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STATE-OF-THE-ART REVIEW

Navigating the Landscape of Translational Medicine of Calcific Aortic Valve Disease

Bridging Bench to Bedside

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ABSTRACT

Calcific aortic valve disease (CAVD) is a prevalent condition characterized by pathological thickening and calcification of the aortic valve, leading to increased pressure overload and cardiac remodeling, particularly in individuals aged 65 and older. This review synthesizes recent advances in understanding the pathogenesis of CAVD, focusing on key mechanisms including hemodynamic alterations, endothelial dysfunction, lipid deposition, inflammation, and fibrotic calcification. We evaluate emerging therapeutic targets based on pivotal basic research and clinical trials, highlighting the potential for mechanism-oriented interventions. Furthermore, we explore the implications of lipid-lowering therapies, anti-inflammatory strategies, and antifibrocalcific agents, as well as novel bioprosthetic designs aimed at enhancing patient outcomes. Additionally, we discuss the inherent genetic and molecular backgrounds influencing individual susceptibility to CAVD, emphasizing the promise of personalized therapy. By bridging the gap between basic science and clinical application, this review aims to guide future research efforts toward more effective prevention and treatment strategies for CAVD. (JACC Asia. 2025;5:503–515) © 2025 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Calcific aortic valve disease (CAVD) is characterized by pathological thickening and calcification of the aortic valve (AoV), which leads to progressive increase of pressure overload and eventually maladaptive cardiac remodeling.[1] As the most common nonrheumatic valvular disease, especially in individuals aged 65 years and older, CAVD constitutes a growing economic and health burden,[2,3] and aortic valve replacement is the only accessible treatment option because effective conservative therapy is absent by far. This led investigators to ponder whether early surgical intervention might provide earlier benefit in some asymptomatic patients with severe aortic stenosis. It turned out that the early surgery group had a significantly lower incidence of all-cause mortality compared with the conservatively treated group, as evidenced in 2 independent clinical trials.[4,5] Stagnation of clinical translation years earlier tipped the scales in the direction of surgical intervention, even as evidence suggested that patients with mild/moderate stenosis might still benefit from early surgical intervention.[6] As basic research progressed in recent years, however, various aspects of the pathogenesis of CAVD are gradually being elucidated, and for each pathogenic segment, there is a corresponding target that is either in need of validation or has already shown preliminary clinical results. Through reviewing the top-cited and most influential basic research and corresponding randomized controlled trials in recent years and

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ABBREVIATIONS AND ACRONYMS

BAV = bicuspid aortic valve

CAVD = calcific aortic valve disease

CHIP = clonal hematopoiesis of indeterminate potential

Lp(a) = lipoprotein (a)

RCT = randomized controlled trial

VEC = valvular endothelial cell

VIC = valvular interstitial cell

assessing them in terms of surveillance, prevention, and treatment, this review aims at providing guidance for subsequent mechanism-oriented therapeutic research, of which we are all drivers and witnesses (**Central Illustration**).

A BRIEF OVERVIEW OF THE PATHOGENIC MECHANISM OF CAVD

After endocardial cushion augmentation, endothelial to mesenchymal transition (EndoMT), and valve primordium remodeling, the postnatal aortic valve presents a slender 3-layered structure consisting of fibrosa, spongiosa, and ventricularis with distinct extracellular matrix (ECM) stratification.[7] Valvular endothelial cells (VECs) encircle the outmost layer, the integrity of which maintains the homeostasis of normal aortic valve. As the most predominant cell population, valvular interstitial cells (VICs) are in fact a highly heterogeneous group, which was once hypothesized to be classified into quiescent, activated, progenitor, and osteoblastic subtypes.[8] Subsequently redefined VIC subtypes could more or less fit into these 4 categories. Taking the example of $CD34^{+}$/PGDFR-α^{+} telocytes in aortic valve, the proportion of this progenitor VIC subpopulation with restorative function decreased progressively with aging.[9] The rest are tissue-resident immune cells, predominantly macrophages, whose function remains uncharted.[10]

CAVD is now perceived as a multistage disease spectrum with sequential yet intertwined pathogenic mechanisms[1] (**Figure 1**). Endothelial dysfunction caused by disrupted blood flow and metabolic abnormalities results in the deposition of lipoprotein, which further stimulates macrophage infiltration, oxidative stress, and the production of proinflammatory cytokines.[11] Changes in valve homeostasis trigger the activation of quiescent VICs into myofibroblasts that produce an excess of collagen.[12] As a result of repeated degradation and redeposition, ECM becomes disorganized and stiff, further promoting osteogenic differentiation of VICs through the mechanosensitive Rho A/ROCK1 signaling pathway.[13] Furthermore, disordered ECM is vulnerable to dystrophic calcification characterized with the nucleation of calcium phosphate crystals.[14] The coupling of passive calcification and active osteogenesis finally resulted in mechanical aortic stenosis (AS).

