$. 

1. The states, territories, and tribal organizations should develop surveillance capacity to support program planning, implementation, and evaluation. Such capacity should include the ability to conduct standardized surveys that would include direct assessments of residents to enable collection of information about prevention, awareness, detection, treatment, and control of obesity, hypertension, dyslipidemia, and diabetes. P I, S I, C $$$. 

2. Indicators and systems for surveillance of policies and environmental conditions related to physical inactivity and unhealthy diet should be developed, tested, and implemented at the national, state, and local levels. P I, S II, C $$. 

**Recommendations for HP2010 Goals 3 (Early Identification and Treatment of Acute Events) and 4 (Prevention of Recurrent Events)**

1. Indicators and systems for surveillance of policies and environmental conditions (eg, proportion of the population covered by enhanced 9-1-1) related to symptom knowledge and recognition, acute healthcare-seeking behavior, availability of automated external defibrillators, and capabilities of the prehospital care system (including first responders and emergency medical services) should be developed, tested, and implemented at the national, state, and local levels. P I, S II, C $$. 

2. Effective surveillance methods should be developed, tested, and implemented to support the collection of data on patients with newly diagnosed heart disease, stroke, CHF, and peripheral arterial disease (PAD) in the outpatient setting, including data on treatment and outcomes. P II, S III, C $$$. 

We have identified specific barriers to obtaining the new data elements that would be required to support the development of a comprehensive surveillance system. These include various methodological challenges, privacy concerns, and the costs associated with supporting new data systems and a comprehensive surveillance system.

The success of efforts to prevent and manage heart disease and stroke is dependent on the availability of surveillance data at the national, state, and local levels to assist federal agencies, state and local health departments, and their partners in assessing prevention and treatment priorities and guiding program planning, implementation, and evaluation. This statement summarizes the information that is needed at the national, state, and local levels to address the HP2010 and AHA goals for 2010; furthermore, this document was designed with a longer-term perspective in mind. When possible, existing data collection efforts have been identified for the addition of new items. Significant gaps (eg, the complete lack of a data source for incidence and recurrence of heart attacks and strokes) and other deficiencies have been identified, and recommendations have been made for enhancement of the surveillance system in the United States. The most far-reaching recommendation may be the proposed designation of heart disease and stroke as reportable conditions across the continuum of care. This approach served to help focus attention on infectious diseases when infection control was the major public health imperative. A similar approach to heart disease and stroke is needed urgently. The other recommendations, although more narrowly focused in many instances, should result in the availability of better information for enhancing heart disease and stroke prevention and management programs. Implementation of all of the recommendations contained in this report would require commitment of substantial additional resources in addition to those already devoted to surveillance; however, some opportunities for greater efficiency were identified that could lead to cost savings, and a staged rollout of these recommendations could mitigate the financial impact. Finally, the return on investment could be substantial in terms of better population health and fewer acute episodes of heart disease and stroke, resulting in fewer inflation-adjusted healthcare dollars being devoted to acute care. Consequently, this statement should serve as a guide to policy makers as they work with public health agencies to develop and implement a surveillance system that can contribute importantly to efforts to prevent heart disease and stroke.

**Introduction**

A strategic goal of the AHA is to reduce heart disease, stroke, and risk for both by 25% and HP2010 established 4 national goals for heart disease and stroke prevention and management. However, the current health tracking systems (surveillance) in the United States cannot track progress toward these goals in a systematic manner. This report provides a brief overview of these goals, prevention and management strategies, and the role of surveillance in monitoring the impact of
prevention and treatment efforts. It also provides a review of the existing surveillance system for monitoring progress toward preventing heart disease and stroke in the United States and recommendations for filling important gaps in that system. This information will serve as an important basis for advocacy to guide the development of a comprehensive surveillance system to support the current HP2010 and AHA goals and the likely future goal of eliminating the epidemic burden of heart disease and stroke.

The primary objectives of this report are to (1) define the key data needed to track progress toward the prevention and optimal management of heart disease and stroke; (2) identify existing data sources and gaps relevant to these data needs; and (3) recommend and prioritize data needs. This effort was motivated by the belief that improvements in knowledge can lead to more effective action. Hence, the goal is to document the potential benefits of having more timely access to important data about heart disease and stroke in the United States by addressing 2 questions: What data are available? What additional data do we need to make better policy and programmatic decisions?

**Public Health Burden of Heart Disease and Stroke**

The major sources of readily available published statistics on heart disease and stroke in the United States include the annual report from the AHA, *Heart Disease and Stroke Statistics,* and the biennial report from the National Heart, Lung, and Blood Institute, *Chart Book on Cardiovascular, Lung, and Blood Diseases.* It is estimated that 71 300 000 Americans have CVD, although many have high blood pressure as their only manifestation of CVD. Heart disease and stroke are the most common of the major forms of CVD, affecting women and men of all racial/ethnic groups and ages. In 2003, 13.2 million Americans had prevalent coronary heart disease, 5.5 million had prevalent stroke, and 5 million had heart failure. With the aging population, the prevalence of heart failure is expected to reach 10 million cases by 2007. More than 8 million adults in the United States are affected by PAD, a condition that increases in prevalence with age and is more prevalent in blacks. Although no surveillance system exists to monitor incidence of heart disease and stroke, estimates have been computed and published by the AHA and the National Heart, Lung, and Blood Institute. The estimated annual incidence of acute myocardial infarction is 565 000, and another 300 000 recurrent attacks occur annually. The estimated incidence of stroke is 500 000 per year, and another 200 000 recurrent strokes occur annually. Among stroke survivors, 15% to 30% are permanently disabled.

Heart disease and stroke have been first and third, respectively, among the causes of death in the United States for several decades. In 2002, CVD accounted for ≈37% of all deaths among US residents and was listed as a primary or contributing cause of death on approximately 1 400 000 death certificates. Although age-adjusted CVD death rates declined considerably from 1979 to 2002, there was only a slight decline in the absolute number of CVD deaths. Additionally, the decline in mortality from heart disease and stroke has not been equal across all racial/ethnic groups; non-Hispanic whites have experienced the greatest declines.

Heart disease and stroke share many of the same modifiable risk factors, such as hypertension, cigarette smoking, diabetes mellitus, obesity, physical inactivity, and, at least for ischemic stroke, dyslipidemia. The percentage of US adults free of these major risk factors decreased from 42% in 1991 to 36% in 2001 based on self-reported data from the Behavioral Risk Factor Surveillance System (BRFSS). It is likely that these data overestimate the proportion of the population free of these major risk factors for CVD, because these data are based on self-report and, in some instances, on access to health care for diagnosis. This trend is yet another indication of the substantial public health burden of heart disease and stroke and the need to implement a coordinated and comprehensive national effort to prevent heart disease and stroke.

Efforts to reduce the burden of heart disease and stroke have been hampered by a lack of knowledge in key areas. Although the emerging obesity epidemic has been developing for several decades, widely spaced episodic surveillance programs contributed to the delay in identification and response. Similar challenges contributed to a delay in recognizing a reversal in the downward trend in prevalence of high blood pressure and an inability to monitor hypertension control. The lack of data on prehospital delay times in patients with symptoms of acute coronary syndromes has hindered evaluation of progress toward the “60 minutes to treatment” goal of the National Heart Attack Alert Program. Current efforts to redesign systems of care for patients with ST-segment elevation myocardial infarction are constrained by a lack of knowledge of the processes of care delivery in various systems. The lack of data has hindered efforts to increase the use of evidence-based therapies (eg, aspirin, β-blockers, and thrombolysis) for patients with myocardial infarction and stroke despite major efforts to disseminate knowledge of the effectiveness of these therapies.

The annual cost associated with CVD in the United States was estimated to be $403.1 billion for 2006. This figure includes health expenditures such as costs of physician, hospital, and nursing home services, as well as lost productivity, but it is likely to be an underestimate because, especially for stroke, the informal care costs and costs of comorbidities may not be included. The cost of CVD is likely to increase dramatically over the next several decades as the “baby boom” population enters the peak heart disease years, putting additional strain on the public health and healthcare delivery systems. It will be increasingly important to conduct surveillance of healthcare costs in addition to outcomes to inform policy makers about the most rapid increases in expenditure lines, whether the return on investment is justifiable, and whether current or new policies are likely to bankrupt the system while trying to help people live longer, healthier lives.

**HP2010 and AHA Goals for Prevention and Management of Heart Disease and Stroke**

HP2010 is a comprehensive set of disease prevention and health promotion objectives for the United States to achieve during the first decade of the 21st century. The overall goals
of HP2010 are to increase the quality and years of a healthy life and to eliminate health disparities. The leading health indicators identified in HP2010 include physical activity, overweight and obesity, and tobacco use. The relevant national health objectives are to increase physical activity, reduce overweight and obesity, and decrease cigarette smoking among adolescents and adults. The 4 goals of HP2010 specific to CVD are shown in Table 1. The 4 goals of HP2010 specific to CVD are shown in Table 1. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health have been charged with leadership responsibility for achieving these goals. The HP2010 Partnership, in which the AHA is a partner, has been established to stimulate progress toward achieving these and other HP2010 goals. The 10-year impact goal of the AHA, to reduce coronary heart disease, stroke, and risk for both by 25% by the year 2010, is aligned with these national health objectives. Specific indicators established by the AHA are shown in Table 2. Efforts are ongoing to develop goals for 2020 and beyond; hence, the recommendations provided in this document are intended to be flexible.

### Opportunities and Approaches to Prevent and Manage Heart Disease and Stroke

Meeting the HP2010 and AHA goals for preventing and managing heart disease and stroke is a challenging but achievable task. Heart disease and stroke are disorders with complex origins and multiple risk factors, so a multifaceted approach to their prevention is crucial to success. With this perspective in mind, the CDC and its key partners and stakeholders, including the AHA, the Association of State and Territorial Health Officials, and the National Institutes of Health (specifically, the National Heart, Lung, and Blood

### Table 1. HP2010 Goals Specific to Heart Disease and Stroke

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<td>Early identification and treatment of heart attacks and strokes</td>
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<tr>
<td>Prevention of recurrent cardiovascular events</td>
</tr>
</tbody>
</table>

### Table 2. Specific Indicators Established by AHA to Track Progress Toward Heart Disease and Stroke Prevention by 2010

- Reduction in death rate due to coronary heart disease and stroke by 25%
- Reduction in prevalence of smoking, high blood cholesterol, and physical inactivity by 25%
- Reduction in rate of uncontrolled high blood pressure by 25%
- Elimination of the growth of obesity and diabetes mellitus
Institute and the National Institute of Neurological Disorders and Stroke) developed A Public Health Action Plan to Prevent Heart Disease and Stroke.22 This plan directly addresses the 4 national goals for heart disease and stroke prevention described in HP2010. The action plan includes an “action framework” (Figure) that serves as both a guide for heart disease and stroke prevention efforts and a useful framework for designing a surveillance system.22

Framework development first required an understanding of the present CVD environment, in which unfavorable social and environmental conditions give rise to the adoption of adverse behavioral patterns that may lead to the development of the major risk factors for heart disease and stroke. Next, first events, many of which are fatal, occur in the population. Survivors are at risk for recurrent events, disability, decompensation, and death. In theory, prevention of heart disease and stroke could be advanced by intervening at any point in this process; however, meeting the 4 goals of HP2010 will require efforts across the full spectrum of cardiovascular health promotion and disease prevention. Prevention of the major risk factors for heart disease and stroke (goal 1) can be achieved only by addressing social and environmental conditions and behaviors. Detection and treatment of the risk factors (goal 2) can be achieved only through efforts that focus on these specific activities, although efforts to prevent the risk factors could, if successful, reduce the magnitude of the challenge inherent in this task. Early identification and treatment of acute events (goal 3) requires population-wide knowledge of symptoms and appropriate (timely) healthcare-seeking behavior, as well as uniform access to high-quality emergency care and acute case management. Prevention of recurrent events (goal 4) requires uniform access to high-quality health care, including rehabilitation services, for all survivors of an acute event. Understanding the strategies required to prevent heart disease and stroke also provides an important basis for considering the requisite scope of a comprehensive surveillance system designed to track progress toward the attainment of the prevention goals set by HP2010.

Role of Surveillance in Efforts to Prevent and Manage Heart Disease and Stroke
Public health surveillance is defined as “the ongoing, systematic collection, analysis, interpretation, and dissemination of data regarding health-related events for use in public health action to reduce morbidity and mortality and to improve health.”23 Comprehensive and accurate disease surveillance systems are critical to the success of efforts to reduce the burden of CVD and stroke. Such systems are particularly important in identifying emerging trends, such as the rise in prevalence of obesity, diabetes mellitus, and chronic (congestive) heart failure3,14; the plateauing of the decline in stroke mortality25; regional and subgroup differences in the decline in myocardial infarction incidence26; or the rise in hospitalizations for atrial fibrillation.27 Comparison of trends across subgroups also helps to identify groups at particularly increased risk28 or that fail to benefit from overall improvements in prevention and treatment.29,30 Reliable surveillance data are essential for identifying public health priorities, tracking the progress of preventive efforts, and intensifying efforts in areas of special need. Guidelines published by the CDC have suggested several criteria for evaluating public health surveillance systems, including simplicity, data quality, acceptability, sensitivity, positive predictive value, representativeness, timeliness, stability, usefulness, flexibility, and cost.23 Although a full review of this information is beyond the scope of this statement, key characteristics of reliable surveillance systems can be grouped into 3 areas: the validity of the data produced, the utility of the resulting information, and the feasibility of implementing the system itself.23,31 It is particularly important for the surveillance system to have sufficient flexibility and nimbleness to enable the incorporation of important new measures in a timely manner.

Surveillance needs at various levels may serve different purposes, but all components of the system should be designed to best inform the strategies for preventing heart disease and stroke that are best implemented at that level. On the national level, surveillance systems should inform policies likely to be set nationwide, such as agricultural subsidies, federal tobacco taxes and other tobacco control policies, Medicare reimbursement for screening and treatment, practice guidelines promulgated by scientific and governmental organizations, and drug or device safety issues addressed by federal agencies. Nationally available data should also permit comparisons between countries, particularly because such comparisons can inform national health policy.

Surveillance data are also critically important at both the state and local level. State and local public health agencies require relevant surveillance data, specific to their state or local area, to use in developing and seeking funding for targeted intervention programs, informing policy makers and guiding policy decisions, and planning and evaluating programs. For example, data are needed to inform state and local decision makers about the impact of current and future policies pertaining to school nutrition and physical education programs, tobacco taxes and other control policies, and Medicaid coverage policies, as well as other prevention programs. Because funding for heart disease and stroke prevention programs is low relative to the public health burden of these diseases in most states and local areas, public health agencies must carefully prioritize their preventive efforts and continually evaluate ongoing programs to assess and improve their impact. State or local populations at particularly high risk for CVD can be identified and targeted for intensive interventions that may not be feasible or efficient on a broader scale.28 State and local public health agencies cannot design, implement, and evaluate such programs without relevant, reliable, accurate, and timely surveillance data.

Several trends are occurring that will influence surveillance capacity in the years to come. Modifications to the surveillance system should be designed to benefit from, or at least accommodate, the likely effects of these influences. The development of geographic information systems technology (eg, geocoding) has enhanced the utility of surveillance data for research, program planning, and evaluation purposes. The development of health information technology, especially the electronic health record, might contribute importantly to the
development of improved insight into the processes and outcomes of healthcare delivery; however, standardization, interoperability, confidentiality safeguards, and the lack of mechanisms to link across data repositories are but a few of the barriers that must be overcome. The implementation of pay-for-performance healthcare reimbursement policies may also influence the availability of data on key performance measures that could be used to track progress toward the prevention and management of CVD. Finally, it is important to recognize that although the present report focuses on heart disease and stroke, enhanced surveillance of other chronic diseases could contribute further to our ability to make better decisions on resource allocation, thereby leading to improvements in the health of our population.

**HP2010 Goals 1 and 2: Risk Factor Prevention, Detection, Treatment, and Control**

Risk factor prevention, detection, and control are addressed in the first and second of the 4 HP2010 goals relevant to heart disease and stroke. Because many components of a surveillance system to monitor risk factor prevention could also provide useful information about risk factor detection and control, surveillance efforts needed to monitor progress toward achieving these goals will be discussed together. Physical inactivity, unhealthy diet, tobacco use, obesity, hypertension, dyslipidemia, and diabetes mellitus are well known as the major modifiable risk factors for heart disease and stroke, and atrial fibrillation is a major risk factor for stroke. Programs that seek to reduce heart disease and stroke incidence, prevalence, and mortality through risk factor prevention, detection, and control must address some or all of these risk factors. Although success in changing current trends in heart disease and stroke incidence, prevalence, or mortality may not be seen for several years after efforts to prevent or control risk factors, program impact on the risk factors themselves may be seen in a shorter time frame, provided that good measures of program progress exist. For these reasons, surveillance systems to track the prevalence, treatment, and control of risk factors over time are needed at the national and state level, and ideally at the local level, as well. In addition, state-based heart disease and stroke prevention programs are working to create policy and environmental changes that will support behavior change and risk factor prevention and control. Therefore, surveillance systems are needed to monitor changes in relevant policies and environmental factors over time. The following sections address the availability of surveillance data relevant to policies and environmental conditions, the major lifestyle risk factors, and the major biological risk factors.

