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Potential drug targets for calcific aortic valve disease

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Abstract

Calcific aortic valve disease (CAVD) is a major contributor to cardiovascular morbidity and mortality and, given its association with age, the prevalence of CAVD is expected to continue to rise as global life expectancy increases. No drug strategies currently exist to prevent or treat CAVD. Given that valve replacement is the only available clinical option, patients often cope with a deteriorating quality of life until diminished valve function demands intervention. The recognition that CAVD results from active cellular mechanisms suggests that the underlying pathways might be targeted to treat the condition. However, no such therapeutic strategy has been successfully developed to date. One hope was that drugs already used to treat vascular complications might also improve CAVD outcomes, but the mechanisms of CAVD progression and the desired therapeutic outcomes are often different from those of vascular diseases. We, therefore, discuss the benchmarks that must be met by a CAVD treatment approach, and highlight advances in the understanding of CAVD mechanisms to identify potential novel therapeutic targets.

Introduction

In 2012, aortic valve disease was directly responsible for >14,000 deaths in the USA, making it the second-leading cause of cardiovascular mortality.¹ Of particular concern, the prevalence of calcific aortic valve disease (CAVD) is projected to increase dramatically owing to the shift in population demographics, with an ageing 'baby-boomer' generation. The latest statistics published by the AHA reveal moderate-to-severe symptoms of CAVD in 2.8% of patients aged >75 years.^{1,2} Given this association with age, for many years, CAVD was presumed to be a passive, degenerative disease that occurred as a result of fatigue or deterioration of the tissue caused by the stresses and strains of cardiac function—the aortic

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Competing interests

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valve opens and closes >3 billion times over the course of an average lifespan. However, the results of many studies on the molecular signalling processes associated with valvular homeostasis have resulted in a paradigm shift, with CAVD now viewed as an active, cellular-driven disease.³ This view offers some hope to patients, because cellular processes can be targeted therapeutically. However, no drug therapies have been developed specifically for CAVD. With aortic valve replacement being the only viable option, physicians often delay intervention until patients present with the late stages of CAVD, which leads to decreased quality of life in the meantime. In fact, only 50% of patients with CAVD are referred for surgery, and only 40% are actually admitted for aortic valve replacement.⁴ The advent of transcatheter aortic valve replacement technologies allows valve replacement in a less-invasive manner than with surgical replacement.⁵ However, this option is currently available only for nonsurgical candidates, remains poor for paediatric patients due to a lack of integration and growth with host tissue, and is also associated with an increased risk of stroke after the procedure,⁶ presumably owing to vascular-wall damage during catheterization. Therefore, in this Review, we discuss potential options for the development of a pharmacological strategy to treat CAVD, which would fulfil a major medical need.

Pathophysiology

Tissue architecture and cellular phenotypes

Normal, healthy aortic valves maintain directionality in blood flow from the left ventricle to the systemic circulation by the co-ordinated action of three thin membranous structures known as leaflets or cusps.⁷ As the heart contracts during systole, the increased pressure in the left ventricle forces the leaflets to open into the aorta, allowing blood to be ejected from the heart (Figure 2a). As the heart enters diastole, the decrease in ventricular pressure allows the adjacent leaflets to coapt and close the valve to prevent retrograde blood flow back into the heart (Figure 2b). Leaflet dynamics throughout the cardiac cycle are controlled solely by the interaction between blood haemodynamics and leaflet biomechanics. Therefore, the structural integrity conferred by the microarchitecture of the leaflets is critical to the appropriate function of the valve.^{8,9} Each leaflet is composed of three distinct, histologically identifiable layers known as the fibrosa, spongiosa, and ventricularis. The fibrosa, proximal to the aorta, is composed largely of circumferentially aligned collagen fibres that provide the necessary tensile strength to withstand diastolic forces and prevent leaflet prolapse into the left ventricle (Figure 2c). On the ventricle side of the leaflet, the ventricularis layer is composed of elastin that allows the leaflets to stretch radially away from the aortic wall to meet and seal the valve.¹⁰ The spongiosa is composed mainly of proteoglycans and glycosaminoglycans and is speculated to serve as a lubricating layer between the outer layers of the aortic valve.¹¹ However, the spongiosa could simply be protein remnants of the cardiac cushions that are precursors to leaflets during heart development. A hallmark of the progression of CAVD is the accumulation of misaligned collagen within the fibrosa layer that results in thickened, fibrotic leaflets and an overall stiffened leaflet with reduced biomechanical functionality. The accumulation of calcific deposits in the later stages of CAVD exacerbates the loss of leaflet compliance as the valve becomes increasingly stenotic. Of note, collagen deposition and calcific nodule formation occur primarily within the fibrosa

region of aortic valve leaflets, leading to an asymmetry in the pathophysiology of CAVD.¹² Importantly, no small animal models of CAVD currently completely recapitulate the disease as observed in humans.

Two resident cellular populations control aortic valve leaflet homeostasis and pathological remodelling: valve endothelial cells (VECs) and valve interstitial cells (VICs).⁷ VECs sheathe the leaflet surface to form a barrier between the internal tissue components and the blood. In this manner, VECs function similarly to endothelial cells that line vessel walls throughout the vasculature. However, VECs exhibit phenotypic differences from vascular endothelial cells.¹³ When exposed to the shear stress of fluid flow *in vitro*, VECs actively align perpendicular to the direction of the flow, whereas vascular endothelial cells align parallel to the flow.^{14,15}

Increased expression of adhesion molecules (such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1), together with the VEC response to changes in shear stresses and cytokines, contributes to the progression of CAVD via recruitment of circulating inflammatory cells to the valve.^{16,17} VEC control of valvular homeostasis might, however, go further than serving as a gateway for inflammatory cells into the leaflets. Studies have indicated that VECs might have an active role in valve remodelling during CAVD by invading the valve through endothelial-to-mesenchymal transition processes that mirror those during valve development.¹⁸ During endothelial-to-mesenchymal transition, VECs lose many common endothelial markers and gain contractile markers similar to those observed in myofibroblast activation of VICs.¹⁹ As with the process that gives rise to VICs during development, these mesenchymal VECs invade the leaflet interstitium. *In vitro* evidence suggests that changes in VECs that lead to endothelial-to-mesenchymal transition are dependent on mechanical strain and activation of Wnt/ β -catenin signalling.¹⁸ VECs on each side of the aortic valve exhibit phenotypic differences as shown by gene array analysis, further indicating that, as with to VICs, VECs might be sensitive to the surrounding biomechanical environment.^{20–22} The predominant blood flow experienced by the fibrosa side of aortic valve leaflets is oscillatory and disturbed, whereas the ventricular side of the leaflets is exposed to steady, laminar shear flows. Additionally, fibrosa VECs show increased activity of downstream markers of signalling pathways associated with CAVD.²³ Perhaps consequently, the fibrosa side of the aortic valve is most susceptible to pathological remodelling that leads to CAVD. This observation might be analogous to the development of atherosclerotic plaques in the vasculature, which predominantly form in regions of haemodynamic oscillatory shear forces. Both VECs and vascular endothelial cells communicate via paracrine signalling to resident cells within each tissue, the differences in phenotype might have an effect on tissue homeostasis, and the expression of microRNAs that might mediate these interactions are regulated in a shear-stress-dependent manner.^{20,24} As an example of the control that VECs might have over VIC responses, co-culture experiments revealed that VECs can prevent phenotypic changes in VICs in the presence of pathological stimuli.^{25–27}