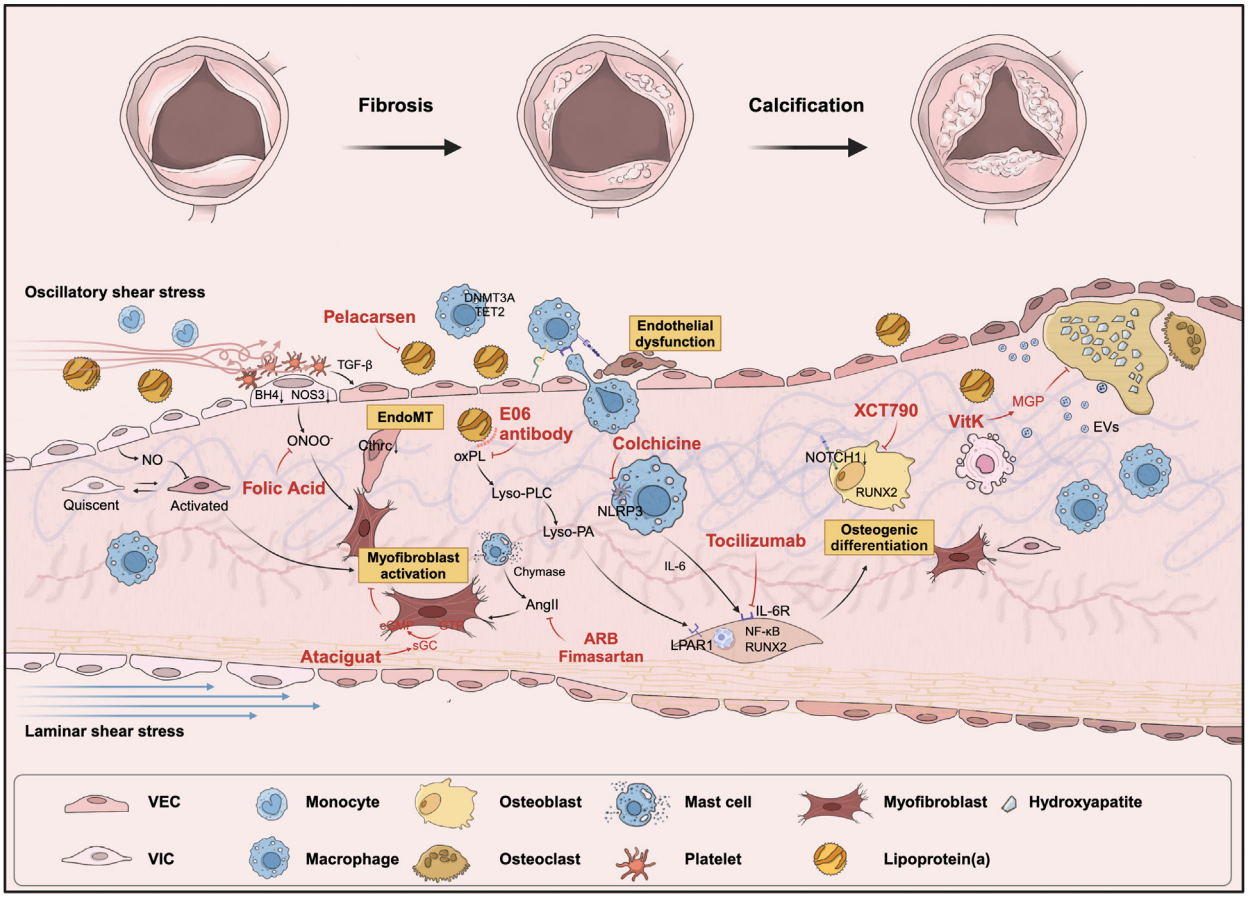
HEMODYNAMICS AND ENDOTHELIAL DYSFUNCTION

Congenital or acquired alteration of hemodynamic pattern is generally considered to be the initiation of CAVD.[1,11] Typically, the ventricular side of the aortic valve is exposed to laminar shear stress peaked at 71 dyn/cm^2 during systole, while the aortic side is exposed to low amplitude oscillatory shear stress (OSS) peaked at only about 20 dyn/cm^2.[15,16] In vitro analogs of OSS facilitated inflammatory activation of VECs, along with Endo-MT.[17,18] Consistent with the difference in stress pattern, the fibrosa layer on the aortic side is predisposed to be the key region of calcification.[19] Scanning electron microscopy images visualized 2 patterns of injury of endothelium in the aortic side-corrugation with microvilli, a sign of endothelial activation, and scattered denudation with platelet aggregation.[20] Once the transvalvular pressure comes to a pathological level arising from, eg, hypertension, and elevates further with the degree of stenosis, high shear stress will be transduced into a “wear and tear” signal and promote endothelial dysfunction.[21]

Currently, the valvular hemodynamic parameter (mean transvalvular pressure gradient and peak aortic jet velocity) is mainly assessed by 2-dimensional Doppler echocardiography based upon hypothetic cross-section.[6] However, when faced with a complex hemodynamic environment, such a hypothesis might lead to oversimplified conclusions. Advances in 4-dimensional flow cardiac magnetic resonance imaging technology made the comprehensive visualization and quantification of the dynamics of aortic blood flow patterns possible in vivo, because of the reconstruction of aortic and the integration of the time dimension.[22] Apart from that, a series of new metrics provided by 4-dimensional cardiac magnetic resonance imaging, such as shear stress and turbulent kinetic energy, allow for more detailed risk and prognostic stratification of CAVD.[23]