**Environmental and Policy Factors**

To support behavior change, risk factor control, and uniform access to high-quality health care, heart disease and stroke prevention programs must address policy, environmental, and systems-level changes in multiple settings (eg, communities, schools, work sites, and healthcare settings). This approach is illustrated by the Figure, which shows policy and environmental change at the far left of the model, with the recognition that policy change is both a way to promote improvements in the built environment that will encourage greater physical activity and a way to improve other behaviors. Although infectious disease interventions have historically focused on policy and environmental changes as effective methods of disease prevention and control, chronic disease prevention and control programs have adopted this approach much more recently. Tobacco prevention and control programs have successfully used policy and environmental strategies to reduce smoking rates (eg, laws and enforcement that limit youth access to tobacco products, cigarette taxes, and insurance coverage of evidence-based nicotine dependency treatment). Unfortunately, aside from policies that address tobacco use and tobacco smoke exposure, there are few surveillance programs pertinent to policy and environmental factors. HP2010 includes several environmental and policy change objectives related to heart disease, stroke, and their risk factors, although many of these objectives were labeled as developmental because surveillance systems were not available to monitor progress. To assist state heart disease and stroke prevention programs in tracking progress, the CDC drafted a list of 31 policy and environmental indicators related to physical activity, nutrition, and tobacco use (Tables 3 through 6).

**Available National Data**

The School Health Policy and Programs Study is conducted at the state, district, school, and classroom level nationwide and includes data on physical activity, nutrition, and tobacco-related policies and environmental factors in schools. The School Health Policy and Programs Study was conducted in 1994 and 2000 and will be conducted again in 2006. Measures include the proportion of schools that require daily physical education for all students, provide access to their physical activity spaces and facilities for all persons outside of normal school hours, provide tobacco-free environments, and make healthy, as opposed to “junk,” foods available. The School Health Policy and Programs Study also provides data on state-level policies related to schools. The National Worksite Health Promotion Survey, listed as the data source for several HP2010 objectives, includes the proportion of worksites that offer nutrition or weight-management classes or counseling, offer employer-sponsored physical activity and fitness programs, have formal smoking policies prohibiting smoking or limiting it to separately ventilated areas, and provide blood pressure screening. This survey was last conducted in 1999. A national survey of airport smoking policies was conducted in 2002, but no ongoing surveillance system of such policies exists.

**Available State Data**

Data from all states on smoking-related policy and environmental factors at the state level are provided by the CDC Office on Smoking and Health’s State Tobacco Activities Tracking and Evaluation System. This system provides current and historical information on indicators such as laws about clean indoor air, preemption laws, and cigarette excise
The Behavioral Risk Factor Surveillance System provides information on smoking policies at respondents’ work sites and homes. The School Health Profiles surveys provide state-level data on school policies and environment related to physical activity, unhealthy diet, and tobacco use. The School Health Profiles are designed and coordinated by the CDC and implemented biennially by some states, territories, and cities (43 states, 1 territory, and 13 cities in 2002). Profiles data come from 2 surveys, a school principal survey and a survey of the lead health education teacher, which are both conducted in each sampled school. At least 1 state (North Carolina) has supplemented the Profiles surveys with state-added questions, including questions related to automated external defibrillator presence and policies. Many state heart disease and stroke prevention programs have also implemented state-level surveys, and even surveillance systems, to measure relevant policies and environmental factors. For example, South Carolina and Georgia have implemented work site surveys. Georgia has conducted a survey of managed care organization policies and practices. North Carolina recently conducted a survey of healthcare practices in the state, examining use of evidence-based guidelines and protocols for treating patients with or at risk for heart disease and stroke. North Carolina also conducted the Stroke Prevention and Treatment Facilities Survey, which measured hospital policies and environments related to treatment and prevention of stroke in 1998 and 2003. This survey is currently being conducted in South Carolina and Georgia, as well. The state-level efforts described here have not developed into true surveillance systems, but the potential exists to develop coordinated state-level surveys that could provide comparable data across states.

**National Gaps**

Although the links between tobacco-related policies and environmental factors and tobacco use and its impact on health have been relatively well established, the research into the influence of policy and environmental factors on a population’s physical activity level and diet is less well developed. In some cases, it is not yet clear which policies or environmental factors most strongly influence physical activity or diet and which show the most promise for public health intervention. These unanswered questions have made it difficult to design surveillance systems at the national and state level. National surveillance systems for school and work settings have been developed; however, the National Worksite Health Promotion Survey is not scheduled to be conducted again. No national surveillance efforts have been developed to monitor risk factor prevention policies and environmental conditions in healthcare or community settings.

**State Gaps**

Few coordinated state-level surveillance systems exist for monitoring policies and environments related to risk factors. The School Health Profiles surveys provide state-level data similar to the national School Health Policy and Programs Study, but some inconsistencies exist between the questionnaires. Although many individual states have conducted surveys in various settings (school, work site, healthcare site, and community), most are not surveillance systems. Coordination of these efforts across states would be required to develop comparable state-
level surveillance systems. Such an effort might also result in the development of better instruments and methods.

**Lifestyle Risk Factors (Physical Inactivity, Unhealthy Diet, and Tobacco Use)**

**Available National Data**

The National Health and Nutrition Examination Survey (NHANES) provides self-reported data on physical activity, such as the frequency and duration of moderate-intensity activity, vigorous activity, and walking or biking to work or school, as well as objective measures of fitness from a submaximal treadmill exercise test.\(^{54,55}\) The NHANES provides data on current physical activity behavior (within the past 30 days) and changes in activity compared with the previous 12 months and a time point 10 years prior. No data source found. Questions from School Health Index could be useful for surveillance if survey mechanism is developed.

Proportion of schools that have adopted tobacco-free school policies that meet CDC recommendations.\(^ {†}\)

Proportion of schools that provide health education instruction that includes the physical education, nutrition, and tobacco use prevention topics listed in School Health Index.\(^ {37}\)

Since 2002, national dietary information has been collected through the “What We Eat in America” survey. This survey integrated 2 earlier nationwide dietary surveys, the Continuing Survey of Food Intakes by Individuals, which was conducted by the US Department of Agriculture, and the dietary survey component of NHANES, conducted by the US Department of Health and Human Services.\(^ {59,60}\) Before this integration, the US Department of Health and Human Services had collected dietary information through NHANES I, II, III, and the yearly NHANES beginning in 1999.\(^ {55}\) The US Department of Agriculture had conducted other surveys of dietary patterns among Americans, such as the Continuing Survey of Food Intakes by Individuals, the Diet and Health Knowledge Survey, and the Nationwide Food Consumption Surveys, but these are no longer conducted.\(^ {61}\)

**TABLE 4. Pilot Indicators and Data Sources for Heart Disease and Stroke Prevention, School Setting, South Carolina and Alabama, 2001**

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Data Sources and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>State policies that require daily physical education or its equivalent in minutes per week, for all students in K–12, with no substitution of other courses or activities for physical education.(^ a)</td>
<td>SHPPS (<a href="http://www.cdc.gov/nccdphp/dash/shpps)%C2%A7">www.cdc.gov/nccdphp/dash/shpps)§</a></td>
</tr>
<tr>
<td>State policies that require schools to assess students on the knowledge and skills specified by the state’s physical education standards, frameworks, or guidelines.(^ b)</td>
<td>SHPPS‡</td>
</tr>
<tr>
<td>State policies requiring that the foods and beverages available at schools outside of school meal programs reinforce the principles of the Dietary Guidelines for Americans.(^ c)</td>
<td>SHPPS‡</td>
</tr>
<tr>
<td>State policies that require newly hired school food service managers to have a nutrition-related baccalaureate or graduate degree and certification/credentialing in food service from either the state or the American School Food Service Association.(^ d)</td>
<td>SHPPS‡</td>
</tr>
<tr>
<td>State policies that require all newly hired staff who teach physical education to be certified, licensed, or endorsed by the state to teach physical education.(^ e)</td>
<td>SHPPS‡</td>
</tr>
<tr>
<td>State policies that require all newly hired staff who teach health education to be certified, licensed, or endorsed by the state to teach health education.(^ f)</td>
<td>SHPPS‡</td>
</tr>
<tr>
<td>State policies that require schools to assess students on the knowledge and skills specified by the state’s health education standards, frameworks, or guidelines.(^ g)</td>
<td>SHPPS‡</td>
</tr>
<tr>
<td>Percent of schools that provide health education instruction that includes the physical education, nutrition, and tobacco use prevention topics listed in School Health Index.(^ {37})</td>
<td>No data source found. Questions from School Health Index could be useful for surveillance if survey mechanism is developed.</td>
</tr>
<tr>
<td>Proportion of schools with School Health Councils.(^ †)</td>
<td>SHEP (<a href="http://www.cdc.gov/nccdphp/dash/profiles">http://www.cdc.gov/nccdphp/dash/profiles</a>). SHEP is completed by a sample of principals and lead health educators in schools having at least 1 of the grades 6–12. No data source available for elementary schools.§</td>
</tr>
<tr>
<td>Proportion of schools that have adopted tobacco-free school policies that meet CDC recommendations.(^ †)</td>
<td>SHEP. See above. SHEP does not include questions to thoroughly assess whether tobacco policies meet recommendations.§</td>
</tr>
</tbody>
</table>

K–12 indicates kindergarten through 12th grade of school; SHPPS, School Health Policy and Programs Study; and SHEP, School Health Education Profile.

\(^ a\)Seven indicators (70%) lack sensitivity (unable to measure incremental change, measured at inappropriate level).

\(^ b\)Two indicators (20%) lack specificity (ambiguous, lac tors (20%) lack precision).

\(^ c\)Seven indicators (70%) have adequate data sources.

\(^ d\)Two indicators (20%) have a data source that could partially measure the indicator.

\(^ e\)Proportion of students that provide health education instruction that includes the physical education, nutrition, and tobacco use prevention topics listed in School Health Index.

\(^ f\)Proportion of schools with School Health Councils.

\(^ g\)Proportion of schools that have adopted tobacco-free school policies that meet CDC recommendations.

\(^ h\)Proportion of schools with School Health Councils.

\(^ i\)Proportion of schools that have adopted tobacco-free school policies that meet CDC recommendations.
The US Department of Agriculture and the Department of Health and Human Services now collaboratively conduct the “What We Eat in America” survey on a continuous yearly basis as part of NHANES. Two 24-hour dietary recalls, for nonconsecutive days, are collected for all respondents with the US Department of Agriculture’s automated multiple-pass method. This survey provides comprehensive data on all foods eaten both at home and away from home and on dietary supplement use. It provides data for estimating energy intake and intake of 60 nutrients and food components, including fat, cholesterol, fiber, carbohydrate, alcohol, and sodium. The “What We Eat in America” survey collects information about current dietary behavior but not about whether respondents are aware of the health effects of their dietary choices.

### TABLE 5. Pilot Indicators and Data Sources for Heart Disease and Stroke Prevention, Work-Site Setting, South Carolina and Alabama, 2001

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Data Sources and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of work sites that have policies supporting the engagement of all employees in physical activity during work time (eg, flexible scheduling, relaxed dress codes).</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of work sites that provide showers and changing facilities to support physically active employees.</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of work sites that provide and promote ongoing on-site employee physical activity programs (eg, walking, stretching, aerobics) during the previous 24 months.</td>
<td>No data source found. National Worksite Health Promotion Survey measures this indicator at the national level, but the sample is too small for state analysis.</td>
</tr>
<tr>
<td>Percent of work sites with vending machines and/or snack bars that offer heart-healthy food and beverage choices, including water or flavored water, 1% or less milk products, 100% juice products, fruits, vegetables, and products labeled low or reduced calorie, low or reduced sodium, or ≤3 g of fat per serving.</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of work sites with cafeterias that offer heart-healthy food and beverage choices, including water or flavored water, 1% or less milk products, 100% juice products, fruits, vegetables, and products labeled low or reduced calorie, low or reduced sodium, or ≤3 g of fat per serving.</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of work sites that offer nutrition or weight management classes or counseling.*</td>
<td>No data source found. National Worksite Health Promotion Survey measures this indicator at the national level, but the sample is too small for state analysis.</td>
</tr>
<tr>
<td>States with laws on smoke-free indoor air that prohibit smoking or limit it to separately ventilated areas in government and private work sites.†</td>
<td>State Tobacco Activities Tracking and Evaluation System (<a href="http://www2a.cdc.gov/nccdphp/osh/state/).%E2%80%A1">http://www2a.cdc.gov/nccdphp/osh/state/).‡</a></td>
</tr>
<tr>
<td>Proportion of work sites (segmented by No. of employees) that cover smoking-cessation programs.*</td>
<td>No data source found.</td>
</tr>
</tbody>
</table>

*Two indicators (25%) lack specificity (ambiguous, lack precision).  
†One indicator (12%) lacks sensitivity (unable to measure incremental change, measured at inappropriate level).  
‡One indicator (12%) has an adequate data source.

### TABLE 6. Pilot Indicators and Data Sources for Heart Disease and Stroke Prevention, Healthcare Setting, South Carolina and Alabama, 2001

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Data Sources and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of managed care organizations that adopt a policy to incorporate nationally accredited guidelines (eg, the AHA Guide to Primary Prevention of Cardiovascular Diseases) as part of their standard-care package.*</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of managed care organizations that adopt a policy to incorporate nationally accredited guidelines (eg, the AHA Guide to Comprehensive Risk Reduction for Patients With Coronary and Other Vascular Disease) as part of their standard-care package.*</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of managed care organizations (eg, health maintenance organizations, independent provider organizations, and preferred provider organizations) that have policies or guidelines to routinely provide or reimburse for assessments and counseling for physical activity, medical nutrition therapy, and tobacco cessation to plan members as part of their standard-care package, according to the Guide to Clinical Preventive Services.</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Percent of health insurance plans that have policies or guidelines to routinely provide or reimburse for assessments and counseling for physical activity, medical nutrition therapy, and tobacco cessation to plan members as a covered benefit, according to the Guide to Clinical Preventive Services.*</td>
<td>No data source found.</td>
</tr>
<tr>
<td>Proportion of current and recent smokers who received advice to quit smoking from a health professional.</td>
<td>BRFSS, optional Tobacco Indicators module (<a href="http://www.cdc.gov/brfss).%E2%80%A0">http://www.cdc.gov/brfss).†</a></td>
</tr>
</tbody>
</table>

*Four indicators (80%) lack specificity (ambiguous, lack precision).  
†One indicator (10%) has an adequate data source.
have been counseled to change their diets, or have made recent changes in what they eat.

The NAMCS and the National Hospital Ambulatory Care Survey (NHAMCS) Outpatient Department Form provide information from healthcare settings about the provision of counseling on physical activity, diet, and tobacco use, as well as data on current smoking status, but these sources do not include measures of physical activity, dietary habits, or the effectiveness of efforts to increase physical inactivity, improve diet, or reduce tobacco use. Furthermore, these data apply only to persons who have access to the healthcare system.

Available State and Local Data
Self-reported data on physical activity behaviors among adults is monitored by the BRFSS. The core BRFSS survey annually collects information by telephone on whether or not individuals have participated in any type of leisure-time physical activity during the past month. During some years (typically biennially), the BRFSS also collects information on the amount of moderate and vigorous physical activity that respondents engage in during a usual week and on their typical occupational activity. In the optional CVD module, the BRFSS collects information on recent increases in physical activity levels to reduce risk for CVD and receipt of healthcare provider advice to increase physical activity to reduce risk for CVD. This information can be used to examine rates of provider counseling (as reported by participants) and self-reported behavior change.

The Youth Risk Behavior Surveillance System (YRBSS) collects information on physical activity among high school students, including the percentage engaging in moderate physical activity, vigorous physical activity, and daily physical education, as well as information on whether respondents have increased their physical activity level in an attempt to lose weight; however, no information is collected on healthcare provider counseling on physical activity. Data on the prevalence of smoking among adults and youth are monitored by BRFSS and YRBSS, respectively. Both of these surveys also provide information on the proportion of smokers who have attempted to quit smoking in the past year, and both surveys enable estimation of the proportion of ever-smokers who have quit smoking as a measure of the success of state-level tobacco control programs. In the optional tobacco module, the BRFSS also collects information about receipt of healthcare provider advice to quit smoking; however, the YRBSS provides no information about healthcare provider counseling on tobacco use. The Youth Tobacco Survey and the Adult Tobacco Survey are conducted in some states; these surveys provide more in-depth information on behaviors and attitudes related to tobacco use, including information on healthcare provider counseling about tobacco use.

Dietary information is quite limited at the state level. Data on daily fruit and vegetable consumption is captured by the BRFSS for adults and by the YRBSS for high school students. The optional CVD module of the BRFSS also includes questions designed to provide information from adults on (1) self-reported changes in diet to eat fewer high-fat or high-cholesterol foods and more fruits and vegetables and (2) receipt of counseling from a health professional to make either of these changes. The YRBSS also captures information on milk consumption among high school students; however, it does not collect information about awareness of or counseling on dietary recommendations.

Gaps at National Level
Before NHANES was redesigned to collect data on a continuous basis beginning in 1999, national estimates relevant to biological risk factors for CVD were based on episodic data collection with widely spaced and variable intervals. The current design of NHANES, if continued, will enable better monitoring of trends in these measures of CVD risk. There are no national sources of data on incidence of behavioral risk factors. The NHANES and NHIS data are limited by biases that affect self-reported data, although the NHANES also collects serum cotinine and objective measures of fitness. No single national database provides fully adequate information on detection, treatment, and control of physical inactivity, unhealthy diet, or cigarette smoking.

