Calcific Aortic Valve Disease: Not Simply a Degenerative Process: A Review and Agenda for Research From the National Heart and Lung and Blood Institute Aortic Stenosis Working Group

Executive Summary: Calcific Aortic Valve Disease – 2011 Update

Nalini M. Rajamannan, Frank J. Evans, Elena Aikawa, K. Jane Grande-Allen, Linda L. Demer, Donald D. Heistad, Craig A. Simmons, Kristyn S. Masters, Patrick Mathieu, Kevin D. O’Brien, Frederick J. Schoen, Dwight A. Towler, Ajit P. Yoganathan and Catherine M. Otto

Circulation. 2011;124:1783-1791
doi: 10.1161/CIRCULATIONAHA.110.006767

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/124/16/1783

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
Calcific Aortic Valve Disease: Not Simply a Degenerative Process

A Review and Agenda for Research From the National Heart and Lung and Blood Institute Aortic Stenosis Working Group

Executive Summary: Calcific Aortic Valve Disease – 2011 Update

Nalini M. Rajamannan, MD*; Frank J. Evans, PhD; Elena Aikawa, MD, PhD; K. Jane Grande-Allen, PhD; Linda L. Demer, MD, PhD; Donald D. Heistad, MD; Craig A. Simmons, PhD; Kristyn S. Masters, PhD; Patrick Mathieu, MD; Kevin D. O’Brien, MD; Frederick J. Schoen, MD, PhD; Dwight A. Towler, MD, PhD; Ajit P. Yoganathan, PhD; Catherine M. Otto, MD

Calcific aortic valve disease (CAVD) encompasses the range of disease from initial alterations in the cell biology of the leaflets to end-stage calcification resulting in left ventricular outflow obstruction. The first detectable macroscopic changes in the leaflets, seen as calcification, or focal leaflet thickening with normal valve function, is termed aortic valve sclerosis, but it is likely that the initiating events in the disease process occur much earlier. Disease progression is characterized by a process of thickening of the valve leaflets and the formation of calcium nodules—often including the formation of actual bone—and new blood vessels, which are concentrated near the aortic surface. End-stage disease, e.g., calcific aortic stenosis, is characterized pathologically by large nodular calcific masses within the aortic cusps that protrude along the aortic surface into the sinuses of Valsalva, interfering with opening of the cusps. There is no disease along the ventricular surface. For decades, this disease was thought to be a passive process in which the valve degenerates with age in association with calcium accumulation. Moreover, although CAVD is more common with age, it is not an inevitable consequence of aging. Instead, CAVD appears to be an actively regulated disease process that cannot be characterized exclusively as senile or degenerative.

The National Heart, Lung, and Blood Institute convened a group of scientists from different fields of study, including cardiac imaging, molecular biology, cardiovascular pathology, epidemiology, cell biology, endocrinology, bioengineering, and clinical outcomes, to review the scientific studies from the past decade in the field of CAVD. The purpose was to develop a consensus statement on the current state of translational research related to CAVD. Herein, we summarize recent scientific studies and define future directions for research to diagnose, treat, and potentially prevent this complex disease process.

Normal Aortic Valve Anatomy and Function

Key Structure-Function Correlations

Heart valves permit unobstructed, unidirectional forward flow through the circulation. Valve components must accomplish the second-to-second movements necessitated by the cardiac cycle and must maintain sufficient strength and durability to withstand repetitive and substantial mechanical stress and strain over many years. The functional requirements of the heart valves are accomplished by a specialized set of cells and heterogeneous extracellular matrix, arrayed in a spatially specific and differentiated tissue structure, that are temporally dynamic and highly responsive to the external biomechanical environment.

The aortic valve (AV) provides a paradigm for valvular structural specialization and tissue dynamics, as viewed by echocardiography and bioreactor models (Figure 1A). The direction of flow during systole allows the valve cusps to open as the blood flows across the open AV leaflets. The inflow surface is located along the direction of flow, as indicated in
Figure 1A. The outflow surface is demonstrated in the diastole as the valves are closed, and there is end-diastolic pressure closing the valve leaflets along the outflow surface. Individual AV cusps attach to the aortic wall in a semilunar fashion, ascending to the commissures and descending to the basal attachment of each cusp. In the closed phase, under the backpressure from the blood in the aorta, the AV cusps stretch and coapt and, thereby, occlude the orifice. Pulmonary valve structure is analogous to the structure of the AV, consistent with the lower-pressure environment. During diastole, the tissue of the cusps is stretched via a backpressure; during systole, the cuspal tissue becomes relaxed and shortens owing to the recoil of elastin, which was elongated and taut during diastole.