Through a novel hierarchical guidewire-induced CAVD mouse model, valve endothelium was proven capable of self-repairing upon mild injury, primarily through noncanonical TGF-β signaling-related up-regulation of collagen triple helix repeat containing 1 (Cthrc1),[24,25] which declined with aging. This capacity diminished even more dramatically for endothelium exposed to OSS because its high turnover rate led to premature replicative senescence.[26] Senescent

CENTRAL ILLUSTRATION **Mechanism-Oriented Therapeutic Target Identification of Calcific Aortic Valve Disease**



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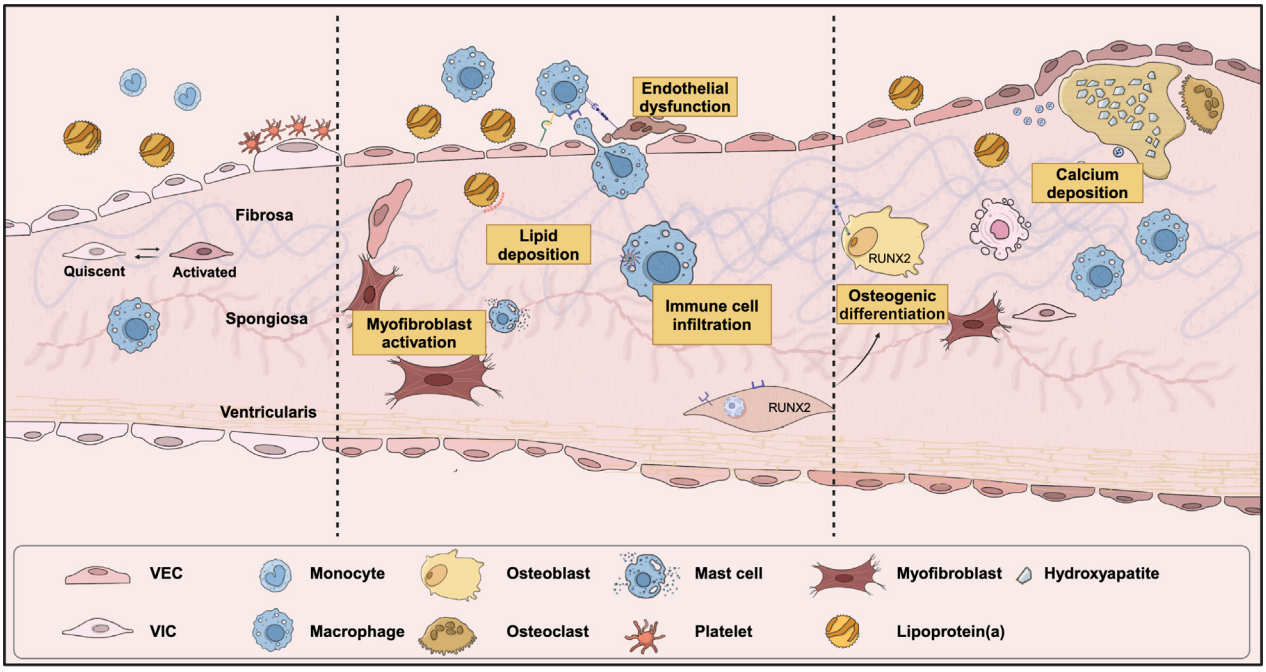
Calcific aortic valve disease (CAVD) is perceived as a multistage disease spectrum with sequential yet intertwined pathogenic mechanisms, classified as endothelial dysfunction, inflammation, lipid infiltration, myofibroblast activation, and calcium deposition. Marked in red are representative therapeutic agents that are currently undergoing clinical trials and have shown preliminary efficacy, along with corresponding therapeutic targets for each pathogenic segment. VEC = valvular endothelial cell; VIC = valvular interstitial cell.

endothelium is accompanied by several other issues, including the release of proinflammatory cytokines and EndoMT,[27] while no studies have yet emerged utilizing senolytics that target senescent endothelium.

In addition to direct endothelial injury, endothelial dysfunction in a broad sense encompasses sorts of maladaptive changes in endothelial functions when presented with pathophysiological stimuli (eg, dyslipidemia, hypertension, obesity, and aging). VEC-derived nitric oxide (NO) guarantees the normal development of AoV and maintains the homeostasis between VECs and VICs through the activation of NOTCH1 signaling pathway.[28] The deficiency of NOS3, which encodes endothelial NO synthase, significantly increased the prevalence of CAVD and bicuspid aortic valve (BAV).[29] Meanwhile, VECs in calcific AoV exhibited profound endothelial NO synthase uncoupling. Normal eNOs depends on tetrahydrobiopterin (BH4) to maintain itself as a coupling dimer. It was demonstrated that the inhibition of de novo or salvage pathway of BH4 biosynthesis resulted in the uncoupling of eNOs, fueling oxidative stress in the endothelium.[30] Together with the deficiency of superoxide dismutase (SOD) in the aortic side endothelium, the persistent oxidative stress ultimately led to myofibroblast activation and calcification of VICs.[31] It is noteworthy that folic acid has been demonstrated to inhibit aortic valve calcification and to reduce the risk of cardiovascular disease by increasing the