Gaps at State and Local Levels
Data on the prevalence of physical inactivity, unhealthy diet, and tobacco use among adults are limited to self-report. To the best of our knowledge, no reports have been published on the validity of the current BRFSS physical activity questionnaire that is used to estimate moderate and vigorous activity. In addition, BRFSS does not currently collect information on cigar smoking. Likewise, physical inactivity, unhealthy diet, and tobacco use data on young people are self-reported. Validity data for the physical activity questionnaire have not been published. Dietary data at the state and local levels are limited to fruit and vegetable intake and milk consumption; the vegetable consumption questions on both BRFSS and YRBSS do not allow measurement of consumption of dark-green or orange vegetables. Not all states participate in the YRBSS, and not all participating states collect data on physical activity, fruit and vegetable consumption, or tobacco use, so data from young people are available for only some states. YRBSS is limited to high school students primarily, although a middle school survey is conducted in some states. No systems exist to routinely collect biological measures of smoking at the state level. Reliable data are available for selected substate-level areas only. The BRFSS optional modules, Adult Tobacco Survey, YRBSS, and Youth Tobacco Survey are not conducted in all states in all years.

Biological Risk Factors (Obesity, Hypertension, Dyslipidemia, and Diabetes Mellitus)
Available National Data
The NHANES collects directly measured and self-reported data relevant to biological risk factors. Obesity-related measures include height and weight (used to calculate body mass index), body circumference, body composition by bioelectrical impedance analysis, skinfold measures, and self-reported height and weight history, weight loss attempts and strategies, and weight change. No information is provided about healthcare provider counseling on weight man-
provide estimates of self-reported overweight and obesity. The prevalence of self-reported diagnosed hypertension among adults has been collected at least biennially since 1984. Since 2005, an optional BRFSS module has provided information on the proportion of adults with high blood pressure who are taking action to control their blood pressure. The prevalence of self-reported diagnosed high blood cholesterol among adults and the proportion of adults who have had their blood cholesterol checked within the preceding 5 years have been estimated from data collected at least biennially since 1987. The prevalence of self-reported diagnosed diabetes among adults has been collected annually since 1988. The optional diabetes module provides information on diabetes treatment and glycohemoglobin testing. The BRFSS provides no information about control of hypertension, dyslipidemia, or diabetes.

Self-reports of height and weight also have been collected through the national and state/local YRBSS since 1999 and are used to calculate body mass index and the prevalence of overweight and those at risk for overweight.66 Some states also participate in the Pediatric Nutrition Surveillance System, which is coordinated by the CDC and collects physical measures of height and weight of low-income children who attend federally funded maternal and child health and nutrition programs. The Pediatric Nutrition Surveillance System provides data on body mass index and the prevalence of overweight and those at risk for overweight among those <5 years of age.74

Gaps at National Level
Before the redesign of NHANES, national estimates relevant to these biological risk factors have been based on episodic data collection with widely spaced and variable intervals. The current design of NHANES, if continued, will enable better monitoring of trends in these measures. There are no national sources of data on incidence of biological risk factors. The NHIS data are limited by biases that affect self-reported data, that is, misclassification due to lack of knowledge and incorrect recall. No national database exists to provide comprehensive information about obesity prevalence, awareness, treatment, and control.

Gaps at State and Local Levels
At the state level, only self-reported data on biological risk factors are routinely available for adults. No coordinated state surveillance efforts have been made to collect direct measures of biological risk factors for data on obesity, hypertension, dyslipidemia, or diabetes prevalence, treatment, and control rates. The New York City Health and Nutrition Examination Survey and the CDC state-based examination survey initiative may lead to greater data availability in the future. In addition, the BRFSS no longer collects data on screening for high blood pressure, so this information is no longer routinely available at the state level. Among youth, the YRBSS relies on self-report of height and weight, which likely leads to an underestimate of the prevalence of overweight.66 Not all states participate in the YRBSS, and not all states that do participate collect these data, so data on youth are only
Surveillance System provides physical measures, this survey is limited to a select population in 13 states, the District of Columbia, Puerto Rico, and several American Indian tribes. Data on weight are available for selected substate-level areas. Data on the incidence of biological risk factors are not available at the state level. Data related to hypertension, high cholesterol, and diabetes are not available for all states for all years and are only available for some substate level areas. There are no coordinated efforts between states to collect information on biological risk factors among youth.

**HP2010 Goals 3 and 4: Early Identification and Treatment of Heart Attacks and Strokes and Prevention of Recurrent Cardiovascular Events**

Accomplishing the early identification and treatment of heart attacks and strokes invokes the vision of an efficient, effective, and coordinated emergency and acute care delivery system, and the need for such a system is obvious. However, it is important to recognize that many silent heart attacks and strokes occur as well as nonhospitalized events that go unrecognized by acute care delivery systems. Consequently, improved identification and treatment of heart disease and stroke in the outpatient setting is increasingly important to prevent first symptomatic events and recurrent events.

**Surveillance of Acute Events**

The ultimate goal of risk factor prevention, detection, and control is to prevent acute events. Consequently, surveillance of cardiac arrests, heart attacks, and strokes is needed to fully assess the impact of risk factor prevention, detection, and control efforts; furthermore, such surveillance would also provide a population in which to assess the impact of efforts to rapidly identify and treat these events (goal 3). Optimal surveillance of acute events requires an understanding of the distinction between incident and recurrent events. Incident events, or first occurrences of heart disease or stroke in persons without known prior heart disease or stroke, differ in important ways from recurrent events, or later attacks in persons with known prior disease. Persons experiencing incident events are less likely to be influenced by preexisting CVD (at other vascular sites) and previous clinical care (other than, potentially, risk factor management). The success of acute treatment influences the size of the population at risk for recurrent events, and subsequent medical care, in addition to other factors, influences that risk. Surveillance systems that enable discrimination between incident and recurrent events provide a much clearer indication of the changing natural history of heart disease and stroke occurrence in the community. Surveillance of incident events is important for evaluating public health measures aimed at maintaining health and function in currently asymptomatic persons. Surveillance of recurrent events also allows the evaluation of efforts aimed at preventing recurrent events (goal 4). Surveillance systems that are limited to the estimation of attack rates, in which incident and recurrent events are not distinguishable, provide data relevant to the early identification and treatment of acute events (goal 3), including the quality and outcomes of acute care, but do not provide sufficient data to evaluate the specific impact of separate efforts targeting prevention of incident and recurrent acute events.

Incident heart disease events can be especially difficult to monitor, however, because cardiac arrest (also known as sudden cardiac death) is often the first manifestation of ischemic heart disease. Surveillance of sudden cardiac death is complicated by the poor level of agreement between sudden cardiac death rates based on vital statistics data and based on adjudicated data. Despite this limitation, roughly half of sudden cardiac deaths are believed to occur in persons without known heart disease, and sudden deaths in persons without heart disease may comprise more than one fifth of all coronary heart disease deaths. Sudden deaths and out-of-hospital deaths, whether due to heart disease or stroke, are often missed by hospital-based surveillance systems. Identification, validation, and classification of out-of-hospital deaths require additional surveillance efforts, including access to death certificate data and contact with next of kin, physicians, coroners, and other informants. In addition, classification of sudden death as an indicator of disease incidence requires exclusion of preexisting, or prevalent, disease, which can be difficult without detailed diagnostic evaluation and/or medical history; nevertheless, surveillance of cardiac death as the initial manifestation of heart disease would provide important insight into the effectiveness of prevention efforts targeting risk factor prevention, detection, and control.

Assessment of incidence is crucial for assessing the population burden of disease and the effectiveness of preventive efforts. Although incidence is typically expressed as a rate per specific population size and time period (such as cases/100,000 population per year), total or absolute numbers of cases are also valuable in assessing total disease burden. Population-based rates, which require reliable data on population size (denominator), are most useful for comparing risk of disease between subgroups, such as those defined by sex, ethnicity, and presence or absence of risk factors. Absolute numbers of cases are useful for evaluating disease burden and planning for distribution and use of healthcare resources.

**Surveillance of Community Indicators**

The early identification and treatment of heart disease and stroke starts in the prehospital phase. Improved in-hospital care and the advent of time-dependent treatments have increased the value of reducing delays from the onset of symptoms to receipt of effective acute care. Delay to treatment can be generically divided into several components: (1) prehospital patient delay (the time from symptom onset to contact with the healthcare system), (2) transport time (time from initial contact with the healthcare system to hospital arrival), and (3) in-hospital delays in diagnosis and treatment. For both heart attack and stroke, the longest of these components is usually prehospital patient delay, which is approximately 2 hours for heart attack and ranges from a median of 3 to 6 hours for stroke. To evaluate progress in reducing all of these time elements, surveillance systems must address both community indicators and hospital indicators related to early identification of events.

The first link in the chain of recovery starts with the recognition of and response to symptoms by patients and
those in their environment. Understanding the level of knowledge about cardiac and stroke symptoms and the appropriate initial response to those symptoms is important for tailoring and improving public health campaigns and programs.

Another community indicator critical for the early and rapid identification of events is the state of emergency medical services systems, including capabilities of 9-1-1 systems and other telecommunications. Emergency medical services and 9-1-1 capabilities vary widely from state to state and within states. Availability of enhanced 9-1-1, with access to technology that facilitates identification of the location of the caller, varies widely. The time a 9-1-1 call is received, dispatch determination, when the emergency medical services personnel are dispatched, arrival time, scene time, transport time, and treatments given at the scene and during transport are just some examples of the key data points that need to be evaluated in the prehospital care phase of acute coronary and stroke events. Other prehospital community-level indicators relevant to early identification and treatment of acute events include public access to and use of automated external defibrillators. Obtaining the information needed to evaluate programs aimed at improving public recognition of and response to symptoms of acute events and access to high-quality prehospital care will require the development of appropriate methods of surveillance.

Surveillance of Healthcare Quality

The Institute of Medicine defines quality of care as “the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge.” Without quality-of-care measurement, it is not possible to know whether current treatment strategies for improving patient survival and well-being are being maximally utilized. Thus, it will be critical to embed quality-of-care measures into any national surveillance efforts that have the goal of reducing the burden of heart disease and stroke. Groups such as the AHA, the Agency for Healthcare Research and Quality, the American College of Cardiology (ACC), the Institute of Medicine, the National Heart, Lung, and Blood Institute, and the Veterans Health Administration have identified cardiovascular quality of care as a “top-tier” priority for national measurement and action to improve care delivery and thereby improve patient outcomes.

Unfortunately, numerous studies have documented significant gaps in the quality of care provided in the United States, to the degree that the Institute of Medicine has identified a “quality chasm” that must be crossed. For example, with regard to heart disease and stroke, a significant proportion of eligible patients do not receive guideline-indicated medications such as aspirin or β-blockers after a heart attack or warfarin for atrial fibrillation. Although much of the focus has been on the underuse of therapies that can help patients, variation in quality of care can also be due to the overuse of therapies that are not necessary or errors in the delivery of therapies that can cause harm to patients. Quality-of-care measurement should therefore include assessment of the delivery of necessary care (eg, guideline-indicated treatments), appropriate care, and safe care. Moreover, quality-of-care measurement should include processes, structures, and outcomes of care. To date, there has been little standardization of quality-of-care measures and limited regional and national quality-of-care surveillance efforts.

Several interventions have been shown to reduce the risk for death and recurrent events in patients with heart disease and stroke; these interventions are included in guidelines from the ACC and AHA. Any quality performance measures to be incorporated into national surveillance of quality care for heart disease and stroke should meet the methodological criteria for performance measures outlined by the ACC/AHA Task Force on Performance Measurement. In brief, measures should be evidence-based (eg, stemming from class I [beneficial] or class III [not beneficial, or harmful] ACC/AHA guideline indications), have clear definitions of which patients are eligible for a given measure, be feasible to measure nationally with reasonable effort, and be actionable, or under the control of hospitals or practitioners so that steps can be taken to improve performance and thereby quality of care. This latter point highlights that national surveillance for quality of care should not be for descriptive purposes only but should be part of a broader national effort to improve quality of care (eg, through feedback of performance with benchmarking). In this way, surveillance becomes an active process in efforts to meet the AHA and HP2010 goals of reducing rates of heart disease and stroke.

To date, groups such as the Centers for Medicare and Medicaid Services, the Joint Commission on Accreditation of Healthcare Organizations, and the Veterans Administration have used some cardiovascular quality performance measures (eg, aspirin and β-blockers on admission and discharge, reperfusion therapy, and lipid-lowering therapy on discharge for eligible heart attack patients), but these measures are defined somewhat differently and are not currently used for national surveillance (eg, the Centers for Medicare and Medicaid Services measures are used only for patients with Medicare coverage). Furthermore, there has been an emphasis on hospital care and little on performance measurement related to longitudinal care and long-term outcomes of heart disease and stroke patients. The ACC/AHA Performance Measurement Task Force has recently developed quality-of-care performance measures for heart failure and acute myocardial infarction. These efforts to measure quality of care should be coordinated to allow the identification of a set of standardized performance measures that can be part of a national surveillance system that will directly promote quality improvement, as do the CDC-funded Paul Coverdell National Acute Stroke Registry programs, currently funded in only 4 states.

Of note, the National Quality Forum (www.qualityforum.org) attempts to bring together consumers, providers, health plans, purchasers, professional societies (including the AHA and ACC), and researchers with regard to national quality performance measures, and it may serve as a source for quality-of-care measures that can be used in national surveillance related to the care of heart disease and stroke patients. To date, the National Quality Forum has released a limited set of performance measures for hospital care, including some relevant to heart attack and heart failure, and has plans to
develop other performance measures for the care of patients with heart attack and heart failure, as well as measures of care coordination. Although efforts like those of the National Quality Forum may lead to identification of standardized national measures of quality of cardiovascular care, additional challenges will be how best to gather the data nationally and how to leverage it toward meeting national quality-improvement goals.

One example of a nongovernmental program with potential for use in national quality-of-care measurement and improvement is the ACC’s National Cardiovascular Data Registry (ACC-NCDR; www.accncdr.com). The ACC-NCDR collects data (based on ACC data standards) on procedures performed in cardiac catheterization laboratories, with feedback on quality measures (eg, complication rates) including comparisons with similar hospitals and national benchmarks. To date, the ACC-NCDR is not nationally representative and lacks data on longitudinal outcomes, but it represents one potential way to promote the collection and use of national surveillance data for improving quality of care, at least for cardiac catheterization laboratory procedures. The AHA National Registry for Cardiopulmonary Resuscitation program (www.americanheart.org) is a potential contributor to safety programs that monitor and reduce in-hospital cardiovascular emergencies. The AHA’s “Get With the Guidelines” (GWTG) programs for coronary heart disease, stroke, and heart failure (www.americanheart.org) are also potential contributors to national surveillance of quality of care and quality improvement for CVDs, but these programs are not currently nationally representative and lack data on longitudinal care and outcomes.

In summary, quality-of-care measures to be included in national cardiovascular surveillance efforts should be methodologically rigorous and standardized under such efforts as the ACC/AHA Performance Measures and the National Quality Forum. Where possible, measurement of quality should be linked to quality-improvement efforts, as is done in the ACC-NCDR and the AHA’s GWTG program. It will be critical to include quality-of-care measures in any national surveillance efforts to reduce the burden of heart disease and stroke and enhance patient outcomes.

**Surveillance of Case Fatality**

Case fatality refers to the mortality rate among persons who experience acute events. Recent trends in coronary heart disease case fatality show steady declines that are attributed to better acute care and reduced severity of the events.\(^{100,101}\) Adjustment for standardized measures of severity could improve the utility of case fatality as an indicator of the impact of acute care. Estimation of case fatality is often limited to deaths that occur during the course of the initial hospital stay; however, variations in the duration of hospital stay complicate interpretation of in-hospital case fatality rates. Consequently, inclusion of all deaths that occur within 30 days of an acute event has become the standard for surveillance efforts. Variations in the proportion of people with acute events who survive to reach hospital care further complicate the interpretation of case fatality; hence, the inclusion of prehospital cardiac deaths may provide a more accurate reflection of the short-term mortality rates related to heart disease. In addition, because trends in case fatality rates may differ across communities in relation to the proportion of events that are incident versus recurrent in that community, the ability to distinguish between incident and recurrent events is also crucial for the interpretation of case fatality trends. Similar considerations apply to stroke.

**Surveillance of Event Severity**

Isolated community-based surveillance studies among people hospitalized with acute myocardial infarction have provided important information on trends in the severity of such events over time. However, reports from the Atherosclerosis Risk In Communities community surveillance,\(^ {101}\) the Worcester Heart Study,\(^ {102}\) and Olmsted County, Minnesota,\(^ {103}\) have reported mixed findings on whether or not the severity of acute myocardial infarctions is decreasing. A clear picture of changes in disease severity is an important component of understanding the relative contributions of medical care and prevention to national trends in CVD mortality. Primary prevention efforts can exert an influence on disease in the face ofunchanging incidence by helping to lessen the severity of clinical events, thereby also reducing case fatality rates. However, surveillance systems that only count the number of incident events fall short of providing in-depth information on the nature of the event (ie, severity indicators). The changing definitions of acute events such as those proposed for acute myocardial infarction by the ACC/European Society of Cardiology joint criteria\(^ {104}\) provide further evidence that the collection of clinical details of the events sufficient to determine severity is important to any complete surveillance system. These new definitions result in the classification of some events as acute myocardial infarctions that previously did not meet standard criteria (based on electrocardiogram and creatine kinase-MB fraction). The resulting “troponin-only” infarcts have been shown to have a higher case fatality rate in some studies\(^ {105}\) and a lower case fatality rate in others.\(^ {106,107}\) Without additional information about severity indicators, incidence rates will be difficult to interpret. Collecting information on severity will improve our ability to track progress toward reaching national goals for reducing heart disease and stroke.