All 4 cardiac valves have a similarly layered architectural pattern composed of cells, including the valvular endothelial cells (VECs) at the blood-contacting surfaces, the deep valvular interstitial cells (VICs), and valvular extracellular matrix (VECM), including collagen, elastin, and amorphous extracellular matrix (predominantly glycosaminoglycans). The AV has a dense collagenous layer close to the outflow surface and continuous with valvar supporting structures, which provide strength: the fibrosa, a central core of loose connective tissue; the spongiosa, rich in glycosaminoglycans; and a layer rich in elastin below the inflow surface, the ventricularis, as shown in Figure 1A. The glycosaminoglycan-rich spongiosa facilitates the relative rearrangements of the collagenous and elastic layers during the cardiac cycle. Moreover, the diverse characteristics of the cell phenotype, such as smooth muscle α-actin, are associated with distinct locations within the valve leaflet in situ. In vitro, this heterogeneity of phenotype is consistently demonstrated in primary cultures of VICs. Only recently, however, has it become possible to identify and characterize the behavior of discrete subpopulations of valvular cells using methods such as cloning and subculturing based on differential adhesion. These approaches have been used to demonstrate that discrete valvular cell subpopulations have unique morphological characteristics, synthesis of extracellular matrix, potential for calcification and ossification, and potential for promoting angiogenesis. These latter 2 characteristics are particularly relevant to calcific valve disease, and hence these methods offer the potential for determining whether selected groups of cells within the entire population undergo specific pathological changes that drive valve remodeling and mediate the progression of disease, which is the calcified valve leaflet as depicted in Figure 1C.

Cardiac Valve Cell Types: Valvular Interstitial Cells

VICs are abundant in all layers of the heart valves, and are crucial to function. VICs synthesize VECM and express...
matrix-degrading enzymes (including matrix metalloproteinases and their inhibitors) that mediate and regulate remodeling of collagen and other matrix components. VICs comprise a diverse, dynamic, and highly plastic population of resident cells. They modulate function among phenotypes in response to changes in stimulation by the mechanical environment or by certain chemicals during valvular homeostasis, adaptation, and pathology. Adult heart valve VICs in situ have characteristics of resting fibroblasts; they are quiescent, without synthetic or destructive activity for extracellular matrix. VICs are activated during intrauterine valvular maturation, by abrupt changes in the mechanical stress state of valves, and in disease states, and VICs continuously repair a low level of injury to the VECM that occurs during physiological functional remodeling of AV tissue.

The Table demonstrates the phenotypic transitions of the VIC cells, which are critical for normal development, homeostasis, and function of the AV, and likely mediate the development of valve calcification. Once activated, VICs can differentiate into a variety of other cell types, including myofibroblasts and osteoblasts, although valve osteoblasts may respond to cellular signals differently than skeletal osteoblasts.

Valvular Endothelial Cells
VECs resemble endothelial cells elsewhere in the circulation in some respects. However, they are phenotypically different from VECs in the adjacent aorta and elsewhere in the circulation. VECs probably interact with VICs to maintain the integrity of valve tissues and potentially mediate disease. Evidence indicates that different transcriptional profiles are expressed by VECs on the opposite (ie, aortic and ventricular) sides of the aortic valve, and some investigators have hypothesized that these differences may contribute to the typical localization of early pathological AV calcification, predominantly near the outflow surface secondary to inhibitors along the inflow surface.

Studies indicate that abnormal hemodynamic forces (such as hypertension, elevated stretch, or shear stresses) experienced by the valve leaflets can cause tissue remodeling and inflammation, which may lead to calcification, stenosis, and ultimate valve failure.

### Normal Cardiac Valve Development

VIC and VEC phenotypes, critical for maintaining valve function, change throughout life in response to environmental stimuli, as demonstrated in recent studies using quantitative histological assessment of human semilunar valves obtained from fetuses, neonates, children, and adults. VECs express an activated phenotype throughout fetal development (eg, vascular cellular adhesion molecule-1, intercellular adhesion molecule-1). Numerous signaling pathways have been proposed and tested in the critical pathways that promote endothelial-mesenchymal transition in the valves. In addition, VIC density, proliferation, and apoptosis are significantly higher in fetal than adult valves. A trilaminar architecture appears by 4–6 weeks of gestation, but remains rudimentary in comparison with that of adult valves. These data of the natural history of cell and matrix changes in valve development extend the paradigm that cardiac valves can adapt to pathological conditions, which suggests similar molecular mechanisms in physiological and pathological cell activation.

