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STATE OF THE ART REVIEW
Valvular heart disease

Calcific aortic valve disease: from molecular and cellular mechanisms to medical therapy

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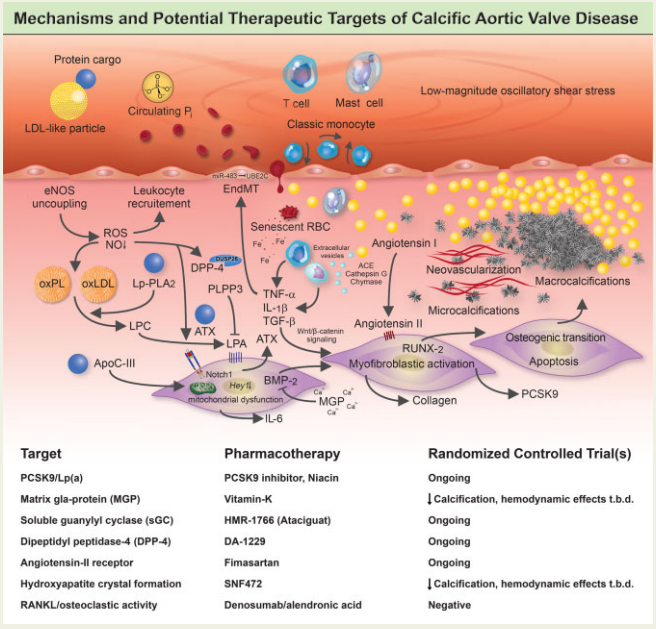
Graphical Abstract



Target	Pharmacotherapy	Randomized Controlled Trial(s)
PCSK9/Lp(a)	PCSK9 inhibitor, Niacin	Ongoing
Matrix gla-protein (MGP)	Vitamin-K	↓Calcification, hemodynamic effects t.b.d.
Soluble guanylyl cyclase (sGC)	HMR-1766 (Ataciguat)	Ongoing
Dipeptidyl peptidase-4 (DPP-4)	DA-1229	Ongoing
Angiotensin-II receptor	Fimasartan	Ongoing
Hydroxyapatite crystal formation	SNF472	↓Calcification, hemodynamic effects t.b.d.
RANKL/osteoclastic activity	Denosumab/alendronic acid	Negative

A complex network of cellular and molecular mechanisms underpins the pathobiology of calcific aortic valve disease. According to the current concept, disruption of the endothelial layer covering the fibrosa promotes the uptake of oxidatively modified lipids (along with the protein-cargo they carry), red blood cells, and immune-cells, thereby promoting an inflammation-calcification feedback loop that results in fibro-calcific remodelling, leaflet stiffening and ultimately narrowing of the left ventricular outflow tract, with its dreadful clinical sequelae such as aortic stenosis, heart failure and premature death. Beyond LDL-C lowering by statins, other previously identified molecules, including PCSK9/Lp(a), mineral-binding matrix Gla protein, soluble guanylate cyclase, dipeptidyl peptidase-4 as well as candidates involved in regulating valvular angiotensin II synthesis and phosphocalcium metabolism, have been targeted pharmacologically in randomized controlled trials. While in some of these studies an attenuation of calcification burden could be observed, effects of target modulation on haemodynamic disease progression, a clinically much more relevant surrogate of disease burden, are uncertain and need to be rigorously assessed in future trials.

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Calcific aortic valve disease (CAVD) is a highly prevalent condition that comprises a disease continuum, ranging from microscopic changes to profound fibro-calcific leaflet remodelling, culminating in aortic stenosis, heart failure, and ultimately premature death. Traditional risk factors, such as hypercholesterolaemia and (systolic) hypertension, are shared among atherosclerotic cardiovascular disease and CAVD, yet the molecular and cellular mechanisms differ markedly. Statin-induced low-density lipoprotein cholesterol lowering, a remedy highly effective for secondary prevention of atherosclerotic cardiovascular disease, consistently failed to impact CAVD progression or to improve patient outcomes. However, recently completed phase II trials provide hope that pharmaceutical tactics directed at other targets implicated in CAVD pathogenesis offer an avenue to alter the course of the disease non-invasively. Herein, we delineate key players of CAVD pathobiology, outline mechanisms that entail compromised endothelial barrier function, and promote lipid homing, immune-cell infiltration, and deranged phospho-calcium metabolism that collectively perpetuate a pro-inflammatory/pro-osteogenic milieu in which valvular interstitial cells increasingly adopt myofibro-/osteoblast-like properties, thereby fostering fibro-calcific leaflet remodelling and eventually resulting in left ventricular outflow obstruction. We provide a glimpse into the most promising targets on the horizon, including lipoprotein(a), mineral-binding matrix Gla protein, soluble guanylate cyclase, dipeptidyl peptidase-4 as well as candidates involved in regulating phospho-calcium metabolism and valvular angiotensin II synthesis and ultimately discuss their potential for a future therapy of this insidious disease.

Keywords Calcific aortic valve disease • Lipoprotein(a) • Notch1 • Ageing • Nitric oxide • Medical therapy

Introduction

Calcific aortic valve disease (CAVD) is the most common valvular heart disease in high-income countries, encompassing a disease spectrum ranging from aortic valve (AV) sclerosis (i.e. fibro-calcific leaflet remodelling without significant impairment in leaflet motion and aortic orifice narrowing) to severe left ventricular (LV) outflow obstruction by calcific AV stenosis (AS). AV sclerosis precedes AS with roughly 9% of sclerosis cases transitioning to AS within a 5-year period,[1] despite marked interindividual differences (*Figure 1*).[2,3] The prevalence of CAVD sharply surges with advancing age, with >25% of people being affected >65 years, and >50% of individuals aged ≥85 years.[4,5] In its preclinical stage, LV outflow is largely unaffected, yet already associated with high risk of adverse events, including stroke, coronary events, and premature death,[5] likely mediated by the frequent co-existence of coronary atherosclerosis.[6]

Despite a decline in mortality,[7] due to an increased use of transcatheter AV implantation (TAVI) in high-risk patients,[8,9] the global disease burden remains substantial with estimated numbers of patients requiring surgical AV replacement (SAVR) or TAVI growing at least two-fold by 2050 in both the USA and Europe.[10–15] Currently, the indication for interventional therapy is driven by AS-related symptoms and a severely reduced AV area, while in asymptomatic patients at low surgical risk SAVR/TAVI can be justified only if profound LV dysfunction (LV ejection fraction <50%) is present.[8,9] Thus, the majority of patients undergo valve replacement when myocardial remodelling and symptoms such as angina, shortness of breath, and impaired exercise performance have already developed. In fact, up to 80% of patients recruited in the prospective, multinational IMPULSE registry had symptomatic disease at baseline, of which >50% already reported severe heart failure symptoms (defined as New York Heart Association class III or IV) at a time when a diagnosis of AS was first established.[16]

Indeed, as CAVD progresses, the elevated LV pressure imposed by the narrowed aortic orifice induces an adaptive response to normalize LV wall stress and to temporarily compensate increased afterload at the price of marked structural changes, ranging from concentric hypertrophy and remodelling to eccentric hypertrophy, the pattern and degree of which is determined by sex, age, comorbidities and hemodynamic disease severity.[17–21] Maladaptive remodelling and LV hypertrophy gradually impair coronary flow reserve (which in turn can induce angina pectoris despite angiographically lesion-free coronary arteries)[22–24] through which subendocardial ischaemia, cardiomyocyte loss, and fibrosis is promoted,[25,26] leading to reduced LV longitudinal strain.[27] LV ejection fraction is generally well preserved in the majority of patients presenting with AS, and hypertrophic changes tend to regress following interventional therapy. Nevertheless, the fibrotic burden within the myocardium remains, putting patients at heightened risk for adverse outcomes, even late after valve replacement therapy.[28,29]

The growing disease burden in the elderly coupled with marked global inequity in access to interventional therapies necessitates effective pharmacological strategies to delay or even cease CAVD progression (*Graphical Abstract*).[8,9,15,30] High levels of low-density lipoprotein cholesterol (LDL-C) and (systolic) hypertension are traditional risk factors that are shared among CAVD and coronary atherosclerosis (affecting 25–50% of CAVD patients),[31] yet aggressive LDL-C lowering consistently failed to impact hemodynamic disease progression or clinical outcomes in well-designed randomized controlled trials (RCTs), implying differential pathogenesis (*Table 1*).[32–35] Similarly, although experimental and observational data support a link between pathways involved in bone metabolism and CAVD pathogenesis,[36,37] neither the receptor activator of nuclear κB ligand (RANKL) inhibitor denosumab nor the bisphosphonate alendronic acid proved effective to blunt the natural course of the disease (*Table 2*).[38]

Multifaceted mechanisms are intricately linked to calcific aortic valve disease pathogenesis

The native AV is an avascular tissue, characterized by a trileaflet architecture, whereby each leaflet comprises three layers, the fibrosa (facing the aorta), the ventricularis (facing the LV outflow tract), and the glycosamino- and proteoglycan-rich spongiosa (residing in-between