FIGURE 1 **Pathogenic Mechanism of Calcific Aortic Valve Disease**



Endothelial dysfunction disrupts the balance of activation and quiescence within the valve, leads to lipid deposition and immune cell infiltration. Furthermore, valvular interstitial cells (VICs) undergo myofibroblast activation and result in collagen accretion presenting as valvular fibrosis. The combination of apoptotic myofibroblasts and actively differentiating osteoblast-like cells leads to eventual calcium deposition. VEC = valvular endothelial cell.

bioavailability of BH4, and related clinical trials have also been registered.[32] Besides, pathological VECs exhibited high expression of a range of major histocompatibility complex II molecules upon proinflammatory stimuli,[33] whereas the subsequent adaptive immune response is scarcely investigated. In addition to known stimuli such as OSS and oxidized lipids, a range of pathogen-associated molecular patterns and damage-associated molecular patterns remain to be explored with the aim of targeting these proinflammatory factors for intervention.[27]

Taken together, alleviation or reversal of endothelial dysfunction is a potentially effective strategy, while it is now targeted only for known risks, such as hyperlipidimia.[34] However, a recent study suggested that statins inhibited the transcriptional activity of EndoMT-regulated genes by decreasing chromatin accessibility at the SOX9 enhancer region.[35] It reveals the significance of endothelium-targeted therapy, complemented by a shear-responsive carrier that enables AoV-specific drug delivery. Meanwhile, various endothelium-specific protective agents also provided guidance for the optimization of bioprosthetic valves. The novel bioprosthetic valve developed by our group with enhanced NO release had better reendothelialization efficacy and better long-term prognostic potential compared to commercially available ones currently.[36]

LIPID DEPOSITION AND LIPID-LOWERING THERAPY

The similarities in the pathophysiology of atherosclerosis and CAVD prompted the clinical trials into the adoption of statins to alleviate CAVD while no benefits were demonstrated.[37,38] The initial conjecture of the potential reason for the failure of statins is the early involvement of lipid deposition in the progression of CAVD, while the majority of the participants were characterized with moderate to severe AS.[37-39] The failure of statins does not modify the consensus about the feasibility of lipid-lowering therapy, rather it has propelled subsequent research toward more precise targets (**Table 1**).

Over the past decade, the association of sequence variation in LPA with atherosclerotic cardiovascular disease and CAVD has been established sequentially.[40,41] Increased plasma Lp(a) level determined

TABLE 1 Representative RCTs With Lipid-Lowing Therapy as the Intervention to Treat CAVD

Study	Phase	Intervention	Assessments	Main Inclusion Criteria	Primary Endpoint	Secondary Endpoint	Preliminary Results	NCT Identifier (Ref. #)
Aortic Stenosis Progression Observation: Measuring the Effects of Rosuvastatin (ASTRONOMER)	3	Placebo or rosuvastatin 40 mg daily	Echocardiography	Mild AS; moderate risk for coronary artery disease	Changes in peak transvalvular aortic velocity, transvalvular gradients, and aortic valve area	Cardiac death; aortic valve replacement	Rosuvastatin did not reduce the progression of AS	NCT00800800 ([37])
Effect of PCSK9 InhibitorS on Calcific Aortic Valve DiseasE (EPISODE)	3	PCSK9i and statin vs Statin	Echocardiography; CT	Asymptomatic CAVS	Change in aortic-jet velocity	Change of aortic valve calcium score	NA	NCT04968509
A Multicenter Trial Assessing the Impact of Lipoprotein(a) Lowering with Pelacarsen (TQJ230) on the Progression of Calcific Aortic Valve Stenosis	2	Pelacarsen (TQJ230) vs placebo	Echocardiography; CT	Mild or moderate CAVS	Change in peak aortic jet velocity; Change in aortic valve calcium score	Change in Lp(a) levels	NA	NCT05646381

AS = aortic stenosis; CAVS = calcific aortic valve stenosis; CT = computed tomography; Lp(a) = lipoprotein (a).

by the variant rs10455872 was causally related to CAVD and AS.[42] Of all the known LPA variants, rs10455872 is representative of the one that leads to increased Lp(a) concentrations, primarily because of the yield advantage from the lower Kringle-IV type-2 region copies.[43]

Concerning the structure of Lp(a), the KIV10 domains of apo(a) are covalently bonded with oxPL.[44] This represents not only the most significant point of divergence in the structural composition of Lp(a) and low-density lipoprotein (LDL) cholesterol, but also the pivotal mechanism underlying the heightened pathogenicity of Lp(a). Lp(a) is the major carrier of oxPL in plasma, as detected by the phosphorylcholine (PC) specific E06 antibody. Inspired by the naturally secreted T15 anti-PC Abs of B-1 cell origin in natural immune defense, this synthesized murine monoclonal IgM antibody that can recognize the phosphorylcholine head group yielded during the oxidation of phospholipids was able to inhibit the uptake of oxLDL by macrophages in vitro.[45] Decades later, Que et al[45] proved that the constitutively expressed scFv fragment of E06 in LDLr$^{-/-}$ mice can effectively attenuate the progression of atherosclerosis, aortic valve calcification, and systemic inflammation.

