Calcific Aortic Valve Disease: Where Are We Now?

Calcific aortic valve disease (CAVD) is the most common valvular heart disease in the aging population of the developed world with projected disease burden expected to increase from 2.5 million in 2000 to 4.5 million in 2030.1 In CAVD, the leaflets become thick, stiff, scarred, and calcified, often covered with nodules on the surface facing the aorta. In general, symptoms of CAVD are minimal even as the valve narrows into aortic stenosis (AS), where pressure overload leads to progressive left ventricular hypertrophy and life-threatening symptoms: angina and syncope. This hemodynamic catastrophe results in heart failure and, without intervention, death within months to years. The mainstay of treatment for CAVD is surgical aortic valve replacement (AVR) with a mechanical or bioprosthetic valve.2 Limitations include increased complications attributable to anticoagulation and the need for reoperation because of the limited lifespan of prosthetic valves.3,4 Transcatheter AVR is currently reserved for patients with excess operative risk, but its indications are now expanding because of the minimally invasive nature of transcatheter AVR compared with surgical AVR.5 If transcatheter AVR becomes more routine, some hope it will reduce the need for repeat surgery. Tissue-engineered valve replacement, particularly in pediatric patients, holds great potential, but remains elusive after years of effort. Currently, there are no medical therapies for CAVD as alternatives to surgery, other than for complications, such as antibiotics for infections and vasodilators for acute decompensation.

How Do We Develop Improved Treatments for CAVD?

Research now implicates osteogenic processes as key mechanisms in CAVD (Figure),4 opening 2 general directions for development of new therapies: (1) inhibition of procalcific regulators and (2) induction of proresorptive regulators in aortic valve tissue. Tissue targeting may be essential to avoid damage to skeletal bone; for example, if osteoclasts or nanoparticles carrying osteoclastic differentiation factors could be targeted to valve tissue, they may reverse CAVD.6 However, even if successful, mineral resorption may leave behind damaged leaflets. Molecular imaging of these events may provide biomarkers for clinical and research monitoring far in advance of critical stenosis. Preventive measures that do not require tissue targeting are under consideration, including...
modification of various factors (dietary phosphorous, vitamins K and D, fetuin, warfarin, bisphosphonates, anti-inflammatory agents, osteopontin, mineralocorticoids, antioxidants, estrogen) and conditions (uremia, metabolic syndrome, hyperglycemia, hyperlipidemia, hypertension). Based on the association of CA VD with hyperlipidemia and atherosclerosis, clinical trials of statins were undertaken, but no benefit was seen.\textsuperscript{7,8} It remains to be determined whether they may have benefit in earlier disease or in patients with hyperlipidemia. Additionally, although atherosclerosis and CA VD have similar risk factors, they are somewhat poor predictors of CA VD, suggesting that an additional set of unknown factors may contribute to the onset and progression of valve disease.

Whether CA VD is a manifestation of an inflammatory disease or whether inflammation is an associated process occurring secondarily in injured and repairing tissue is not entirely known. Thus, an area of interest remains in investigating whether anti-inflammatory agents may prevent and reduce the extent of calcification and osteogenesis, which is fundamentally triggered by inflammatory mediators. To this end, patients with mild–moderate CA VD and coronary artery disease with either diabetes mellitus or metabolic syndrome will be evaluated in the ongoing Cardiovascular Inflammation Reduction Trial (CIRT) to examine whether treatment with low-dose methotrexate, an effective anti-inflammatory drug, affects progression of CA VD.\textsuperscript{9} Evaluation of new therapeutic approaches will require systematically collected, detailed knowledge of the processes of CA VD that include analysis of normal and diseased human aortic valve tissue, development of suitable animal models, functional assays, and clinical and population studies.