**Impact of Changes in Diagnostic and Therapeutic Technology**

Changes in diagnostic and therapeutic technologies may have important implications for interpretation of incidence and mortality trends. The advent of highly sensitive biomarkers for detection of heart attacks, for example, has resulted in substantial increases in heart attack hospitalization rates.\(^ {108}\) The introduction of cranial computed tomographic scanning had a similar impact on stroke trends.\(^ {109}\) The trends toward higher attack rates due to these changes may be accompanied by lower rates of complications and mortality if cases detected by newer biomarkers or imaging studies include milder events that would previously have gone undetected. The concomitant greater use of effective preventive and acute care may also reduce morbidity and mortality. Reliable data on incidence, severity, treatment, and outcomes of acute
Surveillance of Cardiovascular Procedures

Registries of invasive procedures such as thrombolysis, percutaneous coronary intervention, carotid endarterectomy, angioplasty and stenting, and coronary bypass grafting have also been used as indicators of disease burden, but the biases involved in application of these procedures render them of questionable value for purposes of disease surveillance.\(^2\)\(^0\)\(^-\)\(^2\)\(^2\) Data about procedures do provide useful insights, however, into costs of care, as do data on trends in drug usage.\(^1\)\(^1\)\(^3\)\(^-\)\(^1\)\(^5\) Procedures and drug treatment also play a role in modifying rates of case fatality and recurrence, so the collection of accurate data on their use is an important component of a comprehensive heart disease and stroke surveillance system. Surveillance systems must be easy to modify to include data capture of new therapies as they emerge.

Limitations of Hospitalization Data

Despite the common use of hospitalization data for monitoring burden and trends in heart attacks and stroke, hospitalization data have biases related to access that limit their utility. Some people who experience an acute event may not reach the hospital owing to failure to recognize and respond to the event, being in a long-term care facility, or early death, for example. In addition, monitoring trends in acute events is complicated by the recent trend toward observing suspected cases of heart attack and stroke in an acute care setting for \(<\)24 hours, without hospitalization, as a cost-containment strategy. This approach allows ample time for a definitive diagnosis to be made through biomarker evaluation or brain imaging. Persons for whom a heart attack is ruled out may be sent home before the time at which a hospitalization becomes official for billing and surveillance purposes (24 hours). This practice may have contributed to the recent decline in hospitalizations for unstable angina, a phenomenon that complicates efforts to monitor trends in acute coronary syndromes.\(^1\)\(^6\) The issue of outpatient care without admission is especially important as it relates to capturing incidence data and treatment patterns for heart failure. Traditional hospital-based surveillance is not adequate to fully evaluate the impact and burden of heart failure in the community, although the majority (74%) of heart failure patients are hospitalized eventually.\(^1\)\(^7\)

Surveillance of Patient Health Status

Patient health status includes symptom burden, functional status (eg, physical, emotional, and social function), and health-related quality of life, which is the discrepancy between actual and desired function for a given patient.\(^1\)\(^8\) With the significant therapeutic advances in treating CVD over the last 20 years and concomitant improvements in survival, there has been increasing emphasis on patient health status as a primary outcome of care. Assessment of health status directly accounts for the patient’s perspective on how the disease is affecting his or her life, and many patients express a desire for quality of life that is equal to or greater than their desire for quantity of life.\(^1\)\(^9\) Assessment of patient health status is consistent with the Institute of Medicine’s call for more patient-centered care in order to provide the highest quality of care.\(^5\)\(^6\) Thus, health status measurement should be considered for inclusion in any national surveillance of heart disease and stroke to ensure that the surveillance accounts for how well people are living, not just how long they are living.

Health status surveys have been developed that are valid, reliable, sensitive to clinical change, and, in many cases, predictive of subsequent cardiovascular morbidity and mortality.\(^1\)\(^2\)\(^0\)\(^-\)\(^1\)\(^2\)\(^2\) These include both generic surveys that measure overall patient health status and disease-specific surveys that measure how one condition (eg, heart failure) influences a given patient’s symptom burden, functional status, and health-related quality of life. Examples of generic instruments include the Short-Form (SF) 36 health status survey and its even shorter versions (eg, SF-12) and the EQ-5D, which is a utilities measure that allows derivation of quality-adjusted life-years when combined with economic assessment. Examples of disease-specific instruments include the Seattle Angina Questionnaire and the Mac-New questionnaire for patients with ischemic heart disease, the Kansas City Cardiomyopathy Questionnaire and the Minnesota Living with Heart Failure Questionnaire for heart failure patients, and the Stroke-Specific Quality of Life Scale (SS-QOL) and National Institutes of Health Stroke Scale for stroke patients.\(^1\)\(^2\)\(^3\)\(^-\)\(^1\)\(^2\)\(^8\)

Although health status surveys have been used extensively in research studies (eg, to assess the impact of new therapies or interventions on quality-of-life outcomes), they have generally not been used as part of cardiovascular surveillance. Surveys by the CDC and the Agency for Healthcare Research and Quality have addressed health behaviors and risk factors but have not explicitly measured patient health status, nor have they focused on clinical conditions.\(^8\)\(^8\) The Veterans Administration has undertaken large-scale patient health status surveys using generic instruments like the SF-36 but has not used ongoing health status surveillance or tied the health status data to clinical conditions or events.

In summary, patient health status is an essential measurement for adequately assessing the impact of heart disease and stroke, as well as of the therapies and interventions for these conditions, on patients’ lives. Validated health status surveys (including disease-specific surveys for ischemic heart disease, heart failure, and stroke) are available and can provide clinically relevant and prognostic information that cannot be obtained in other ways.\(^1\)\(^8\) To date, patient health status has, for the most part, not been part of the cardiovascular surveillance system. Future efforts toward establishing national surveillance for heart disease and stroke should incorporate patient health status assessment and thereby directly promote patient-centered care of the highest quality.

Available National Data

National data are available on attack rates of acute events (NHDS, Nationwide Inpatient Sample [NIS])\(^1\)\(^2\)\(^9\)\(^,\)\(^1\)\(^3\)\(^0\); however, it is not possible to distinguish between incident and recurrent events. In-hospital case fatality rates can be determined from

<table>
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<th>Measure</th>
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<tr>
<td>Aspirin at arrival</td>
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<tr>
<td>Aspirin prescribed at discharge</td>
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<tr>
<td>ACE inhibitor or angiotensin receptor blocker for left ventricular systolic dysfunction</td>
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<tr>
<td>Adult smoking-cessation advice/counseling</td>
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<tr>
<td>(\beta)-Blocker prescribed at discharge</td>
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<tr>
<td>(\beta)-Blocker at arrival</td>
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<tr>
<td>Mean time to thrombolysis</td>
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<tr>
<td>Thrombolytic agent received within 30 minutes of hospital arrival</td>
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<td>Mean time to PCI</td>
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<td>PCI received within 120 minutes of hospital arrival</td>
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<tr>
<td>Inpatient mortality†</td>
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<tr>
<td>LDL Cholesterol assessment (optional test measure)*</td>
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<tr>
<td>LDL Cholesterol testing within 24 hours after hospital arrival (optional test measure)*</td>
</tr>
<tr>
<td>Lipid-lowering therapy at discharge (optional test measure)*</td>
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ACE indicates angiotensin-converting enzyme; PCI, percutaneous coronary intervention.
*Centers for Medicare and Medicaid Services only.
†Joint Commission only.

these sources; however, linkage to other data sources such as the National Death Index is not possible. Consequently, assessment of 30-day case fatality rates is not possible, except through administrative data sources such as the Centers for Medicare and Medicaid Services. Data on use of cardiovascular procedures are available, but linkage of these data to outcomes beyond the hospital stay is difficult.

Although representative data are not available to monitor the quality of secondary prevention at the national level, several data sources provide insight pertinent to selected measures in selected populations. The Centers for Medicare and Medicaid Services and the Joint Commission on Accreditation of Healthcare Organizations have adopted a uniform set of quality measures and publish data on several secondary prevention measures among hospitalized patients (Tables 7 and 8). For appropriate stroke patients, the Centers for Medicare and Medicaid Services also provide data on warfarin use for atrial fibrillation. The Veterans Administration reports similar information. The National Committee for Quality Assurance reports data from participating managed care organizations. These data are reported by the participating organizations on a voluntary basis and are collected to inform quality-improvement efforts. Measures include use of \(\beta\)-blockers at discharge after a myocardial infarction, cholesterol screening and control in patients hospitalized for coronary heart disease, control of hypertension and diabetes, and smoking cessation counseling.

The AHA National Registry for Cardiopulmonary Resuscitation program is a hospital-based program that provides information from participating hospitals on the quality of care and outcomes of patients who experience cardiac arrest. Measures include monitored status, performance of cardiopulmonary resuscitation, timely defibrillation, and hospital outcome. Recent extensions of the National Registry for Cardiopulmonary Resuscitation program include measures of patients who have risk factors for in-hospital cardiac arrest and receive interventions to reduce this risk. The AHA GWTG Coronary Artery Disease program is a hospital-based quality-improvement effort that provides information from participating hospitals on patients hospitalized for coronary heart disease. Measures include use of aspirin, \(\beta\)-blockers, angiotensin-converting enzyme inhibitors, lipid-lowering therapy and blood pressure–lowering therapy at discharge, smoking-cessation counseling, and referral to cardiac rehabilitation. In addition, GWTG programs for stroke and heart failure have been initiated. GWTG for Stroke provides information on smoking-cessation counseling, cholesterol-lowering therapy, use of antiplatelet agents, weight and exercise management, use of anticoagulants for atrial fibrillation, and diabetes management among appropriate patients hospitalized for stroke. GWTG for Heart Failure provides information on discharge instructions, measurement of left ventricular function, and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker and \(\beta\)-blocker use among appropriate patients at discharge.

Professional society–run registries also exist, such as the ACC-NCDR for cardiac catheterization/percutaneous coronary intervention, implantable cardioverter defibrillators, and carotid stenting and the Society of Thoracic Surgery’s registry for cardiothoracic surgery. Finally, industry-sponsored registries exist for cardiovascular conditions such as acute coronary syndromes (CRUSADE [Can Rapid Stratification of Unstable Angina Patients Suppress Adverse Outcomes with Early Implementation of the ACC/AHA Guidelines?] and NRMI [National Registry of Myocardial Infarction]) and heart failure (ADHERE [Acute Decompensated Heart Failure National Registry]). CRUSADE is a quality-improvement program that involves >440 hospitals across the United States. It provides information on use of aspirin, \(\beta\)-blockers, angiotensin-converting enzyme inhibitors, and lipid-lowering therapy at discharge. ADHERE is a registry of patients hospitalized with acutely decompenated heart failure at 260 participating US hospitals that provides information on the use of angiotensin-converting enzyme inhibitors and \(\beta\)-blockers at discharge, as well as smoking-cessation counseling. At this time, none of these programs or registries are truly nationally representative; however, each has unique strengths and limitations, and they all have the potential for use in national surveillance.

TABLE 8. 2005 Heart Failure National Quality Measures: Centers for Medicare and Medicaid Services and the Joint Commission on Accreditation of Healthcare Organizations

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<th>Measure</th>
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<td>Discharge instructions</td>
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</tr>
<tr>
<td>Adult smoking-cessation advice/counseling</td>
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Available State and Local Data
State-based data on attack rates and procedure use are available from the 37 states that participate in the NIS. A handful of population-based research studies of the incidence of acute coronary disease and stroke are currently active in selected communities: the Minnesota Heart Survey and Minnesota Stroke Survey in Minneapolis and St. Paul, Minn; the Rochester (Minnesota) Epidemiology Project; the Atherosclerosis Risk in Communities study (Washington County, Md; Minneapolis, Minn; Jackson, Miss; and Forsyth County, NC); the Worcester (Massachusetts) Heart Attack Study; the Northern Manhattan Stroke Study; and the Greater Cincinnati/Northern Kentucky Stroke Study. Some of these studies also feature out-of-hospital death investigation that allows for an estimate of incidence of fatal out-of-hospital events. Most of these hospital-based surveillance programs also collect data on use of procedures and other treatments and severity of acute events; however, all of these programs are research projects rather than public health surveillance programs. Four states (North Carolina, Massachusetts, Georgia, and Illinois) have established Paul Coverdell National Acute Stroke Registries that monitor acute care and preventive care at discharge for 4 states, but these registries are not designed to provide information about stroke incidence, severity, 30-day case fatality, or recurrence of acute coronary or stroke events in either the inpatient or outpatient settings. Available data on the quality of acute care and preventive care provided to these patients apply to selected subgroups of patients or are reported on a voluntary basis. Performance measures are not consistent across databases. No data are available on patient health status. No data are available on community indicators relevant to early identification and response to symptoms or access to high-quality care in the prehospital setting.

Gaps at National Level
No nationally representative data are available on incidence, severity, 30-day case fatality, or recurrence of acute coronary or stroke events in either the inpatient or outpatient settings. Available data on the quality of acute care and preventive care provided to these patients apply to selected subgroups of patients or are reported on a voluntary basis. Performance measures are not consistent across databases. No data are available on patient health status. No data are available on community indicators relevant to early identification and response to symptoms or access to high-quality care in the prehospital setting.

Gaps at State and Local Levels
State and local regions also lack representative data on acute event incidence, severity, 30-day case fatality, recurrence, quality of acute care (in the prehospital and hospital settings), and community indicators of early identification of and response to symptoms. No systematic data are available on secondary prevention efforts at the state or local level. The Paul Coverdell National Acute Stroke Registries will provide data on stroke severity and quality of acute stroke care and preventive care at discharge for 4 states, but these registries are not designed to provide information about stroke incidence or 30-day case fatality. No data are available on patient health status.

Surveillance of CVD Prevalence
Prevalence data provide an indicator of the overall disease burden in the population. Potentially, prevalence data could be based on the combination of overt disease diagnosed in the healthcare setting and disease that has not yet come to the attention of the medical system, although prevalence based on the former is more often assessed. Prevalence data could include information on persons who are living in the community as well as those who are institutionalized and bear a disproportionate share of the burden of heart disease and stroke. Although many data sources provide estimates of prevalent disease, no systematic methods have been developed for combining individual and unique data sets to provide an estimate of the overall disease burden. In addition, privacy issues make such information difficult to obtain, because linkage between different data sources, although feasible, is often proscribed. Persons who are institutionalized, particularly in nursing homes, constitute a large portion of the disease burden but are rarely included in population surveys. Finally, much prevalent disease is subclinical and undiscovered by the healthcare system and can only be detected in population surveys.

Available National, State, and Local Data
Many data sets that touch on elements of prevalence are available. The NHANES and NHS provide prevalence data representative of the noninstitutionalized national population. The National Nursing Home Survey, last conducted in 1999, collects information on up to 7 medical diagnoses at admission and at the time of the survey; however, the National Nursing Home Survey does not collect standardized data elements specific to CVD diagnoses. The BRFSS provides prevalence data representative of the noninstitutionalized populations of the states; however, many data elements are lacking, especially for various institutionalized populations, and no common system is in place for merging the existing data to provide an overall picture.

Gaps at National, State, and Local Levels
Population surveillance of prevalent disease should include institutionalized individuals and methods of detecting subclinical disease. To be useful at the state and local levels, such a system would require a large sample size, because even common CVDs are not widely prevalent in the general population.

Surveillance of Other CVD Conditions
Although not addressed specifically in the goals set forth in HP2010, CHF (historically labeled congestive heart failure) and PAD contribute substantially to national morbidity, mortality, and healthcare costs. Consequently, our ability to make good decisions about allocation of public health and healthcare resources will be improved by having access to better data on the changing burdens of these diseases.

Chronic Heart Failure
CHF is epidemic in the United States and other industrialized countries. It is estimated that 5 million Americans currently have CHF (2003), with 550 000 new cases added each year. From 1993 to 2003, deaths due to heart failure increased 20%. Five-year survival of CHF patients is <50%. In addition, many hospital discharges list CHF as an associated condition. In 2003, there were 1 093 000 hospitalizations with CHF as a primary diagnosis, a 174% increase over
1979, and approximately twice as many listed CHF as a secondary diagnosis. More than 20% of acute hospitalizations of all individuals >65 years of age list CHF as a primary or secondary diagnosis. Outpatient visits for CHF are in the millions. An estimated total of $29.6 billion will be spent for CHF care in 2006.

Several reasons are suggested for the increasing burden of CHF in the US population. The first, improved survival after acute myocardial infarction, results in more patients with damaged myocardium who are prone to developing CHF. Second, the number of untreated or inadequately treated patients with high blood pressure remains high. Third, the proportion of elderly adults, the principal sufferers of this condition, has steadily increased in the population owing to increasing lifespan. Finally, improvements in medical and surgical care for patients with CHF have probably changed the natural history of disease, prolonging the lives of many.

Although general agreement exists that the burden of CHF has increased, precise estimates are not available. Mild manifestations of a failing heart are not easily diagnosed, and debate exists over standard criteria for population studies. In addition, it has been suggested that diagnostic-related groups for Medicare reimbursement (which increase compensation for listing CHF as a primary or complicating condition) have led to diagnostic upgrading of discharge reports to augment hospital reimbursement. Finally, although many CHF patients are hospitalized, a growing proportion of those patients first diagnosed receive treatment in the outpatient setting. Few data exist on outpatient care for CHF, yet some have suggested that the prognosis is equally poor as for those admitted to the hospital.

The need for ongoing and systematic surveillance of CHF is clear. Prevalence is increasing, as is the consumption of medical resources in the diagnosis and treatment of the condition, and the prognosis is poor.

Available National, State, and Local Data
In the United States, the currently available national data sets that contain information on CHF include the NHDS and NIS, which include discharge diagnoses and surgical procedures; NAMCS and NHAMCS, which include outpatient diagnoses; and NHANES, which is based on self-reported data. As mentioned above, ADHERE and GWTG for Heart Failure are registries that provide information about quality of care for patients hospitalized with CHF; however, these registries are not representative of the US population. No data are collected on CHF at the state level in the BRFSS.