### Pathobiology of CAVD

Calcific AV stenosis has characteristic pathological features of an osteoblast phenotype. The calcific process begins deep in the valvular tissue, near the margins of attachment. In advanced disease, the nodules extend through the outflow surfaces of the cusps and are nearly transmural. An early morphological stage of the calcification process is called AV sclerosis. In the later stage, AV stenosis, the functional valve area is decreased sufficiently to cause measurable obstruction to outflow and a significant gradient from the left ventricle to the aorta.

Lipids also play an important initiating role in the cell signaling of vascular and valvular calcification. Surgical pathological studies have shown the presence of oxidized low-density lipoprotein (LDL) in calcified valves. Patients with homozygous familial hypercholesterolemia provide an opportunity to test the hypothesis that lipids play a role in the development of calcific aortic stenosis, because these patients have extremely elevated levels of LDL choles-
terol without other traditional risk factors for coronary artery disease.17–20

Renin-Angiotensin Signaling Pathway
Angiotensin-converting enzyme is expressed and colocalizes with LDL in calcified AVs.21 In addition, an observational study showed the slowing of progression of AV disease in patients taking angiotensin-converting enzyme inhibitors in comparison with those not taking this therapy.22 This study is the first to demonstrate this novel signaling pathway in CAVD, which is still preliminary and somewhat controversial, but it is promising for the future potential to target this pathway with angiotensin-converting enzyme inhibitors and angiotensin receptor blockade in the early stages of the disease.

Initiating Events: Oxidative Stress
In the presence of cardiovascular risk factors, similar to vascular atherosclerosis, an early event is the presence of abnormalities in oxidative stress. This has been demonstrated in abnormal endothelial nitric oxide synthase function, which decreases normal physiological levels of nitric oxide along the valve endothelium.23,24 In atherosclerotic plaques, increased oxidative stress seems to be due primarily to increases in NAD(P)H oxidase activity.25 In calcified stenotic human26 and murine AVs,27 levels of superoxide and hydrogen peroxide are markedly increased. In addition, the uncoupling of nitric oxide synthase23–26 may play an important role in the generation of superoxide in calcified AVs.

Calcifying Phenotype: Myofibroblast Osteoblastogenesis
The initial confirmation of pathological bone in the AV was demonstrated by bone histomorphometry28 and osteogenic gene expression13 in diseased human valves. The likely sources of the myofibroblasts and osteoblasts that appear and persist in CAVD include native VICs, which contain mesenchymal progenitor-like cells that are highly plastic,3 and small numbers of circulating progenitors29 and mesenchymal cells that transition from endothelial cells.30 Possible triggers for VIC pathological differentiation or dysfunction include abnormal biomechanical forces (such as hypertension,10 elevated stretch,11 altered shear stresses,11 or altered VECM stiffness31), reactive oxygen species, inflammatory cytokines32–35 and growth factors,36 and the cellular environment caused by other disease states. Delivery of antagonists to pathological stimuli or otherwise inhibiting VIC proliferation or apoptosis can significantly reduce calcification. In vitro administration of an ERK pathway inhibitor significantly decreased VIC proliferation, apoptosis, and nodule formation, enabling these cells to retain a quiescent phenotype.37 Meanwhile, delivery of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) in both in vitro and in vivo animal models decreased VIC proliferation, apoptosis, and calcification.38–41 Statins represent a particularly intriguing avenue to pursue in regulating VIC function, because these drugs have demonstrated clinical but controversial slowing of the progression of CAVD.42

Calcification-Bone Formation
Calcification is largely responsible for the hemodynamic progression of AV stenosis. Recent descriptive studies from patient specimens have demonstrated the cell changes associated with AV calcification, including osteoblast expression, cell proliferation, and atherosclerosis.13,28,43,44 Furthermore, these studies have also shown that specific bone cell phenotypes present in calcifying valve tissue from human specimens45–49 demonstrate the potential for vascular cells to differentiate into calcifying phenotypes.