VICs reside within the leaflet of the valve and directly remodel and maintain the microarchitecture of the leaflets by synthesis and degradation of extracellular-matrix components.^{28,29} In healthy adult valves, VICs remain in a quiescent state with

characteristics similar to fibroblasts. However, these specialized cells show a high degree of phenotypic plasticity, with VIC differentiation observed during periods of valve development and disease.²⁸ Notably, activation of VICs to a myofibroblast phenotype—characterized by an increased expression of smooth muscle cell protein markers—is observed within developing valve leaflets and within fibrotic and calcific valve leaflets. This activated phenotype is believed to be responsible for the synthesis and remodelling of the extracellular matrix, and overactive VICs might be responsible for fibrotic accumulation of collagen during the initial stages of CAVD.^{30,31} Additionally, markers of osteoblastic differentiation of VICs have been observed in CAVD and might be responsible for the osteogenic nodules observed in CAVD leaflets.³² High frequencies of both mesenchymal and osteogenic progenitors have been identified in freshly isolated porcine VICs.³³ However, the relationship between these activated and osteoblastic phenotypes is uncertain, because investigators have been unable to produce both phenotypic changes within the same *in vitro* culture conditions. The rational design of a therapeutic strategy for CAVD begins with the identification of targets that can be used to modulate the molecular pathways that lead to CAVD. To accomplish this, we must first understand the tissue and cellular level changes that occur with CAVD, and the desired therapeutic end points that would be reached with the development of a successful drug.

Cardiovascular calcification and risk factors

Cardiovascular calcification is a general term that encompasses CAVD, calcific aortic valve stenosis, and calcification within various regions of the vasculature. Both CAVD and vascular calcification correlate with features of metabolic syndrome, such as obesity, diabetes mellitus, chronic kidney disease, hypertension, and chronic inflammation.^{34,35} Additionally, both types of calcification are more common in men than in women, and the incidence of cardiovascular calcification increases with age and lifestyle-associated risk factors, such as smoking.^{36,37} Despite various overlapping risk factors, however, many patients present with either CAVD or vascular calcification, but not both.³⁶ Additionally, the traditional risk factors mentioned above are fairly poor predictors of CAVD compared with the predictive power of these factors for vascular disease.³⁷ These discrepancies might indicate an additional set of unidentified factors that contribute to the onset and progression of CAVD.

Fibrosis and calcification

CAVD has been termed a fibrocalcific disease owing to the increased collagen content found in the valve leaflets of small animal models.³⁸ A hallmark of CAVD initiation is fibrotic collagen accumulation, which leads to thickened, stiffened valve leaflets and a deterioration in function.³⁹ Calcific nodules forming within these sclerotic leaflets exacerbate the loss of tissue compliance, ultimately leading to aortic valve stenosis.³⁹ However, whether this fibrotic response is inseparable from the formation of calcific nodules, or whether the fibrotic and calcific processes occur in parallel during the progression of CAVD remains uncertain. The association observed between collagen accumulation and calcification in CAVD is reminiscent of features of atherosclerosis, in which a collagen-rich fibrotic cap encapsulates an underlying calcification that forms within the plaque.⁴⁰ From a therapeutic viewpoint, a major difference exists in the desirability of

this fibrotic response in CAVD compared with atherosclerosis. After an atherosclerotic lesion develops, collagen accumulation is desirable, to stabilize the plaque and minimize the potential for rupture and thrombosis.^{41–43} Stability has historically been quantified by the thickness of the collagen in the fibrotic cap,⁴⁴ and biomechanical analyses indicate that underlying calcifications might further stabilize the plaque.⁴⁵ The major benefit of statins to atherosclerotic plaque stability seems to arise from collagen accumulation within the fibrous cap. Additionally, reports have suggested that statins do not decrease the prevalence of vascular calcification.^{46,47} Therefore, the heightened plaque stability observed in the fibrous cap of patients taking statins might be conferred by thickening of the cap. The extent to which growth of the atherosclerotic plaque is desirable is limited by stenosis of the vessel. However, the overall aim in the management of atherosclerosis tends towards a heightened fibrocalcific response.⁴⁴ Conversely, aortic valve function is highly dependent upon the appropriate biomechanical properties of the valve leaflets and, therefore, fibrotic and calcific extracellular-matrix content that interferes with leaflet biomechanics and function are entirely undesirable.

Inflammation

A hallmark of CAVD and calcification in atherosclerotic plaques is the presence of inflammatory cells, such as macrophages and T cells, within and surrounding calcified regions of the tissue (Figure 1).^{48–51} This feature is in contrast to calcification associated solely with chronic kidney disease, such as medial calcification within the arterial wall in which inflammation is not present.⁵² Intravital molecular imaging of cardiovascular tissues from *Apoe*^{-/-} mice reveals that the infiltration of inflammatory cells is an early event in the calcification of aortic valve leaflets and atherosclerotic lesions.^{53,54} These cells might have a role in initiating the remodelling processes that lead to calcification, because they actively release proteases that degrade extracellular-matrix components, priming the tissue for new matrix secretion. In physiological processes associated with wound repair, this type of inflammation is necessary to restore tissue homeostasis. However, in pathological situations, persistent inflammation has detrimental effects. In atherosclerotic plaques, collagen degradation owing to excessive inflammation leads to a decrease in plaque stability.⁴³ This mechanism is hypothesized to generate the anti-inflammatory effects associated with statins: fibrotic collagen accumulation that increases plaque stability. Along with proteases, these cells release proinflammatory cytokines that have been shown to promote pathological differentiation of valvular cells.³⁴ NAD-dependent protein deacetylase sirtuin-1, a regulator of macrophage biology, was found to decrease the expression of resistin.⁵⁵ This hormone leads to the accumulation of LDL cholesterol in arterial walls and is positively correlated with CAVD in elderly patients.⁵⁶ Therefore, in addition to the release of proinflammatory cytokines that influence the behaviour of valvular cells, inflammation might also be associated with lipid changes that influence further valvular remodelling. Additionally, in procalcific environments, macrophages within the atherosclerotic plaque might actively participate in the deposition of calcium phosphate minerals,⁵⁷ and similar processes might contribute to CAVD.

