Cellular mechanisms of valvular thickening in early and intermediate calcific aortic valve disease

Pauli Ohukainen[a], Heikki Ruskoaho[a,b] and Jaana Rysä[c]

[a] Computational Medicine, Faculty of Medicine, University of Oulu and Biocenter Oulu, Oulu, Finland

[b]Drug Research Program, Division of Pharmacology and Pharmacotherapy, University of Helsinki, Finland

[c]School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Finland

Running title: Early and intermediate valvular thickening in CAVD

Address for correspondence:

Associate Professor Jaana Rysä, PhD
University of Eastern Finland
School of Pharmacy,
POB 1627,
70211 Kuopio, Finland
Phone: +358-50-3164105
Fax: +358-17-162424
e-mail: jaana.rysa@uef.fi

Keywords: Aortic valve, aortic stenosis, calcification, atherosclerosis, inflammation
Subject codes: Valvular heart disease
Word count: 5477
Total number of figures and tables: 2

Abstract

Calcific aortic valve disease is common in an aging population. It is an active atheroinflammatory process that has an initial pathophysiology and similar risk factors as atherosclerosis. However, the ultimate disease phenotypes are markedly different. While coronary heart disease results in rupture-prone plaques, calcific aortic valve disease leads to heavily calcified and ossified valves. Both are initiated by retention of low-density lipoprotein particles in the subendothelial matrix leading to sterile inflammation. In calcific aortic valve disease, the process towards calcification and ossification is preceded by valvular thickening, which can cause the first clinical symptoms. This is attributable to the accumulation of lipids, inflammatory cells and subsequently disturbances in the valvular extracellular matrix. Fibrosis is also increased but the innermost extracellular matrix layer is simultaneously loosened. Ultimately, the pathological changes in the valve cause massive calcification and bone formation - the main reasons for the loss of valvular function and the subsequent myocardial pathology. Calcification may be irreversible, and no drug treatments have been found to be effective, thus it is imperative to emphasize lifestyle prevention of the disease. Here we review the mechanisms underpinning the early stages of the disease.

Nonstandard Abbreviations

apoB	apolipoprotein B
Ang	angiotensin
AT1R	type 1 angiotensin receptor
CAVD	calcific aortic valve disease
ECM	extracellular matrix
LDL	low-density lipoprotein
MMP	matrix metalloproteinase
oxLDL	oxidized LDL
RAS	renin-angiotensin system
TIMP	tissue inhibitor of matrix metalloproteinase
VIC	valve interstitial cell

Introduction

Calcific aortic valve disease (CAVD) is an active inflammatory disease characterized by several hallmarks of atherosclerosis. The main difference is the ultimate phenotype – CAVD does not lead to vulnerable plaques but rather, heavily calcified and ossified aortic valves. As the accumulation of calcium and bone seems to be irreversible, early prevention is of paramount importance. Due to the active nature of the disease, it seems plausible that there would be a causal chain of events leading to the final disease phenotype. It will be necessary to clarify the early stages of the disease if we are to prevent the progression. In this review, we summarize the mechanisms of early valvular thickening – the stage in which CAVD may become symptomatic.

Cellular and subcellular events of early CAVD take place in valves consisting of a three-layered extracellular matrix (ECM) (Figure 1). Each layer has its own characteristic main structural protein[1]. The main layers are termed ventricularis, spongiosa and fibrosa with their corresponding main components being elastin, glycosaminoglycans and collagen. While these layers are rather stable, some studies in porcine models have indicated that the ultrastructural organization may change with aging[2]. The three layers also have somewhat different functional roles. In the ventricularis, elastin provides the necessary stretching ability, but it also serves as a scaffold to maintain valvular collagen in place[3,4]. The spongiosa layer distributes the hemodynamic stress across the valve leaflets, acting as a shock- and vibration-absorbing cushion[5,6]. Finally, the fibrosa has a highly dynamic pressure-dependent circumferential collagen structure, and it is the main load-carrying layer in the valve[7].