### Clues to CAVD Mechanisms From Human Population Studies

Several large population cohorts have sought to identify the key epidemiological factors contributing to CA VD. The Cardiovascular Health Study (CHS), focusing on risk factors for coronary artery disease, identified age, male sex, and lipoprotein (a) levels as showing the strongest correlates of prevalent aortic valve sclerosis and stenosis; hypertension, tobacco use, and low-density lipoprotein (LDL) cholesterol levels gave weaker signals.\textsuperscript{10,11} Advanced age remains the leading risk factor for CA VD. In all age categories, aortic valve sclerosis and stenosis were more prevalent in male subjects compared with women.\textsuperscript{10} The Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated that the prevalence of CA VD was highest in non-Hispanic whites, followed by Hispanics and blacks,\textsuperscript{12} and confirmed a significant role for age and male sex with the development of CA VD.\textsuperscript{13} By comparison, impaired kidney function is only modestly associated with aortic valve calcification.\textsuperscript{14} Metabolic syndrome was independently associated with the development of CA VD,\textsuperscript{15} potentially attributable to similar factors participating in their pathogenesis, including circulating oxidized LDL and small dense LDL particle phenotype.\textsuperscript{15} In addition, metabolic syndrome was independently associated with the progression of AS.\textsuperscript{16} A polymorphism in the lipoprotein (a) gene associated with serum apolipoprotein (a) peptide levels has been identified as critical in the pathogenesis of CA VD.\textsuperscript{17,18} In addition, recent investigations have identified...
lipoprotein-associated phospholipase A2 (Lp-PLA2) expression in stenotic aortic valves and vulnerable plaques. Lp-PLA2 produced within the aortic valve hydrolyzes oxidized phospholipids from lipoproteins which have proinflammatory effects that promote valve mineralization. Thus, this lipoprotein may mediate unique aspects of atherosclerosis and CAVD.

Mechanical, Hemodynamic, and Genetic Aspects of Bicuspid Aortic Valve and CAVD

Bicuspid aortic valve (BAV) is the most common congenital valvular heart disease (0.6%–1.0% of the adult population), the primary cause of ≈50% of isolated severe AS requiring AVR, and is frequently associated with aortic aneurysm and aortic dissection. BAV patients exhibit wide variation in the clinical progression of AS and CAVD. BAV-associated AS commonly requires AVR between the fourth and ninth decades of life, although many patients never require surgery. To date, the age of presentation for AVR in BAV patients has not been associated with genetic or environmental factors, nor variants in lipoprotein (a) previously associated with CAVD. Clinical symptoms often presage the diagnosis of BAV, thus complicating studies of the relationship between BAV and CAVD and hindering identification of effective therapies of CAVD. Tenable hypotheses for the markedly increased incidence and severity of early-onset CAVD seen in BAV patients include the abnormal mechanical strain and flow patterns across the BAV that may initiate the cellular processes of CAVD. Alternatively, the genetic or cellular differences that promote BAV may lead to CAVD, independent of the hemodynamic insult.

Although differences in the transcriptional profile and histology of aortic aneurysms between tricuspid valves and BAVs are well documented, there is no evidence for functional transcriptional differences between bicuspid and tricuspid aortic valves in end-stage CAVD. This implies that CAVD is a common pathway resulting from initiating insults in BAV, but does not guide us as to these initiating events, be they hemodynamic, cellular, or both. This also states the need for examining BAV early in the CAVD process, a difficult field of study. Increased biomechanical stress has been predicted based on modeling of human BAV versus normal tricuspid valves, but whether these forces contribute to accelerated CAVD has not yet been determined. Little is known about the developmental origins of human BAV, but animal models support the dual importance of embryonic cardiac neural crest cells and the second heart field that could underlie cellular mechanisms of CAVD.

These models provide opportunities to test genetic and biological mechanisms of BAV in CAVD, but are limited by differences in anatomy, developmental pathways, and distribution of pathological calcification between rodents and humans. Thus, incomplete and conflicting evidence from molecular, embryological, animal model, and clinical studies have not yet resolved the roles of biomechanical and cellular mechanisms.