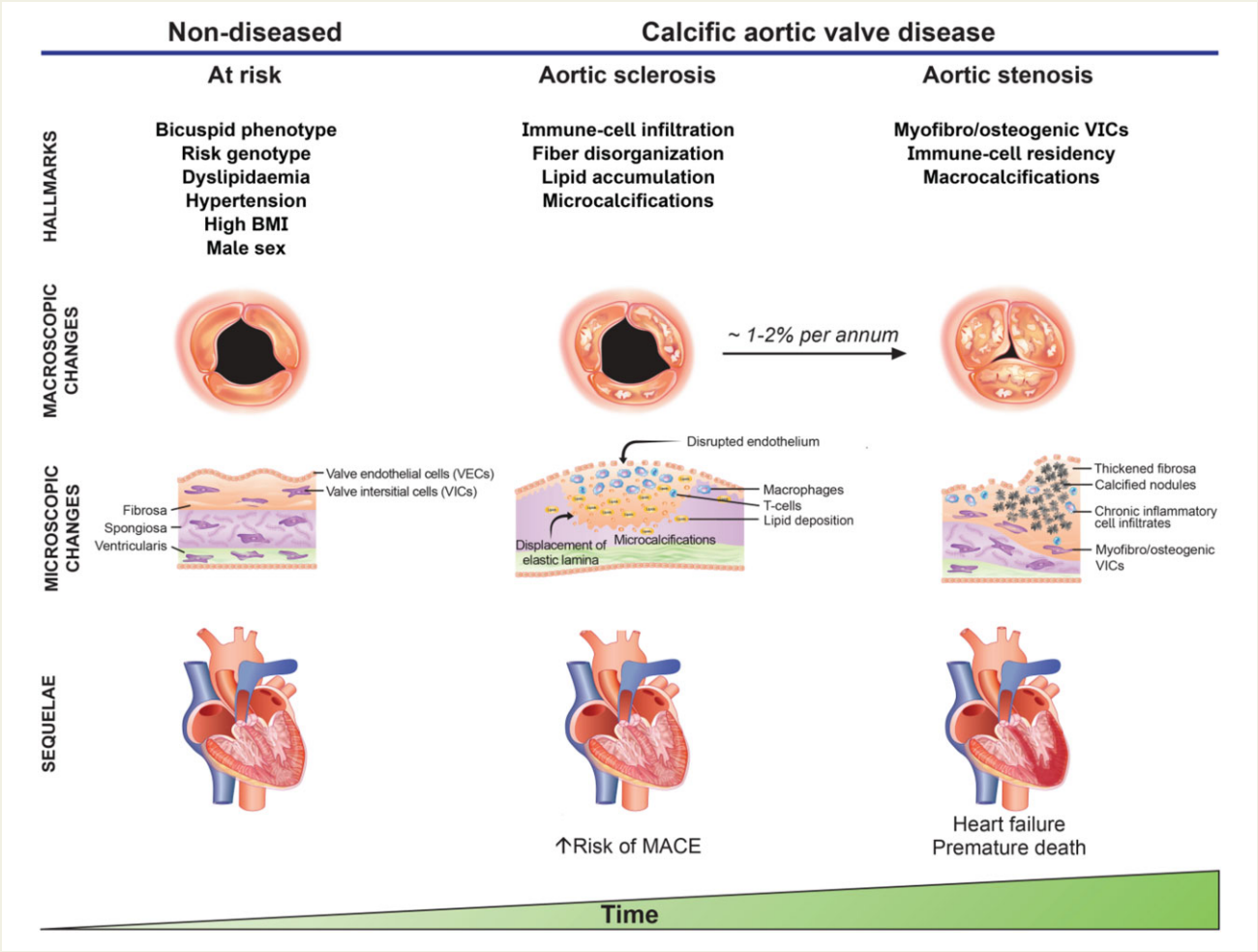

Figure 1 Risk factors, structural changes and sequelae of calcific aortic valve disease at different disease stages. A variety of risk factors, including a bicuspid phenotype, dyslipidaemia, hypertension, diabetes, and increased body mass index enhance the risk to develop calcific aortic valve disease. Endothelial disruption, lipid accumulation, immune-cell infiltration and collagen fibre disorganization occur early in the disease process, with fine-stippled mineralisations being a hallmark of early disease stages. Of note, patients without left ventricular outflow obstruction but sclerotic changes of the aortic valve are at increased risk for major adverse cardiovascular events, likely mediated by the frequent co-existence of coronary atherosclerosis. While the rate of transition from aortic sclerosis to symptomatic aortic valve stenosis varies considerably between patients, findings from the population-based Cardiovascular Health Study[1] suggest that 1–2% of patients with aortic sclerosis progress to aortic stenosis annually, of which three-quarter develop heart failure, undergo valve replacement or die within 2 to 5 years of follow-up.

the former two). The ventricularis is abundant in radial elastin, and the fibrosa is rich in circumferentially aligned type-I collagen fibres, which provides leaflet structural integrity. In non-diseased AVs, all three layers are populated by valvular interstitial cells (VICs),[53] a heterogeneous cell pool comprising at least five phenotypes,[54] with the majority representing quiescent fibroblast-like cells.[55] VICs residing within the AV largely originate from cells of the endocardial cushion that undergo endocardial-to-mesenchymal transition during valvulogenesis, a version of which is reactivated as CAVD evolves (reviewed by Ma *et al.*[56]). During disease initiation and progression, VICs undergo myofibroblastic and osteogenic differentiation, thereby evoking extracellular matrix (ECM) remodelling, collagen deposition, nucleation loci formation (via apoptotic bodies or extracellular vesicles) and eventually osteoblastic VIC-mediated bone formation.[57] The osseous metaplasia is rare and found only in 10–13% of surgically removed AVs.[58] By harnessing single-cell RNA sequencing on normal vs. CAVD tissues, Xu *et al.*[59] identified 14 different cell subtypes populating AV tissues, with resident VICs comprising at least 3 subpopulations. The intricacy of VIC differentiation and possible crosstalks of myofibro- and osteogenesis are further highlighted by the study of Hjortnaes *et al.*[60], showing that the osteoblastic differentiation of VICs loaded into three-dimensional (3D) hydrogel constructs is preceded by and, at least in part, depends on their myofibroblastic activation. This is in line with a recent -omics-based study, showing that the fibrotic stage represents an intermediate gene expression profile between non-diseased and calcific tissues.[61]

Circumferentially aligned valvular endothelial cells (VECs) sit on the surface of the AV, where they form a physical barrier, sense environmental changes, and through their paracrine actions, maintain tissue homeostasis, which involves nitric oxide (NO) signalling,

among others.[62] Although our understanding of their involvement in disease initiation is ambiguous, it is a well-accepted concept that injurious insults on the endothelial layer abet the uptake of lipids along with the protein cargo they carry, immune cells and red blood cells (RBCs), which coincides with the nucleation of calcium and phosphorus within the AV, collectively perpetuating a pro-inflammatory milieu during which VICs become progressively activated, culminating in fibro-calcific leaflet remodelling and eventually LV outflow obstruction (*Figure 2*).[63–66] While early phases of CAVD are characterized by lipid and collagen depositions (typically accompanied by stippled microcalcifications that originate within the base of the fibrosa), macrocalcifications predominate more advanced disease stages, with prominent sex-specific differences in the burden of AV calcifications, reflecting in lower Agatston unit thresholds in women compared to men for a diagnosis of severe AS.[8,9,17,67–70] Indeed, artificial intelligence based models applied on the transcriptomic data of AV tissues obtained from propensity score matched AS patients of both sexes revealed a marked enrichment of fibrotic pathways in calcified vs. non-diseased AV tissues in females, indicating that the transcriptomic signature of CAVD is strongly determined by sex, which may underpin differential disease phenotypes.[71] While multifaceted aspects certainly contribute to sex-specific dissimilarities in fibro-calcific leaflet remodelling, including differences across pro-inflammatory, pro-apoptotic, pro-angiogenic and pro-fibrotic pathways,[71–73] it is thought-provoking that female AV leaflets exhibit accentuated baseline expression of the ECM-embedded endogenous calcification inhibitor mineral-binding matrix Gla protein (MGP).[73] Presently, mechanistic insight into and potential therapeutic implications of sex-specific aspects of human CAVD is scant, yet the hitherto available studies unequivocally suggest that differences among both pro-fibrotic and anti-calcific mechanisms account for the observed dissimilarities in CAVD pathobiology (reviewed in detail by Summerhill *et al.*[74]).