Dystrophic and osteogenic processes

Cardiovascular calcification is characterized by the deposition of calcium phosphate mineral within the extracellular matrix of tissues. This mineralization can be present as dystrophic calcification, a disorganized crystal structure that is often associated with precipitation of calcium phosphate on debris remaining after cell death, or as a bone-like, organized crystal lattice of hydroxyapatite, which is indicative of osteogenic processes.³² Calcification with dystrophic characteristics seems to predominate in 83% of explanted leaflets; however, 13% of leaflets show bone-like structures indicative of active osteogenesis.³² Histological analyses of CAVD progression indicate that osteogenic mineral deposition might follow the initial wave of dystrophic calcification.⁵⁸ Similarly, osteogenic reprogramming is well described in vascular (particularly medial) calcification.^{59,60} Bone morphogenetic protein 2 (BMP-2)-dependent signalling has been shown to lead to activation of osteoblast-associated transcription factors and the resultant increased expression of osteogenic proteins.^{61–63} Using advanced material-based analytical methods, investigators identified spherical particles containing calcium phosphate mineral in calcified aortic valves and calcific regions of the vessel wall.⁶⁴ The origins of these calcifying structures in both tissues remain uncertain; however, evidence suggests that cellular-derived matrix vesicles contribute to the initiation of hydroxyapatite mineralization within atherosclerotic plaques.^{57,65} As with processes observed in bone formation, these vesicles are released by living resident cells (such as macrophages or smooth muscle cells) and are enriched in calcium-binding proteins that serve as initiating sites for nucleation of calcium phosphate crystals. The role of matrix vesicles has not been explored in the context of CAVD and warrants further investigation to improve understanding of early calcification events.

In vitro evidence suggests that the release of calcifying matrix vesicles is promoted by changes in cell phenotype caused by elevated calcium and phosphate in cellular growth media.⁶⁵ Cellular phenotypic changes in CAVD seem less straightforward than those identified in the regulation of vascular calcification. Much has been learned about the processes that lead to vascular calcification using a common protocol in which vascular smooth muscle cells are cultured in an osteogenic environment for up to 21 days and phenotypic changes and monitored,⁶⁶ but VICs seem to be less sensitive to this osteogenic reprogramming protocol.⁶⁶ VICs have been shown to have a high degree of mechanosensitivity that leads to uncontrollable phenotypic changes in 2D culture systems.⁶⁷ Therefore, the development of an *in vitro* system that allows for independent modulation of the VIC phenotype is a challenging problem in CAVD research.

Although vascular and valvular diseases might involve overlapping risk factors and present similar pathophysiological features, growing evidence indicates a divergence in the underlying cellular signalling and phenotypic changes that lead to pathological alterations of the tissue.³⁸ Furthermore, the desired therapeutic end points differ owing to differences in the biomechanical environment and resulting functions of valves and vessels. CAVD therapeutics will need to address both fibrosis and calcification to restore normal valvular function. Therefore, we focus on known CAVD mechanisms to initiate discussion of potential specific therapeutic strategies that might target the condition independently of vascular calcification.

Potential therapeutic targets

Lipids

Dyslipidaemia-induced valvular changes—CAVD correlates with dyslipidaemia, and investigators in early studies identified substantial lipid accumulation in CAVD leaflets, particularly in early lesions.⁶⁸ The presence of plasma-derived and locally produced lipoprotein(a), apolipoprotein B, and apolipoprotein E was associated with the majority of extracellular lipids in the CAVD leaflets. These findings led to the assumption that, as with atherosclerotic lesions, lipid accumulation within the valve leaflets might drive an inflammatory response and lesion development in early CAVD. In support of this theory, the circulating level of oxidized LDL, a known promoter of atherogenesis, was found to be significantly correlated with fibrocalcific remodelling of the aortic valve.⁶⁹ Accumulation of oxidized LDL is associated with the expression of lipoprotein lipase and small, dense HDL particles within stenotic valve leaflets, and is positively correlated with leaflet remodelling.⁷⁰ *In vitro* studies have shown that oxidized LDL stimulates an increase in the expression of both the osteogenic cytokine BMP-2 and a known mediator of the deposition of calcium phosphate mineral, sodium-dependent phosphate transporter 1.⁷¹ Interestingly, inhibition of this transporter was found to prevent oxidized-LDL-induced increases in BMP-2 expression, suggesting that the lipid signalling might alter VIC phosphate handling, which in turn promotes osteogenic signalling. Given the strong associations between lipids and CAVD, lipid lowering would seem to be an appropriate CAVD therapeutic strategy. However, this strategy has so far failed to produce improved valvular outcomes.

Statins—Several preclinical studies have suggested that drugs successfully developed for vascular complications might also prevent CAVD,^{72–75} but controlled clinical trials have not shown a therapeutic benefit for patients with CAVD.⁷⁶ 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, are some of the most-commonly prescribed drugs in the USA, and have resulted in a significant decrease in morbidity and mortality from cardiovascular events.^{77,78} In addition to cholesterol lipid lowering, part of the clinical success of statins might result from their beneficial anti-inflammatory effect.⁷⁹ Statin use seems to enhance atherosclerotic plaque stability by promoting collagen accumulation within the fibrous cap through a reduction in protease-secreting macrophages.⁸⁰ The association between inflammation and CAVD suggests that statins might also have a positive therapeutic effect in aortic valves. In further support of this notion, *in vitro* studies have shown that statins might also inhibit pathological changes in valvular cell phenotypes.⁸¹ Initial retrospective studies that were published in 2001 indicated that patients using statins had improved CAVD outcomes: a significant decrease in peak systolic pressure across the aortic valve, and significantly larger aortic valve leaflet areas compared with patients not receiving statin treatment.^{73,75} The promising results obtained from these fairly small, retrospective studies led to prospective studies on the efficacy of lipid lowering with statins for patients with CAVD.

In the first investigation of prospectively recorded data, Bellamy and colleagues studied a small cohort of 158 patients with CAVD, of whom 38 were receiving statins.⁸² The statin group had a significantly smaller decrease in aortic valve leaflet area than patients not taking

a statin. These results were supported by findings from the RAAVE study,⁷⁴ in which 50% of 121 patients with moderate-to-severe CAVD received statins, and the treated group had a reduction in the haemodynamic progression of CAVD, as determined by the velocity of blood flow across the aortic valve. Both of these studies contributed to the growing enthusiasm for statins as a therapeutic option for CAVD. However, the studies involved fairly small patient cohorts. In 2005, results from the SALTIRE trial,⁸³ a study with a randomly allocated patient population and placebo controls, showed no significant benefit in terms of blood-flow velocity across the aortic valve or gross leaflet calcium scoring for patients with CAVD receiving statins compared with those receiving placebo.

The SALTIRE trial⁸³ results were supported by those from the SEAS study⁸⁴ in 2008, in which 1,873 patients with mild-to-moderate asymptomatic CAVD were given either statin treatment or a placebo and monitored for major cardiovascular events. A significant decrease in the rate of ischaemic events not associated with CAVD occurred in patients treated with a statin—further indicating the beneficial role of statins in vascular disease. However, statin treatment did not reduce CAVD-associated events. Furthermore, statin treatment did not significantly reduce the number of required aortic valve replacements compared with placebo. A follow-up report from the SEAS study divided the patients into tertiles of CAVD severity on the basis of peak blood velocity across the aortic valve.⁸⁵ Statins did not prevent major aortic valve events even in patients with mild CAVD, indicating that statins cannot be used as a treatment for CAVD, even if administered to patients early in the course of the condition.