Another important cargo carried by Lp(a) is phospholipase A2 (PLA2). PLA2 can hydrolyze oxPL at the sn-2 position and generate lysophosphatidylcholine (Lyso-PLC). The discrepancy though was that with the higher serum level of Lp(a) came the weaker catalytic ability of Lp-PLA2, thus leading to increased oxPL level.[43] In addition, autotaxin carried by Lp(a), demonstrated by in situ proximity ligation assay, can hydrolyze Lyso-PLC into lysophosphatidic acid (Lyso-PA). Lyso-PA was found to promote nuclear translocation of the p65 subunit of NF-κB through LysoPA receptor1, further activating the expression of interleukin (IL)-6, and ending in the osteogenic transition of VICs through BMP2-dependent pathway.[46,47] At the same time, the down-regulation of phospholipid phosphatases (PLPPs), which is constitutively present in the membrane to degrade LysoPA, amplified the effect of LysoPA during CAVD.[48] Autotaxin was also secreted by VIC itself in response to inflammatory stimuli, creating a vicious cycle of response to Lp(a).[46]

Of note, however, a study with a median follow-up of up to 14 years (Q1-Q3: 13.9-14.2 years) demonstrated that Lp(a) was significantly associated only with baseline and new-onset CAVD, but not with the progression of CAVD,[49] which further revealed the potential of Lp(a) as an early-stage biomarker. The statin regulatory spectrum is predominantly oriented toward LDL, and an additional potential explanation for its ineffectiveness against CAVD is its inability to effectively regulate Lp(a), which may even lead to its paradoxical up-regulation.[50] It is therefore imperative that novel and effective strategies for lowering lipids be sought. In a secondary analysis of 63 patients in the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial, the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor evolocumab reduced median Lp(a) and LDL-C synchronously at 48 weeks, and 1 year treatment significantly lowered the risk of AS events.[51] Notably, one of the loss-of-function mutations of PCSK9 was proven associated with a lower prevalence of CAVD.[52] A phase 3 clinical trial utilizing a PCSK9 inhibitor as

JACC: ASIA, VOL. 5, NO. 4, 2025
APRIL 2025:503–515

an intervention is currently in the subject recruitment stage (Table 1). Great advances were made toward significant and specific inhibition of Lp(a) recently. Of them, Pelacarsen (Ionis Pharmaceuticals),[53] an antisense oligonucleotide drug, and Olpasiran (AMG 890; Amgen),[54] a siRNA drug, both target the mRNA product of LPA. Another one was the first oral drug, muvalaplin (LY3473329),[55] which specifically reduced Lp(a) by mimicking a natural variant of apo(a) and preventing its noncovalent interaction with apo B100. Although all of these therapies reduce Lp(a) by more than 80%, clinical studies linking them to cardiovascular events are still ongoing (Table 1), and the results of these studies will be critical in translating them into primary prevention or secondary treatment options.

INFLAMMATION AND DRUG REPOSITIONING

Inflammation plays a predominant role in the early stage of CAVD.[56] With ^{18}F-fluorodeoxyglucose as the representative tracer, positron emission tomography has become the sophisticated technique to identify valvular inflammation in a noninvasive manner.[57] Preliminary in-human evidence indicated a significantly increased ^{18}F-fluorodeoxyglucose uptake in patients with mild to moderate AS compared with no AS groups, while the severe AS group did not manifest remarkable inflammation.[58] Another promising tracer is ^{68}Ga-DOTATATE,[59] but they both exist only in clinical trials because of their steep cost and limited availability.

Genome-wide association studies incorporating larger samples identified that polymorphisms in IL6 and NLRP6 were associated with CAVD,[60,61] further validating the pivotal role of the inflammation. Given the long insidious phase of CAVD, elucidating the causes and pathogenic mechanisms of inflammation is essential for clinical translation. Recent research indicated that clonal hematopoiesis of indeterminate potential (CHIP), which is defined as the presence of an expanded somatic blood cell clone without other hematological abnormalities, increased with age and was linked to CAVD.[62] Acquired somatic mutations in the most frequently mutated CHIP-driver genes (DNMT3A and TET2) were associated with an increase in pro-inflammatory leukocyte subsets,[63,64] suggesting the potential of CHIP as an early indicator.

Various predisposing conditions such as aging, hypertension, dyslipidemia, smoking, and obesity can induce leukocyte recruitment into the aortic valve. The recruited leukocytes release proinflammatory cytokines such as tumor necrosis factor-α, IL-1β, and IL-6, which trigger the osteogenic differentiation of VICs. As the predominantly infiltrated immune cell type in normal and calcific valves, macrophages play a crucial role in modulating the local immune response.[65,66] In addition to the remotely acting secretion function, several studies recently confirmed that macrophages may promote fibrosis/calcification through direct contact with VICs in a cdh11-dependent manner.[67,68] Conventional notion identifies macrophages by simple dichotomization (M1 and M2), and similarly, with the advancement of multi-omics studies, inflammation in the AoV was thought to be the result of the synergistic action of recruited macrophages and tissue-resident macrophages.[10] Preliminary evidence suggested that niche-dependent resident macrophage dysfunction is an important cause and the major histocompatibility complex-II^{lo}Lyve1^{+} subtype with anti-inflammation gene expression showed a significant decrease in calcified AoV.[69] Among the array of secretory profiles of macrophages, the role of TGF-β remains equivocal, with some studies suggesting that it promoted osteogenesis,[70] while others suggested that its effects only persist in the fibrogenesis phase.[71] This suggests that subsequent studies would require the tissue-mimicking 3-dimensional model to minimize background activation to achieve relatively consistent results.