Alterations in paracrine nitric oxide signalling occur early in the disease process

Endothelium-dependent vasomotion is profoundly impaired in early-stage CAVD,[75] and altered NO signalling is implicated in accelerated disease progression.[2] In non-diseased AVs, endothelial nitric oxide synthase (eNOS) protein abundance is almost five times lower on the aortic compared with the ventricular side,[76] possibly owing to shear stress that corresponds to ≈20–30% of the magnitude encountered by the ventricular surface,[77] coinciding with increased propensity of VICs to undergo myofibro-/osteogenesis.[62,78–81] Endothelial NO deficiency is further aggravated by eNOS uncoupling (i.e. switch from its classical NO synthesis function to superoxide production due to tetrahydrobiopterin depletion),[82] which contributes to enhanced valvular reactive oxygen species (ROS) formation (reviewed in detail by Greenberg *et al.*[83]), as it is typically observed in pericalcific regions in human CAVD tissues.[84,85] Consequently, VEC-derived NO has emerged as an important mediator to maintain valvular homeostasis by modulating the behaviour of VICs in a paracrine fashion, as previously reported.[62,76,81,86]

For instance, Gould *et al.*[81] showed that the myofibroblastic potential of porcine VICs seeded on polyethylene glycol (PEG) hydrogels with varying degrees of elasticity was diminished if co-cultured with VECs, and that these effects were reversible upon L-NAME exposure. Notably, blockage of soluble guanylyl cyclase activity, the main downstream effector of NO promoting GTP transformation to cyclic GMP (cGMP), or pharmacological ROCK stimulation abolished the protective effects conferred by VECs, implying ROCK-dependent mechanisms. Mice null for *eNOS* [≈25–45% of which present bicuspid aortic valve (BAV) pathology][62,78] elicit profound AV fibrosis, irrespective of valvular phenotype, with accelerated AV calcification being confined to BAVs, suggesting that disturbed flow represents an important trigger for valvular calcifications.[79] Bosse *et al.*[62] were the first to establish a link between NO and NOTCH1 signalling, a transcriptional regulator utterly essential for proper AV development, with *NOTCH1* mutations enhancing the susceptibility for CAVD in humans.[87] Indeed, endothelial-derived NO inhibits RUNX2 dependent calcification,[76] at least in part by NOTCH1 activation and subsequent Hey1 down-regulation.[88] Recently, Majumdar *et al.*[86] deepened mechanistic insight by showing that VEC-derived NO inhibits VIC-driven calcification through S-nitrosylation of USP9X, stabilization of MIB1, activation of NOTCH1, and in turn diminished activation of RUNX2. Importantly, AV tissues obtained from patients undergoing SAVR displayed blunted S-nitrosylation of USP9X, diminished MIB1 levels, and increased nuclear localization of the NOTCH1 intracellular domain (NICD), while the amount of S-nitrosylated USP9X inversely correlated with CAVD severity, providing insights into new pathways during human CAVD pathogenesis.[86]

Beyond the downstream mechanism outlined above, Choi *et al.*[89] recently reported that endothelial dysfunction evoked by NO depletion enhances dipeptidyl peptidase-4 [DPP-4; a multifunctional protein whose stability is regulated by dual-specificity phosphatase 26 (DUSP26)][90] expression in VICs, in turn limiting autocrine insulin-like growth factor-1 (IGF-1) signalling, and thus accelerating CAVD progression. These findings were recapitulated *in vivo* using *eNOS*-deficient mice and a rabbit model, in which a CAVD-like phenotype was established by high cholesterol diet coupled with daily vitamin D2 supplementation. Taken together, these studies indicate that depletion of VEC-derived NO, as it occurs already early in the disease process, fuels several pro-fibrotic and pro-calcific processes involving, at least in part, ROCK-, NOTCH1-, and IGF-1-dependent mechanisms. Considering that NO homeostasis is perturbed already early in the disease process, coupled with the evolving availability of pharmaceutical approaches to specifically interfere with downstream acteurs of the NO signalling pathway (e.g. HMR1766; *Table 2*), an increased understanding of its role in CAVD pathogenesis is crucial for the appropriate design of future RCTs (see section 'Pharmacotherapies: moving from past to contemporary clinical trials').

Mechanical stress disrupts endothelial structure and function

Seminal histological studies on early-diseased AV tissues revealed that leaflet thickening and the formation of microcalcifications preferentially affect the aortic side, with the endothelium covering the lesion being disrupted, and the underlying elastic lamina displaced.[91] Subendothelial lipid deposits superimposed by immune cells tend to align parallel to the valvular endothelium,[91,92] and subjacent microcalcifications predominantly evolve in regions where disturbed flow

Table 1 **Completed randomized controlled trials on the effects of statin-induced lipid-lowering on calcific aortic valve disease progression**

Trial, year published	Sample size, *n*	Inclusion criteria	Intervention	Follow-up period	AVA at baseline	Increase in mean and/or peak transaortic gradient during follow-up	Decrease in AVA during follow-up
ASTRONOMER,[32] 2010	269	Asymptomatic patients aged 18–82 years, peak aortic valve velocity 2.5–4.0 m/s	Rosuvastatin (40 mg od) vs. placebo	Median, 3.5 years (IQR 2.1–4.5)	1.49 ± 0.71 vs. 1.56 ± 0.70 cm^2	Mean, 10.7 (95% CI, 8.4–13.0) vs. 9.6 (95% CI, 7.5–11.7) mmHg[a] Peak, 18.3 (95% CI, 14.0–22.6) vs. 15.4 (95% CI, 11.0–19.0) mmHg[a]	-0.19 vs. -0.16 cm^{2a}
SALTIRE,[33] 2005	155	Patients >18 years with AS and aortic-jet velocity ≥2.5 m/s	Atorvastatin (80 mg od) vs. placebo	Median, 25 months (range, 7–36)	1.03 ± 0.40 vs. 1.02 ± 0.41 cm^2	Peak, 6.48 ± 7.43 vs.6.56 ± 7.10 mmHg/year[a]	-0.079 ± 0.107 vs. -0.083 ± 0.107 cm^2/year[a]
SEAS,[34] 2008	1873	Asymptomatic patients aged 45–85 years with peak aortic-jet velocity of 2.5–4.0 m/s	Simvastatin (40 mg od) plus ezetimibe (10 mg od) vs. placebo	Median, 52.2 months	1.29 ± 0.48 vs. 1.27 ± 0.46 cm^2	Mean, 2.7 ± 0.1 vs. 2.8 ± 0.1 mmHg/year[b]	-0.03 ± 0.01 vs. -0.03 ± 0.01 cm^2/year[b]
TASS,[35] 2008	47	Asymptomatic patients aged >18 years with AS, mean systolic gradients ≥15 mmHg and peak velocity ≥2.0 m/s	Atorvastatin (20 mg od) vs. placebo	Mean, 2.3 ± 1.2 years	N/A	Mean, 29.2 ± 9.1 (baseline) to 31.3 ± 12.3 (at 24 months) vs. 25.6 ± 9.3 (baseline) to 29.9 ± 14.8 (at 24 months) mmHg[b]	N/A

± values are mean ± SD.
AU, denotes arbitrary units; AVA, aortic valve area; CI, confidence interval; ns, not significant; N/A, not applicable; IQR, interquartile range.
[a]Difference in the change from baseline in the treatment vs. placebo arm with $P > 0.05$.
[b]No *P*-value provided but reported as ns.

Table 2 Exemplar pharmaco-therapeutic randomized controlled trials beyond low-density lipoprotein cholesterol lowering to delay calcific aortic valve disease progression

Trial name, year registered	Key inclusion criteria	Primary outcome measure	Secondary outcome measures	Agent/pathways targeted	Status/major findings
ALFA,[39] 2012	• Normotensives aged 20–75 years • Peak aortic jet velocity of 3.0–4.5 m/s, mean pressure gradient of 25–49 mmHg or AVA 0.76–1.5 cm^2 • NYHA functional class <III	Change of VO_{2max} in cardiopulmonary exercise test	• Change of peak aortic jet velocity in echocardiography • Change of mean pressure gradient across AV • Diastolic function—LV area (cm^2), E/E' value • LV mass index • Development of AS symptoms or LVEF <50% • AVR • 6-min walk distance • Adverse events • Safety endpoint	Fimasartan vs. placebo AT-II antagonism	Unknown
AVADEC,[40] 2017	• Participants in the Danish Cardiovascular Screening Trial • AV calcification score >300 AU, but without AS	Change in AV calcification	• Change in compiled arterial calcification (CT) • Change in aortic diameter • Change in plaque burden in coronary and carotid arteries (CT) • Change in AVA (echocardiography) • Change in bone mineral density (CT) • Change in MGPs and osteocalcin • Change in quality of life	Menaquinone-7 plus vitamin D vs. placebo MGP activation	Ongoing
BASIK-2,[41] 2016	• BAV • Calcified mild to moderate AS on prior echocardiography	Change in AV calcium metabolism (^{18}F-NaF PET/CT)	• Change in AV calcium score (CT) • Progression of AS (echocardiography)	Vitamin K2 vs. placebo MGP activation	Ongoing
CAVS,[42] 2015	• Age >50 years • AVA >1.0 cm^2 but <2.0 cm^2 • AV calcium >300 AU (CT) • EF >50%	Changes in aortic valve calcium levels (CT)	• Change in levels of plasma interleukin-6 • Change in AV function: AVA/mean transvalvular pressure gradient • Change in LV function • Change in plasma tumour necrosis factor alpha	Ataciguat (HMR-1766) vs. placebo Nitric oxide-independent soluble guanylate cyclase activator	Completed
CaLIPSO,[43] 2016	• Aged 18–80 years	Change in log CAC volume score from	• Change in log CAC volume score • Change in log CAC Agatston score		Published[44]