The ASTRONOMER trial,⁸⁶ the latest randomized, prospective study on the effects of statins in CAVD, was published in 2010. A total of 269 patients were randomly allocated into two groups with 134 patients receiving 40 mg per day of rosuvastatin, and 135 patients receiving a placebo. At follow-up (median 3.5 years), the peak transvalvular pressure gradient was found to have worsened significantly in both groups, and no benefit from statin use was observed.⁸⁶ In a subsequent analysis of 168 patients included in the ASTRONOMER study, statin treatment did not attenuate valvular diastolic dysfunction, regardless of the initial severity of the dysfunction.⁸⁷

The results of the prospective studies on the effects of statins in patients with CAVD (Table 1) were aggregated in a meta-analysis by Teo and colleagues.⁷⁶ This analysis clearly showed a lack of therapeutic benefit of statins in CAVD, and further emphasized the need to focus on valve-specific therapeutic targets. A major problem with using statins to treat CAVD is that the molecular target of these drugs in the valve is unclear, because hyperlipidaemia and inflammation are not always the causes of this disorder.³⁷ Additionally, calcification is detected using PET–CT imaging in 45% of patients with aortic valve sclerosis,⁴⁹ suggesting that, in patients with mild or severe aortic stenosis, calcification might already have progressed to irreversible stages and cannot be modulated by statin therapy.

Future of lipids and CAVD—In light of the latest statin-treatment guidelines from the ACC and AHA,⁸⁸ clinicians should re-examine and closely monitor valvular effects of statin treatment. Under these guidelines, statin treatment is encouraged for patients with a substantial burden of cardiovascular risk factors, regardless of cholesterol levels. If statin

treatment is effective in preventing the onset of CAVD, the treatment regimen as prescribed under these new guidelines might lead to a lower rate of CAVD in patients who are at risk of the condition. Additionally, potential detrimental valvular changes should also be closely monitored, because the number of patients taking statins is increased under the new guidelines. *In vitro* evidence showed that, although statins decrease myofibroblastic activation of VICs, simvastatin treatment led to a significant increase in the expression of osteoblastic markers in cultured VICs.⁸⁹ Furthermore, data from a substudy of the ASTRONOMER trial showed that patients with metabolic syndrome taking statins tended to have increased rate of CAVD progression than placebo-treated individuals with or without metabolic syndrome.⁹⁰ Young patients with metabolic syndrome had the fastest progression of CAVD. These patients should be monitored closely for valvular changes under the new statin guidelines. If statins increase the rate of CAVD progression in some patients, the guidelines might need to be altered to exclude these patients.

The positive correlation between lipids and CAVD seems to be contradicted by the negative results obtained from the statin trials. Therefore, other methods of lipid modulation should be investigated. Particularly, investigators have identified a genome-wide correlation between a single nucleotide polymorphism in the lipoprotein(a) locus and CAVD in a population of 6,942 participants.⁹¹ Correspondingly, the genetically determined lipoprotein(a) level was associated with CAVD.⁹¹ Statins are ineffective in lowering the lipoprotein(a) level.⁹² Therefore, an important study has started on the effect of niacin treatment, a known regulator of the lipoprotein(a) level, in patients with CAVD. The results of this study might give new insights into the role of lipids in the progression of CAVD. In addition to this study, pioglitazone, an agonist of peroxisome proliferator-activated receptor γ , has shown promise in attenuating lipid deposition and subsequent apoptosis-driven calcification in hypercholesterolaemic mice.⁹³ Interestingly, pioglitazone also reduced markers of osteogenesis in valve leaflets, but not in the aortic wall.⁹³

Alternatively to lipid lowering, studies have suggested that raising the HDL-cholesterol level might have positive valvular outcomes.^{94–97} The level of apolipoprotein (apo)A-I, the major component of HDL, was found to be lower in stenotic aortic valves than in healthy valves, and treating mice and rabbits with an apoA-I mimetic peptide led to a reduction in fibrotic remodelling and calcification.^{95–97} Importantly, the ApoA-I mimetic was given after the animals had developed symptoms of CAVD, and the peptide was successful in reversing pathophysiological changes in the valves. These alternative pathways might be better strategies than statins to combat dyslipidaemia-induced CAVD.

TGF- β

VICs regulate the structural homeostasis and biomechanics of aortic valve tissue by controlling the composition of the extracellular matrix, and changes in VIC phenotype are associated with the pathological leaflet alterations characteristic of CAVD. The mechanisms by which VICs undergo myofibroblastic or osteoblastic phenotypic changes are uncertain. However, signalling pathways involving the transforming growth factor (TGF)- β superfamily of cytokines might have a major part in this process.⁹⁸ TGF- β_1 is secreted by cells and stored in the extracellular matrix as part of a latent complex. After liberated from

its latent state, the active form of TGF- β_1 has been shown to control the expression of proteins associated with VIC myofibroblast activation.^{99–101} Interestingly, TGF- β_1 is often thought to have beneficial effect in vascular diseases, because it promotes the smooth muscle cell phenotype and prevents pathological differentiation of these cells.¹⁰² However, the acquisition of smooth muscle cell characteristics leads to pathological responses in fibroblastic VICs. When exposed to active TGF- β_1 *in vitro*, VICs exhibit increased expression of smooth muscle α -actin and production of type I collagen.¹⁰¹ TGF- β_1 -induced myofibroblast activation of VICs also leads to formation of apoptotic-driven dystrophic calcific nodules *in vitro* that result from increased contractility and intercellular tension.^{81,103} Furthermore, the combination of active TGF- β_1 and mechanical strain applied to aortic valve leaflets in an *ex vivo* bioreactor synergistically increases responses associated with CAVD,¹⁰⁰ and exposing VICs to mechanical strain after incubation with TGF- β_1 exacerbates the formation of dystrophic calcific nodules *in vitro*.¹⁰⁴

The mechanical influence of myofibroblastic activation on VICs is also observed by changing the stiffness of the cell culture substrate.^{67,105} Culturing VICs on commonly used tissue culture polystyrene plates pushes VICs towards a pathological myofibroblastic phenotype, independently of added biochemical stimuli. Similarly, culturing VICs on polyacrylamide-based substrates with elastic moduli—a measure of stiffness—corresponding to the stiffer fibrosa region of the aortic valve leaflets leads to an increase in TGF- β_1 -induced expression of myofibroblast markers. Activation of the Wnt/ β -catenin signalling pathway was found to be responsible for the increase in myofibroblastic activation on stiffer polyacrylamide gels¹⁰⁵ and, notably, Wnt/ β -catenin activation has been observed in the fibrosa layer of excised human aortic valve leaflets. Additionally, Wnt/ β -catenin-induced enhancement of VIC activation was independent of canonical TGF- β_1 signalling pathways. Canonical transcription of TGF- β_1 -associated proteins is controlled by activation and nuclear localization of a specific set of transcription factors, Smad2 and Smad3 (mothers against decapentaplegic homologs 2 and 3).¹⁰⁶ However, study results have indicated that TGF- β_1 can also activate MAP kinase signalling pathways.¹⁰⁷ This effect is interesting, because the normal serine–threonine kinase activity of the TGF- β_1 receptors is transduced into tyrosine kinase activity that phosphorylates a specific MAP kinase, p38.¹⁰⁸ We showed that phosphorylation of p38 is required for TGF- β_1 -induced myofibroblastic activation of VICs.¹⁰⁹ These findings might indicate that various TGF- β_1 outcomes can be independently modulated by targeting specific pathways and, thereby, overcome the therapeutic problems associated with the ubiquity of TGF- β_1 signalling.