The ECM of aortic valve cusps consists of a heterogeneous class of mesenchymal cells, called valve interstitial cells (VICs, reviewed in[8,9]). VICs have several functions, including the maintenance of valve ECM as well as contributing to certain physical characteristics of the valve cusps, and their responses to injury. VICs and other mesenchymal cells share many similarities and display non-specific features of both smooth muscle cells and myofibroblasts. Valvular VICs seem to have a distinct surface antigen expression profile and an ability to respond to vasoactive agents and in these respects differ from the mesenchymal cells present in pericardium and skin[10]. Five different phenotypes of VICs have been postulated[11]: embryonic progenitor endothelial/mesenchymal cells, quiescent VICs (qVICs), progenitor VICs (pVICs), activated VICs (aVICs) and osteoblastic VICs (obVICs).

Comparing the early initiation of atherosclerosis and CAVD

In the first seminal human study investigating the early stages of CAVD, autopsy samples were histochemically characterized in order to examine the pathophysiological features associated with this disease[12]. Several hallmarks of atherosclerosis were seen already in non-stenotic yet thickened valves. The basement membrane below the endothelium was found to be disorganized and the tissue had become infiltrated by neutral lipids (mainly triglycerides, as revealed by Oil Red O staining). Thickening of the valve was found to be due to increased ECM of the fibrosa layer. These areas also contained macrophages, foam cells and T lymphocytes, whereas only scattered macrophages were found in the control valves. Small calcifications were also found in the early thickened regions.

More recently, newly formed lesions have also been found to contain apolipoproteins B (apoB), (a) and E[13]. In atherosclerosis, the attachment of apoB-containing low-density lipoprotein (LDL) -particles in the intima-layer of coronary arteries is both sufficient and essential to trigger the initiation of atherosclerosis[14]. A significant discovery supporting this concept is that if the electrical interactions between apoB primary structure and proteoglycan are removed, transgenic mice become highly resistant to atherosclerosis despite significantly elevated serum LDL levels[15]. Because of their similar histologies, it is reasonable to propose that an analogous process initiates CAVD.

Retained apoB containing particles are predisposed to undergo a variety of oxidative modifications. Oxidized LDL (oxLDL) particles are strong triggers evoking an inflammatory response and the valves in the early stages of CAVD also exhibit oxLDL[16]. Certain proteoglycans such as decorin[17] and biglycan[18] colocalize with oxLDL, indicating that they can bind these particles within the valvular ECM. Recent studies have also shown that isolated LDL particles from aortic valves have larger diameters and are oxidatively modified, demonstrating that they aggregate and become modified within the valves, very likely contributing to the early inflammatory signals[19]. In conclusion, the data suggest that the early events in both atherosclerosis and CAVD are very similar (Figure 2).

Localization of coronary plaques and the effects of hemodynamics also provide important clues to the similarities between early CAVD and atherosclerosis. Plaques are mostly located in the branching regions and arterial bends, where local shear stress is lower (reviewed in[20,21]). This causes endothelial cells to express an inflammatory response which includes the induction of several vascular adhesion molecules (vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and E-Selectin), ECM breakdown, increased uptake and permeability of LDL particles, apoptosis, widening of intercellular junctions (which allows inflammatory cells to infiltrate through the endothelium), oxidative stress, increased thrombogenicity and impaired vasodilatation.

Many of these processes remain to be explored in CAVD but several independent lines of evidence suggest that local hemodynamics also affect valvular tissue in a similar, pro-atherogenic fashion: 1) The aortic side of the valves is subject to turbulent and transiently reduced shear stress (reviewed in[22]). This is also the side where early lesions are invariably found[12]; 2) The bicuspid aortic valve (BAV) causes hemodynamically different conditions characterized by larger differences in shear stress compared to the more typical tricuspid valve (reviewed in[23]). BAV usually leads to a faster progression of CAVD and earlier clinical manifestation by about two decades[24,25]; and 3) In tricuspid valves, the right- and left-coronary cusps are adjacent to the ostia leading to the coronary arteries. This causes these cusps to be also subjected to more laminar shear stress. The non-coronary cusp has a different hemodynamic environment and is usually affected most by early valvular thickening the[26].

Inflammation in early atherosclerosis and CAVD

It has become widely recognized that in atherosclerosis, LDL retention in the arterial intima is the initiating event for plaque development. The subsequent inflammatory

response is likely intended to clear the ectopic cholesterol from the arterial wall. If the macrophage-derived foam cells are successful, the initial fatty streaks are removed. However, if the exposure to pro-atherosclerotic factors persists, inflammation becomes chronic, leading to the formation of an atheroma. In addition to macrophages, other cells of the immune system, including T-cells, mast cells and B cells are also present in atherosclerotic lesions (this process has been thoroughly reviewed[27–31]).