Continued

Table 2 *Continued*

Trial name, year registered	Key inclusion criteria	Primary outcome measure	Secondary outcome measures	Agent/pathways targeted	Status/major findings
	• CAC volume score 100–3500 AU (Agatston score) • Either ≥55years or history of diabetes mellitus • On haemodialysis for ≥6 months	baseline to Week 52 for the combined dose groups vs. placebo	• Number of subjects with <15% progression in CAC Agatston score • Number of subjects with ≥15% progression in CAC Agatston score • Change in log thoracic aorta calcification volume score • Change in log thoracic aorta calcification Agatston score • Change in log AV calcification volume score • Change in log AV calcification Agatston • Number of participants with the composite safety endpoint • Mortality rate (all-cause)	SNF472 (myo-inositol hexaphosphate) vs. placebo Selective inhibition of hydroxyapatite crystal formation	SNF472 attenuated the progression of AV calcification in patients with end-stage kidney disease receiving haemodialysis (14% vs. 98%; $P < 0.001$)
DECAV-K2,[45] 2017	• AV calcium score >300 • Absence of AS requiring operation	Hemodynamic disease progression on echocardiography (change in pressure gradients, AVA, peak aortic jet velocity)	• Activation of the MGP • Change in AV calcification (CT) • Improvement of dyspnoea • Improvement of the quality of life	Menaquinone-7 plus vitamin D3 vs. vitamin D3 MGP activation	Recruiting
DIP-CAVD,[46] 2019	• Patients aged >19 years • 2.0 m/s ≤ peak aortic jet velocity <4.0 m/s OR AV calcium score ≥300 AU on echocardiography/CT	Change in AV calcium volume (96 weeks)	• Change in aortic valve calcium volume change (48 weeks) • Change in aortic valve calcium score (48 weeks, 96 weeks) • Relative change in aortic valve calcium volume (48 weeks, 96 weeks)	DA-1229 at two different dosages vs. placebo Attenuated DPP-4-dependent insulin-like growth factor-1 inhibition	Recruiting
EAVaLL,[47] 2014	• Age >50 and <85 years • Aortic sclerosis (thickening or calcification of aortic leaflets with normal leaflet excursion and peak aortic jet velocity <2 m/s) OR mild AS (peak aortic jet velocity 2–3 cm/s, AVA >1.5 cm^2, mean gradient <25 m) • Lp(a) >50 mg/dL	Calcium score progression by cardiac CT	• Mean change in Lp(a) levels • Rates of valve disease progression (echocardiography) • Drug compliance • Side effects and adverse event	Niacin vs. placebo Downregulation of the transcriptional activity of the *LPA* promoter; Attenuated apoB100 synthesis	Unknown

Continued

Table 2 *Continued*

Trial name, year registered	Key inclusion criteria	Primary outcome measure	Secondary outcome measures	Agent/pathways targeted	Status/major findings
PCSK9 Inhibitors in the Progression of Aortic Stenosis,[48] 2017	• Mild-to-moderate AS	Progression of calcium score (CT and NaF-PET)	• Efficacy of inhibition in calcium score progression (Agatston score) by the presence of Lp(a) SNPs • Mean change in Lp(a) levels between treatment arms • Mean change in lipid panel • Change in AVA (echocardiography) • Change in aortic valve peak velocity (echocardiography) • Adverse events	PCSK9 inhibitor vs. placebo PCSK-9 inhibition/LDL-C and Lp(a) lowering	Unknown
SALTIRE-II,[49] 2014	• Age >50 years • Peak aortic jet velocity >2.5 m/s on echocardiography • Grade 2–4 calcification of the AV on echocardiography	Change in AV calcium score	• Change in AV ^{18}F-NaF uptake • Change in aortic jet velocity • Change in thoracic aortic and CAC score • Change in thoracic spine bone mineral density • Change in quality of life determined by Short Form Questionnaire	Alendronic acid vs. placebo; Denosumab vs. placebo Inhibition of osteoclast-mediated bone-resorption; RANKL inhibition	Published[38] Neither denosumab nor alendronic acid affected AV calcification, peak aortic jet velocity or ^{18}F-NaF aortic valve uptake at 24 months
SLOW,[50] 2020	• Asymptomatic AS • AVA >1 cm^2 • Peak aortic jet velocity <4 m/s	• Change in AV calcification (MSCT) • Correlation with mitral annulus and ascending aorta calcification	N/A	Menaquinone vs. placebo MGP activation	Recruiting
Vitamin K Supplement for Inhibition of the Progress in Aortic Valve Calcification,[51] 2008	• Peak aortic jet velocity >2 m/s on echocardiography • eGFR ≥60 mL/min/1.73 m^2 • Not on VKA	• Change in AV calcium score	• Changes of dp-ucMGP plasma levels	Phytomenadione vs. placebo MGP activation	Published[52] Vitamin K attenuated AV calcium volume score progression in patients with AS (10.0% vs. 22.0%; $P < 0.001$)

AS, denotes aortic stenosis; AU, arbitrary units; AV, aortic valve; AVA, aortic valve area; AVR, aortic valve replacement; BAV, bicuspid aortic valve; CAC, coronary artery calcium; CT, computed tomography; EF, ejection fraction; eGFR, estimated glomerular filtration rate; NYHA, New York Heart Association; Lp(a), lipoprotein (a); LDL-C, low-density lipoprotein cholesterol; LV, left ventricular; LVEF, left ventricular ejection fraction; MGP, matrix Gla protein; MSCT, multislice computed tomography; N/A, not applicable; NYHA, New York Heart Association; PCSK9, proprotein convertase subtilisin/kexin type 9; PET, positron emission tomography; RANKL, receptor activator of NF-κB ligand; VKA, vitamin K antagonist; VO_{2max}, maximum oxygen consumption.