In addition to TGF- β_1 , elevated levels of BMP-2 and BMP-4 expression have been observed in stenotic CAVD leaflets.^{110,111} BMP-2 and BMP-4 are members of the TGF- β superfamily of cytokines that are associated with osteogenic processes. The addition of these cytokines to isolated VICs increases the expression of common markers of osteogenic processes.¹¹² Furthermore, the combination of TGF- β_1 and cyclic strain applied to aortic valve leaflets in an *ex vivo* osteogenic environment leads to increased expression of both BMP-2 and BMP-4 within the leaflets.¹¹³ *Ex vivo* and *in vitro* experiments showed that the combination of BMP-4 and mechanical strain leads to pathological differentiation and mineralization of VICs that can be blocked using the BMP-4 inhibitor Noggin.¹¹³ True osteogenic

differentiation of VICs, however, has been difficult to achieve using typical *in vitro* culture conditions. VICs cultured on soft substrates can be induced to differentiate into osteoblast-like cells when supplied with an osteogenic growth media, leading to the formation of ossified calcific nodules *in vitro* (Figure 3).⁶⁷ These *in vitro* studies, coupled with the *ex vivo* observations of both types of calcification, might indicate that both osteogenic and myofibroblastic differentiation pathways contribute to CAVD progression. However, further studies are needed to clarify their particular roles. From a therapeutic viewpoint, direct modulation of the VIC phenotype by targeting TGF- β cytokines might cause unwanted off-target effects owing to the systemic nature of these signalling pathways. Therefore, a strategy would be to identify targets that can interfere with TGF- β pathways in a valve-specific manner.

G-protein-coupled receptors

G-protein-coupled receptors are the most-targeted receptors in pharmacological therapies today.¹¹⁴ These cell-surface receptors often provide tissue-specific targets to avoid unwanted effects associated with inhibition of systemic signalling pathways. Two specific molecular pathways associated with G-protein-coupled receptors have received attention for their capacity to modulate valvular homeostasis: serotonergic and angiotensin signalling.

Serotonergic signalling—Studies data have suggested that targeting serotonergic signalling pathways might inhibit fibrotic responses in cardiopulmonary tissues—perhaps offering a localized therapeutic approach to CAVD.¹¹⁵ The first link between serotonin (5-hydroxytryptamine, 5-HT) and valvular alterations was noted in 1931 by the Dutch pathologist A. J. Scholte, who observed that a patient with a carcinoid tumour also had thickened tricuspid valve leaflets.¹¹⁶ Carcinoid tumours arising from oncogenic metastasis of enterochromaffin cells to the liver lead to the release of excess serotonin into the systemic circulation. Enterochromaffin cells are reservoirs of serotonin and deliver serotonin to platelets for controlled, physiological distribution. However, when they metastasize to the liver, excess serotonin is delivered directly to the systemic circulation. This serotonin is carried to the right side of the heart through the inferior vena cava, where interaction with serotonergic receptors on VICs in the tricuspid and pulmonary heart valves leads to the extracellular-matrix secretion and the thickening of the leaflets that is characteristic of valve disease. After the blood enters pulmonary circulation, monoamine oxidases convert serotonin into 5-hydroxyindole acetic acid, rendering the molecule inactive before the blood enters the left side of the heart. Therefore, carcinoid heart disease is one of the few disorders that selectively affects the valves on the right side of the heart.

For over six decades, the specific serotonergic receptor responsible for carcinoid heart disease was not known owing to the fact that serotonin has a similar affinity for many of its receptors, and several of the subtypes are expressed throughout the cardiovascular system. Identification of the 5-HT_{2B} receptor as the major heart valve target of serotonin occurred as a result of inadvertent targeting of the 5-HT_{2B} receptors with the popular anorexigen combination of fenfluramine and phentermine.^{117–119} Short-term usage of this drug was found greatly to increase the risk of valve disease via agonist signalling of norfenfluramine (a fenfluramine metabolite) at 5-HT_{2B} receptors on VICs.^{118,119} In response to the

unintended valvular responses to particular serotonergic drugs, the US National Institute of Mental Health Psychoactive Drug Screening Program screens large numbers of drug compounds for agonist activity at 5-HT_{2B} receptors to identify compounds with the potential to have valvular adverse effects.¹²⁰ Subsequently, two drugs that were widely used to treat Parkinson disease, cabergoline and pergolide, were also found to exhibit agonist signalling at 5-HT_{2B} receptors and have been associated with heart valve disease.¹²⁰ Agonist-dependent signalling via 5-HT_{2B} receptors has been found to lead to increased VIC proliferation in the valves,¹²¹ and an increase in TGF- β ₁ secretion in the pulmonary artery.¹²²

The interaction between 5-HT_{2B} receptors and TGF- β ₁ led us to hypothesize that 5-HT_{2B} receptors might be a localized target to disrupt TGF- β ₁ signalling in VICs that would avoid the adverse effects of targeting TGF- β ₁ directly.¹⁰⁹ We found that antagonism at 5-HT_{2B} receptors abrogated p38 phosphorylation by TGF- β ₁, which prevented myofibroblast activation and subsequent dystrophic calcific nodule formation of VICs *in vitro*.¹⁰⁹ TGF- β ₁ activates p38 signalling via proto-oncogene tyrosine-protein kinase Src, and antagonism of 5-HT_{2B} receptors physically arrests the movement of Src in VICs, which prevents its association with p38.¹⁰⁹ The physical sequestration of Src might be the result of receptor internalization—a process known to involve activated Src in similar receptors—after 5-HT_{2B}-receptor antagonist treatment. The potential therapeutic benefit of 5-HT_{2B}-receptor antagonism for CAVD should be further investigated in **placebo** controlled studies. As the role of serotonin in aortic-valve homeostasis is defined, therapeutics might be developed specifically to target these pathways.

Angiotensin signalling—Jaffré and colleagues demonstrated that 5-HT_{2B} receptors work in combination with angiotensin II type 1 receptors to mediate common hypertrophic signalling pathways, such as IL-2, IL-6, and tumour necrosis factor, in cardiac fibroblasts.¹²³ These results indicate that, in cardiac fibroblasts, the function of both of these G-protein-coupled receptors is required for the activity of either one. Consequently, by inhibiting one receptor, the action of the other receptor is likewise inhibited. Interestingly, stenotic aortic valves have significantly higher expression levels of enzymes associated with angiotensin II production, such as angiotensin-converting enzyme (ACE) and chymase, as well as the angiotensin II type 1 receptor.¹²⁴ Additionally, angiotensin receptors have been proposed as potential targets owing to their interaction with pathways that are involved in both CAVD and other diseases. Specifically, angiotensin II-receptor blockers (ARBs) are used to treat Marfan syndrome.¹²⁵ ARBs mitigate the excessive canonical TGF- β ₁ signalling associated with this syndrome.^{126,127} ARB therapy is more localized and has fewer adverse effects than those that target TGF- β ₁, which is systemically ubiquitous.