In early CAVD, many of these cells are also encountered e.g., macrophages and foam cells[12], T lymphocytes[12,32], mast cells[33] and B cells[34]. Macrophages are localized close to lipid depositions[16] whereas T lymphocytes are found near both lipids[16] and calcifications[32]. Mast cells are more evenly distributed throughout the valves although with a slight preference for calcific nodules[33] and finally B cells are situated close to the macrophages[34].

In addition to diverse cell types, several molecular components of an inflammatory response have also been found in CAVD. For example, the levels of vascular cell adhesion molecule 1, intercellular adhesion molecule 1 and E-selectin are upregulated in diseased valves compared to controls[35,36]. Mechanistic studies have elucidated some of the inflammatory pathways in more detail. Increased expression of interleukin-1β has been detected in the leukocytes present in stenotic human aortic valves[37]. VICs were exposed to interleukin-1β, resulting in increased production of matrix metalloproteinases (MMPs) -1 and -2. Their presence has also been confirmed immunohistochemically in diseased valves. In addition, tumor necrosis factor-α has been detected in valvular macrophages and shown to upregulate the expression of MMPs in VICs[38]. These studies emphasize that not only is inflammation clearly present in early CAVD but that it also contributes to ECM remodeling early on in the disease spectrum.

Active extracellular matrix remodeling

During the intermediate stage of CAVD, there is more pronounced valvular thickening. Histologically, this is characterized, in part by an initial disorganization and then a loosening of the spongiosa layer of valvular ECM[39]. In clinical terms, the valvular thickening contributes to the loss of function, creating an increased myocardial strain. In addition, thickening also promotes CAVD progression. ECM integrity appears to be critical for the physiological function of the VIC because its disruption causes apoptosis and upregulation of several disease-related markers, such as α-smooth muscle actin, alkaline phosphatase, and osteocalcin[40]. Paradoxically, the overall amount of collagen in the valves decreases with CAVD progression, despite upregulated type I collagen production[41]. This indicates that CAVD also possesses a significant component of active ECM degradation, which is also evident from the increased expression of MMPs. Concordant degradation and synthesis of ECM are general features of an active remodeling in developmental processes and several diseases (for a general review, see[42]). In the following chapters, these simultaneous processes are examined separately.

Activation of the local renin-angiotensin-system and other pro-fibrotic factors

The renin-angiotensin-system (RAS, or renin-angiotensin-aldosterone-system, RAAS) is known to be involved in many illnesses and systemic physiologic processes. Certain

parts of RAS are found locally within many tissues which means that angiotensin II (Ang II) is also an intracrine signaling molecule (reviewed in[43–45]). ‘Local RAS’ has significant roles in fibrosis and inflammation and has therefore been extensively researched in the cardiovascular system (reviewed in[46–51]).

Components of the local RAS are expressed in healthy and diseased aortic valves. For example, cultured VICs are able to produce angiotensinogen, and angiotensin-converting enzyme (ACE) *de novo*[52]. ACE has also been found to physically interact with LDL particles in the plasma and with apoB proteins in CAVD, which suggests that should LDL be retained in the valve, it may also carry ACE along with it. Ang II has also been found to colocalize with apoB and ACE, which implies that the latter is enzymatically active[53]. Degranulated mast cells are also a source for Ang II in aortic valves. They can secrete chymase, which is another peptidase able to cleave Ang I into Ang II[33].

Expression of type 1 angiotensin receptor (AT1R) on VICs has also been reported[53]. This may have pathological significance as Ang II exerts its pro-fibrotic effect via AT1R. Cardiac myofibroblasts displayed upregulation of the production of LDL-binding biglycan when cultured with Ang II[54,55], which could make it feasible to postulate that a similar response could also occur in VICs. The synthesis of type I collagen is significantly increased around calcific nodules[41]. This elevated synthesis of type I collagen and biglycan can be hypothesized to be significant contributors to the fibrotic phenotype of intermediate CAVD. Support for the clinical features of this putative Ang II-mediated valvular fibrosis emerged from a bioreactor-study, in which porcine aortic valves became significantly less flexible upon incubation in Ang II-containing media[56].