Recent observations in ex vivo human tissue suggest that rapid advancement in our understanding of the basic mechanisms involved in the initiation and progression of vascular and valvular calcification is now possible. If an osteoblast phenotype is present, then the factors important in the regulation of bone development and regeneration must be considered in the understanding of calcification of the AV. It is well known that cardiovascular calcification is composed of hydroxyapatite deposited on a bone-like matrix of collagen, osteopontin, and other minor bone matrix proteins,28,43,50 and regulation occurs via activation of specific transcription factors, including MSX2,51 Runx2,13,45 and Sox9.45 Calcified AVs removed from surgical valve replacement show bone formation (osseous metaplasia).13,28,45 Further characterization of this phenotype has proven, in calcified bicuspid AVs, that immunohistochemistry staining shows the expression of osteopontin.50 In addition, osteopontin expression has been demonstrated in the mineralization zones of heavily calcified AVs obtained at autopsy and surgery.13,28,43,45

In Vivo Models of CAVD
Studies in the field of vascular calcification have set the stage for the experimental studies in valvular heart disease. Elevated LDL and its oxidative modification represent one of the major factors of CAVD. Therefore, addressing the mechanisms of CAVD in hypercholesterolemic animal models is a reasonable and essential approach. Development of CAVD has been shown in both apoE and LDL receptor-deficient mice.27,29,52 Aortic valves in hypercholesterolemic mice and rabbits,23,41,44 characterized by thickened leaflets with macrophage-rich subendothelial lesions in early stages and the formation of calcific deposits on the aortic site of the valve in late stages, reproduce key pathological features found in human valve disease. In addition, clinicopathological studies of stenotic AVs in humans identified lesions similar to those in inflamed atherosclerotic plaques.15,16 Cholesterol lowering in such models improves various features associated with atherogenesis and AV disease.23,41,44,53 These animal models are important and need to be characterized further with regard to CAVD. However, these models also have limitations in that no one model recapitulates the human disease process completely, but each published model to date provides incremental mechanistic insight into the human disease process. The evidence that compares the osteogenic process in the valve with the bone is the most compelling to dissect the molecular mechanism and to demonstrate the foundation for both of these cellular processes and the potential for medical therapy.49,54–56 Figure 2 demonstrates a current working model of the signaling events published in the field of CAVD.
Clinical Studies

Prevalence, Genetics, and Cardiovascular Risk Factors

The presence or progression of CAVD has been associated with several clinical, genetic, and anatomic factors. Bicuspid AV valve disease is the most common congenital heart abnormality. A congenitally bicuspid AV is present in 50% of adults undergoing valve replacement for severe CAVD, and nearly all patients with a bicuspid valve will eventually need valve surgery, either for regurgitation in young adulthood or for stenosis in the fifth or sixth decade of life. Bicuspid valve disease appears to be inherited in an autosomal dominant pattern in some families, and a mutation in the NOTCH1 gene segregates with both bicuspid valve anatomy and premature valve calcification and complex congenital heart defects.57 Calcification of trileaflet AVs also may be affected by genetic factors, based on population studies and case-control comparisons for specific polymorphisms, including the Vitamin D receptor,58 estrogen receptor,59 apolipoprotein E4,60 and interleukin 10 alleles.61 Mild CAVD, called aortic sclerosis, is present in ~25% of adults >65 years of age, and is associated with adverse cardiovascular outcomes with about a 50% increased risk of cardiovascular events over 5 years.62

Similar to the cardiovascular risk factors defined by the Framingham study for vascular atherosclerosis, clinical factors associated with the presence of CAVD in the Cardiovascular Health Study included older age, male sex, serum lipoprotein(a) and LDL levels, height, hypertension, metabolic syndrome, and smoking, as shown in Figure 3.62–78 The association with elevated LDL is relatively weak in those >65 years old, the group at greatest risk of progressing to aortic stenosis.