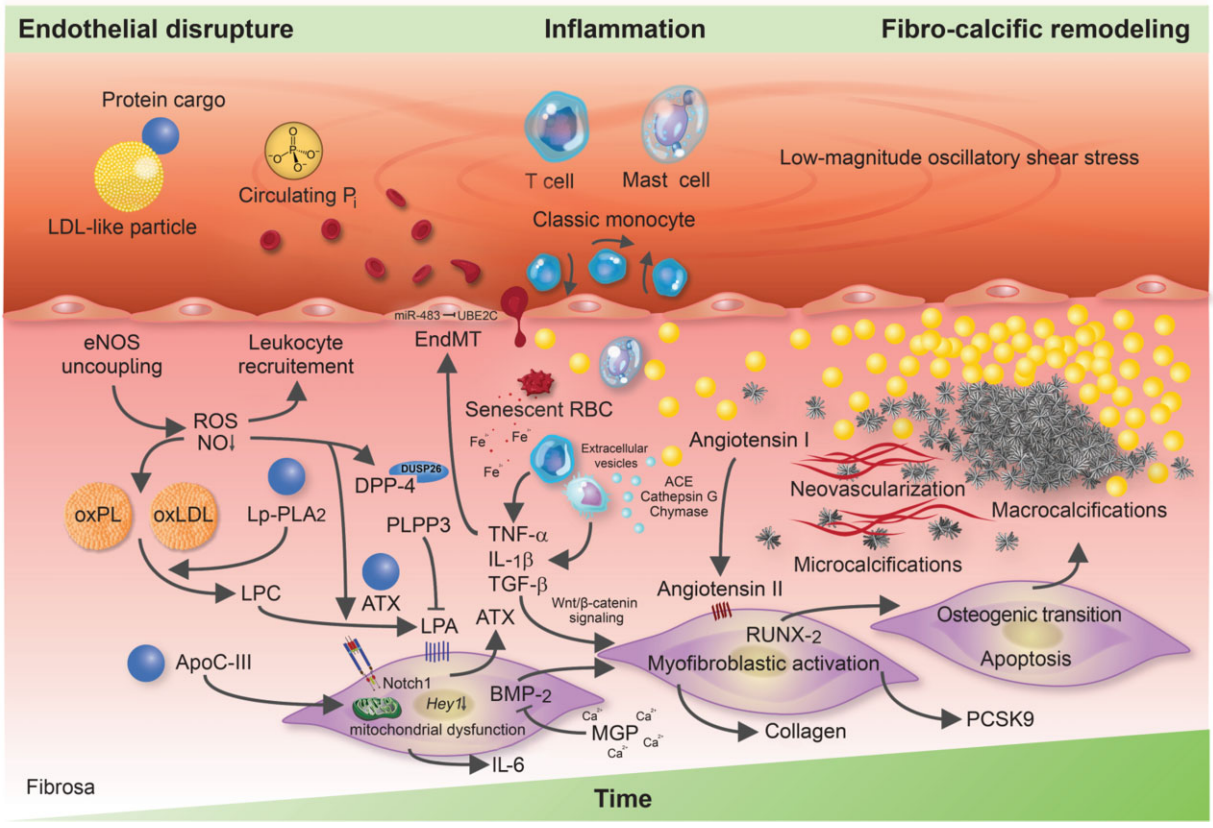

Figure 2 Molecular and cellular mechanisms involved in calcific aortic valve disease pathogenesis. The injured endothelium covering the fibrosa fosters the uptake of immune-cells, red blood cells as well as low-density lipoprotein-like particles and their protein cargo, such as autotaxin and lipoprotein-associated phospholipase A_2. Reactive oxygen species formation, enhanced by nitric oxide synthase uncoupling, aggravates the oxidative modification of lipids, promotes endothelial immune-cell trafficking and induces valvular interstitial cell apoptosis—yielding apoptotic bodies which may form additional nidi for the deposition of calcium and phosphorus crystals. While lipoprotein-associated phospholipase A_2 hydrolyses the ester bond of oxidized phospholipids, autotaxin—which is secreted by valvular interstitial cells—catalyzes lysophosphatidic acid synthesis by choline group removal. Importantly, apoC-III colocalizes with calcific regions, promotes mitochondrial stress and increases interleukin-6 and bone morphogenetic protein-2 expression in human valvular interstitial cells. Matrix metalloproteinase/tissue inhibitors of matrix metalloproteinases imbalances disrupt extracellular matrix homeostasis and promote leaflet stiffening, while bone morphogenetic protein-2 drives osteogenic transition of valvular interstitial cells through increased expression of pro-osteogenic transcription factors, such as RUNX2. Infiltrated mast cells release chymase which facilitates angiotensin II synthesis, thereby promoting valvular interstitial cell-mediated collagen production and thus stiffening of aortic valve leaflets—a potent promoter of osteogenic valvular interstitial cell differentiation. Neovascularization, fuelled by vascular endothelial growth factor secretion, exacerbates immune-cell recruitment and cytokine secretion, which in turn boosts the fibro-calcific response.

occurs,[93–95] collectively suggesting endothelial injury as a prime driver of CAVD. For instance, individuals with a BAV, a congenital condition with incomplete cusp separation during embryogenesis,[96] are at accentuated risk to develop CAVD prematurely, and despite a low prevalence of 0.5–1.5%, account for up to 50% of patients undergoing SAVR.[97] Although mechanisms beyond hemodynamics likely contribute to the high prevalence and exacerbated disease progression, it is interesting to note that shear stress abnormalities are most pronounced near the base of the fused cusps,[94] where calcifications most frequently occur.[98]

The laminar shear stress encountered by the surface of the ventricular side reaches up to 64–91 dyne/cm^2,[77] whilst shear stress on the aortic side shows both anterograde and retrograde components (i.e. oscillatory) and peaks already at 19 dyne/cm^2 (*Figure 3*),[93] known to induce endothelial dysfunction, hamper barrier function, and to shape the expression of key mediators governing the transition of VICs into a myofibro- and osteoblastic phenotype, respectively.[99–101] Indeed, by employing a physiologically relevant bioreactor system, Mahler *et al.*[102] provided compelling evidence that decreasing shear stress magnitudes upregulates ICAM-1 and nuclear factor κB (NFκB) expression in porcine VECs, with low-magnitude oscillatory shear stress promoting their invasion and transdifferentiation into myofibroblastic VICs, a process termed endothelial-to-mesenchymal transition (EndMT). The genetic lineage tracing study of Gee *et al.*[103] proposed that induction of EndMT mainly relies on NFκB activation and might be activated in the postnatal stage solely during diseased conditions, despite former studies ascribing EndMT a physiological function.[104,105] The findings of Fernandez Esmerats *et al.*[99] further

implicated miR-483 and in turn ubiquitin E2 ligase C (UBE2C) in this process, and highlighted the importance of endothelial inflammation, as disturbed flow regulated EndMT via enhanced UBE2C-mediated activation of the pro-inflammatory hypoxia-inducible factor 1α (HIF1α) pathway. Other inflammatory mediators, such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), have also been shown to accelerate EndMT, likely acting through the Akt/NFκB pathway.[104] Increased rates of EndMT, triggered by the mechanisms outlined above, can perturb endothelial structure and thus hamper its barrier function, thereby allowing blood-derived cargo to invade the valvular interstitium.[106]

In contrast to the ventricular side, the endothelium covering the disease-susceptible fibrosa shows areas of denudation,[63,107] which fosters the intraleaflet accumulation of RBCs during early phases of CAVD, while neovessel formation may act as an additional source of intraleaflet RBCs at more advanced disease stages.[63] Impairments in endothelial barrier function coupled with the enhanced expression of endothelial scavenger receptors (SR) also promotes the uptake of lipoproteins. For instance, the SR-A1 and the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) are abundantly expressed in CAVD tissues, with LOX-1 showing high affinity to oxidatively modified LDL-like particles.[108,109] Both LDL-C and lipoprotein(a) [Lp(a)] emerged as important risk factors for CAVD, with the latter also spurring disease progression.[1,110–113] Structurally, Lp(a) consists of an LDL-like moiety covalently bound to apolipoprotein(a), with strong fibrin and lysine binding sites, likely facilitating its valvular interaction upon endothelial injury (*Figure 4*).[114–117] These apolipoprotein B100-containing lipoproteins bind oxidized phospholipids (oxPLs), autotaxin (ectonucleotide pyrophosphatase/phosphodiesterase 2), angiotensin-converting enzyme (ACE), apoC-III, and lipoprotein-associated phospholipase A_2 (Lp-PLA_2), all of which are mechanistically implicated in CAVD pathogenesis.[66,110,118–122] The importance of mechanical stress in CAVD pathogenesis is further underscored by the independent association of elevated systolic blood pressure (SBP) and accelerated AV calcification,[123] and by a recent population-based Mendelian randomization (MR) study showing an up to three-fold increased risk for incident AS per 20-mmHg increase in SBP.[124] While the mechanisms linking heightened SBP and CAVD warrant further study, it is tempting to speculate that amplified tensile stress experienced by the fibrosa coupled with changes in quality and magnitude of shear stress may contribute to this phenomenon.

Inflammation drives fibro-calcific leaflet remodelling

Impaired endothelial structure and function evoked by the mechanisms outlined above promotes the uptake of numerous blood-derived components (including lipoproteins, the protein cargo they carry and RBCs), which, coupled with alterations in paracrine signalling, perpetuates an inflammation-calcification feedback loop that culminates in LV outflow obstruction by fibro-calcific leaflet remodelling. Chronic inflammation causes valvular calcifications in hyperlipidaemic mice,[125,126] a phenomenon also well established in humans by fluorodeoxyglucose (FDG) imaging and histology.[65,127] Indeed, years before symptoms manifest, the human AV already harbours a variety of immune cells in the subendothelium of the fibrosa, including macrophages, mast cells, and $CD8^+$ T cells, with microcalcifications being largely confined to the lesion's base, suggesting their transendothelial recruitement.[91,128,129]

Infiltrated macrophages, predominantly from the M1 subtype,[130] secrete pro-inflammatory cytokines, including TNF-α and IL-6, which contribute to ECM remodelling, ignite EndMT, and drive the evolution of micro- and eventually macrocalcifications.[131] Indeed, IL-6 expression correlates with CAVD severity in humans, and exposures of human VICs to recombinant IL-6 induces their osteoblastic activation, while its knockdown alleviates VIC-driven calcification.[36,132] Recently, Schlotter *et al.*[66] explored the CAVD-specific apolipoproteome and found that apoC-III, an apolipoprotein known to interact with Lp(a),[120] is abundantly expressed in the disease-prone fibrosa and accelerates VIC-driven calcification *in vitro*, likely also via increased IL-6 production. Of note, a recent genome-wide association study using data of four European cohorts identified *IL6* as a novel risk loci for incident AS.[133] Activity of Lp-PLA_2 is enhanced during such pro-inflammatory states,[134] and its expression is increased in CAVD tissues,[122] implying accentuated enzymatic activity. Mechanistically, Lp-PLA_2 transforms oxPL, a highly atherogenic molecule bound to Lp(a), into lysophosphatidylcholine, upon which it undergoes autotaxin-mediated conversion to lysophosphatidic acid. Lysophosphatidic acid is a strong promotor of osteoblastic transition of VICs and, thus, AV calcification, with its degrading enzymes (i.e. phospholipid phosphatases) being increasingly acknowledged as pivotal drivers of its activity.[110,118,135,136] Furthermore, activated macrophages release extracellular vesicles (providing a scaffold for nucleation of calcium-phosphate crystals)[137–139] and express matrix metalloproteinase (MMP)-1, -2, -3, -9, and -10,[140–142] able to modulate ECM elasticity, which in turn determines VIC activation in response to biochemical cues.[143] Activated VICs (along with invaded mast cells) are an important source of pro-angiogenic factors such as VEGF-A,[144,145] thereby stimulating neovessel formation and thus further accelerating the uptake of blood-derived components, such as lipoproteins and immune cells. In support of this notion, deprivation of the antiangiogenic chondromodulin-I elicits high *Vegf-A* expression, lipid deposition, immune-cell invasion, and AV calcification in aged mice.[146]