Novel components of RAS have been discovered in recent years. One of these is an ACE homologue, ACE2, which is able to cleave Ang I into a distinct nona-peptide, Ang(1-9)[57]. This is also an alternative substrate for ACE which turns Ang(1-9) into Ang(1-7) that has a specific receptor called Mas[58]. Compared to AT1R, Mas seems to have opposing downstream effects[59], much like the angiotensin type 2 receptor (AT2R) (reviewed in[60]). Together, AT2R, Mas and ang(1-7) can be considered to be part of a “compensatory arm” of RAS which counterbalances the vasoconstrictive and pro-fibrotic effects of Ang II and AT1R (reviewed in[61–63]). In calcific aortic valves, Mas and AT2R are downregulated, which is consistent with the proposal that the Ang II- and AT1R-mediated pro-fibrotic local RAS is the dominating arm in this disease[64].

Another somewhat novel RAS component is the (pro)renin receptor[65]. It binds renin and prorenin and it also mediates a pro-fibrotic response (reviewed in[66]). The study by Peltonen et al. (2011) suggested that the (pro)renin receptor is expressed in neovessels of diseased valves. However, in overall valve tissue, its mRNA was not significantly downregulated. If confirmed in subsequent studies, this would imply that while the receptor has pro-fibrotic effects in certain cells, its total contribution to CAVD may be time and location-dependent.

Many non-RAS components also promote active fibrosis. If VICs are cultured in the presence of transforming growth factor beta-1, they begin to express a pro-fibrotic

phenotype[67]. Both endothelin-1, a fibrosis-inducing factor (reviewed in[68–70]) and its receptor are upregulated in CAVD[71]. Further mechanistic studies will be required to confirm the role of transforming growth factor beta-1 and endothelin-1 in CAVD.

Downregulation of anti-fibrotic factors may be another way for fibrosis to become dominant in CAVD. C-type atrial natriuretic peptide (CNP) is one of these anti-fibrotic factors; it has been shown to inhibit fibrosis in *in vitro*[72] as well as after experimental MI *in vivo*[73]. The expressions of CNP and its receptors are downregulated in CAVD[74], but more detailed research of the contribution of CNP for CAVD development is needed.

Extracellular matrix degradation

Loss of the collagen content and loosening of the spongiosa layer of the valvular ECM in CAVD are directly opposing processes to fibrosis. This must be mediated by specific ECM-degrading enzymes. One of the most studied are the MMPs which have various functions in cardiovascular diseases (reviewed in[75,76]). In CAVD, increased expression of MMPs -1, 2, -3 and -9 has been reported[38,39,77,78]. In order to maintain their physiological functions, the activities of the MMPs are counterbalanced by the presence of specific tissue inhibitors (tissue inhibitor of metalloproteinases, TIMPs). Although it may seem paradoxical, it appears that expressions of TIMP-1 and -2 are significantly increased in stenotic valves[38,78,79]. However, there seems to be a significant overproduction of MMPs with respect to the TIMPs in diseased valves[78]. This suggests that while TIMPs may be able to inhibit some level of ECM degradation, an overwhelming persistent inflammation will eventually cause the tissue degradation to become prevalent. Indeed, in milder stages of the disease, TIMP expression tends to be dominant, which is also a feature seen in atherosclerosis[80].

In CAVD, MMPs may originate from VICs, since they appear to be capable of expressing MMP-2 (and TIMP-1 and -2), in primary cell culture conditions[39]. After stimulation by pro-inflammatory TNF-α, the VICs upregulate MMP-1 production while TIMP-1 expression remains unchanged, supporting the imbalance hypothesis[38]. In addition to the VICs, macrophages/monocytes are also able to express a variety of MMPs (reviewed in[81]). These data also strongly implicate inflammation as a direct causal driver of ECM degradation.

Another ECM-modulating pathway, similar to the MMP-TIMP-system, involves a family of cysteine proteases called cathepsins and their tissue inhibitors. Similar to the MMP-TIMP-system, cathepsins and their inhibitors seem to be significant contributors to many cardiovascular diseases, from atherosclerosis to aneurysms (reviewed in[82–84]). In CAVD, a significant upregulation has been reported in the levels of cathepsins S, K and V as well as in their inhibitor, cystatin C[85]. Cathepsin V was detected in close proximity to valvular neovessels, where it may be able to degrade the ECM to make way for new blood vessels. While more studies are needed, it appears that similar to the situation with the MMP-TIMP-system, also with cathepsins and their inhibitors, the ultimate disease phenotype is caused by their degradative properties overcoming their inhibition.