Gaps at the National, State, and Local Levels
The validity of data on discharges and visits for CHF in NHDS, NIS, NAMCS, and NHAMCS is not well established. Population surveillance of CHF is difficult because the diagnosis frequently depends on clinical signs and symptoms that are poorly measured and recorded in medical charts, and tests such as chest radiographs have limitations and are frequently unavailable. Having valid data would permit surveillance of time trends, with the understanding that any method of tracking patients in the health system does not necessarily reflect true population prevalence. Inclusion in NHANES of additional information on signs, symptoms, and treatment of heart failure would improve the comprehensiveness of CHF surveillance. For future surveillance of CHF, agreement on a standard method of defining CHF is required. The addition of items related to CHF to the BRFSS would improve the ability to conduct surveillance of CHF at the state level; however, this information would be limited to self-reported data.

Peripheral Arterial Disease
PAD in the legs is due to atherosclerosis causing narrowing or obstruction in the major arteries serving the lower limbs. PAD suffers may (1) remain asymptomatic, (2) develop intermittent claudication, or (3) develop the severe complication of critical limb ischemia.

Asymptomatic disease that causes a significant disruption to blood flow is usually detected by a low ratio of ankle-to-arm systolic pressure (ankle-brachial index [ABI]), a measurement that requires use of a Doppler flow probe in addition to a sphygmomanometer. This procedure is easily conducted in appropriately equipped primary care settings. An ABI <0.9 is deemed conventionally to be indicative of disease, although the precise validity in asymptomatic subjects is unknown. Other noninvasive means of detecting asymptomatic PAD, such as duplex scanning and magnetic resonance angiography, are too complex for routine surveillance.

The symptom of intermittent claudication is characterized by pain on exercise, normally in the calf and relieved within a few minutes of stopping the exercise. Standardized questionnaires are available to detect claudication. The World Health Organization/Rose questionnaire has been used traditionally, but sensitivity is poor (60%), and more recently, questionnaires with improved sensitivity, such as the Edinburgh or San Diego claudication questionnaires, have been used. In symptomatic subjects, an ABI <0.9 is accurate confirmation of the presence of PAD.

Chronic critical limb ischemia is characterized by persistent leg pain at rest, gangrene, or ulceration. This severe manifestation of PAD occurs uncommonly, with an annual incidence of ~5 to 10 per 10,000 adults. The diagnosis is made on the basis of clinical symptoms and signs, with most, but not all, patients having a very low ankle pressure of <50 mm Hg.

The routine surveillance of PAD in the population is not straightforward:

1. Mortality data are unhelpful, because patients do not die of PAD per se but are at high risk for death due to coronary heart disease or stroke.
2. Hospital discharge data are not a useful indicator of prevalence because of considerable variation in admission policies; furthermore, discharge diagnostic codes, such as those from the International Classification of Diseases, are too imprecise to capture true discharge rates for PAD.
3. Surgical interventions for PAD, including angioplasty, bypass surgery, and amputation, can be classified more precisely than diagnoses and tend to be recorded more accurately. These can give a good indication of surgical and radiological workload, but because of considerable
variation in clinical practice, they do not necessarily reflect the population prevalence of PAD.

4. In primary care settings, recording visits for intermittent claudication can provide an indication of physician workload, but differences in patient self-referral and physician diagnoses mean that rates may not be a good proxy for population prevalence. Time trends may be useful if data are collected consistently. Critical limb ischemia occurs too infrequently to be measured in primary care settings.

5. Surveys of intermittent claudication in the population by use of standardized questionnaires are the best method of assessing population prevalence of symptomatic disease.

6. Surveys of ABI in the population may give an indication of the frequency of asymptomatic PAD. Although the ABI is simple to perform and the variability is similar to that for routine blood pressure measurements, no standard method has been agreed on for measuring the ABI, nor which ankle and arm pressures should constitute a subject’s result.

Available National, State, and Local Data
In the United States, the currently available national data sets that contain information on PAD include the NHDS, NIS, and National Survey of Ambulatory Surgery, which include discharge diagnoses and surgical procedures; NAMCS and NHAMCS, which include outpatient diagnoses; and NHANES, which since 1999 to 2000 has measured ABI in subjects ≥40 years of age.6 No information is collected about PAD at the state level.

Gaps at the National, State, and Local Levels
The validity of data on discharges, procedures, and visits for PAD in the NHDS, NIS, National Survey of Ambulatory Surgery, NAMCS, and NHAMCS is not well established. Having valid data might permit surveillance of time trends, with the recognition that any method of tracking patients in the health system does not necessarily reflect true population prevalence. Inclusion in NHANES of a questionnaire on claudication, as well as continuing measurement of the ABI, would improve the comprehensiveness of PAD surveillance. For future surveillance of asymptomatic PAD, agreement on a standard method of measuring ABI is required. Addition of items related to PAD to the BRFSS would improve the ability to conduct surveillance of PAD at the state level; however, this information would be limited to self-reported data.

Surveillance of CVD Mortality
Although surveillance of CVD mortality, including heart disease and stroke, does not correspond directly to any of the HP2010 goals for heart disease and stroke, monitoring total and cause-specific mortality rates is core to our understanding of the health of populations. Furthermore, surveillance of CVD mortality is necessary for monitoring progress toward reaching the AHA goal of reducing coronary heart disease, stroke, and risk for both by 25% by 2010.7 Information on the occurrence of a death is collected nationally with a standardized death certificate, a process that has existed for decades. Cause of death is recorded by trained nosologists using the standardized international classification published by the World Health Organization.153 Death certificate data are collected initially at the local level and forwarded to county and state health departments. From there, they are transferred to the National Center for Health Statistics, which manages the National Death Index.154

Although the fact of death is undeniable and comprehensively collected, the cause of death is frequently misclassified. This is particularly true for CVDs in the instances of out-of-hospital death, death among the elderly with multiple comorbidities, and deaths in some racial/ethnic groups. Although the system for classification of deaths is clear and systematic, data required to make appropriate classifications are frequently absent at the time of certification. In addition, data are not available in a timely fashion. State and national data are not available for ≥1 year after the end of any calendar year.

Available National, State, and Local Data
Actual death certificates are collected by the state and are computerized in many instances. They contain multiple identifiers, as well as the circumstances and classified cause of death, and these data are available at the national, state, and local levels.

Gaps at the National, State, and Local Levels
The death certification system is a comprehensive national resource; however, improvements in several areas could increase its utility. These improvements include the following:

1. Validation of cause of death. The accuracy of cardiovascular causes of death is frequently suspect, and misclassification is a problem. A systematic sampling with validation of cause of death that uses medical records, family interviews, and other data would enhance our ability to provide valid estimates based on the available data.

2. Timeliness. Death certificates are sent to the appropriate health department shortly after the fatal event; however, comprehensive data sets may not be available until years later. Improving the timeliness of classification and computerization could make the death certificate system a more useful tool in understanding disease trends.

3. Linkage. The inability to link death certificate data to outpatient and inpatient medical records limits our ability to understand the effect of medical care on the final outcome. Privacy regulations have placed further barriers on an already difficult situation. Common identifiers, such as a health identifier, could enable the linkage of death information with medical care information.

Death certification, including information surrounding the event and its likely causes, is central to disease surveillance. In the United States, we are fortunate to have a common system for collecting and classifying these data; however, improved validation of cause of death, more timely availability of data, and linkage of mortality data to healthcare data would considerably enhance the utility of the valuable death certification data.
Surveillance of Healthcare Costs

Surveillance of healthcare costs related to various approaches to heart disease and stroke prevention and management is crucial to understanding where costs are increasing and whether the resources were well spent. The Medical Expenditure Panel Survey, a survey cosponsored by the Agency for Healthcare Research and Quality and the CDC’s National Center for Health Statistics, provides nationally representative estimates of healthcare use, expenses, sources of payment, and insurance coverage for the US population living in communities. These data are not available at the state or local level. State-specific information on Medicare reimbursement for traditional fee-for-service hospital care can be obtained from the Medicare claims files submitted to the Centers for Medicare and Medicaid Services; however, no public-use data set exists for this information. Information on Medicaid reimbursement for health care for the low-income population is also available at the state level in most states; however, no standardized method or policy for reimbursement exists that would allow national pooling of these state data.

Recommendations

Recommendations are categorized as overarching (fundamental recommendations that cut across goal areas) or as goal-specific. They are further classified according to priority, staging, and cost. Priority was classified as high or moderate (no low-priority recommendations were made); staging was classified as early (1 to 2 years), intermediate (2 to 4 years), or later; and cost was classified as low (<$10 million/y), intermediate ($10 million to $100 million), or high (>=$100 million).

Overarching Recommendations

1. A National Heart Disease and Stroke Surveillance Unit should be established to produce annual reports on key indicators of progress in the prevention and management of heart disease and stroke.

The establishment of a National Heart Disease and Stroke Surveillance Unit, perhaps modeled after the CDC’s National Diabetes Surveillance System, is a top priority. This entity should be charged, on a continuing basis, with assembling the most currently available and relevant data, identifying critical gaps in knowledge and data systems, and proposing modifications to existing surveillance components or development of new ones to fill these gaps. This recommendation was judged to be a high priority for early staging and only moderately costly, requiring a staff of perhaps 3 or 4 appropriately trained individuals. This need is currently being addressed in part by the efforts of volunteers and staff of the AHA, as well as by professional staff in various parts of the Department of Health and Human Services (Agency for Healthcare Research and Quality; CDC [including the National Center for Health Statistics and the Division for Heart Disease and Stroke Prevention, among others]; National Heart, Lung, and Blood Institute; and the National Institute of Neurological Disorders and Stroke).

2. CVD, including cardiac arrests, acute coronary syndromes (heart attack and unstable angina), stroke, CHF, and related interventional procedures, should be classified as reportable conditions. The reporting system should

a. incorporate
   i. distinction between incident and recurrent events;
   ii. validation of diagnosis, at least in a subsample, to enable the estimation of valid rates over time;
   iii. adjustment for changes in diagnostic technology over time; and
   iv. collection of information on severity of the event and quality of prehospital care, acute care, procedure use, and preventive care at discharge; and
b. enable
   i. surveillance of 30-day case fatality through linkage with the National Death Index;
   ii. monitoring of healthcare quality as part of efforts to prevent recurrent events after discharge; and
   iii. monitoring of patient health status after discharge.

Classification of CVD as a reportable condition would remove many of the barriers to timely surveillance. Standard definitions exist for these diseases and for most of the relevant data elements related to quality of care and outcomes; hence, an efficient surveillance system could be developed and implemented based on a reportable event model. This recommendation was judged to be a high priority and highly costly; however, additional developmental work is required. Consequently, this recommendation was considered for later staging. It is recognized that efforts to promote healthcare quality, including pay-for-performance and accreditation programs, are creating an environment that is increasingly primed for classification of cardiovascular conditions as reportable events. Given the reality that hospitals will be reporting data on many of these conditions as part of pay-for-performance and accreditation programs, hospital reporting of CVD events may be a more feasible approach than physician reporting. Reporting of cardiac arrests will require development and integration of additional reporting mechanisms that involve emergency medical systems and other sources of data for events that occur outside of hospitals.

3. Data collection on patients’ encounters with the healthcare system should be revised to include collection of data on lipoprotein cholesterol concentrations, blood sugar, and glycohemoglobin values.

Data collection on these elements is critical to our understanding of risk factor identification and control before and after the diagnosis of CVD; hence, this recommendation addresses goals 1, 2, and 4. Because surveillance programs currently exist to monitor patient encounters with the healthcare system (eg, NAMCS and NHAMCS), early staging of implementation of this high-priority recommendation could be accomplished at low cost.

4. Data elements should be standardized across surveys, and unnecessary duplication in data sources should be avoided.

We identified multiple examples of duplication in data collection activities. Improved coordination of effort, with greater standardization and less redundancy, could result in significant cost savings, thereby freeing up resources to support enhanced surveillance in critical areas. This recom-
recommendation was judged to be a high priority for early staging and potentially cost saving, although some cost would be incurred in the short term to evaluate and develop a coordination plan for the existing surveillance programs. The proposed National Heart Disease and Stroke Surveillance Unit should be charged with this task.

5. The design and conduct of nationally representative surveillance programs should be revised to facilitate oversampling by states, territories, and tribal organizations and to provide meaningful estimates on ethnic subgroups in the populations. Sampling within states, territories, and tribal organizations should be designed to facilitate oversampling by counties.

Modification of national surveys to facilitate the ability of the states, territories, and tribal organizations to leverage resources through funding of supplemental samples is critical to their ability to plan and evaluate their heart disease and stroke prevention and management programs. Likewise, the states, territories, and tribal organizations should implement their surveillance programs in a manner that facilitates the ability of localities to leverage resources to attain supplemental samples to support local efforts to prevent and manage heart disease and stroke. Several ethnic subgroups in the population are disproportionately affected by heart disease and stroke; hence, it is especially important to collect sufficient data to produce meaningful estimates for these populations. This recommendation was judged to be a high priority but will require developmental work; hence, intermediate staging may be more appropriate. This effort was estimated to be moderately costly in the near term, with long-term costs potentially much greater depending on the extent of oversampling implemented. The cost implications relate to the need to modify current sampling strategies to ensure that all states, territories, and tribal organizations are represented in all nationally representative surveys. This change would result in a modest reduction in data collection efficiency at the national level.

6. Mechanisms should be developed to enable linkage between healthcare data systems, including the national surveillance programs (eg, NAMCS, NHDS, and National Death Index), and electronic health records.

To facilitate surveillance, it is critical that federal and state efforts ensure that health information systems, including the national surveillance programs (eg, NAMCS, NHDS) and electronic health records are interoperable, utilize harmonized data standards, and have appropriate safeguards in place. Discussions between public and private stakeholders are currently taking place to determine how best to achieve these goals, including within the American Health Information Community. At present, health records and surveillance systems lack linkable unique health identifiers for individuals. This shortcoming has limited our ability to gain insights about the health of the public from these records and systems. Creative strategies will be necessary for linking information between systems in a manner that safeguards confidentiality. One such model worth noting is the eHealth Initiative record locator service, which facilitates the secure linkage of patient health records. The record locator service stores enough data to allow a person’s health records to be tied back to a master index. The record locator service is currently being tested by several communities. This model and others should be evaluated for their utility in supporting surveillance efforts. This high-priority recommendation could be highly expensive to implement, especially in the early stages; however, in the long term, the system would likely be of intermediate cost to maintain. Given the developmental work required, this recommendation was considered for intermediate staging.

7. Studies are needed to establish the validity of multiple measures collected by self-report and provider report in national databases.

Many of the data elements collected in current surveillance activities are based on self-report or provider report, and little information is available on the validity of these data. Given the complexity of the current surveillance system, validation efforts will be more expensive than necessary. Implementation of this recommendation might be more efficient after redundancies in the system have been minimized. This recommendation was judged to be a moderate priority for intermediate staging and to incur intermediate cost.

Recommendations for HP2010 Goals 1 and 2

8. Data collection in national surveys should be expanded to include important measures that are currently missing from the data collection process, including information on awareness, detection, treatment, and control of physical inactivity, unhealthy diet, cigarette smoking, and obesity.

Efforts to prevent and control lifestyle risk factors and obesity are impeded by lack of information on public knowledge of health risks and the progress of risk factor detection and control programs. If we were tracking awareness of unhealthy lifestyle habits, we could focus our awareness efforts more effectively. Similarly, if we were tracking detection, treatment, and control efforts based, for example, on self-reported or provider-reported data, we could focus our quality-improvement efforts more effectively. This recommendation was judged to be a high priority for early staging because of the importance of lifestyle factors in the origin of heart disease and stroke, as well as many other chronic diseases. Implementation of this recommendation would be relatively low cost, because the data systems already exist through which these elements could be collected (eg, NHANES and NAMCS).

9. The states, territories, and tribal organizations should develop surveillance capacity to support program planning, implementation, and evaluation, including the ability to conduct standardized surveys that include direct assessments of residents to enable collection of information on prevention, awareness, detection, treatment, and control of obesity, hypertension, dyslipidemia, and diabetes.

The data currently available at the state level on risk factor prevalence, detection, treatment, and control are based on self-report. Consequently, only persons who are aware of their risk factor status can provide information about their control status. Given that the states have been charged with developing programs to prevent and manage heart disease and stroke, directly measured data on risk factor prevalence, detection, treatment, and control are critical to program planning, imple-
mentation, and evaluation. This high-priority recommendation is judged to be high cost, owing to the cost of covering all states, territories, and tribal organizations. Strong efforts should be made to implement this recommendation at the earliest possible stage, because progress in reducing the burden of heart disease and stroke is highly dependent on effective action at the state and local level.

10. Indicators and systems for surveillance of policies and environmental conditions related to physical inactivity and unhealthy diet should be developed, tested, and implemented at the national, state, and local levels.

As an example, the State Tobacco Activities Tracking and Evaluation system is an electronic data warehouse that contains up-to-date and historical state-level data on prevention and control of tobacco use. The main topic areas presently being offered are behaviors, demographics, economics, funding, health consequences and costs, and legislation. The State Tobacco Activities Tracking and Evaluation system has provided the basis for generating important reports about state laws on tobacco control, thereby providing information to support tobacco-control efforts. Similar systems should be developed to provide data on physical inactivity and diet. This high-priority recommendation is judged to be appropriate for intermediate staging. It is likely to be low cost, potentially requiring only several staff members to collate information, maintain World Wide Web–based databases, and generate reports.

Recommendations for HP2010 Goals 3 and 4

11. Indicators and systems for surveillance of policies and environmental conditions (eg, proportion of the population covered by enhanced 9-1-1 systems) related to symptom knowledge and recognition, acute healthcare-seeking behavior, availability of automated external defibrillators, and capabilities of the prehospital care system (including first responders and emergency medical services) should be developed, tested, and implemented at the national, state, and local levels.