To date, however, retrospective epidemiological studies into the effectiveness of ARB treatment for CAVD have been inconclusive. Early reports indicated that ARBs were ineffective in preventing the progression of CAVD in high-risk, elderly patients,¹²⁸ but subsequent studies have shown a therapeutic effect of ARBs, with patients having reduced atrioventricular remodelling,¹²⁹ and improved overall cardiovascular outcomes from atrioventricular stenosis.¹³⁰ In the latest retrospective analysis of the effect of angiotensin inhibition on aortic stenosis, 338 patients were divided into four groups: patients without

hypertension, but given no angiotensin-associated treatment; patients with hypertension, but given no angiotensin-associated treatment; patients with hypertension and treated with an ACE inhibitor; and patients with hypertension and treated with an ARB.¹³¹ Patients treated with ARBs had significantly slower progression of CAVD and a reduced mortality than those in any of the other groups. Patients treated with ACE inhibitors had a slower rate of CAVD progression than patients with hypertension who were not receiving angiotensin inhibition, but not compared with patients without hypertension who were not receiving treatment. These results indicate that ACE inhibition might abrogate CAVD responses associated with increased strain on the valve leaflets during hypertension. However, ARB treatment might activate antifibrotic responses beyond the changes caused by the hypertensive state. Rabbits treated with a specific ARB olmesartan showed decreased levels of valvular markers of myofibroblastic and osteoblastic phenotypes, as well as enhanced overall valvular function.¹³²

Notably, ARB treatment mitigates TGF- β_1 -induced dilated mitral valve remodelling that is also observed in patients with Marfan syndrome.¹³³ This observation suggests that ARB treatment might be more effective in inhibiting extracellular-matrix degradation than inhibiting the fibrotic extracellular-matrix accumulation that is a hallmark of CAVD progression. The initiating events in CAVD might involve extracellular-matrix degradation that precedes and then accompanies fibrotic changes, and using ARBs at early points might arrest CAVD progression. However, the treatments might be less effective after fibrosis has begun. Further studies are needed to clarify the effectiveness of ARBs in CAVD therapy, but later stages of the disease might be responsive to ARB therapy in combination with an antifibrotic strategy to restore valvular homeostasis. Angiotensin II type 1 receptors and 5-HT_{2B} receptors might modulate different, but overlapping, processes involved in extracellular-matrix remodelling: inhibition of the angiotensin II type 1 receptor prevents matrix degradation, whereas antagonism of 5-HT_{2B} receptors prevents collagen accumulation and fibrosis. Future studies might explore the targeting of both pathways to prevent fibrotic remodelling in CAVD.

Cadherin-11

Cadherin-11 is associated with fibrotic tissue remodelling in pulmonary fibrosis and rheumatoid arthritis.^{134–136} As with CAVD, these diseases are characterized by both inflammation and myofibroblast-associated fibrosis. Cadherin-11 was originally named OB-cadherin, in reference to its elevated expression in osteoblasts.¹³⁷ Subsequently, cadherin-11 expression was found to be associated with fibroblast-to-myofibroblast transitions, in which the conversion of adherens junctions from N-cadherin to cadherin-11 leads to an increase in intercellular adhesion strength.¹³⁸ Increased intercellular adhesion might help with coordinated cellular contractile mechanisms that are important in extracellular-matrix remodelling involved in wound healing. However, as with other processes involved in matrix remodelling, overactivation leads to fibrosis. In CAVD, cadherin-11 expression was shown to be associated with calcific regions and is also colocalized with aortic smooth muscle α -actin in histological sections of calcified leaflets from patients.¹³⁹ The formation of TGF- β_1 -induced calcific nodules by VICs *in vitro* is dependent on an increase in VIC expression of cadherin-11 that causes a substantial accumulation of intercellular tension.

Treating VICs with TGF- β_1 increases cadherin-11 expression in a manner that is dependent on signalling via mitogen-activated protein kinases 1 and 3 (commonly known as extracellular signalling-regulated kinases 1 and 2, Erk1/2).¹³⁹ The combination of increased contractility associated with elevated smooth muscle α -actin and increased intercellular adhesion through cadherin-11 leads to elevated tension in TGF- β_1 -treated VIC monolayers.¹³⁹ The addition of mechanical strain to these cultures creates a force imbalance that results in the formation of dystrophic calcific nodules. The exact *in vivo* translation of this *in vitro* process remains uncertain. However, cadherin-11 might be at the interface of the inflammatory, biomechanical, fibrotic, and calcific processes that have been identified in CAVD.

NOTCH1 mutations

In a seminal study, Garg and colleagues discovered that *NOTCH1* haploinsufficiency results in CAVD with 100% penetrance in humans.¹⁴⁰ The *NOTCH1* mutation is of particular interest given its role in bicuspid aortic valve and CAVD in humans. The most-common congenital heart valve defect is the bicuspid aortic valve, which is observed in 1–2% of the population, and mutations in notch signalling pathways have been associated with a proportion of these patients.¹⁴¹ Although bicuspid aortic valve disease and CAVD seem to have divergent mechanisms,^{142,143} notch 1 signalling might be involved in both. The notch signalling pathway, which includes notch receptor proteins 1–4, protein jagged and delta-like protein, and the notch1 intracellular domain, is an important regulator of cell fate in the cardiovascular system,¹⁴⁴ and multiple other organs.¹⁴⁵ Signalling via the four notch receptors has been found to synergize with,^{146–148} or antagonize,^{149–152} TGF- β_1 signalling in a context-dependent and cell-type-dependent manner. However, *Notch1*^{+/-} leads to increased synthesis, signalling, or both, of BMP-2 and TGF- β_1 in the aortic valve leaflets of mice,^{153,154} which results in the early signs of CAVD. Additionally, toll-like receptor 4 (TLR-4) has been shown to be associated with BMP-2 expression in CAVD leaflets,^{155–157} and *in vitro* studies with human VICs showed that activation of TLR-4 leads to increased BMP-2 expression via a process dependent on the cleavage and nuclear translocation of notch 1.¹⁵⁷ TLR-4 is commonly activated in response to inflammatory stimuli; therefore, notch 1 signalling might be an important initiator of VIC phenotypic changes in response to the inflammation observed in CAVD. *Notch1*^{+/-} mice provide a clinically relevant animal model to test therapeutic strategies for CAVD. However, the calcific regions mainly develop on the ventricularis side of the leaflet in mice,¹⁵³ which is not where calcification occurs in humans. However, why calcification develops in the aortic valve, but not the pulmonary valve, which should experience similar shear stress, is uncertain. Changes in both biomechanical stress and molecular signalling might synergistically contribute to pathological valve remodelling, but whether one predominates and why is currently uncertain.