All of the previously mentioned evidence suggests that inflammatory modulation could be a potential target for the treatment of CAVD. IL37 has shown promising results in animal models and in vitro experiments,[72,73] but there are no effective results of in-human trials yet. Researchers dedicated to this field can take inspiration from recent advances in the treatment of atherosclerosis, whereby current anti-inflammatory drugs can be repositioned given the prolonged cycle for new drug development. Drug repurposing is not an aimless screen per se, by leveraging large-scale genomic and transcriptomic data, the spotlight can be focused on the most promising ones early on.[74] A recently published Mendelian randomization study suggested the genetically proxied tocilizumab (IL-6R inhibitor) was significantly associated with reduced risk of AS.[75] Besides, colchicine was evaluated in several clinical trials for the treatment of cardiovascular diseases caused by its efficient inhibition of NLRP3 inflammasome, with generally favorable results,[74] and clinical trials of colchicine as the treatment of AS are currently underway (Table 2).

However, the foreseeable consequence of the global adoption of these drugs is a decrease in host

TABLE 2 RCTs With Colchicine as the Intervention

Study	Phase	Intervention	Assessments	Main Inclusion Criteria	Primary Endpoint	Secondary Endpoint	Preliminary Results	NCT Identifier
Colchicine and inflammation in aortic stenosis (CHIANTI)	3	Colchicine vs Placebo	CT	Asymptomatic moderate aortic valve stenosis	Change in aortic valve calcium score	Difference in aortic valve 18F-NaF uptake; change in echocardiographic parameter for aortic stenosis	NA	NCT05162742
Effect of Colchicine on the Progression of Aortic Valve Stenosis – A Pilot Study (COPAS-Pilot)	2	Colchicine vs Placebo	18F-NaF PET; CT	Mild to moderate aortic stenosis	Change in aortic valve calcification activity	Change in aortic valve calcification	NA	NCT05253794

CT = computed tomography; PET = positron emission tomography; RCT = randomized controlled trial.

immunity, so the search for drug targets should be accompanied by an effort toward targeted delivery and disease-stage-specific application.

FIBROTIC CALCIFICATION AND THE CALCIFICATION PARADOX

It is customary to assume that before the onset of calcification, a long and insidious phase of fibrosis occurs,[76] against which angiotensin receptor blockers and angiotensin convertase enzyme inhibitors are currently under clinical trials[77] (Table 3). The pathogenic role of renin-angiotensin-aldosterone system (RAAS) has long been well-documented. In brief, activated mast cells facilitate the accumulation of angiotensin II accumulation by secreting chymase, which initiates myofibroblast activation of VICs.[78] Indeed, before investigating the potential for RAAS inhibition to mitigate AS, it was already known that this approach could be beneficial in the context of compensated left ventricular hypertrophy caused by AS. A number of clinical trials have demonstrated that RAAS inhibition is an effective treatment for myocardial fibrosis.[79] Besides, C-type natriuretic peptide was proven to significantly inhibit myofibroblast activation through the activation of cyclic guanosine monophosphate signaling,[80] and the activator of soluble guanylate cyclase, Ataciguat (HMR1766), is currently in a phase 2 clinical trial (Table 3).

In contrast to the conventional, stepwise progression of CAVD, growing evidence suggests that fibrosis and calcification appear to be concomitant. Iqbal et al[81] identified the pathological role of sortilin in the global spectrum of CAVD and thus identified a novel combined inflammatory myofibroblastic-osteogenic VIC phenotype, and specific antagonists targeting sortilin probably hold broad promise.

At the terminal phase, mineralization activity becomes dominant in disease progression.[82] Albeit echocardiology remains the first-line AS grading strategy, one of its major defects is the ambiguity regarding the actual extent of the calcification,[6] while the computed tomography-based Agatston scoring has

TABLE 3 RCTs Targeting the Fibrosis Stage

Study	Phase	Intervention	Assessments	Main Inclusion Criteria	Primary Endpoint	Secondary Endpoint	Preliminary Results	NCT Identifier
Angiotensin Receptor Blockers in Aortic Stenosis (ARBAS)	4	Angiotensin receptor blockers vs placebo	CT	Mild to moderate aortic stenosis (peak aortic jet velocity ≥2.5 and <4 m/s)	Change in the anatomic progression of aortic stenosis	Change in peak aortic jet velocity	NA	NCT04913870
A Randomized Trial of Angiotensin Receptor bLocker, Fimasartan, in Aortic Stenosis (ALFA)	4	Fimasartan vs placebo	Echocardiography	Moderate to severe AS	Change of VmaxO2 in cardiopulmonary exercise test	Change of peak aortic jet velocity in echocardiography	NA	NCT01589380
A Study Evaluating the Effects of Ataciguat (HMR1766) on Aortic Valve Calcification (CAVS)	2	Ataciguat vs placebo	CT	Moderate CAVS	Changes in aortic valve calcium levels	Change in levels of plasma interleukin-6	Ataciguat significantly lowered aortic valve calcium	NCT02481258

Abbreviations as in Table 1.