Osteogenic Mechanisms in CAVD

Valve endothelial cells likely play a key role in initiation of valve injury. Valve endothelial cells are uniquely positioned to sense hemodynamic forces and encounter systemic circulating factors which in turn play a critical role in the paracrine regulation of valve interstitial cells (VICs). VICs comprise a diverse and highly plastic population of resident cells responsible for remodeling and integrity of extracellular matrix. Various VIC phenotypes have been identified in human heart valves, including quiescent fibroblast-like VICs, which on pathological cues can differentiate into activated myofibroblast-like VICs, smooth muscle cells located subendothelially and at the base of the leaflet, and osteoblast-like VICs, which are responsible for the active deposition of calcium in CAVD (Figure). The initiating processes of CAVD have been proposed to stem from endothelial dysfunction where increased permeability results in the nonspecific diffusion of cytokines and cells into valve leaflets that subsequently initiate a phenotypic switch of quiescent VICs to a myofibroblast-phenotype and thereby promotes aortic valve sclerosis and stiffness. However, the progression from aortic valve sclerosis to AS is not well elucidated, though it seems to relate to active processes of biomineralization and ossification of osteoprogenitor cells derived from multiple sources, including activated VICs, endothelial-to-mesenchymal transition of valve endothelial cells, and bone marrow myeloid cells.

Histological studies report the formation of bone nodules in stenotic aortic valves as a significant contributor to CAVD. Subsequently, regulatory pathways of bone mineralization in the skeletal system have been shown to be activated in CAVD. These include the wingless-type MMTV integration site family members (Wnt)/β-catenin and bone morphogenetic protein (BMP) signaling pathways, transcription factors runt-related transcription factor 2 (Runx2), sex determining region Y-box 9 (Sox9), and msh homebox 2 (Msx2), as well as proteins involved directly in mineralization, such as alkaline phosphatase, Glα proteins, proteoglycans, osteopontin, and bone sialoprotein. Osteogenic gene induction occurs in CAVD of distinct pathogeneses and may represent a final common pathway in aortic valve mineralization. Inflammation is one of the causes of osteogenic gene induction through the production of cytokines (eg, tumor necrosis factor-α and interleukin-6) and generation of intracellular oxidative stress, which activates both runt-related transcription factor 2 and Msx2/Wnt signaling cascades providing a milieu conducive for mineralization and ectopic bone formation. Another important mechanism by which inflammation promotes CAVD is via the formation of mineralizing matrix vesicles. The proinflammatory cytokine S100A9, an endogenous agonist of the receptor for advanced glycation end products and the toll-like receptor 4, is involved in the secretion of calcifying matrix vesicles from macrophages via formation of a phosphatidylinerse–annexin5–S100A9–hydroxyapatite complex.

Endocrine Mechanisms of CAVD

Although vascular calcification and CAVD have many similarities, it is probable that they are not precisely the same in terms of the cell types or inductive mechanisms driving mineralization, which may affect optimization of treatment strategies. Likewise, similarities between CAVD and bone mineralization could be exploited therapeutically. Intriguing differences are emerging in cellular ontogeny and regulation of early-phase CAVD mineralization and skeletal mineralization. Inflammatory oxylipins, innate immune signaling, and the integrated tissue response fundamentally differ between skeletal and vascular...
parenchymal cells with distinct origins. This difference may also extend to endocrine regulation. In diabetic, dyslipidemic low-density lipoprotein receptor-deficient mice (LDLR−/−), administration of the bone anabolic hormone parathyroid hormone (PTH) increases bone formation while inhibiting arterial calcification.33 Reductions in oxidative stress, oxylipid production, and serum phosphate signaling by PTH/PTH-related protein receptor (PTH1 receptor) activation may be important underlying mechanisms.34 Yet, the net impact of PTH1 receptor signaling on CAVD remains unclear. Although PTH1 receptor is expressed throughout the vasculature, and the risk for CAVD is increased in patients with primary hyperparathyroidism,35 the impact of chronic PTH exposure on valve PTH1 receptor signaling is unknown. Recent studies suggest that PTH is regulated by the renin–angiotensin system via angiotensin II that directly stimulates PTH secretion.36 Moreover, angiotensin-converting enzyme is expressed in human aortic valve stenosis,37 and angiotensin receptor blockade inhibits aortic valve pathology in hypercholesterolemic rabbits.38 Clearly, the endocrine and metabolic regulation of CAVD deserves additional inquiry. Strategies focused on understanding oxylipid signaling, tissue-specific innate immune responses, and its endocrine regulation are likely to afford therapeutic opportunities.