Early identification and treatment of acute episodes of heart disease and stroke are limited by patient recognition and response to symptoms and by the capacity of the prehospital care system to respond rapidly and appropriately to patients’ conditions. Information on the implementation and effectiveness of public education campaigns about symptom recognition and response, state and local policies pertinent to liability issues with the use of automated external defibrillators, the implementation and effectiveness of automated external defibrillator programs, and the capabilities of the prehospital care system (eg, enhanced 9-1-1 system, 12-lead ECGs in the field, dispatch and transport policies for suspected acute episodes of heart disease and stroke) are essential for the success of efforts to enhance early identification and treatment. This high-priority recommendation is judged to be appropriate for intermediate staging because it may be more easily accomplished after the establishment of the surveillance unit described in recommendation 1. This task is likely to be low cost, potentially requiring only several staff members to collate information, maintain World Wide Web–based databases, and generate reports. Additional pertinent information could be collected at low cost through the inclusion of additional questions (eg, about symptom knowledge) in existing surveys.

12. Effective surveillance methods should be developed, tested, and implemented to support the collection of data on patients with newly diagnosed heart disease, stroke, CHF, and PAD in the outpatient setting, including data on treatment and outcomes.

Increasingly, patients with heart disease and stroke (including CHF and PAD) are being diagnosed and treated in the outpatient setting. Reliance on hospital surveillance hinders efforts to monitor the burden of disease, including human suffering and other costs. Decreasing hospitalization rates may mislead policy makers into thinking that heart disease rates are decreasing when care may be simply shifting to the outpatient setting. Information on the broader spectrum of heart disease and stroke will enable policy makers to make better decisions on the need for outpatient care facilities and sustained prevention programs. This moderate-priority recommendation was judged to be moderately costly in the near term owing to the need for development work. Developmental work should begin as early as possible to support implementation at a later stage. Long-term costs will be difficult to estimate until surveillance models have been developed and tested but are likely to be high. If it proves possible to collect this information through the reportable disease system described above (recommendation 2) or through existing surveys (NAMCS), the long-term cost implications might represent only a moderate increase over the costs otherwise committed to the surveillance program.

Barriers

We have identified specific barriers to obtaining the new data elements that would be required to support the development of a comprehensive surveillance system. These include various methodological challenges, privacy concerns that have surfaced since the implementation of the Health Insurance Portability and Accountability Act (HIPAA), and the costs associated with supporting new data systems and a comprehensive surveillance system.

Methodological Challenges

Methodological challenges to establishing and supporting a comprehensive national surveillance system can be grouped into the following categories: limited data availability in the current surveillance systems, lack of standardized surveillance indicators, limitation of some current surveillance systems to persons living in households (exclusion of nursing home residents and other institutionalized persons), inability to link across data sources, and other limitations of current data elements related to validity, reliability, or specificity.

Interagency Coordination

Cultural barriers exist between governmental and nongovernmental agencies that can pose barriers to the processes of sharing data and functioning cooperatively. In some instances, competition might exist for future funding or even future existence. Models of cooperation, such as the HP2010 Partnership and others, should be examined, and the lessons learned should be applied to this effort to avoid these potential barriers.

Health Insurance Portability and Accounting Act

The implementation of HIPAA on April 14, 2003, created new barriers to the development of a comprehensive surveil-
lance system. Under HIPAA, the privacy rule establishes minimum federal standards for protecting the privacy of individually identifiable health information, and some states have more restrictive rules. The privacy rule applies to covered entities, which are health plans, healthcare clearinghouses, and healthcare providers who electronically transmit any health information in connection with transactions for which the Department of Health and Human Services has adopted standards. The privacy rule defines protected health information as individually identifiable health information, held or maintained by a covered entity or its business associates acting for the covered entity, that is transmitted or maintained in any form or medium (including the individually identifiable health information of non-US citizens). The rule confers certain rights on individuals, including rights to access and amend their health information and to obtain a record of when and why their protected health information has been shared with others for certain purposes.

The rule defines a set of 18 variables that could be used to identify an individual or the individual’s relatives, employers, or household members, including age, address, dates (eg, birth date, hospital admission and discharge dates, and date of death), telephone numbers, Social Security numbers, and medical record numbers. Protected health information may be shared by a covered entity under the following circumstances: (1) an individually signed privacy authorization form is obtained; or (2) data are deidentified by removing all 18 variables; or (3) a limited data set is created by removing all 18 variables except for dates and address, limited to town or city, state, and zip codes, in association with a data use agreement, that is, an agreement between the covered entity and the intended recipient that establishes the way in which the information in the limited data set may be used and how it will be protected; or (4) sharing is mandated by law (eg, cancer data for registries in some states).

The privacy rule adds time and costs to the development of a comprehensive CVD surveillance system. Privacy authorization forms are generally not available, so data management resources must be available at the covered entity to create deidentified or limited data sets. Limited data sets are preferable to deidentified data sets because they can contain event dates; only the year of the event is allowed in a deidentified data set, which makes it impossible, for example, to determine the sequence of events, including recurrences, within a year. Limited data sets require the development of data use agreements, which add complexity to the data acquisition and, in some instances, may require significant negotiation processes. Both deidentified and limited data sets exclude Social Security numbers so that it would be impossible to link provided data, such as hospitalizations, with other databases that contain the Social Security number, such as the National Death Index.

Some concern exists that hospitals and other health systems will be reluctant to provide data because HIPAA is perceived by them as a barrier to the use of any patient data. However, the act also states that “without individual authorization, a covered entity may disclose protected health information to a public health authority [or to an entity working under a grant of authority from a public health authority] that is legally authorized to collect or receive the information for the purposes of preventing or controlling disease, injury, or disability including but not limited to reporting of disease, injury, and vital events (eg, birth or death) and conducting public health surveillance, investigations, and interventions.” Thus, state health departments, the federal government, and entities working under their authorization for the purpose of public health surveillance should be able to convince health systems that reporting patient data for surveillance purposes may not require patient authorization under the privacy rule. It would be to the benefit of state health departments to request assistance from the state’s legal services to develop a written document that highlights interpretation of HIPAA, as well as the additional and separate issue of informed consent, to share with potential sources of hospital and medical systems data.

Costs

Cost represents a significant barrier to the establishment of a comprehensive surveillance system for heart disease and stroke. We have not provided a detailed estimate of cost in this publication because development of a surveillance system to support prevention of heart disease and stroke could be based on enhancements of current efforts; however, we have provided comments on the relative magnitude of costs associated with the major recommendations. Although the incremental cost is difficult to estimate, it is unlikely to represent >0.1% of the societal costs of CVD, estimated at $403.1 billion in 2006. If surveillance data were used to inform the planning, ongoing implementation, and evaluation of strategies to prevent heart disease and stroke, it is likely that the return on investment would be substantial in terms of both human health and healthcare costs.

Conclusions

The success of efforts to prevent and manage heart disease and stroke is dependent on the availability of surveillance data at the national, state, and local levels to assist federal agencies, state and local health departments, and their partners in assessing prevention and treatment priorities and guiding program planning, implementation, and evaluation. This statement summarizes the information that is needed at the national, state, and local levels to address the HP2010 and AHA goals for 2010; furthermore, this document was designed with a longer-term perspective in mind. When possible, existing data collection efforts have been identified for addition of new items. Significant gaps (eg, the complete lack of a data source for incidence and recurrence of heart attacks and strokes) and other deficiencies have been identified, and recommendations have been made for enhancement of the surveillance system in the United States. The most far-reaching recommendation may be the proposed designation of heart disease and stroke as reportable conditions across the continuum of care. This approach served to help focus attention on infectious diseases when infection control was the major public health imperative. A similar approach to heart disease and stroke is needed urgently. The other recommendations, although more narrowly focused in many instances, should result in the availability of better information for enhancing heart disease and stroke prevention and management programs. Implementation of all of the recommendations contained in this report would require commitment of substantial additional resources in addition to those already devoted to surveil-
lance. However, some opportunities for greater efficiency were identified that could lead to cost savings, and a staged rollout of these recommendations could mitigate the financial impact. Finally, the return on investment could be substantial in terms of better population health and fewer acute episodes of heart disease and stroke, resulting in fewer inflation-adjusted healthcare dollars being devoted to acute care. Consequently, this statement should serve as a guide to policy makers as they work with public health agencies to develop and implement a surveillance system that can contribute importantly to efforts to prevent heart disease and stroke.

Disclosures

Writing Group Disclosures

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<th>Other</th>
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<tbody>
<tr>
<td>David C. Goff Jr</td>
<td>Wake Forest University School of Medicine</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Lawrence Brass†</td>
<td>Yale University</td>
<td>Bristol Myers Squibb; Sanofi/Synthelabo</td>
<td>None</td>
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<tr>
<td>Lynne T. Braun</td>
<td>Rush University Medical Center</td>
<td>None</td>
<td>None</td>
<td>AstraZenea; Disnexus; Pfizer</td>
<td>None</td>
<td>None</td>
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<td>Nexcura Editorial Board</td>
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<tr>
<td>Janet B. Croft</td>
<td>CDC</td>
<td>None</td>
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<td>Judd D. Flesch</td>
<td>CDC Foundation</td>
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<td>Francis J.R. Foxkes</td>
<td>The University of Edinburgh</td>
<td>Sanofi/Synthelabo</td>
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<tr>
<td>Yuling H. K. Hong</td>
<td>American Heart Association and Penn State College of Medicine</td>
<td>None</td>
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<tr>
<td>Virginia Howard</td>
<td>University of Alabama at Birmingham</td>
<td>None</td>
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<td>NIH</td>
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<tr>
<td>Sara Hudson</td>
<td>University of North Carolina at Chapel Hill</td>
<td>None</td>
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<td>Stephen F. Jencks</td>
<td>Centers for Medicare and Medicaid Services</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Russell Luepker</td>
<td>University of Minnesota</td>
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<tr>
<td>Teri Manolio</td>
<td>National Heart, Lung, and Blood Institute</td>
<td>None</td>
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<td>Wayne Rosamond</td>
<td>University of North Carolina School of Public Health</td>
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<td>John Rumsfeld</td>
<td>Denver VA Medical Center</td>
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<td>Stephen Sidney</td>
<td>Kaiser Permanente</td>
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<td>Zhi-Jie Zheng*</td>
<td>National Heart, Lung, and Blood Institute</td>
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This table represents the relationships of writing group members that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit.

*Dr Zheng was affiliated with the Centers for Disease Control and Prevention at the time this statement was written.
†Deceased.

Reviewer Disclosures

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<tr>
<th>Reviewer</th>
<th>Employment</th>
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<th>Other Research Support</th>
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<tr>
<td>Stephen P. Fortmann</td>
<td>Stanford University Medical School</td>
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<td>None</td>
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<td>Robert J. Goldberg</td>
<td>University of Massachusetts Medical School</td>
<td>None</td>
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<td>Dilip K. Pandey</td>
<td>University of Illinois at Chicago</td>
<td>None</td>
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<tr>
<td>Ralph Sacco</td>
<td>Columbia University</td>
<td>NINDS*</td>
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NINDS indicates National Institute of Neurological Disorders and Stroke.

This table represents the relationships of reviewers that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all reviewers are required to complete and submit. A relationship is considered to be “significant” if (1) the person receives $10,000 or more during any 12-month period, or 5% or more of the person’s gross income; or (2) the person owns 5% or more of the voting stock or share of the entity, or owns $10,000 or more of the fair market value of the entity.

*Significant.
References


KEY WORDS: AHA Scientific Statement heart disease stroke peripheral vascular disease
An asymptomatic 54-year-old man, who had been on hemodialysis for 11 years because of chronic renal failure caused by chronic glomerulonephritis, was observed in our hospital. In November 2002, an aortic valve replacement was performed because of severe aortic stenosis. Preoperative echocardiography demonstrated mitral annular calcification (MAC) at the base of the posterior mitral annulus (Figure, A). Computed tomography of the heart also identified the MAC (Figure, B). In November 2004, follow-up echocardiography revealed enlargement of the MAC, which was detected as a spherical dense echogenic calcified mass (36×21 mm) with a central echolucent area (Figure, C). Trivial mitral regurgitation was observed. Electrocardiographic gated cardiac multislice computed tomography confirmed that the mass had a high density but also contained a low-density area that was thought to be soft tissue (Figure, D). Multislice computed tomography coronary angiography showed that the mass had no feeding artery. These findings suggested that this mass was a liquefaction necrosis of the MAC. Additional treatment was not performed because liquefaction necrosis of MAC usually has a benign prognosis. After 11 months, echocardiography and multislice computed tomography revealed that the size of the mass was reduced (16×6 mm), especially in the central liquefaction region (Figure, E and F). Doppler echocardiography also revealed moderate mitral regurgitation attributable to incomplete coaptation. Although the pathogenesis of MAC in chronic renal failure is related to calcium–phosphorus homeostasis, the levels of total serum calcium, serum phosphorus, and calcium–phosphorus product were not changed significantly during the observation period. These findings clearly demonstrated that liquefaction necrosis was a reversible form of MAC. Because mitral regurgitation with various grades could have been caused by the varying size of the MAC, further follow-up study was necessary for this case. Serial assessment was important in this particular disease.

Disclosures

None.
Two-dimensional echocardiography of the parasternal long-axis view and multislice computed tomography demonstrating mitral annular calcification (A and B). After 2 years, the parasternal long-axis view revealed a large echo-dense mass at the base of the posterior mitral leaflet (C). The mass had a central echolucent area. A high-density mass with a low-density area within it was seen on multislice computed tomography at the base of the posterior mitral leaflet (D). Echocardiography (E) and computed tomography (F) performed 11 months later showed that the size of the mass and lucent area within the mass had decreased. The abnormal calcified mass is identified on the echocardiogram with an arrow head (A, C, and E) and on multislice computed tomography with an arrow (B, D, and F).
A 60-year-old man with an acute stroke of unknown origin was referred to our institution for further diagnostic evaluation. Contrast-enhanced ECG-gated 64-slice spiral computed tomography depicted a well-defined, pedunculated, mobile, spherical lesion (density 69 ± 21 Hounsfield units) attached to the commissure of the left coronary and noncoronary aortic valve leaflet. The lesion showed a relatively homogenous inner structure with a slightly villous outer margin (Figure 1A through 1D). ECG-gated magnetic resonance imaging exhibited a solid lesion with intermediate signal intensity on both T1- and T2-weighted spin-echo sequences (Figure 2A and 2B). Transthoracic and multiplane transesophageal echocardiography also demonstrated a spherical solid structure within the aortic root, confirming the computed tomography and magnetic resonance imaging findings (Figures 3A and 3B and 4A through 4C). Dynamic computed tomography files (using the temporal information of the recorded 4D computed tomography dataset), cine magnetic resonance imaging (using dynamic steady-state free-precession gradient-echo sequences), and color-coded Doppler echocardiography demonstrated a competent aortic valve with a highly mobile, slightly deformable intraluminal mass (Movies I through IV). Surgery was performed and the tumor was completely removed. The gross pathological specimen appeared as a translucent, gelatinous mass (Figure 5A). Histological examination revealed a benign tumor with multiple papillary fronds of different size that consisted of an acellular matrix surrounded by a single layer of endothelial cells, leading to the definite diagnosis of papillary fibroelastoma (Figure 5B and 5C).

Disclosures
None.
Figure 1. Contrast-enhanced ECG-gated multislice spiral computed tomography. Color-coded, semitransparent, 3-dimensional reconstruction in angulated coronal (A), transverse (B), and sagittal (C) planes and color-coded endoluminal view (D) demonstrating a well-defined, pedunculated, spherical mass (arrows) located in both the left and noncoronary sinus slightly above the aortic valve.
Figure 2. ECG-gated magnetic resonance imaging. T1-weighted (A) and T2-weighted (B) spin-echo sequences showing a solid lesion (arrows) with intermediate signal intensity.

Figure 3. Transthoracic echocardiography (A) and multiplane transesophageal echocardiography (B) depicting a spherical echogenic structure (arrows) within the aortic root.
Figure 4. Submillimeter, multiplanar, reformatted computed tomography image across the aortic root (A) in direct comparison with corresponding images obtained with a steady-state free-precession gradient-echo magnetic resonance imaging sequence (B), and multiplane transesophageal echocardiography (C), all demonstrating a solid, intraluminal mass (arrows) attached to the commissure of the left coronary and noncoronary aortic valve leaflet.

Figure 5. A, The macroscopic specimen appeared as a translucent, gelatinous mass. B, The histological specimen in the hematoxylin & eosin stain (magnification ×10) shows a benign lesion with multiple papillary fronds. C, The papillary fronds consist of 3 layers comprising a collagenous core with low elastin content, an amorphous intermediate layer, and a delicate coat of single-layer endothelium as demonstrated in elastica–van Gieson stain (magnification ×50).
A 28-year-old man presented to the emergency department with progressive shortness of breath complicated by large hemoptysis. At 3 months of age, he had been diagnosed with a “hole in his heart” in the Ukraine and had undergone 3 coronary catheterizations (2 in Russia, 1 in India; results unavailable). His parents had declined options for treatment. After stabilization in the emergency department, he was transferred to coronary care, where echocardiography demonstrated a persistent ductus arteriosus (PDA) measuring 0.9 × 2.0 cm with a right-to-left shunt consistent with Eisenmenger syndrome. Left ventricular function was severely impaired (ejection fraction = 21%).