Purinergic signalling

Investigators have identified an important role for extracellular ATP in the regulation of VIC mineralization. Culturing VICs in a high level of phosphate led to a depletion in extracellular ATP via increased expression of ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (E-NPP 1).¹⁵⁸ This ATP depletion resulted in

dystrophic VIC mineralization in an apoptotic-driven process. ATP promotes VIC survival via signalling at the P2Y purinoreceptor 2 (P2Y₂). E-NPP 1 hydrolyses ATP, and a genetic polymorphism leading to elevated E-NPP 1 expression was found to be associated with CAVD.¹⁵⁸ Interestingly, E-NPP 1 signalling has also been associated with lipid handling in VICs. CAVD leaflets were found to contain elevated expression of phospholipase A₂, an enzyme that hydrolyses phospholipids to generate lysophosphatidylcholine.¹⁵⁹ This compound promotes the mineralization of VICs in an E-NPP 1-dependent manner. Therefore, E-NPP 1 might be involved in both phosphate-mediated and lipid-mediated VIC mineralization. Agonism of P2Y₂ receptors using an ATP mimic reversed E-NPP 1-mediated mineralization *in vitro*,¹⁵⁸ and might, therefore, be a therapeutic target for CAVD. However, data from another study indicated that an elevated level of ATP can actually promote osteogenic mineralization by increasing VIC alkaline phosphatase activity *in vitro*,¹⁶⁰ an indicator of osteoblastic differentiation. Future studies should address the osteogenic versus dystrophic mineralization responses to ATP signalling to determine whether this pathway can be targeted *in vivo*.

Elastin and cathepsin S

The ventricularis layer of the aortic valve is composed mainly of elastin that allows the valve leaflets high radial strain in response to diastolic loading. Disruption of this elastin layer has been shown to result in pathological remodelling associated with CAVD.^{161–163} Chronic renal disease is strongly correlated with CAVD in humans, and CAVD processes in *Apoe*^{−/−} mice are accelerated by inducing chronic renal disease through resection of five-sixths of the total kidney volume.¹⁶¹ Interestingly, induction of chronic renal disease in *Apoe*^{−/−} mice leads to a marked increase in activity of the elastolytic enzyme cathepsin S, whereas deletion of cathepsin S in these mice mitigates CAVD progression.¹⁶¹ Cathepsin S remodelling in this mouse model was found to be closely associated with the presence of inflammatory cell markers within the leaflets.¹⁶¹ Additionally, elastin degradation peptides, also known as matrikines, are highly biologically active molecules and might induce VIC osteogenic differentiation in aortic valves.¹⁶¹ Even in the absence of chronic renal disease, elastin haploinsufficient mice have an increased level of myofibroblastic VICs and progressive valvular remodelling, resulting in aortic regurgitation.¹⁶² Application of pathological cyclic strain to aortic valve leaflets in an *ex vivo* bioreactor increased the activity of cathepsin S and a similar protease, cathepsin K, within the leaflets.¹⁶⁴ This finding might indicate that the induction of matrix remodelling processes is a function of inflammatory and biomechanical factors, and that the remodelling response itself can induce VIC differentiation. Therefore, inhibiting these remodelling processes early and focusing on elastin preservation might be very important to successful CAVD therapies.

Conclusions

To address the need for a CAVD therapy, emphasis should be placed on targeting valvular-specific signalling pathways and achieving a desirable biomechanical outcome that restores normal aortic valve function (Box 1). Treatment strategies for CAVD differ from those for atherosclerotic plaques: stabilizing atherosclerotic plaques to prevent thrombosis by promoting a thick fibrotic cap might be an appropriate strategy for many patients with

vascular calcification. However, the dynamic environment of the aortic valve dictates that physiological tissue structure be restored (Figure 4). Both the fibrotic and calcific mechanisms that underlie CAVD must be addressed, and one strategy might be to use a combination of therapies, or to tailor therapies to the stage of CAVD. In addition to the molecular pathways that focus on protein-based targets presented in this Review, other studies have identified potential microRNA regulators of valve cell phenotype and resultant tissue homeostasis.^{20,110} These novel regulators warrant further investigation, because they might be specific targets to control VIC and VEC behaviour.

Box 1

Potential therapeutic targets for CAVD

Inflammation: reduce remodelling and release of cytokines involved in early CAVD. Might be valid only if targeted at initiating CAVD events^{32,48,53,54}

Sodium-dependent phosphate transporter 1: mediates calcium phosphate mineral deposition. Oxidized LDL has been shown to induce osteogenic signalling via activation of this transporter; therefore, inhibition might reverse these effects⁷¹

PPAR γ : pioglitazone, a PPAR γ agonist, has shown potential in mitigating lipid-deposition and CAVD in hypercholesterolaemic mice. Action might be valve-specific⁹³

Lipoprotein(a): a single nucleotide polymorphism in the gene encoding lipoprotein(a) is associated with CAVD. The level of lipoprotein(a) cannot be modulated with statins, but lowering by other means might be beneficial⁹¹

HDL cholesterol: a high level of HDL cholesterol is associated with improved valvular outcomes. An apolipoprotein A-I mimetic peptide might reverse CAVD-associated leaflet remodelling^{94–97}

Angiotensin II type 1 receptor: block this receptor to inhibit TGF- β_1 expression and proteolytic enzymes involved in matrix remodelling. Might have to be combined with antifibrotic strategies^{129–132}

5-Hydroxytryptamine receptor 2B: inhibit TGF- β_1 -induced activation of VICs and subsequent fibrotic remodelling. Might function in combination with angiotensin II receptors to modulate homeostasis¹⁰⁹

Cadherin-11: inhibit myofibroblast differentiation and calcific nodule morphogenesis of VICs. Might be involved in both calcific and fibrotic cellular responses¹³⁹

Notch 1: activation of notch 1 signalling might inhibit both TGF- β_1 (fibrotic) and bone morphogenetic protein 2 (calcific) responses. Might also have a role in cellular mechanotransduction^{139,152,153}

P2Y purinoreceptor 2: ATP promotes VIC survival via signalling at the P2Y purinoreceptor 2. Agonism of this receptor might provide a strategy to prevent apoptotic-driven dystrophic calcification^{158,159}

Cathepsin S: inhibit elastin degradation that induces both myofibroblastic and osteogenic reprogramming of VICs. Might be involved in early remodelling or inflammatory processes^{161–163}

Abbreviations: CAVD, calcific aortic valve disease; PPAR γ , peroxisome proliferator-activated receptor γ ; TGF- β_1 , transforming growth factor β_1 ; VIC, valve interstitial cell.

The development of a therapeutic agent for CAVD might dovetail with the need for detection strategies to identify early changes in the leaflets. Reversibility of calcific deposition in valves is uncertain. However, the early extracellular-matrix remodelling responses that presage gross fibrosis and calcific nodule formation might respond to therapy.¹⁶⁵ Traditional detection of valvular dysfunction using echocardiography cannot be used to identify these early leaflet changes, because regurgitant blood flow and leaflet thickening occur in subsequent stages of CAVD remodelling and calcification. PET–CT imaging modalities have emerged as a means to detect inflammation and mineralization within valve leaflets of patients,⁴⁹ and optical molecular imaging strategies have been successfully used to visualize the progression of CAVD in mice.⁵⁴ These technologies are highly sensitive to small changes in the leaflets, and might offer the resolution necessary to identify patients who will respond to therapy. Using these imaging strategies might also give better insight into the temporal aspects of CAVD, and might lead to an improved understanding of the optimal therapeutic strategy at each stage of CAVD.