TABLE 4 Representative RCTs Targeting Osteogenesis and Calcification Stage

Study	Phase	Intervention	Assessments	Main Inclusion Criteria	Primary Endpoint	Secondary Endpoint	Preliminary Results	NCT Identifier (Ref. #)
Study Investigating the Effect of Drugs Used to Treat Osteoporosis on the Progression of Calcific Aortic Stenosis (SALTIRE II)	2	Denosumab; alendronic acid vs placebo	CT; 18F-NaF PET	Age >50 y, peak aortic jet velocity of >2.5 m/s, grade 2-4 calcification of the aortic valve	Change in aortic valve calcium score	Change in aortic valve 18F-NaF uptake	Neither denosumab nor alendronic acid affected progression of aortic valve calcification	NCT02132026 ([94])
Vitamin K Supplement for Inhibition of the Progress in Aortic Valve Calcification	3	Vitamin K2 vs placebo	CT; echocardiography	CAVD	Decrease of aortic valve calcification	Progression of diastolic and systolic dysfunction	Vitamin K significantly attenuated AVC progression	NCT00785109 ([87])
The Aortic Valve DECalcification (AVADEC) Trial (AVADEC)	NA	MK-7 and VitD vs placebo	CT; echocardiography	aortic valve calcification score above 300; no aortic valve stenosis	Change in aortic valve calcium score	Change in compiled arterial calcification	MK-7 plus vitamin D supplementation did not influence AVC progression	NCT03243890 ([88])
Bicuspid aortic valve stenosis and the effect of vitamin K2 on calcium metabolism on 18F-NaF PET/MRI (BASIK2)	2	Vitamin K2 vs placebo	18F-NaF PET; CT	Bicuspid aortic valve with mild to moderate aortic stenosis	Change in calcium metabolism, measured as uptake of the 18F-NaF tracer on an 18F-NaF PET/CMR scan	Change in aortic valve calcium score, measured on CT	NA	NCT02917525 ([85])

CMR = cardiac magnetic resonance imaging; other abbreviations as in Tables 1 and 2.

been recognized as a valuable supplementary indicator.[83] Furthermore, a unique tracer of calcification activity, 18F-NaF, was employed to visualize developing calcium thus predicting the location of macrocalcific deposits.[84] Although it is not yet available in clinical practice, it was utilized as a measurement in several randomized controlled trials (Table 4).[85]

The generally termed valvular "calcification" is manifested as either dystrophic calcification characterized by disorganized calcium phosphate crystal on apoptotic debris, or organized hydroxyapatite crystal lattice reflecting active osteogenic processes.[86] Collagen disruption caused by massive apoptosis of myofibroblasts and increased expression of matrix metalloproteinases provided an optimal site for the aggregation of hydroxyapatite. Earlier studies demonstrated the effectiveness of vitamin K2 which enhanced the anticalcific effects of matrix-Gla protein to alleviate CAVD progression.[87] However, the result of the AVADEC (Aortic Valve DECalcification) trial extinguished the nascent hope—2 years of vitamin K2 plus vitamin D did not postpone CAVD progression.[88] The major discrepancy between these 2 trials lies in the inclusion criteria, where the enrolled patients in AVADEC had heavier baseline calcification (AVC score >300). Subsequent studies are in urgent need to

TABLE 5 Representative BAV/CAVD-Related Genetic Variants

Gene	Phenotype	Roles in AoV Development	Genetic Evidence	Mouse Model	Ref. #
NOTCH1	BAV/CAVD	Cell proliferation and apoptosis	Linkage analysis; GWAS	$Notch1^{+/-}$ mTR^{G2}	104
GATA4	BAV	EndoMT	GWAS	NA	114
GATA6	BAV	ECM remodeling	Linkage analysis	$Gata6^{+/-}$	115
ROBO4	BAV	Axon guidance and cell adhesion	Linkage analysis	$Robo4^{tm1Lex/tm1Lex}$	106
NOS3	BAV/CAVD	Nitric oxide production	NA	$NOS3^{-/-}$	100
ADAMTS5	BAV	ECM remodeling	NA	$Adamts5^{-/-}$	116
ADAMTS19	BAV/CAVD	ECM modeling and EndoMT	Linkage analysis	$Adamts19^{-/-}$	107
ADAMTS16	BAV/CAVD	ECM remodeling	Linkage analysis	$Adamts16^{+/-}$/ $Adamts16^{+/H355Q}$	108
PALMD	BAV/CAVD	Cytoskeletal regulation	GWAS; eQTL	$PALMD^{-/-}$	110,113

BAV = bicuspid aortic valve; CAVD = calcific aortic valve disease; eQTL = expression quantitative trait locus; GWAS = genome-wide association study.

determine the actual effectiveness of vitamin K. As for the studies of the mechanism into osteogenic differentiation, several previous reviews have summarized in detail.[1,12] Among these potential mechanisms, inflammation-induced expression of dipeptidyl peptidase-4 (DPP-4) appeared to be an essential mediator of osteogenesis,[89] and targeted inhibition or promotion of its ubiquitination could significantly alleviate AVC in ApoE KO mice.[90]