Dysregulated Mineral Metabolism and Osteoclast Deficiency in CAVD

Patients with end-stage kidney disease have accelerated progression of CAVD compared with patients with early-stage chronic kidney disease or the general population.39 This increased risk has been attributed to nontraditional risk factors related to mineral imbalances, including abnormalities in serum Pi, Ca, and vitamin D that may drive bone formative processes.40 Indeed, therapies aimed at normalizing mineral and mineral-regulating hormone balance, such as phosphate binders and low-phosphate diets, vitamin D receptor agonists, and calcimimetics, have been shown to have some benefit in reducing vascular and valvular calcification in human and experimental studies.41 In fact, in several studies, vascular calcification actually decreased from baseline with treatment, suggesting that mechanisms for ectopic mineral regression do exist.42,43 Other factors that may also contribute to the high rate of CAVD in these patients are high levels of phosphate that promote apoptosis via the pituitary-specific transcription factor (Pit1) signaling pathway,44,45 undercarboxylated matrix Gla protein potentially as a result of vitamin K deficiency, fetuin deficiency, and abnormalities in fibroblast growth factor 23 (FGF23)/klotho and receptor activator of nuclear factor-xB (RANK)/RANK ligand/osteoprotegerin systems.39

The RANK/RANK ligand/osteoprotegerin system plays a major role in regulating bone resorption. RANK ligand binding to RANK stimulates osteoclast formation and skeletal bone resorption, whereas the RANK decoy receptor osteoprotegerin inhibits this process. Although bone-like anabolic processes seem to be turned on, there is a paucity of data for active, bone-like catabolic processes in CAVD, because few osteoclasts have been observed in these lesions.46 Mounting evidence suggests that the inflammatory milieu in CAVD actually favors inhibition of osteoclastogenesis, because osteoprotegerin is upregulated early in disease progression in valve tissue and in serum, where it predicts mortality in patients with symptomatic CAVD.47 This finding is also true in late-stage chronic kidney disease patients, in whom osteoprotegerin levels are elevated and directly correlate with the presence of vascular calcification and the risk of cardiovascular mortality.48 Together, these data suggest that enhancing osteoclast-like mineral resorptive activities might represent a novel approach to inhibiting and potentially regressing CAVD. In support of this, osteoclasts derived from rat bone marrow were capable of removing mineral from calcified elastin in vitro and limited elastin calcification in rat subdermal implants.49 Furthermore, new techniques to engineer monocytes that conditionally differentiate into osteoprotegerin-resistant osteoclasts have been recently developed.50 Thus, approaches to control osteoclast differentiation and locally deliver autologous osteoclasts represent potential novel therapeutic modalities to treat or even regress CAVD.

Developmental Signaling and Gene Regulatory Mechanisms in CAVD

Human genetic analyses and experimental models have demonstrated that multiple cell signaling pathways active in valve development also are active in CAVD.50,51 The Notch signaling pathway was among the first pathways associated with CAVD based on the association of mutations in human NOTCH1 with BAV and CAVD.52 Indeed, Notch signaling protects against valve mineralization in studies in mice and cultured VICs.53,54 In contrast, Wnt/β-catenin signaling is induced in human CAVD and has been hypothesized to promote progression of disease through activation of osteogenic differentiation.55,56 BMP signaling, evident in increased ligand expression and expression of phospho-mothers against decapentaplegic (Smad)1/5/8, is activated in human and mouse CAVD.57 However, the mechanisms of BMP2 induction are not known, and the potential for BMP signaling inhibition in the treatment of CAVD has not yet been demonstrated. The intersection of Notch and BMP signaling is crucial during the early stages of embryonic valvulogenesis and also in aortic VIC calcification in culture.53,58 Thus, multiple signaling pathways linked to valve and bone development likely act to promote or inhibit CAVD in distinct valve cell populations under different physiological or genetic conditions.