The infiltration of both macrophages and mast cells, along with LDL- and VIC-derived ACE,[147,148] also accelerates local angiotensin II synthesis. In fact, ACE-, chymase, and cathepsin G are highly expressed in calcific vs. normal AV tissues, thereby promoting enhanced angiotensin II production.[121,149] Notably, exposure of rat VICs to angiotensin II increases type I collagen synthesis, likely via binding to the angiotensin II type 1 receptor (AT-1R).[147] Accordingly, high-dose angiotensin II administration to $Apoe^{-/-}$ mice induces myofibroblastic activation of VICs and subsequent AV thickening, effects that can be suppressed by concomitant olmesartan but not by hydralazine administration, suggesting that angiotensin II exerts pro-fibrotic effects via AT-1R independent of blood-pressure lowering,[150] findings corroborated in hypercholesterolaemic rabbits.[151] Côté *et al.* found that preoperative plasma levels of angiotensin II correlate strongly with the tissue expression of TNF-α and IL-6 in excised CAVD tissues,[152] and showed in a follow-up study that the use of

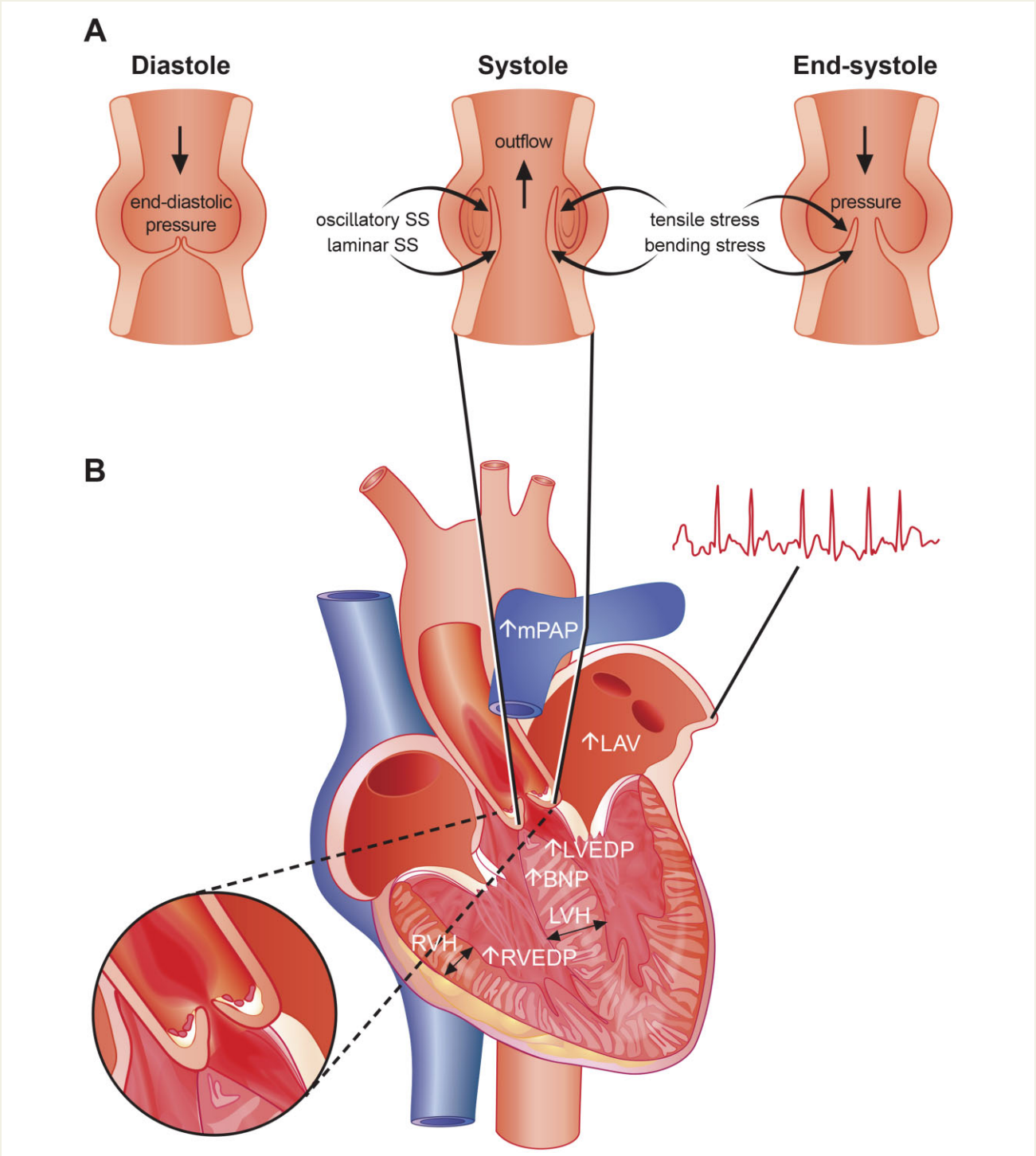

Figure 3 Hemodynamic flow across the aortic valve and myocardial alterations occurring with advanced calcific aortic valve disease. (*A*) The hemodynamic forces aortic leaflets are exposed to are shown. Note that disturbed hemodynamic flow can perturb tissue homeostasis by acting on pro-inflammatory and pro-fibrotic signalling, thereby promoting calcific aortic valve disease progression and eventually the development of aortic stenosis. (*B*) As calcific aortic valve disease progresses and impediments in left ventricular outflow occur, left ventricular hypertrophy and myocardial fibrosis evolves leading to reduced left ventricular longitudinal function, although left ventricular ejection fraction typically remains unchanged in the majority of patients. If left untreated, the left atrium enlarges, enhancing the susceptibility to atrial fibrillation. Due to left ventricular hypertrophy and the reduced diastolic pressure gradient, coronary flow reserve can substantially decrease leading to cardiomyocyte loss further perpetuating processes underlying myocardial fibrosis. At late disease stages, secondary pulmonary hypertension and right-ventricular dysfunction evolves.