Review criteria

PubMed and Google Scholar databases were used to obtain initial articles of interest by using the search terms: calcific aortic valve disease, vascular calcification, aortic valve stenosis, fibrocalcific remodeling, valve interstitial cells, valve endothelial cells. We then selected relevant texts from all years, and special attention was given to recent articles that focused on the molecular mechanisms of CAVD. Reference lists from these papers were then used for further leads and to help identify previous studies on which current paradigms have been established.

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Key points

Calcific aortic valve disease (CAVD) seems to be distinct from vascular calcification in terms of both disease mechanisms and progression, although some risk factors are shared

Valve-specific drug targets are likely to be required to prevent or treat CAVD, given that drug strategies for vascular disease have not proven successful when applied to CAVD

Ubiquitous targeting of cytokines that are thought to lead to valve disease is not likely to be feasible or beneficial because they are required for wound healing elsewhere in the body

Targeting specific combinations of G-protein-coupled receptors might simultaneously prevent myofibroblast activation and collagen accumulation, which are thought to be two of the main initiators of, and contributors to, CAVD

Functional blocking of cadherin-11 might be an effective target to prevent mechanotransduction between valve cells that is believed to exacerbate cytokine signalling and synergistically lead to calcification

Lipid lowering might be an effective strategy for CAVD treatment; however, a targeted approach centred on lowering the lipoprotein(a) level and increasing the HDL-cholesterol level might be required

Figure 1.

Cardiovascular inflammation and calcification. a | Mouse aortic valve leaflets have a similar gross morphology to human leaflets.¹⁶¹ b | Molecular imaging can be used to identify a high level of inflammation in the leaflets.¹⁶¹ c | This inflammation is closely associated with regions of calcification.¹⁶¹ d | The association between calcification and inflammation is observed throughout cardiovascular tissues.⁵³ Panels a–c reprinted from Aikawa, E. *et al.* Arterial and aortic valve calcification abolished by elastolytic cathepsin S deficiency in chronic renal disease. *Circulation* **119** (13), 1785–1794 (2009). Panel d reprinted from Aikawa, E. *et al.* Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging *in vivo*. *Circulation* **116** (24), 2841–2850 (2007).

Figure 2.

Structure–function relationship in aortic valve biomechanics. a | Systolic contraction of the left ventricle forces the aortic valve leaflets to open, allowing blood to enter the aorta. b | The reversed pressure gradient, created when the heart rests in diastole, causes the aortic valve leaflets to close, preventing retrograde blood flow into the heart. The circumferential alignment of collagen allows the leaflets to stretch in the radial direction so that apposing leaflets meet and seal the valve annulus, while providing the tensile strength required to prevent leaflet prolapse. c | Imaging of picrosirius red stained collagen fibres using polarized light illustrates the alignment of collagen fibres within the aortic valve leaflets.¹⁶⁶ Panel c reprinted from Aikawa, E. *et al.* Human semilunar cardiac valve remodeling by activated cells from fetus to adult: implications for postnatal adaptation, pathology, and tissue engineering. *Circulation* **113** (10), 1344–1352 (2006).

Figure 3.

The evolution of *in vitro* calcific nodules. a | Early studies reported calcific nodules formed by apoptotic VICs in culture after treatment with transforming growth factor β_1 .¹⁰³ b | Subsequent studies showed the formation of dystrophic calcific nodules in real-time owing to aggregation of myofibroblastic VICs.⁸¹ c | This aggregation process is exacerbated by the addition of mechanical strain, where a necrotic core (red fluorescence) is surrounded by a ring of apoptotic VICs (green fluorescence).^{104,139} d | Calcific nodules with osteogenic characteristics (osteopontin staining shown) can be generated on soft culture substrates.⁶⁷ These nodules have a similar morphology to dystrophic nodules with VICs radiating from the nodule. e | By contrast, alizarin red staining of calcification in smooth muscle cell cultures is observed in association with intact smooth muscle cells (arrow). Abbreviation: VIC, valvular interstitial cell. Panel a reprinted from *Ann. Thorac. Surg.* **75** (2) Jian, B. *et al.* Progression of aortic valve stenosis: TGF-1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. 457–765 © 2003, with permission from Elsevier. Panel b reprinted from Benton, J. A. *et al.* Statins block calcific nodule formation of valvular interstitial cells by inhibiting α -smooth muscle actin expression. *Atheroscler. Thromb. Vasc. Biol.* **29** (11), 1950–1957 (2009). Panel c reprinted from Hutcheson, J. D. *et al.* Cadherin-11 regulates cell–cell tension necessary for calcific nodule formation by valvular myofibroblasts. *Atheroscler. Thromb. Vasc. Biol.* **33** (1) 114–120 (2013). Panel d reprinted from Yip, C. Y. *et al.* Calcification by valve interstitial cells is regulated by the stiffness of the extracellular matrix. *Atheroscler. Thromb. Vasc. Biol.* **29** (6), 936–942 (2009).

Figure 4.

Biomechanical consequence of cardiovascular calcification. a | Aortic valve leaflets rely on appropriate biomechanical responses to open during systole and seal the valve during diastole. Similarly, vessels expand under systolic pressure. b | However, fibrocalcific remodelling is detrimental to aortic valve function. Leaflet stiffening in calcific aortic valve disease impairs the opening and closing of the leaflets. Localized calcification is not detrimental to vessel function, and fibrotic collagen accumulation is often desired to stabilize atherosclerotic plaques and prevent thrombosis.

Table 1

Summary of studies involving statins in CAVD

Study	Design	Outcomes
Retrospective study (2001) ⁷³	180 participants aged >60 years; peak systolic pressure gradient measured as marker of aortic valve function	Significant decrease in peak systolic pressure gradient in patients taking statins
Retrospective study (2001) ⁷⁵	174 patients with mild-to-moderate CAVD (57 statin-treated, 117 not taking statins); measured changes in aortic valve area	Patients taking statins had a slight improvement in aortic valve remodelling
Prospective analysis (2002) ⁸²	156 patients of whom 38 received statin treatment; monitored changes in aortic valve area	Patients taking statins had a slight improvement in aortic valve area
SALTIRE trial (2005) ⁸³	Double-blind, placebo-controlled trial of 155 patients given atorvastatin (mean follow-up 25 months)	No significant difference between atorvastatin and placebo in aortic jet velocity or valve calcification
RAAVE trial (2007) ⁷⁴	Prospective study of 121 patients with moderate-to-severe CAVD; 61 patients received rosuvastatin and 60 received no treatment	Statin treatment seemed to slow haemodynamic progression of CAVD, as indicated by changes in aortic jet velocity
SEAS trial (2008) ⁸⁴	1,873 patients with mild-to-moderate asymptomatic aortic stenosis receiving either simvastatin or placebo daily	Statin treatment did not reduce cardiovascular events associated with CAVD
SEAS follow-up (2010) ⁸⁵	1,763 patients from the SEAS trial divided into tertiles according to CAVD severity on the basis of peak aortic jet velocity	Statins did not improve CAVD outcomes regardless of initial severity
ASTRONOMER trial (2010) ⁸⁶	Double-blind study of 269 patients randomly allocated to receive rosuvastatin or placebo	Statin treatment did not reduce deterioration of peak aortic pressure gradient

Abbreviation: CAVD, calcific aortic valve disease.