Role of lp(a) (lipoprotein a) in early lesion development

Epidemiological studies have highlighted that elevated serum levels of lp(a) are a strong risk factor for CAVD[86–89]. Strong evidence for causality has been implied in a Mendelian Randomization study[90]. It has also been shown that the oxidized phospholipids carried by lp(a) (OxPL) are associated with a faster progression of CAVD[91].

In more mechanistic experimental studies, OxPLs on lp(a) particles have been postulated to be the drivers of pathogenesis. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is enriched in lp(a)[92] and it has the ability to hydrolyze OxPLs into lysophosphatidylcholine (LPC) which has been reported to activate mineralization in VICs[93]. Lp(a) particles also possess another enzyme, autotaxin (ATX), which hydrolyzes LPC into lysophosphatidic acid (LPA). LPA is also a potent promoter of calcification[94]. ATX was also found to be expressed by VICs, indicating that although serving as an important additional source, lp(a) particles are not required for ATX-mediated calcification pathway. While macroscopically larger deposits of calcium emerge in the later stages of CAVD, it should be noted that these lp(a)-dependent primary mechanisms are likely present already during disease initiation. This emphasizes the need for early prevention.

Summary

Several early hallmarks of CAVD are very similar to those encountered in atherosclerosis; LDL retention, infiltration of inflammatory cells and subsequent ECM remodeling. Valvular thickening, which may cause many of the first clinical symptoms, is ultimately the result of an accumulation of foam cells into the valve as well as loosening of the spongiosa layer of valvular ECM. The valve's mechanical properties are also compromised by the increased fibrosis occurring in other areas. All of these are active processes that precede much of the irreversible calcification. Lifestyle interventions should always be the first line of prevention of cardiovascular disease. In the case of CAVD, this is the only feasible approach, since no drug treatments have been found to be effective. These interventions are best targeted towards classical atherosclerotic risk factors.

Acknowledgements: None

Sources of funding: P. Ohukainen acknowledges the Aarne Koskelo and Emil Aaltonen Foundations for financial support. H. Ruskoaho acknowledges the Academy of Finland (grant no. 266661), the Sigrid Jusélius Foundation and Finnish Foundation of Cardiovascular Research for financial support. J Rysä acknowledges the Academy of Finland (grant no. 276747) and Finnish Foundation of Cardiovascular Research for financial support.

Disclosures: None

References

1. Schoen FJ. Evolving concepts of cardiac valve dynamics: the continuum of development, functional structure, pathobiology, and tissue engineering. *Circulation*. 2008;118(18):1864-1880. doi:10.1161/CIRCULATIONAHA.108.805911.
2. Stephens EH, Saltarrelli JG, Baggett LS, et al. Differential proteoglycan and hyaluronan distribution in calcified aortic valves. *Cardiovasc Pathol*. 2011;20(6):334-342. doi:10.1016/j.carpath.2010.10.002; 10.1016/j.carpath.2010.10.002.
3. Vesely I. The role of elastin in aortic valve mechanics. *J Biomech*. 1998;31(2):115-123. http://www.ncbi.nlm.nih.gov/pubmed/9593204. Accessed July 7, 2015.
4. Scott M, Vesely I. Aortic valve cusp microstructure: the role of elastin. *Ann Thorac Surg*. 1995;60(2 Suppl):S391-4. http://www.ncbi.nlm.nih.gov/pubmed/7646194. Accessed July 7, 2015.
5. Tseng H, Grande-Allen KJ. Elastic fibers in the aortic valve spongiosa: a fresh perspective on its structure and role in overall tissue function. *Acta Biomater*. 2011;7(5):2101-2108. doi:10.1016/j.actbio.2011.01.022.
6. Stella JA, Sacks MS. On the biaxial mechanical properties of the layers of the aortic valve leaflet. *J Biomech Eng*. 2007;129(5):757-766. doi:10.1115/1.2768111.
7. Sacks FM, Moye LA, Davis BR, et al. Relationship between plasma LDL concentrations during treatment with pravastatin and recurrent coronary events in the Cholesterol and Recurrent Events trial. *Circulation*. 1998;97(15):1446-1452.
8. Taylor PM, Batten P, Brand NJ, Thomas PS, Yacoub MH. The cardiac valve interstitial cell. *Int J Biochem Cell Biol*. 2003;35(2):113-118. http://www.ncbi.nlm.nih.gov/pubmed/12479860. Accessed July 14, 2015.
9. Mulholland DL, Gotlieb AI. Cell biology of valvular interstitial cells. *Can J Cardiol*. 1996;12(3):231-236. http://www.ncbi.nlm.nih.gov/pubmed/8624972. Accessed July 15, 2015.
10. Taylor PM, Allen SP, Yacoub MH. Phenotypic and functional characterization of interstitial cells from human heart valves, pericardium and skin. *J Heart Valve Dis*. 2000;9(1):150-158. http://www.ncbi.nlm.nih.gov/pubmed/10678389. Accessed July 15, 2015.
11. Liu AC, Joag VR, Gotlieb AI. The emerging role of valve interstitial cell phenotypes in regulating heart valve pathobiology. *Am J Pathol*. 2007;171(5):1407-1418. doi:10.2353/ajpath.2007.070251.
12. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of "degenerative" valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation*. 1994;90(2):844-853.
13. O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of "degenerative" valvular aortic stenosis. *Arterioscler Thromb Vasc Biol*. 1996;16(4):523-532.
14. Tabas I, Williams KJ, Borén J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation*. 2007;116(16):1832-1844. doi:10.1161/CIRCULATIONAHA.106.676890.
15. Skålén K, Gustafsson M, Rydberg EK, et al. Subendothelial retention of