Cardiac 64-slice multidetector computed tomography (MDCT) was undertaken to provide optimal depiction of the PDA and main pulmonary artery (PA) and confirmed a widely patent PDA measuring 2.0 cm in largest diameter (Figure 1A). Functional MDCT cine multiphase reconstructions of the PDA revealed a bidirectional shunt (Movie I). Main, left, and right PAs were markedly enlarged (Figure 1B). Coronary MDCT evaluation demonstrated severe left main coronary artery (LM) compression (>90%) between the aortic sinus and the enlarged PA (Figure 1C). Additionally, coronary MDCT demonstrated a downward angulation of the LM with the left sinus of Valsalva of 11° (Figure 1D). Functional MDCT cine multiphase reconstructions demonstrated dynamic compression of the LM between the aortic sinus and PA during systole (Movie II and III) and confirmed severe global left and right ventricular dysfunction (Movie IV). Noncardiac findings included pulmonary hemorrhage and pulmonary parenchymal peripheral vascular pruning that were consistent with severe pulmonary hypertension and Eisenmenger syndrome (Figure 1E).

Coronary angiography confirmed LM stenosis (Figure 2; Movie V), and hemodynamic evaluation revealed a Qp/Qs of 1.05. Three days after catheterization, the patient suffered a cardiac arrest and underwent percutaneous transcatheter intervention with stent placement across the LM. The patient declined heart–lung transplantation and was discharged in stable condition. The patient remains event free at 4-month follow-up.

Left coronary artery compression syndrome was first described in 1957 and is characterized by compression of the LM between the aorta and an enlarged main PA.1 It is usually seen with a congenital cardiac defect, most commonly an atrial septal defect, ventricular septal defect, or tetralogy of Fallot.2 The association between an isolated PDA and left coronary artery compression is rare.3 Both the degree of LM compression and its angle with the left sinus of Valsalva <30° are thought to increase the likelihood of significant myocardial ischemia. A main PA/aorta diameter ratio >2 is considered an additional risk factor. Cardiac 64-slice MDCT provides a noninvasive method for evaluating the degree of dynamic LM compression throughout the cardiac cycle, angulation of the LM relative to the left sinus of Valsalva, evaluation of left and right ventricular function, and depiction of pulmonary pathology, making it a valuable investigation in the workup of patients suspected of left coronary artery compression.

None.

References

Disclosures
None.

The online-only Data Supplement, consisting of movies, is available with this article at http://circ.ahajournals.org/cgi/content/full/115/1/e7/DC1.
Figure 1. A, Oblique coronal multiplanar reformat demonstrated a large persistent ductus arteriosus (solid arrow) between the aorta and main pulmonary artery (hollow arrow), which is massively enlarged. B, Axial oblique multiplanar reformat demonstrated the enlarged main pulmonary artery (solid arrow) and right (hollow arrow) and left pulmonary arteries (curved arrow). Main pulmonary artery/aorta ratio was >2, which is consistent with pulmonary hypertension. C, Oblique sagittal image demonstrated severe compression of the left main coronary artery (arrow) between the aorta and the main pulmonary artery. D, The angle between the left main coronary artery and left sinus of Valsalva is 11° (normal subjects=90°). E, Pulmonary parenchymal evaluation revealed consolidation and ground glass opacity consistent with severe pulmonary hemorrhage (black arrow). Note the marked peripheral pulmonary vascular pruning (hollow arrows) and large central arteries (curved arrow).

Figure 2. Invasive coronary angiogram confirmed a short, compressed left main coronary artery.
Letter by Selvaraj and Chauhan Regarding Article, “Upsurge in T-Wave Alternans and Nonalternating Repolarization Instability Precedes Spontaneous Initiation of Ventricular Tachyarrhythmias in Humans”

To the Editor:

We read with interest the article by Shusterman et al1 concerning repolarization dynamics preceding spontaneous initiation of ventricular tachyarrhythmias in humans. Two related time-domain methods measured a larger magnitude of “alternans” at 30 minutes compared with 60 to 120 minutes preceding arrhythmia onset. Spectral analysis of T-wave amplitude showed a nonuniform increase in power in all frequency ranges. The authors interpret these findings as a surge in alternans and nonalternating repolarization complexity preceding the arrhythmia.

An increase in sympathetic activity preceding the onset of ventricular arrhythmia was reported by the authors in the same population.2 This can lead to increased noise in the ECG recordings from perspiration and an increase in motion and respiratory artifact.3

The modified moving average and, to a larger extent, the intrabeat average measurements are affected by noise that cannot be completely removed by preprocessing.3 Using these time-domain methods, the authors have previously emphasized the need to measure surrogate alternans in the isoelectric T end to P onset segment to show that an increase in T-wave alternans magnitude is not artifactually attributable to noise.3 It is unclear why the authors did not use a similar analysis of surrogate alternans in the present study1 to ensure that noise was not a confounder.

Using the spectral method, white noise can spuriously increase the spectral power in the alternans frequency, and subtraction of the spectral power in a “noise band” is required to measure true alternans.4 Therefore, the increase in spectral power seen in all frequency ranges could be explained by an increase in noise rather than an increase in “nonalternating repolarization complexity.” The possibility of subharmonics influencing the measured power in the alternans range is another important confounder, particularly respiratory subharmonics (0.15 to 0.25 cpb), which increase in spectral power before the tachyarrhythmia. The authors feel the contribution of respiratory subharmonics is improbable because the increase in T-wave alternans magnitude has been confirmed by 3 independent methods. However, the concordant findings do not exclude the confounding effects of subharmonics, because these methods are all inherently influenced by subharmonics.3 Although the increase in alternans may not be accounted for entirely by respiratory subharmonics, this is likely an important contribution and is not unexpected, because respiratory excursions that influence T-wave amplitude can increase during sympathetic surges preceding tachyarrhythmia. Thus, we feel that white noise and respiratory oscillations may significantly contribute to the apparent increase in alternating and nonalternating repolarization instability.

Disclosures
None.

Raja J. Selvaraj, MD
Vijay S. Chauhan, MD, FRCPC
Division of Cardiology
University Health Network
Toronto, Canada

Response to Letter Regarding Article, “Upsurge in T-Wave Alternans and Nonalternating Repolarization Instability Precedes Spontaneous Initiation of Ventricular Tachyarrhythmias in Humans”

We thank Drs Selvaraj and Chauhan for their interest in our work and discussion of the possible impact of white noise and respiration. We do not feel that white noise is a significant problem, although respiration (along with other physiological factors) may play a role, as emphasized in our report.

Impact of Noise

We used the “surrogate” analysis (ie, test of baseline stability) to control for white noise, respiration, movement, and other sources of artifacts. In addition, we removed noisy segments of data and used sectional (T-wave) averaging to reduce the level of white noise. Each data segment was carefully reviewed at high magnification at each processing step. The residual white noise was small (<3 μV), was relatively constant over time, and could not explain the dynamics of T-wave alternans (TWA) or other frequency components. The level of TWA was at least 2 times greater than all sources of noise combined. In addition, the assumption that changes in TWA could be a mere result of noise caused by elevated heart rate and sympathetic activity contradicts the observation that the magnitude of TWA before the arrhythmia was higher than at similar or faster heart rates during arrhythmia-free periods. Therefore, changes in repolarization before the onset of the arrhythmia cannot be explained by an increase in noise at faster heart rates or elevated sympathetic drive.

Impact of Respiration

We explicitly defined the “respiratory” range and emphasized that the greatest increase occurred in that range. Thus, the connection to respiration is clear. However, because the data on respiratory patterns were not available, we avoided speculations about the exact impact of respiration. Furthermore, changes in heart rate in the studied group were relatively small (8%), which does not support the assumption of major respiratory changes.

It is well known that spectral estimates of TWA in real-life recordings are affected by respiration. The magnitude of this effect, which is different for spectral and nonspectral techniques because of the different filtering properties of modified moving average and intrabeat average, needs to be estimated. Therefore, we have provided an explicit estimate of the possible impact of respiratory oscillations on TWA. This estimate shows that the increase in TWA could not be completely explained by this factor. Adding white noise would not change the relationship between spectral components.

Thus, we believe that our presentation of the results is appropriate. Our goal was to describe (not “interpret”) the high-risk periods before the onset of tachyarrhythmias. Although respiration may play some role, the exact role of each contributor requires further study.

Disclosures

Dr Shusterman has ownership interest in PinMed, Inc. A. Goldberg and Dr London report no conflicts of interest.

Vladimir Shusterman, MD, PhD
Anna Goldberg, BSc
Barry London, MD, PhD
Cardiovascular Institute
University of Pittsburgh
Pittsburgh, Pa

AHA Issues New Products
The following new products for the public and the healthcare professional are available through your local American Heart Association or by calling 1-800-AHAUSA1.

- **AHA Science Advisory: Detection of Chronic Kidney Disease in Patients With or at Increased Risk of Cardiovascular Disease.** This advisory presents recommendations for the detection of chronic kidney disease in patients with cardiovascular disease. Chronic kidney disease can be reliably detected with the combined use of the Modification of Diet in Renal Disease equation to estimate glomerular filtration rate and a sensitive test to detect microalbuminuria. Product code 71-0372.

- **AHA Science Advisory: Indications for Renal Arteriography at the Time of Coronary Arteriography.** This multispecialty consensus document describes the rationale for patient selection for screening renal angiography at the time of cardiac catheterization. Product code 71-0375.

- **AHA Scientific Statement: Assessment of Coronary Artery Disease by Cardiac Computed Tomography.** This scientific statement reviews the scientific data for cardiac computed tomography related to imaging of coronary artery disease and atherosclerosis. Product code 71-0373.


- **AHA Scientific Statement: Physiological Assessment of Coronary Artery Disease in the Cardiac Catheterization Laboratory.** This statement provides a logical approach to the use of coronary physiological measurements in the catheterization lab to assist both clinicians and investigators in improving patient care. Product code 71-0370.

- **AHA Scientific Statement: Promoting Physical Activity in Children and Youth: A Leadership Role for Schools.** This statement focuses on physical activity and describes a renewed and expanded role for schools in the area of physical activity. It also addresses the current state of affairs and summarizes the evidence supporting schools’ potential for effectively providing and promoting physical activity. This statement recommends several key changes in school policy and practice. Product code 71-0367.

- **AHA Scientific Statement: Reducing Delay in Seeking Treatment by Patients With Acute Coronary Syndrome and Stroke.** This statement summarizes the evidence that (1) demonstrates the benefits of early treatment, (2) describes the extent of the problem of patient delay, (3) identifies the factors related to patient delay in seeking timely treatment, and (4) reveals the inadequacies of our current approaches to decreasing patient delay. Suggestions for clinical practice and future research are also presented. Product code 71-0364.

- **AHA Scientific Statement: A Taxonomy for Disease Management.** This statement presents a taxonomy for disease management that describes critical program attributes and allows for comparisons across interventions. Routine application of the taxonomy may facilitate better comparisons of structure, process, and outcome measures across a range of disease management programs and should promote uniformity in the design and conduct of studies that seek to validate disease management strategies. Product code 71-0371.

- **AHA/ACC Guidelines for Secondary Prevention for Patients With Coronary and Other Atherosclerotic Vascular Disease: 2006 Update.** Since the 2001 update of the AHA/ACC consensus statement on secondary prevention, important evidence from clinical trials has emerged that further supports and broadens the merits of aggressive risk-reduction therapies for patients with established coronary and other atherosclerotic vascular disease, including peripheral arterial disease, atherosclerotic aortic disease, and carotid artery disease. This growing body of evidence confirms that aggressive comprehensive risk factor management improves survival, reduces recurrent events and the need for intervention procedures, and improves quality of life for these patients. Product code 71-0361.

- **AHA/ACC Science Advisory: Influenza Vaccination as Secondary Prevention for Cardiovascular Disease.** Evidence from cohort studies and a randomized clinical trial indicates that annual vaccination against seasonal influenza prevents cardiovascular morbidity and all-cause mortality in patients with cardiovascular conditions. Product code 71-0374.

- **AHA/ASA Guideline: Primary Prevention of Ischemic Stroke.** This guideline provides an overview of the evidence on various established and potential stroke risk factors and provides recommendations for the reduction of stroke risk. Product code 71-0356.
Meetings Calendar

AHA Meetings
These meetings are sponsored by the American Heart Association (AHA) and its scientific councils. For information, contact AHA, Scientific Meetings, 7272 Greenville Avenue, Dallas, TX 75231-4596; Fax 214-373-3406; E-mail scientificconferences@heart.org; or visit the Web site http://my.americanheart.org/portal/professional/conferencesevents

2007

Feb 7–9: International Stroke Conference 2007. San Francisco, Calif. The International Stroke Conference features more than 550 presentations that highlight the most recent advances in the basic sciences of cerebral circulation and brain function, clinical stroke research and outcomes, rehabilitation science, and surgery, as well as exhibitors showcasing the newest advancements in stroke products and services. This conference provides a forum to present recent scientific work related to stroke and cerebrovascular disease. More than 550 posters, oral presentations, and lectures will be featured. See Web site: http://strokeconference.americanheart.org

Feb 28–Mar 3: 47th Annual Conference on Cardiovascular Disease Epidemiology and Prevention in association with the Council on Nutrition, Physical Activity, and Metabolism. Orlando, Fla. The 47th Annual Conference on Cardiovascular Disease Epidemiology and Prevention in association with the Council on Nutrition, Physical Activity, and Metabolism is a scientific program that provides participants with the opportunity to learn about: (1) causes, mechanisms, and risk factors for atherosclerosis and other vascular diseases; (2) population trends in cardiovascular diseases and their risk factors; (3) results of cardiovascular disease treatment and prevention trials; (4) methods of population surveillance for cardiovascular disease and risk factors; (5) techniques in preventive cardiology; (6) the role of nutrition in cardiovascular disease; and (7) outcomes research in cardiovascular disease. See Web site: http://www.americanheart.org/presenter.jhtml?identifier=3038389

Apr 19–21: Arteriosclerosis, Thrombosis and Vascular Biology Annual Conference 2007. Chicago, Ill. This conference is sponsored by the Council on Arteriosclerosis, Thrombosis, and Vascular Biology and the Council on Nutrition, Physical Activity, and Metabolism. The meeting will focus on new developing research opportunities in the areas of arteriosclerosis, thrombosis, and vascular biology. Special lectures, discussion, and oral and poster presentations are planned. The meeting format provides opportunities for intense interaction among participants during sessions and breaks. The goal of this meeting is to bring together diverse disciplines within the arteriosclerosis, thrombosis, and vascular biology research communities to allow investigators to explore areas of cross-disciplinary interest. The program encourages cross-fertilization by examining new and emerging areas in lipids and lipoproteins, arteriosclerosis, thrombosis, and vascular biology in an informal setting. Sessions planned by each of the representative areas—that is, arteriosclerosis, thrombosis, vascular biology, and nutrition/physical activity/metabolism—will provide an opportunity for oral presentations of abstracts. See Web site: http://www.americanheart.org/presenter.jhtml?identifier=3039918

May 9–11: Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke Conference 2007. Washington, DC. The 8th AHA Scientific Forum on Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke is the premier meeting dedicated to the studying of patients’ experiences with cardiovascular care and leveraging these insights to improve the quality of cardiac and stroke care. The conference features several formats to facilitate interactive learning, sharing, and networking with international leaders in the field. Before the conference, workshops introducing the methods of quantifying and improving healthcare quality and advanced seminars on evolving research approaches are presented. The conference includes state-of-the-art plenary sessions, smaller and more interactive “breakout” sessions, and poster abstracts of the latest science in the field. A major focus of the conference is to support the career development of future leaders in quality of care and outcomes research. Thus, the conference actively fosters informal interaction among attendees and provides networking opportunities for early-career investigators. The meeting will focus on new and developing opportunities, initiatives, projects, policies, and research relevant to measuring and improving quality of care and outcomes for persons with or at risk for cardiovascular disease and stroke. Novel research methods for quantifying outcomes, new findings from clinical trials and observational studies, and translational research will also be presented. See Web site: http://www.americanheart.org/presenter.jhtml?identifier=3041715

Jul 28–Aug 1: 6th Hypertension Summer School. Fort Collins, Colo. Co-sponsored by the American Heart Association’s Council for High Blood Pressure Research and the Council on the Kidney in Cardiovascular Disease. This three-and-a-half-day school will focus on areas of epidemiology and treatment of
hypertension, basic research in hypertension and clinical research in hypertension. To be considered for selection, candidates are required to submit a completed and signed application form. See Web site: http://www.americanheart.org/presenter.jhtml?identifier=3043738

Jul 29–Aug 10: 33rd 10-Day Seminar on the Epidemiology and Prevention of Cardiovascular Disease. Tahoe City, Calif. The primary goal of the seminar is to provide an intensive introduction to the epidemiology and prevention of major cardiovascular diseases for qualified health professionals planning careers in research, teaching or practice in this area. Candidates must be at the postdoctoral level with some residency training or its equivalent. Consideration for selection requires: (1) a completed and signed application form; (2) a letter of nomination from a sponsor well acquainted with the candidate’s experience and interest; sponsor must be from the candidate’s institution and indicate the availability of financial support for travel and accommodations; (3) a letter prepared by the candidate indicating the basis for his or her interest in the seminar; and (4) the candidate’s curriculum vitae. See Web site: http://www.americanheart.org/presenter.jhtml?identifier=3043315.