Mouse models have been useful in identifying specific regulatory mechanisms of CAVD. Apolipoprotein-deficient (ApoE−/−) and LDLR−/− mice fed a high-fat diet model hyperlipidemic cardiovascular disease in the context of cardiovascular inflammation and lipid deposition.59 Notch signaling-deficient mice exhibit aortic valve calcification, but, unlike humans, there is little or no association with BAV.54 Klotho-deficient mice exhibit aortic valve calcification associated with premature aging and hyperphosphatemia attributable to kidney disease.60 These various mouse models may be useful in dissecting mechanisms of CAVD with distinct causes and progressive mechanisms. Currently, the cellular origins of CAVD are not well defined. Thus, lineage tracing may reveal the specific cellular origins and contributions to CAVD using mouse models. Such studies will likely be useful in developing new-targeted pharmacological approaches for CAVD based on specific molecular or cellular mechanisms.
MicroRNAs as Biomarkers and Potential Therapeutics for CAVD

MicroRNAs control the signaling proteins involved in CAVD and have diagnostic, prognostic, and therapeutic potential. The stable extracellular microRNAs found in plasma, urine, and saliva are excellent biomarkers that reflect the physiological or pathological conditions of specific tissues. Although microRNA expression profiling is being widely investigated in coronary diseases, specific data about CAVD is limited. Unique microRNA signatures have been reported within the valves themselves or in aortic aneurysms and plasma from patients with BAV. Distinct flow and side-related microRNA patterns are found when endothelial cells isolated from the fibrosa, which is prone to calcification, are compared with those from the ventricularis side of the valve. Understanding microRNA patterns associated with CAVD resulting from different pathogeneses may provide predictive clues or biomarkers of the pathophysiology of each patient’s disease. In addition, microRNAs, singly or in combination, could have therapeutic potential through inhibition of signals driving CAVD. For example, the microRNA-34 family of microRNAs, which repress the procalcific BMP2, improved heart function in models of cardiomyopathies and could be applied to CAVD treatment. It remains to be determined whether microRNA profiles distinguish between patients with the common risk factors for both atherosclerosis and CAVD.

Existing Gaps in Imaging Characterization of CAVD

By the time CAVD comes to clinical attention, it is often in advanced disease stages. Aortic valve abnormalities are often diagnosed incidentally on echocardiograms performed for unrelated clinical indications. Based on current management guidelines, these subjects are followed with intermittent imaging studies until symptoms appear. This approach is based on the absence of an appropriate medical therapy to slow or reverse the disease progression in asymptomatic subjects. The development of an approach to identify the subjects who are at high risk for CAVD can facilitate the development and validation of such medical therapies and help track the effectiveness of therapeutic interventions in individual subjects. Besides detecting the extent of calcification, a modest determinant of progression, classical imaging modalities (eg, echocardiography) do not provide any information on CAVD propensity for progression. In addition, long clinical trials with interventional monitoring of disease progression are largely impractical. By detecting and quantifying the earliest molecular and cellular events involved in CAVD development, molecular imaging can address this important gap in clinical care. Furthermore, molecular imaging can help address aspects of biology that are not readily detectable through common biological techniques and thus advance our understanding of basic CAVD pathobiology. The feasibility of molecular imaging of CAVD in humans has been shown in a few recent clinical studies using Food and Drug Administration–approved radiotracers, [18F]fluorodeoxyglucose and [18F]sodium fluoride. In parallel to inflammation and calcification, it is important to focus on other relevant aspects of valvular pathobiology (eg, proteolytic activity), which could provide complementary information about the pathogenesis and progression of CAVD.