atherogenic lipoproteins in early atherosclerosis. *Nature*. 2002;417(6890):750-754. doi:10.1038/nature00804.
16. Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. *Arterioscler Thromb Vasc Biol*. 1999;19(5):1218-1222.
17. Mahmut A, Boulanger M-C, Fournier D, et al. Lipoprotein lipase in aortic valve stenosis is associated with lipid retention and remodelling. *Eur J Clin Invest*. 2013;43(6):570-578. doi:10.1111/eci.12081.
18. Derbali H, Bossé Y, Côté N, et al. Increased biglycan in aortic valve stenosis leads to the overexpression of phospholipid transfer protein via Toll-like receptor 2. *Am J Pathol*. 2010;176(6):2638-2645. doi:10.2353/ajpath.2010.090541.
19. Lehti S, Käkelä R, Hörkkö S, et al. Modified lipoprotein-derived lipid particles accumulate in human stenotic aortic valves. *PLoS One*. 2013;8(6):e65810. doi:10.1371/journal.pone.0065810.
20. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA*. 1999;282(21):2035-2042. http://www.ncbi.nlm.nih.gov/pubmed/10591386. Accessed August 10, 2015.
21. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol*. 2007;49(25):2379-2393. doi:10.1016/j.jacc.2007.02.059.
22. Balachandran K, Sucosky P, Yoganathan AP. Hemodynamics and mechanobiology of aortic valve inflammation and calcification. *Int J Inflam*. 2011;2011:263870. doi:10.4061/2011/263870.
23. Siu SC, Silversides CK. Bicuspid aortic valve disease. *J Am Coll Cardiol*. 2010;55(25):2789-2800. doi:10.1016/j.jacc.2009.12.068.
24. Beppu S, Suzuki S, Matsuda H, Ohmori F, Nagata S, Miyatake K. Rapidity of progression of aortic stenosis in patients with congenital bicuspid aortic valves. *Am J Cardiol*. 1993;71(4):322-327.
25. Pachulski RT, Chan KL. Progression of aortic valve dysfunction in 51 adult patients with congenital bicuspid aortic valve: assessment and follow up by Doppler echocardiography. *Br Heart J*. 1993;69(3):237-240.
26. Cujec B, Pollick C. Isolated thickening of one aortic cusp: preferential thickening of the noncoronary cusp. *J Am Soc Echocardiogr*. 1988;1(6):430-432. http://www.ncbi.nlm.nih.gov/pubmed/3272793. Accessed August 26, 2015.
27. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2012;32(9):2045-2051. doi:10.1161/ATVBAHA.108.179705.
28. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111(25):3481-3488. doi:10.1161/CIRCULATIONAHA.105.537878.
29. Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420(6917):868-874. doi:10.1038/nature01323.
30. Libby P, Ridker PM, Hansson GK, Atherothrombosis LTN on. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol*. 2009;54(23):2129-2138. doi:10.1016/j.jacc.2009.09.009.
31. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat Rev Immunol*. 2010;10(1):36-46. doi:10.1038/nri2675.
32. Olsson M, Dalsgaard CJ, Haegerstrand A, Rosenqvist M, Ryden L, Nilsson J.

Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves. *J Am Coll Cardiol*. 1994;23(5):1162-1170.
33. Helske S, Lindstedt KA, Laine M, et al. Induction of local angiotensin II-producing systems in stenotic aortic valves. *J Am Coll Cardiol*. 2004;44(9):1859-1866. doi:S0735-1097(04)01637-7 [pii]; 10.1016/j.jacc.2004.07.054 [doi].
34. Natorska J, Marek G, Sadowski J, Undas A. Presence of B cells within aortic valves in patients with aortic stenosis: Relation to severity of the disease. *J Cardiol*. June 2015. doi:10.1016/j.jjcc.2015.05.002.
35. Ghaisas NK, Foley JB, O'Briain DS, Crean P, Kelleher D, Walsh M. Adhesion molecules in nonrheumatic aortic valve disease: endothelial expression, serum levels and effects of valve replacement. *J Am Coll Cardiol*. 2000;36(7):2257-2262. http://www.ncbi.nlm.nih.gov/pubmed/11127470. Accessed October 13, 2015.
36. Mazzone A, Epistolato MC, De Caterina R, et al. Neoangiogenesis, T-lymphocyte infiltration, and heat shock protein-60 are biological hallmarks of an immunomediated inflammatory process in end-stage calcified aortic valve stenosis. *J Am Coll Cardiol*. 2004;43(9):1670-1676. doi:10.1016/j.jacc.2003.12.041.
37. Kaden JJ, Dempfle C-E, Grobholz R, et al. Interleukin-1 beta promotes matrix metalloproteinase expression and cell proliferation in calcific aortic valve stenosis. *Atherosclerosis*. 2003;170(2):205-211. http://www.ncbi.nlm.nih.gov/pubmed/14612199. Accessed October 13, 2015.
38. Kaden JJ, Dempfle C-E, Grobholz R, et al. Inflammatory regulation of extracellular matrix remodeling in calcific aortic valve stenosis. *Cardiovasc Pathol*. 2005;14(2):80-87. doi:10.1016/j.carpath.2005.01.002.
39. Fondard O, Detaint D, Iung B, et al. Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors. *Eur Heart J*. 2005;26(13):1333-1341. doi:10.1093/eurheartj/ehi248.
40. Rodriguez KJ, Piechura LM, Porras AM, Masters KS. Manipulation of valve composition to elucidate the role of collagen in aortic valve calcification. *BMC Cardiovasc Disord*. 2014;14:29. doi:10.1186/1471-2261-14-29.
41. Eriksen HA, Satta J, Risteli J, Veijola M, Väre P, Soini Y. Type I and type III collagen synthesis and composition in the valve matrix in aortic valve stenosis. *Atherosclerosis*. 2006;189(1):91-98. doi:10.1016/j.atherosclerosis.2005.11.034.
42. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol*. 2011;3(12):10.1101/cshperspect.a005058. doi:10.1101/cshperspect.a005058; 10.1101/cshperspect.a005058.
43. Kumar R, Thomas CM, Yong QC, Chen W, Baker KM. The intracrine renin-angiotensin system. *Clin Sci (Lond)*. 2012;123(5):273-284. doi:10.1042/CS20120089.
44. Re RN, Cook JL. Studies of intracellular angiotensin II. *Methods Mol Biol*. 2015;1234:1-8. doi:10.1007/978-1-4939-1755-6_1.
45. De Mello WC, Frohlich ED. On the local cardiac renin angiotensin system. Basic and clinical implications. *Peptides*. 2011;32(8):1774-1779. doi:10.1016/j.peptides.2011.06.018.
46. Pacurari M, Kafoury R, Tchounwou PB, Ndebele K. The Renin-Angiotensin-aldosterone system in vascular inflammation and remodeling. *Int J Inflam*.