Echocardiographic Imaging for CAVD

Because of its ability to detect and quantify valve-related hemodynamic obstruction, echocardiography long has been recognized as a useful clinical tool for monitoring aortic stenosis (AS), the later, obstructive stage of CAVD.79 Echocardiography can reliably visualize aortic valve anatomy, although once severe calcification is present, distinguishing a bicuspid from a trileaflet valve can be difficult. Echocardiographic measures of AS severity have been well validated in numerous studies and now are the clinical standard for patient management. Guidelines recommend measurement of aortic jet velocity, mean pressure gradient, and continuity equation valve area. Although clinically robust, these measures are subject to several sources of error, including physiological changes, recording technique, and measurement variability.80

In addition, there is marked variability between patients in the rate of hemodynamic progression and the degree of stenosis that results in clinical symptoms. Aortic sclerosis is defined on echocardiography as focal areas of leaflet thickening without significant obstruction to left ventricular outflow, with an aortic velocity <2.6 m/s.81 Echocardiographic measures of aortic jet velocity and leaflet calcification have been shown to be robust predictors of clinical outcome.81,82 In the design of clinical trials, we will need to consider the effects of the variability of echocardiographic data on sample-size
calculations and define the imaging standards and protocols for the use of this tool to quantify noninvasively the level of disease in the patients.

**Computed Tomography in Quantifying Calcification in CAVD**

The early stage of CAVD, aortic sclerosis, is characterized by aortic valve calcium (AVC) accumulation, but not hemodynamic obstruction. Because echocardiography does not have the resolution for quantifying AVC, it is less useful for monitoring early-stage CAVD. In contrast, computed tomography (CT) is a relatively sensitive and precise tool for quantifying AVC. Thus, CT has emerged as a useful tool for studying aortic sclerosis, complementing the utility of echocardiography in studying AS. Moreover, because the aortic valve leaflets lie in the same anatomic plane as the coronary arteries, AVC can be quantified by any CT scan obtained for the purpose of quantifying coronary artery calcium. Taking advantage of these issues, investigators have used CT to study traditional and novel risk associations for AVC in the Multiethnic Study of Atherosclerosis (MESA), a 6780-participant study of risk factors for subclinical coronary artery disease. In MESA, the metabolic syndrome is a strong risk factor for prevalent and early-stage disease. Thus, metabolic syndrome appears to be an adverse risk factor in all stages of CAVD.

**Clinical Trials: 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Pathway**

The first randomized, prospective study testing the effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in AV disease was published in 2005. In this double-blind, placebo-controlled trial, patients with calcific AS were randomly assigned to receive either 80 mg of atorvastatin daily or a matched placebo. Aortic valve stenosis and calcification were assessed with the use of Doppler echocardiography and helical CT, respectively. The Scottish Aortic Stenosis Lipid Lowering Therapy Impact on Regression (SALTIRE) investigators demonstrated a trend in slowing the progression of the AV stenosis, but it was not a statistically significant study for primary end points. The SALTIRE investigators concluded that intensive lipid-lowering therapy does not halt the progression of calcific AS or induce its regression, and the reason for this negative trial might be the timing of therapy.

In the Rosuvastatin Affecting Aortic Valve Endothelium (RAAVE) trial, performed a prospective trial of AS with Rosuvastatin targeting serum LDL, slowed progression of echo hemodynamic measurements, providing the first clinical evidence for targeted therapy using an 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in patients with asymptomatic moderate AS in a hypothesis-driven open-label study. These results are small and controversial, but test the lipid hypothesis. The next clinical trial, Simvastatin and Ezetimibe in Aortic Stenosis (SEAS), examined intensive lipid-lowering with Simvastatin and Ezetimibe in Aortic Stenosis. This trial was a randomized, double-blind trial involving 1873 patients with mild-to-moderate, asymptomatic AS. Again, the investigators concluded that the medication did not reduce the composite outcome of combined AV events in patients with AS, including echo progression and vascular end points. Finally, the most recent trial, Aortic Stenosis Progression Observation Measuring Effects on Rosuvastatin (ASTRONOMER), randomly assigned patients to Rosuvastatin versus placebo in patients with moderate AV disease and bicuspid AV disease. This study also did not demonstrate slowing of the progression of this disease. These 4 clinical trials have different results, which may be due to a number of reasons, including differences in trial designs, differences in enrollment criteria, differences in statin medication, or timing of therapy. Although the 3 randomized trials did not demonstrate slowing of the progression of AS, the largest trial, SEAS, did demonstrate improvement in primary end points of ischemic vascular disease. The future of clinical valve trials may need further analysis of the trial design, the type of medications, and the duration of the trials, but for now there is no primary indication for statin therapy in patients with valvular heart disease to slow progression of this disease. Treatment of all cardiovascular patients with risk factors remains appropriate according to the guidelines as described by the American Heart Association and American College of Cardiology.