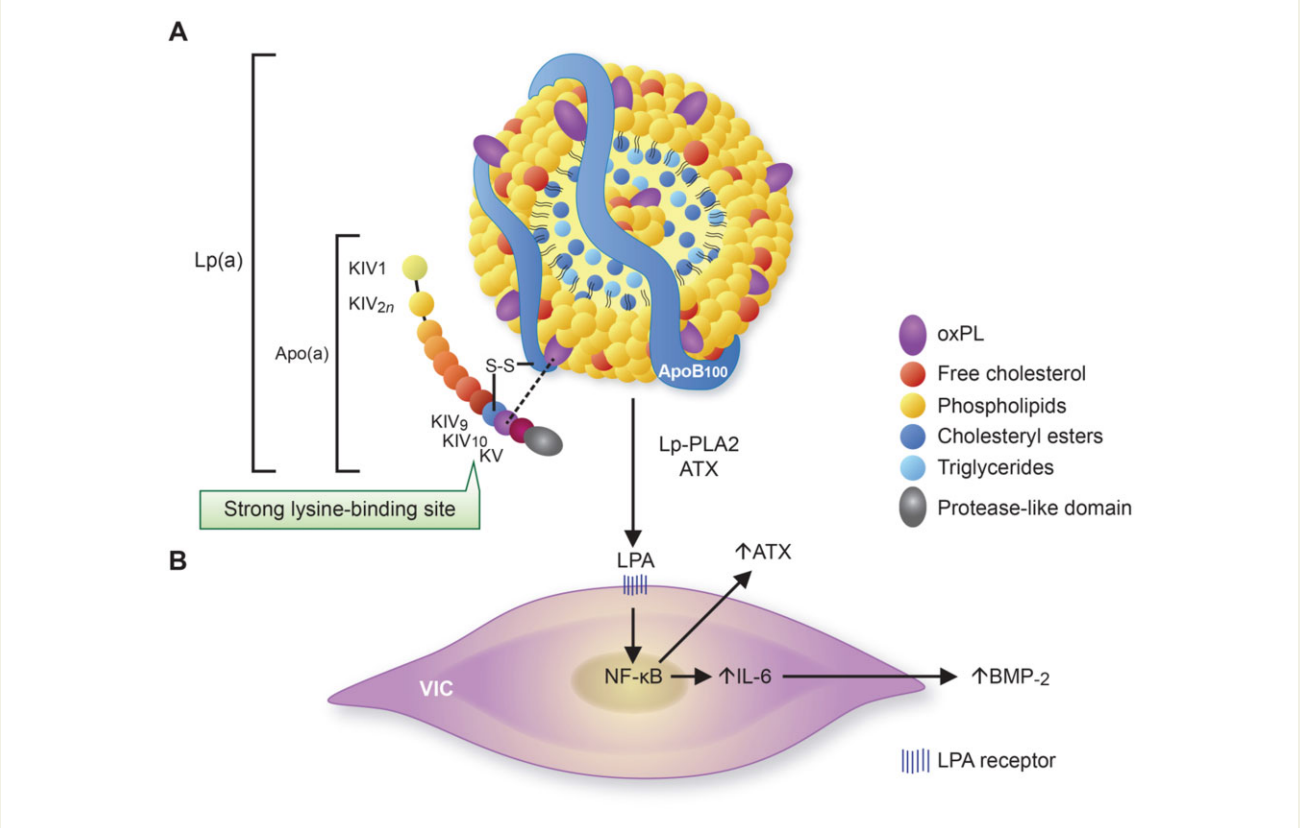

Figure 4 Structure of lipoprotein(a) and its pro-osteogenic effects on valvular interstitial cells. (*A*) Lipoprotein(a) is characterized by a low-density lipoprotein-like particle (note the single apoB100 molecule) that is covalently linked to the unique apolipoprotein(a) glycoprotein which is encoded by the *LPA* gene. While its lipid core consists mainly of cholesteryl esters and (some) triglycerides, its outer shell is mainly composed of phospholipids and free cholesterol. Although the majority of oxidized phospholipid is bound to apolipoprotein(a), lipids can also be covalently linked to apoB100 or even found freely in the lipid-shell. Twelve domains form apolipoprotein(a), with 10 (i.e. KIV1–KIV10) being homologous to plasminogen kringle-IV and one representing a kringle-V-like domain (i.e. KV) which is followed by an inactive protease-like domain. Different functions have been ascribed to each, with KIV10 being characterized by a strong lysine-binding site crucial for oxidized phospholipid binding. (*B*) Lipoprotein-associated phospholipase A_2 and autotaxin can transform oxidized phospholipid to lysophosphatidylcholine and lysophosphatidic acid, respectively, thereby promoting endogenous interleukin-6 and autotaxin production through NF-κB activation. Interleukin-6 can induce increased bone morphogenetic protein-2 expression in a paracrine manner resulting in osteogenic transition of adjacent valvular interstitial cells and eventually aortic valve calcification.

angiotensin receptor blockers (ARBs) associates with lower IL-6 expression and fibrotic remodelling.[36] Although the valvular renin–angiotensin system (RAS) and its implications in CAVD pathogenesis warrants further study, it is interesting to note that a very recent report highlighted a link between the activity of the angiotensin II-degrading enzyme ACE2 and the degree of AV calcification but failed to establish an association with hemodynamic disease severity,[153] opening an exciting avenue for future research on the role of the valvular RAS in CAVD pathogenesis beyond its effects on blood pressure regulation. Given the so-far contradictory results obtained across different clinical studies (see section 'Pharmacotherapies: moving from past to contemporary clinical trials'),[154–158] we must deepen our mechanistic understanding of the valvular RAS in human CAVD. This may provide the basis for the right timing and proper selection of both the study population as well as type of intervention in future RCTs to eventually convincingly assess the impact of RAS modulation on CAVD progression.

Dysregulated calcium-phosphate metabolism promotes aortic valve mineralization

Besides pathogenic processes directed by the mechanisms outlined above, dysregulation in systemic phospho-calcium metabolism/homeostasis, as it occurs during chronic kidney disease (CKD) or osteoporosis,[159] is also implicated in CAVD. This pathway acts through distinct mechanisms (reviewed in detail by Bäck and Michel[160]) but exhibits multiple points of crosstalk that may operate simultaneously within the same AV.[53] CAVD is a common comorbidity of CKD, hallmarked by premature manifestation and accelerated

disease progression, with alterations in systemic calcium-phosphate metabolism being implicated in its pathogenesis. Similarly, osteoporosis and enhanced bone resorption activity have been consistently linked to CAVD,[37,161] suggesting that deranged calcium-phosphate homeostasis is involved in its pathogenesis.

Phosphates represent essential structural (nucleic acids, phospholipids) and functional (purinergic system and pyrophosphate metabolism) building blocks for proper cell function, involving its inorganic (P_i) and biologically active (organic) form. As metabolic alterations occur, such as CKD or osteoporosis,[161–163] phosphates are shifted from their organic to inorganic form, loose their intracellular predominance, and can initiate the mineralization process within the valvular ECM.[160] A variety of sources contribute to their extracellular abundance, including plasma P_i, phospholipids (derived from lipoproteins, cell- or exosome-derived membranes), and nucleotides,[160] with the former two playing a predominant role in CKD-associated CAVD.[159,164] Calcium typically precipitates on exposed phosphates when its product ($Ca \times P_i$) approximates its saturation point. Therefore, hyperphosphataemia, for instance due to CKD, may enhance the propensity for hydroxyapatite deposition within valvular and vascular tissues, yet endogenous inhibitors that act on systemic (fetuin-A, Klotho) or local levels (MGP, osteopontin) may oppose calcium-phosphate precipitation.

For instance, liver-derived fetuin-A limits calcium-phosphate precipitation by forming colloidal calciprotein particles, and interferes with Wnt/β-catenin signalling.[165] Since fetuin-A deprivation elicits more profound calcifications in hyperphosphataemic mice,[166–168] it might well be that fetuin-A exerts protective effects only when calcium-phosphate metabolism is disturbed,[169] which may explain the conflicting results obtained across different observational studies.[170,171] In contrast, Klotho—a fibroblast growth factor-23 (FGF-23) co-receptor—regulates P_i by diminishing renal phosphate reabsorption, and its loss evokes high valvular RUNX2 expression *in vivo*, indicating the osteoblastic activation of VICs.[172] At the valvular level, VICs synthesize MGP that, following vitamin K-dependent post-translational γ-carboxylation of its glutamic acids, is incorporated in the valvular ECM.[173] Carboxylated MGP limits calcium-phosphate precipitation mainly by calcium binding[174] but may also suppress bone morphogenetic protein (BMP)-2 and BMP-4 expression.[175,176] Loss of murine MGP leads to severe arterial calcifications,[177] and its expression is upregulated in human calcified AVs—concomitantly with osteocalcin and Gla-rich protein, suggesting a pivotal role in CAVD.[178]

Beyond these mechanisms, CKD patients accumulate endogenous toxins, such as indoxyl sulphate, and have heightened Lp(a) as well as oxLDL levels,[179] highlighting important crosstalks to phosphate-independent mechanisms. In addition, other aspects that contribute to the almost tripled prevalence of CAVD among CKD patients may include frequently observed (systolic) hypertension, chronic volume overload, and accentuated mechanical stress across the AV due to the presence of an arteriovenous fistula/graft and regular hemodialysis.[164,180]

Pharmacotherapies: moving from past to contemporary clinical trials

Low-density lipoprotein cholesterol

Despite the wealth of data supporting a causal role for LDL-C in CAVD,[181–183] aggressive LDL-C lowering has consistently failed to blunt disease progression in well-designed RCTs (*Table 1*).[32–35] Indeed, in the *SALTIRE* study enrolling 155 patients with AS (defined as AV calcification on echocardiography and aortic-jet velocity ≥2.5 m/s) atorvastatin reduced LDL-C by 53% over a median follow-up of 25 months, but failed to impact disease progression.[33] Similarly, in the large-scale SEAS study, in which 1873 patients with mild-to-moderate AS were randomized to receive simvastatin (40 mg od) plus ezetimibe (10 mg od) or placebo, no effect on the primary outcome was observed during a median follow-up of 52.2 months, despite a mean reduction in LDL-C of 53.8%.[34] Also, in the smaller ASTRONOMER trial, rosuvastatin-mediated LDL-C lowering of 54.5% in relatively young patients with similar AS severity had no effect on disease progression over a median follow-up of 3.5 years.[32] Likely, the insufficient macrophage-driven lipid removal mechanism in CAVD, a well-documented pathophysiologic process in atherogenesis, contributes to these findings.[66,184] Also, off-target effects of statins, ranging from perturbed glucose homeostasis[185,186] and increased Lp(a) levels[187] to pro-osteogenic properties,[188,189] could counterbalance their LDL-C lowering effects. Finally, and in stark contrast to Lp(a), LDL-C does not associate with hemodynamic disease progression in observational studies,[1,110,190,191] questioning the effectiveness of pharmaceutical strategies directed at this target when AS has already evolved.

Lipoprotein(a)

Landmark MR studies imply a causal role for Lp(a) in CAVD,[111,192,193] with preclinical studies providing mechanistic insights into its role as a carrier of culprits involved in VIC-driven calcification, including autotaxin and oxPLs.[119,135,194] A *post hoc* analysis of the FOURIER trial[195] suggests that proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors may exert protective effects, likely because they not only lower LDL-C but also reduce Lp(a) by up to 25–30% and interfere with pathways directly involved in valvular remodelling.[196–201] Yet, based on estimates to lower the risk and improve outcomes of coronary artery disease, a greater Lp(a) reduction might be required to achieve benefit.[202,203] In this context, novel antisense oligonucleotides targeting hepatic *LPA* mRNA might represent promising tools for aggressive Lp(a) lowering,[204] as interventions currently applied in ongoing RCTs (*Table 2*), such as niacin, only modestly lower Lp(a) by up to 30%. On the other hand, overreliance on Lp(a)-directed pharmacotherapies should be avoided, as current evidence highlights that high Lp(a) (exceeding 175 nmol/L) only accounts for up to 7% of AS cases,[205] thus likely benefitting only a minority of patients afflicted by CAVD.