Unanswered Questions and Future Directions

Despite recent progress in clinical, genetic, and animal studies, many important questions remain in terms of optimal diagnosis, treatment strategies, and mechanistic understanding of CAVD. The causes of CAVD are not fully understood, but valve pathogenesis can occur with congenital malformation, atherosclerotic disease, advanced kidney disease, diabetes mellitus, or aging. Additional factors that influence the incidence of CAVD include sex and race, but exactly how they affect CAVD initiation or progression is unknown. It seems likely that the molecular mechanisms and cellular contributions of CAVD differ depending on the initial pathogenic stimulus and comorbidities. For example, the specific localization and molecular progression of CAVD resulting from hyperphosphatemia versus that resulting from inflammation may not be the same. For congenitally malformed valves, such as BAV, biomechanics may be a driving force in the disease process, but the specific stimuli and molecular mechanisms by which this occurs are in need of further investigation. The relationship between vascular and valvular calcification is unclear; each may result from different pathogeneses and molecular mechanisms depending on patient comorbidities. In addition, it is recognized that, in the human population, it is likely that an individual may have multiple risk factors for CAVD, further affecting the heterogeneity of disease manifestation and progression, making determination of optimal treatment strategies a challenge.

It is difficult to identify patients at risk for CAVD before symptom onset, but the population of patients with congenital BAV, who are highly vulnerable to CAVD, may serve as a valuable study group. The current standard of care is surgical valve replacement, but this is usually a temporary solution. Thus, development of improved bioprosthetic valves and methods of delivery such as transcatheter AVR, although costly, are priorities. Current clinically important questions include the determination of when a stenotic valve should be replaced and how best to manage the risk of replacement. Emerging molecular imaging technologies and identification of biomarkers that detect the earliest stages of valve mineralization will be important in the early diagnosis and monitoring of progression of CAVD. Studies in animal models support the reversibility of CAVD in some cases. With improved diagnosis and mechanistic insights into CAVD, the development of new therapeutic approaches that will eliminate the need for surgical replacement in patients with CAVD may be possible.

Acknowledgments

We thank Frank Evans of the National Heart, Lung, and Blood Institute for his valuable suggestions.

Sources of Funding

This work was supported by grants R01 HL114682 to K.E. Yutzey, R01 HL114709 to L.L. Demer, R01 HL114823 to S.C. Body, R01 HL114794 to G.S. Huggins, R01 HL114806 to D.A. Towler, R01 HL114611 to C.M. Giachelli, R01 HL114821 to M.A. Hofmann-Bowman, R01 HL114751 to D.P. Mortlock and M.B. Rogers, R01 HL114703 to M.M. Sadeghi, and R01 HL114805 to E. Aikawa.
Disclosures

D.A. Towler is a paid consultant for Eli Lilly and Merck & Co. The other authors report no conflicts.

References

Calcific aortic valve disease (CAVD) is the most common valvular heart disease in the aging population with a disease burden of millions worldwide. Despite recent progress in clinical, genetic, and animal studies, many important questions remain in terms of optimal diagnosis, treatment strategies, and mechanistic understanding of CAVD. Inflammatory, endocrine, osteogenic, biomechanic, and developmental factors contribute to CAVD progression and heterogeneity related to the initial pathogenic stimulus and comorbidities. Clinically important questions include the determination of when a stenotic valve should be replaced and how best to manage the risk of replacement. Emerging molecular imaging technologies and identification of biomarkers that detect the earliest stages of valve mineralization will be important in the early diagnosis and monitoring of progression of CAVD. With improved diagnosis and mechanistic insights into CAVD, the development of new therapeutic approaches that will eliminate the need for surgical replacement in patients with CAVD may be possible.