What is more, the calcification paradox initially defined the association between ectopic arterial mineralization and reduced bone mineral density,[91] and over the last few years, this concept has been extended to valvular calcification. Reduced bone mineral density and osteoporosis were both found to be significantly associated with accelerated progression of AS.[92,93] These findings certainly brought up the subsequent question of whether antiosteoporotic drugs could be used to treat CAVD. It turned out that even up to 2 years of medication (denosumab or alendronate) failed to make any substantial progress in the alleviation of valvular calcification.[94] One potential explanation for the result of SALTIRE (Study Investigating the Effect of Drugs Used to Treat Osteoporosis on the Progression of Calcific Aortic Stenosis) is the long-neglected role of osteoclasts in aortic valve, which is excusable since they were barely found and tended to be dysfunctional.[95] Hence, activating osteoclasts via RANKL signaling rather than inhibiting might be the only underlying biological approach to reverse calcification. In addition to this, recently a noninvasive ultrasound device demonstrated spectacular benefits by delivering energy to soften the calcified portion in valve leaflets, thus enhancing its mobility.[96]

INHERENT BACKGROUND AND PERSONALIZED THERAPY

Growing evidence pointed to a significant genetic background of CAVD.[97] In particular, BAV is representative of hereditary abnormalities linked with CAVD. Altered aortic root hemodynamics caused by BAV accelerated routine wear-and-tear thus causing earlier-onset CAVD. The representative BAV/CAVD-related genetic variants identified to date were detailed in **Table 5**.

NOTCH1 mutations were the first reported genetic variants causing familial BAV and the series of research performed by Garg et al[98] could serve as a paradigm for the subsequent unraveling. Since the first familial with NOTCH1 mutation was identified, various embryological and biomolecular experiments have elucidated the role of NOTCH signaling in valve development.[99] Briefly, following the augmentation of endocardial cushion, the activation of NOTCH1 is essential for the initiation of EndoMT.[100] Once the cellularization of the cardiac cushion is completed, the primary function of NOTCH shifted to "decellularization"- the proliferation-apoptosis balance of VICs was disrupted through the selective activation of tumor necrosis factor-α and the valve primordium was finally sculpted into thin valve leaflets.[101] VEC-specific Notch1 deficient mice resulted in the excessive fusion of the outflow tract. Intriguingly, mutation of NOTCH1 alone results in a maximum of 30% BAV penetration, whereas the addition of NOS3 perturbation considerably increased to 64%,[100] further indicating the complex polygenic background of BAV. Human induced pluripotent stem cells offered an intuitive platform for further study.[102] NOTCH1-haploinsufficient human induced pluripotent stem cell-derived endothelial cells were proven with disrupted epigenetic architecture and resulted in the activation of latent pro-osteogenic and pro-inflammatory gene networks,[103] which might be corrected by a small molecular, XCT790.[104] Linkage analysis of BAV pedigrees also identified genetic loci including GATA6,[105] ROBO4,[106] ADAMTS19,[107] and ADAMTS16,[108] whose pathological mechanism remains to be further elucidated. However, caused by the fact that the majority of BAV cases were sporadical, prophylactic screening for first-degree relatives of BAV patients has not yet been implemented in clinical practice. The intertwined genetic backgrounds of those sporadical cases are also gradually being elucidated. The most extensive genome-wide association study conducted thus far confirmed the contribution of risk loci TEX41, GATA4, and MUC4 to BAV.[109]

Despite the fact that congenital AoV malformation obscures the connection between the pathogenic genes listed above and CAVD, recent progress in genomics allowed us to better identify direct inherited risk factors for CAVD. Combining the genome-wide association study and valve expression quantitative trait locus data sets, the first transcriptome-wide association analysis identified PALMD, encoding palmdelphin, an essential component of cytoskeleton, as a susceptibility gene of CAVD.[110] Risk allele at rs6702619 suppressed the expression of PALMD via inhibiting the recruitment of NFATC2,[111] leading to the inability of VECs to generate a perinuclear actin cap resisting mechanical stress when exposed to shear stress.[112] PALMD$^{-/-}$ mice demonstrated exacerbation of CAVD through the activation of NF-κB signaling, which could be moderated by the injection

HIGHLIGHTS

- Limited therapeutic options for CAVD urgently require the development of therapeutic targets.
- Mechanism-driven target identification has led to clinical trials of various therapeutic agents for CAVD.
- The development of novel monitoring techniques and personalized therapy is still in progress.

of AAV-PALMD,[113] suggesting its potential as a therapeutic target.

Despite major limitations in genetically modified models, targeted gene deletions in mice, zebrafish, and cultured aortic valve cells have offered important insights into potential gene involvement in aortic valve formation.[97] These approaches may be turned into high-throughput assays to evaluate potential genes for BAV generation or disease progression, thus providing a solid basis for personalized therapy for the specific mutant carriers.

CONCLUSIONS

CAVD is a disorder of complex traits caused by interactions between genetic and metabolic risk factors (**Central Illustration**). Several molecular mechanisms and modifiable risk factors for CAVD have been identified over the past 2 decades. Targeting AoV-specific signaling pathways and attaining desired biomechanical results to restore normal aortic valve function should be the main emphasis in order to satisfy the objective of a CAVD therapy and it is imperative to tackle the processes of fibrosis and calcification that result in CAVD concurrently. To fulfill the unmet clinical needs, additional innovative research paradigms integrating multiomics, preclinical and clinical investigations will be required.

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