Sep 26–29: 61st High Blood Pressure Research Annual Conference 2007. Tucson, Ariz. The two-and-one-half-day scientific program gives physicians and research investigators an opportunity to enhance their knowledge, advance their skills, and learn about the latest developments in research pertaining to hypertension, stroke, kidney function, obesity, and genetics. The program will include state-of-the-art lectures and more than 350 oral and poster abstract presentations and discussions led by authorities. Registration opens May 2, 2007. See Web site: http://www.americanheart.org/presenter.jhtml?identifier=3043476

Nov 4–7: Scientific Sessions 2007. Orlando, Fla. Scientific Sessions encompasses 4 days of invited lectures and investigative reports. Simultaneous presentations represent all fields of cardiovascular and related disciplines. The program will include more than 3500 basic, clinical, and population science abstract presentations; plenary, special, and how-to sessions, morning programs, and cardiovascular seminars; clinical practice sessions focusing on current standards of care for practicing clinicians; translational science sessions that bring together basic scientists and clinicians; and “Ask the Experts” luncheons and in-depth subspecialty updates. There will also be pre-Sessions symposia. See Web site: http://scientificsessions.americanheart.org

Other Meetings of Interest—Domestic

2007

Feb 15–18: 23rd Annual Computed Body Tomography 2007: The Cutting Edge. Orlando, Fla. This seminar will provide a comprehensive review of recent advances in computed body tomography focusing on 16- and 64-slice MDCT. A series of focused lectures have been designed to concentrate on specific topics in depth. Participants will have the opportunity to expand their knowledge of the latest concepts in multidetector-row CT, CT angiography, the value of high-resolution CT in the chest, the uses of CT in the GI tract, cardiac CT and coronary artery imaging, and PET/CT in oncology. There will be time for questions and discussion. Optional hands-on workstation training will be available. For more information, e-mail cmenet@jhmi.edu. See Web site: http://www.hopkinscme.net

Feb 22–24: Cardiovascular Topics at Johns Hopkins. Baltimore, Md. This activity is clinically oriented, including case presentations, updates on clinical practice and reviews of multicenter trial data, but with an emphasis on the scientific underpinnings of cardiology practice. Subjects range from ion channels to outcomes research, and presentations are given by faculty who are in day-to-day practice, drawn from the Hopkins cardiology division and related specialties of pediatric cardiology, neurology, renal medicine, and cardiac and vascular surgery. “Meet the Professor” sessions offer the opportunity to bring interesting or problem cases to stump or share with the clinical experts. For more information, e-mail cmenet@jhmi.edu. See Web site: http://www.hopkinscme.net

Mar 27–Apr 1: Metabolic Syndrome and Cardiovascular Risk (Z2). Steamboat Springs, Colo. This meeting aims to integrate approaches from basic and translational science, with an overall goal of understanding (1) how to translate this science into more optimal lifestyle management and (2) the prospects for therapeutic intervention. Keystone Symposia is a nonprofit organization dedicated to “Connecting the Scientific Community.” See Web site: http://www.keystonesymposia.org

Apr 10–14: National Kidney Foundation 2007 Clinical Meetings. Orlando, Fla. CM.07 offers innovative educational opportunities for physicians, fellows and residents, pharmacists, physician assistants, nurse practitioners, nephrology nurses and technicians, renal and clinical dietitians, and nephrology social workers. The program is designed to create an interactive environment to encourage the flow of ideas between all members of the kidney healthcare team. The National Kidney Foundation is the major voluntary health
The American Heart Association welcomes announcements of interest to physicians, scientists, researchers, and others concerned with cardiovascular and cerebrovascular medicine. All copy is reviewed by the Scientific Publishing Department and Science and Medicine Resources. Content may be edited for style, clarity, and length. Copy should be sent to Publications—AHA News & Meetings Calendar, American Heart Association, Scientific Councils, 7272 Greenville Ave, Dallas, TX 75231-4596; Fax 214-691-6342; E-mail Scientific.Publishing@heart.org

organization dedicated to preventing kidney and urinary tract diseases, improving the health and well-being of individuals and families affected by these diseases, and increasing the availability of all organs for transplantation. See Web site: http://www.nkfclinicalmeetings.org

Other Meetings of Interest—International

2007

Mar 8–10: Carotid Disease and Stroke Symposium—Bringing Basic Science Into Clinical Practice. Stockholm, Sweden. The Department of Vascular Surgery at the Karolinska University Hospital and Institute has arranged three symposia under the heading “Bringing Science Into Clinical Practice.” The meeting is based on lectures by invited experts in the field of cerebrovascular disease and is the fourth symposia in a series. It will focus on vascular reconstruction for cerebrovascular disease. Vascular surgeons, as well as specialists in adjacent fields, will be provided with the basis for current knowledge of treatment for cerebrovascular disease in general and carotid stenosis in particular. Sessions include the symptomatic patient, imaging of symptomatic and asymptomatic carotid disease, surgical treatment of carotid stenosis, and endovascular and medical treatment. See Web site: http://www.congress.se/vascular2007

Oct 7–10: 7th International Congress on Coronary Artery Disease—From Prevention to Intervention (ICCAD 7). Venice, Italy. The meeting will follow the format of the very successful previous ICCAD Coronary Artery Disease meetings and will provide a comprehensive update on coronary disease in all its aspects. Keynote lectures will be delivered by a distinguished international faculty, while a large number of selected free communications will report new data from basic research laboratories and clinical centers around the globe. The program will include sessions on molecular mechanisms, gene therapy and cell therapy, epidemiology and prevention, and clinical aspects. There will be a major focus on new frontiers in interventional cardiology and on the surgical management of coronary disease. A new feature will be a fast track for recent and “about-to-break” clinical trials. For more information, contact coronary@kenes.com or phone +41-22-908-0488. See Web site: http://www.kenes.com/cad7

Oct 14–16: 5th International Meeting on Intensive Cardiac Care. Tel Aviv, Israel. Three parallel sessions, with more 100 presentations, will be featured. A parallel nursing stream will also be provided. For details, contact seminars@isas.co.il. See Web site: http://isas.co.il/cardiac-care2007

Nov 8–11: Fifth International Congress on Vascular Dementia. Budapest, Hungary. Attendees shall have an opportunity to deliberate on large- and small-vessel brain diseases and how they contribute to cognitive decline. There will also be an opportunity to identify the specific psychological markers, if any, of vascular dementia, and also the genetic factors involved. The overlap with Alzheimer’s disease will be a central issue, as will be the white matter changes frequently seen in vascular dementia. For details, contact vascular@kenes.com. See Web site: http://www.kenes.com/vascular
Cardiology, like life in general, is changing rapidly in Latvia. Just 15 years ago, the country separated itself from the Soviet empire and gained independence (Figure 1). “It has totally changed after the Soviet time,” says Dr Andrejs Erglis, chief of the Latvian Centre of Cardiology at Paul Stradins Clinical University Hospital. “With independence has come an increase in cardiology problems. Mortality from cardiovascular diseases was higher in the mid-1990s compared to the beginning of that decade.”

Dr Erglis believes that this change occurred because of a bad economic and medical situation. He says, “In Latvia, as in all Eastern European countries, everything had totally changed by the mid-1990s. It was very difficult psychologically for people to go from the Soviet system to capitalism.” He continues, “Social protection was suddenly much reduced. And, of course, the collection of statistics had probably improved, perhaps skewing the picture slightly.”

Dr Erglis explains, “For countries in the West, like England, 10 years is not such a big time frame. But for Latvia, 1996 and 2006 are just not comparable because the improvements are happening so fast.” For example, new houses are being built in large numbers, with so many plans being submitted that there is a crisis in the market.

There is a shortage of nurses in the Latvian health service, but plenty of doctors. But Dr Erglis says, “We are afraid young doctors will go to better countries.” Job offers are coming in from other parts of Europe, including Sweden and England.

Medicine From the Pre-Soviet Era to Date.
Historically, medicine and cardiology have been good in Latvia. During the period from 1920 to 1940, medical care was of a high standard. “We were in fourth place in Europe as regards medical students per million of the population,” says Dr Erglis. “At this time, the quality of medical practice was quite good. Medical education was not so bad, but in the Soviet era we had absolutely no technology. But we know that modern cardiology is based on technology. For this reason, we had practically no modern cardiology in Latvia when we started trying to catch up in 1990.” The situation began to change in the mid-1990s when the country gained its first digital angiography machine.

Percutaneous coronary intervention (PCI) began in Latvia in 1990, and the numbers quickly shot up. There were 1000 PCIs in 2001, and in 2006 there are expected to be 4500. Dr Erglis points out, “So you can see that it is practically impossible to compare the situation with that in Western Europe.” This late development allowed Latvia to avoid some of the mistakes made by other countries. “We had nothing,” says Dr Erglis. “It is easier to build than to renovate. If you start to build in an empty place, you can do it faster.”

Latvia could see what was happening in cardiology in the rest of the world and wanted that for itself. Two years ago, a special programme for the development of cardiology in the country was begun with the government. Six angiographic systems were bought through a government-organised tender. An educational programme was begun to train staff in angiography and PCI. One of the goals was to move things from the centre out to the periphery.

Cardiology Training
Dr Erglis did his cardiology training in Latvia. At the age of 25, in 1990, he carried out his first angioplasty there. He says,
For years cardiology has focused on treatment with drugs and interventions, but now the focus is turning more towards prevention and rehabilitation. With the increasing interest in these topics, the European Society of Cardiology (ESC) formed the European Association for Cardiovascular Prevention and Rehabilitation (EACPR). The EACPR was officially launched at the ESC Congress in Munich in August 2004. It was formed from a merger of 2 ESC Working Groups, Cardiac Rehabilitation and Exercise Physiology (WG1) and Epidemiology and Prevention (WG13).

EACPR president Dr Hugo Saner, who is professor of

Developments in European Preventive Cardiology

The cardiology spotlight in Europe is moving from drugs and interventions to prevention and rehabilitation. Hugo Saner, MD, president of the European Association for Cardiovascular Prevention and Rehabilitation, talks to Ingrid Torjesen, BSc.

Figure 2. Dr Erglis with his interventional cardiology team. “PCI is now good in Latvia,” he says.

Figure 3. Waiting lists for angiography are long, but the situation is improving as new centres open.

not ready for an organised flow of patients from other countries. Morbidity is still high in Latvia, and we still have a problem just caring for our own patients. Waiting lists are long, more than 56 weeks for angiography, but the situation is improving as new centres open,” says Dr Erglis (Figure 3).

The Latvian Health System

With the healthcare system in Latvia, the government pays for treatment and there is a budget that allows for procedures. Several years ago, prices for each procedure were defined, and each year the amount that can be spent is specified. At present, each PCI is priced at €5000. “We had so many discussions with politicians in official and unofficial ways,” says Dr Erglis. “For this reason, I can say that in the last 2 years, the budget for PCI procedures was doubled.”

The next big project planned is the setting up of a government foundation that will maintain a registry of hospitals, conditions, treatments, and outcomes. This registry will enable audits to be carried out, so that areas for further improvement can be identified.

One of the main contributors to the high mortality from cardiovascular diseases in Latvia is diet. “The right diet is expensive,” says Dr Erglis. “In the mid-1990s, rural Latvians ate too much fat, with milk, cottage cheese, butter, and meat, rather than fish, being the primary culprits.” Smoking and alcohol abuse also contribute. The situation is improving with education via newspapers and television, but Dr Erglis points out that the improvement is happening slowly. “It’s very difficult to change traditions and change minds. The most difficult thing is to change our thinking.”

Jennifer Taylor is a freelance medical writer
cardiovascular prevention and rehabilitation at the Swiss Cardiovascular Centre, Bern, Switzerland, explains that although the association is called the European Association for Prevention and Rehabilitation, it is focused on prevention. “With prevention you always think about primary prevention, but with rehabilitation we really mean secondary prevention,” he says.

“In Europe, secondary prevention is not paid for. Health insurance companies pay for rehabilitation programmes but not for prevention programmes. Therefore we have to still stick with this name and provide secondary prevention through rehabilitation programmes.” However, he predicts that if attitudes change, the association is likely to be renamed the European Association for Preventive Cardiology.

Although a young organisation, the EACPR already has around 1500 members. It is the umbrella for all the groups within the ESC that deal with cardiovascular prevention and rehabilitation. Dr Saner says, “We now have a common mission to promote excellence in research practice, education, and policy in cardiovascular prevention and rehabilitation in Europe, and we have a clear horizontal and vertical structure for our activities.”

The association is divided into 6 nuclei: sports cardiology, exercise physiology, rehabilitation, basic science, prevention and health policy, and epidemiology and public health. Each nucleus has a core of 10 to 12 distinguished specialist members in the field, who come together and share their expertise on EACPR committees such as the scientific committee, guideline committee, and education committee. “This means that if we deliver educational programmes, guidelines, or scientific statements or are organising our congress, we really have an in-depth discussion that includes all specialists involved in cardiovascular prevention and rehabilitation,” says Dr Saner. He continues, “This will give much more importance and weight to our activities and our statements.”

The EACPR provides guidelines and recommendations and sets standards through accreditation and its fellowship programme. It also develops and validates risk assessment methods and is working on instruments to evaluate physical activity, nutrition, psychosocial risk factors, and quality of life to help ensure that reliable and comparable data is produced in Europe. The EACPR has selected lifestyle as its main focus and will direct its efforts towards improving physical activity and nutrition and cutting smoking and psychosocial stress. Initially, it is concentrating on physical activity.

Dr Saner explains, “We have selected physical activity as a target for the moment because that is where we have the most experts in our association, including experts on both exercise physiology and sports cardiology. This also makes us an interesting partner for companies dealing with exercise and sports, such as Nike, Adidas, and Reebok.”

The lifestyle focus will give the EACPR an opportunity to gain more independence from pharmaceutical companies, which to date have been the main sponsors of the association’s activities.

Dr Saner says, “The EACPR’s main objective for next year is to give clear structure and clear tasks to the association and to start making connections with European health ministers, within the ESC, and with general practitioners, so that at all levels we make contacts and start cooperation with the corporate world.” The EACPR plans to write to every European health ministry at least once a year to update them on prevention and rehabilitation: what should be done, how it could be done, and which corporate partners would be appropriate. In addition, the EACPR plans to send all general practitioners in Europe a lifestyle newsletter in their local language at least 3 times a year to inform them about what is new in lifestyle management and prevention.

Dr Saner has always been convinced that there is a place for lifestyle change, as well as drugs and interventions, in cardiology. He started the first comprehensive cardiovascular prevention and rehabilitation programme in Europe in Olten, Switzerland around 20 years ago. “I was convinced that in order to motivate the patients you have to have a broad perspective and do something that they can continue to do in daily life,” he recounts. “I tried to integrate my preventive interventions into the daily life of the patients. So, besides gymnastics and machines, we started to do hiking with the patients and bicycling in order to encourage them to cycle to work, school, or whatever. We also have cooking classes and things like that.”

Patients attend the 12-week programme 3 times a week and it has turned out to be a successful model, copied more than 30 times in Switzerland and also elsewhere in Europe: first in Vienna, Austria and then in Cologne, Germany.

Ten years ago Dr Saner joined the University of Bern, when the university became the first in Europe to integrate cardiovascular prevention and rehabilitation in the cardiology department and in the training and teaching of residents and fellows. Most universities still do not integrate these subjects into cardiology training.

The University of Bern now has the most important cardiovascular prevention and rehabilitation programmes in Europe. Its 12 different rehabilitation programmes include
ones for regular patients, senior patients, and patients with heart failure, peripheral arterial disease, diabetes, metabolic disease, or obesity. These programs are a model for the EACPR, which has a goal that, within 10 years, there will be a prevention centre at every medium and large hospital in Europe. Every 2 years, the University holds a European fellows training course on how to set up a cardiac rehabilitation and prevention programme and how to improve an existing programme. This course usually attracts attendees from more than 20 European countries.

Dr Saner is pleased by the growing interest in prevention and rehabilitation in cardiology. At the last ESC World Congress in Barcelona, Spain in September 2006, almost 20% of all sessions involved some aspect of prevention or rehabilitation. “It is not a declaration that the ESC wants to make this topic more important; it is simply a fact that we get increased attendances for our topics,” he says. He predicts that this interest will continue to grow now that many are questioning the safety of drug-eluting stents. “It is just the right time to discuss changing lifestyle combined with optimal drug treatment instead of relying on these interventions,” Dr Saner says. “It is very fashionable.”

Dr Saner takes encouragement from these developments saying, “It is a nice time for me, because 20 years ago I was like a preacher in the desert, perhaps even 10 years ago. Everyone was fascinated by the technical interventions, and I was regarded as less interesting and not so important. But now over the past few months this attitude has changed considerably.” And Dr Saner practises what he preaches. Every week he still goes back to the small hospital in Olten where he launched and still runs a prevention and rehabilitation programme, and every week he hikes with his rehabilitation patients to set a good example.

Ingrid Torjesen is a freelance medical writer.

The opinions expressed in Circulation: European Perspectives in Cardiology are not necessarily those of the editors or of the American Heart Association.

15–16 February
Annual Meeting of the Belorussian Scientific Society of Cardiologists
Minsk, Belarus
For more information, contact bssc@cardio.by

20–23 February
34th Annual Congress of the Egyptian Society of Cardiology
Cairo, Egypt
For more information, contact fathiaalsaid@link.net

23–24 March
7th Annual Spring Meeting on Cardiovascular Nursing: “Changing Practice to Improve Care”
Manchester, United Kingdom
For more information, contact cardiology@conferencesearch.co.uk

28–31 March
6th International Workshop on Interventional Pediatric Cardiology
San Donat Milanese (Milan), Italy
For more information, contact info@workshopIPC.com

12–16 February
Cardiology Update 2007: Educational Programme
Davos, Switzerland
For more information, contact uwe.fritz@congressorg.ch

Editor: Thomas F. Lüscher, MD, FRCP, FACC
Managing Editor: Keith Barnard, MB, BS, MRCS, LRCP
We welcome your comments. E-mail the managing editor at Keith.Barnard@wolterskluwer.com