**Recommendations**

Based on this review of the current state of knowledge as summarized in this article, the Working Group make the
Recommendations are presented as being of equal weight, not in priority order.

1. Identify genetic, anatomic, and clinical risk factors for the distinct phases of initiation and progression of CAVD to identify individuals at higher risk, to determine interactions between risk factors, and to determine whether the severity of AS is a risk factor for surgical AV replacement. These factors should encompass the unique contributions of atherosclerosis, metabolic syndrome, hypercholesterolemia, type II diabetes mellitus, and chronic kidney disease. New, larger epidemiological studies and existing epidemiological datasets in which CT scans, echocardiograms, or possibly MRI scans have been obtained could be used in this effort.

2. Develop high-resolution and high-sensitivity imaging modalities that can identify early and subclinical CAVD, including molecular imaging and other innovative imaging approaches. Continue research to define the state-of-the-art for detecting early calcification not identified by traditional echocardiographic imaging.

3. Understand the pathogenesis and pathophysiology of bicuspid AV, especially to establish correlations between phenotype and genotype, and to clarify the key features of this disease process that potentiate calcification.

4. Understand the basic valve biology (eg, early events, mechanisms, and regulatory effects) of CAVD, including signaling pathways and the roles of valve interstitial and endothelial cells and the autocrine and paracrine signaling between them, the extracellular matrix and matrix stiffness, the role of age-related changes in both valve cells and extracellular matrix, the interacting mechanisms of cardiovascular calcification and physiological bone mineralization, and microscale mechanotransduction and macro-scale hemodynamics.

5. Develop and validate suitable multiscale in vitro, ex vivo, and animal models. Improved models are needed that realistically duplicate the conditions in which human CAVD develops. Metabolic studies are needed, from the cellular level through the patient level, to define those conditions.

6. Identify the relationship between calcification of the AV and bone and the reciprocal regulation of these processes.

7. Encourage, promote, or establish tissue banks that make valve tissue from surgery, pathology, and autopsy unsuitable or unneeded for transplantation—with and without CAVD—available for research. Human valve cell lines should be derived including immortalized VICS.

8. Conduct clinical studies specific to CAVD to determine the feasibility of earlier pharmacological intervention in aortic AV sclerosis versus stenosis. Determine the correct design of the clinical trials to test the hypothesis, ascertain whether it includes measurement of calcification, and to further understanding of the biology of the AV as measured by the continuity equation by Doppler echocardiography.

9. Determine the risk factors and optimal timing of surgical valve replacement in view of the current state of the data defining the biological mechanisms of CAVD.

Disclosures

The working group was commissioned by the National Heart, Lung, and Blood Institute, and support for the meeting was directly from the National Institutes of Health (NIH). Dr Schoen is a consultant for Edwards LifeSciences, Medtronic, Pi-R-Sq, Sadra Medical, Sorin Medical, St. Jude Medical, and Sulver Carbomedics. Dr Rajamannan is the inventor of a patent for methods to slow progression of valvular heart disease. This patent is owned by the Mayo Clinic, and the author does not receive any royalties from this patent. Dr Heistad receives funding from a grant from Amgen Corporation. The other authors report no conflicts.

Sources of Funding

Dr Yoganathan is supported by the Division of Engineering Education and Centers (EEC) at NSF-9731643. Dr Rajamannan is supported by NIH grants 5R01HL085591 and 3R01HL085591S1. Dr Towler is supported by NIH grants HL069229, HL081138, and HL088651, and by the Barnes-Jewish Hospital Foundation. Dr Simmons is supported by the Canadian Institutes of Health Research (MOP-102721) and the Heart and Stroke Foundation of Ontario (NA6654) and the Canadian Research Chair. Dr Grande-Allen is supported by NIH grant 1R21HL081558 and Whittaker Foundation Biomedical Engineering Grant the Human Frontiers Science Program. Dr O’Brien is supported by NIH grant 1R21HL081558. Dr Aikawa is supported by the American Heart Association 0835460N. Dr Heistad is supported by Carver Research Program of Excellence, Amgen Inc (Industry Grant), and HL 62984 (NIH). Dr Demer is supported by NIH grant 1R01 HL081202. Dr Masters is supported by 1R01 HL093281.

References


**Key Words:** aortic valve • aortic valve calcification • aortic valve stenosis • calcification • cardiac calcifications