Mineral-binding matrix gla protein

Considering the largely non-vascularized architecture of the native AV, boosting the activity of ECM-embedded calcification inhibitors, such as MGP (which is intriguingly highly expressed in fibrotic areas of CAVD tissues[61]), could represent a promising avenue for future drug development. In support of this theory, loss of MGP evokes profound valvular calcification in mice,[177] whereas liver-derived fetuin(a) deficiency necessitates a hyperphosphataemic mileu.[168] In line with previous reports,[206–208] a retrospective substudy of the population-based DANCAVAS trial[209] with 15 048 participants revealed that vitamin-K antagonist (VKA) use confers heightened propensity for AV calcification, with an increase in computed tomography (CT)-

detected AV calcium by 6%/year on VKA. However, the retrospective character and unavailability of surrogates of MGP carboxylation status (e.g. plasma-derived as used in previous studies)[210] are strong limitations and thus warrant further study. A small prospective proof-of-concept study found that daily vitamin K supplementation slightly blunted the progression of AV calcification during 12 months of follow-up (10.0% in patients undergoing treatment vs. 22.0% in the placebo arm)[52] but unfortunately lacked power to assess effects on more clinically relevant parameters of disease progression. The DECAV-K2 trial[45], designed to assess the effects of vitamin K supplementation on hemodynamic disease progression with a sample size 1.5 times larger than the afore-mentioned trial, started recruiting patients in 2017, with results likely available by 2022/2023.

Renin–angiotensin system

Angiotensin II is abundantly expressed in CAVD tissues and induces the myofibroblastic activation and collagen deposition of VICs *in vitro*,[147] while contributing to valvular ECM remodelling and eventually leaflet thickening *in vivo*.[150] Independent of its effects on blood pressure, AT-1R blockage attenuated myofibroblastic VIC activation and AV thickening in two independent preclinical models,[150,151] suggesting that AT-1R inhibition exerts anti-fibrotic effects. In their retrospective study, Côté *et al.* reported that preoperative ARB use associated with diminished AV remodelling,[154] while in another study, ARBs slowed hemodynamic disease progression, whereas ACE inhibitors have failed.[155] This is in line with the initial report of Rosenhek *et al.*[156] showing neutral effects of ACE inhibition on hemodynamic disease progression. Yet, O'Brian *et al.*[157] reported that the use of ACE inhibitors is associated with diminished increase in CT-detected AV calcium load,[157] while in the prospective RIAS trial[158] only a trend toward slower hemodynamic disease progression could be established. In aggregate, these studies suggest that ARBs may diminish valvular remodelling, while ACE inhibition has thus far yielded conflicting results across different reports. Likely, the abundance of mast cell- or macrophage-derived chymase/cathepsin G necessitates targeted downstream inhibition to provide benefit. Prospectively designed studies, such as the currently running ALFA trial[39], are urgently warranted to convincingly assess the efficacy of RAS-modulating agents to impact CAVD progression.

Targets involved in nitric oxide and IGF-1 signalling

Minute amounts of endothelial-derived NO activate soluble guanylate cyclase (sGC) via its prosthetic haem moiety (whose reduced form binds NO), thereby inducing the conversion of GTP to cGMP, a key player of VIC quiescence and maintenance of valvular homeostasis.[81] Non-sGC sources of AV cGMP, e.g. via the particulate guanylate cyclase *Npr2*, also contribute to valvular homeostasis, embryonic development, and inhibition of fibrosis/calcification in mice—pointing towards a broader importance of valvular cGMP levels in maintaining AV health.[211] Early-stage CAVD is associated with systemic endothelial dysfunction,[75] and derangements in paracrine NO signalling drives pro-fibrotic and pro-calcific processes that underpin CAVD pathogenesis.[62,81,86] HMR-1766, an sGC activator that acts independently of NO and preferably interacts with oxidized sGC,[212] was shown to exert anti-fibrotic effects in a rat model of myocardial infarction,[213] while a preliminary report also implies a role in valvular BMP-2 signalling.[214] The CAVS[42] trial, in which patients with AS were randomized to receiving 200 mg HMR-1766 daily or matching placebo, is undergoing analysis currently. VEC-derived NO depletion, as it occurs during CAVD progression, enhances DPP-4 expression, and induces the osteoblastic activation of VICs via accelerated IGF-1 degradation.[89] The DIP-CAVD trial[46] will test whether selective DPP-4 inhibition by orally administered evogliptin (DA-1229) once daily can alter the progression of AV calcification over 96 weeks, with first study results likely being available by 2024. These RCTs open an exciting avenue for future research to study drugs to reinstate paracrine VEC/VIC homeostasis, a process likely deranged early in the disease process.

Phosphate/calcium-metabolism-associated targets

Epidemiological and preclinical evidence linking CAVD with dysregulated phosphocalcium metabolism has stimulated RCTs to assess the effectiveness of pharmaceutical strategies directed at targets interfering with hydroxyapatite crystal formation (SNF472), the RANKL/RANK/osteoprotegerin axis (denosumab), or osteoclastic activity (bisphosphonates). As noted earlier, the almost tripled prevalence of CAVD in CKD patients is secondary to a combination of factors, with a predominant role of deranged mineral metabolism.[160] The landmark CaLIPSO trial[44] showed that 52-week treatment with SNF472, a myo-inositol hexaphosphate that selectively inhibits hydroxyapatite formation,[215] significantly attenuated AV calcium volume score progression in CKD patients on long-term haemodialysis and adjunct therapies (57% of which had AV calcifications at baseline), equalling a progression of 98% with placebo vs. 14% with active treatment. Although further studies are needed to study the effects on hard cardiovascular endpoints, including hemodynamic disease progression, the clinical implications could be huge, particularly in patients with high propensity for CAVD, yet at high risk for adverse outcomes following interventional valve replacement therapies.[164]

The RANKL/RANK/osteoprotegerin axis regulates bone turnover and is mechanistically implicated in osteoporosis pathogenesis, a condition linked to high CAVD prevalence. RANKL is upregulated in calcific lesions of the AV,[216] and promotes matrix calcification and osteoblastic activation of VICs,[217] while its inhibition by osteoprotegerin attenuates CAVD in $Ldlr^{-/-}$ $ApoB^{100/100}$ mice.[218] Observationally, bisphosphonate use is associated with reduced hemodynamic AS progression and lower prevalence of AV calcifications,[37,219] while bone density correlates inversely with incident AS.[161] In the recently published SALTIRE II trial,[38] 150 AS patients with a mean peak aortic jet velocity of 3.36 m/s were randomized to receiving denosumab, placebo injection, alendronic acid, or placebo capsule and were subjected to serial echocardiography, CT AV calcium scoring, and ^{18}F-NaF positron emission tomography (PET)/CT during 24-month follow-up. A decline in serum C-terminal telopeptide by >50% confirmed efficacy of both active drugs, yet neither a change in AV calcium load/activity nor peak aortic jet velocity could be established, highlighting the need for the identification of novel therapeutic targets beyond the RANKL/RANK/osteoprotegerin axis.

Conclusions

Several mediators, including Lp(a) (mainly via bound oxPLs), NO, RAS, DPP-4/IGF-1, MGP, autotaxin (via enhanced lysophosphatidic acid), and IL-6, have emerged as pivotal drivers of CAVD, some of which are already being therapeutically targeted in ongoing trials (*Table 2*). Despite the progress made, our understanding of the mechanisms operative in valvular tissues in response to hemodynamic and biochemical cues is still incomplete, with well-designed randomized controlled trials targeting LDL-C or key players of bone metabolism showing disappointing results.[32–35] However, phase II studies harnessing interventions of MGP carboxylation (by oral vitamin K supplementation) or hydroxyapatite crystal formation inhibition (by intravenous myo-inositol hexaphosphate administration) have yielded promising results in specific patient populations. Whether these interventions impact hemodynamic disease progression and, in turn, the necessity for interventional therapy, in both male and female patients, needs to be shown in larger RCTs, some of which are currently ongoing (e.g. AVADEC,[40] BASIK2,[41] and DECAV-K2[45]). Lastly, there is a pressing need to design tailored RCTs investigating the effects of aggressive Lp(a) lowering on CAVD progression, as such an approach might represent a promising remedy for patients with elevated Lp(a).[220]

In parallel, preclinical efforts aimed at characterizing the pathobiology of different CAVD stages should be continued incessantly. For the discovery of novel mediators and final common pathways of CAVD initiation and progression, the application of spatiotemporally resolved omics studies coupled with the rigorous validation of promising therapeutic targets in *ex vivo*/*in vivo* models merit consideration.[221–224] Finally, the identification of patients best suited for medical therapy intertwined with the development of more sensitive screening modalities for the detection of early fibro-calcific changes, which markedly differ between sexes, will be key if interventional trials are to be efficiently conducted and novel drugs shown to be effective are to be broadly applied. As indicated, the disease-causing mechanisms may change as CAVD evolves, with women typically showing a more fibrotic phenotype compared to men.[17,67,68] Therefore, novel composite endpoints—depending on the study population recruited and disease stage targeted—need to be established, as emerging surrogates of inflammation and fibro-calcific remodelling (e.g. assessed by PET/CT with ^{18}F-NaF/^{18}F-FDG) can provide incremental information on top of echocardiography, and likely represent more accurate measures of disease activity.

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