2014;2014:689360. doi:10.1155/2014/689360.
47. Yamazaki T, Komuro I, Yazaki Y. Role of the renin-angiotensin system in cardiac hypertrophy. *Am J Cardiol*. 1999;83(12A):53H-57H. http://www.ncbi.nlm.nih.gov/pubmed/10750588. Accessed November 26, 2015.
48. Bader M. Role of the local renin-angiotensin system in cardiac damage: a minireview focussing on transgenic animal models. *J Mol Cell Cardiol*. 2002;34(11):1455-1462. http://www.ncbi.nlm.nih.gov/pubmed/12431444. Accessed November 26, 2015.
49. Lan T-H, Huang X-Q, Tan H-M. Vascular fibrosis in atherosclerosis. *Cardiovasc Pathol*. 22(5):401-407. doi:10.1016/j.carpath.2013.01.003.
50. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol*. 2007;292(1):C82-97. doi:10.1152/ajpcell.00287.2006.
51. De Mello WC, Danser AH. Angiotensin II and the heart : on the intracrine renin-angiotensin system. *Hypertension*. 2000;35(6):1183-1188. http://www.ncbi.nlm.nih.gov/pubmed/10856260. Accessed November 26, 2015.
52. Katwa LC, Tyagi SC, Campbell SE, Lee SJ, Cicila GT, Weber KT. Valvular interstitial cells express angiotensinogen and cathepsin D, and generate angiotensin peptides. *Int J Biochem Cell Biol*. 1996;28(7):807-821. http://www.ncbi.nlm.nih.gov/pubmed/8925411. Accessed November 27, 2015.
53. O'Brien KD, Shavelle DM, Caulfield MT, et al. Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma. *Circulation*. 2002;106(17):2224-2230.
54. Tiede K, Stöter K, Petrik C, et al. Angiotensin II AT(1)-receptor induces biglycan in neonatal cardiac fibroblasts via autocrine release of TGFbeta in vitro. *Cardiovasc Res*. 2003;60(3):538-546. http://www.ncbi.nlm.nih.gov/pubmed/14659799. Accessed November 27, 2015.
55. Ahmed MS, Øie E, Vinge LE, et al. Induction of myocardial biglycan in heart failure in rats--an extracellular matrix component targeted by AT(1) receptor antagonism. *Cardiovasc Res*. 2003;60(3):557-568. http://www.ncbi.nlm.nih.gov/pubmed/14659801. Accessed November 27, 2015.
56. Myles V, Liao J, Warnock JN. Cyclic pressure and angiotensin II influence the biomechanical properties of aortic valves. *J Biomech Eng*. 2014;136(1):11011. doi:10.1115/1.4026041.
57. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res*. 2000;87(5):E1-9. http://www.ncbi.nlm.nih.gov/pubmed/10969042. Accessed January 8, 2016.
58. Santos RAS, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A*. 2003;100(14):8258-8263. doi:10.1073/pnas.1432869100.
59. Kostenis E, Milligan G, Christopoulos A, et al. G-protein-coupled receptor Mas is a physiological antagonist of the angiotensin II type 1 receptor. *Circulation*. 2005;111(14):1806-1813. doi:10.1161/01.CIR.0000160867.23556.7D.
60. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev*. 2000;52(3):415-472.

61. Xu P, Sriramula S, Lazartigues E. ACE2/ANG-(1-7)/Mas pathway in the brain: the axis of good. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(4):R804-17. doi:10.1152/ajpregu.00222.2010.
62. Lazartigues E, Feng Y, Lavoie JL. The two fACEs of the tissue renin-angiotensin systems: implication in cardiovascular diseases. *Curr Pharm Des*. 2007;13(12):1231-1245. http://www.ncbi.nlm.nih.gov/pubmed/17504232. Accessed January 9, 2016.
63. Mendoza A, Lazartigues E. The compensatory renin-angiotensin system in the central regulation of arterial pressure: new avenues and new challenges. *Ther Adv Cardiovasc Dis*. 2015;9(4):201-208. doi:10.1177/1753944715578056.
64. Peltonen T, Napankangas J, Ohtonen P, et al. (Pro)renin receptors and angiotensin converting enzyme 2/angiotensin-(1-7)/Mas receptor axis in human aortic valve stenosis. *Atherosclerosis*. 2011;216(1):35-43. doi:10.1016/j.atherosclerosis.2011.01.018.
65. Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest*. 2002;109(11):1417-1427.
66. Nguyen G, Danser AH. Prorenin and (pro)renin receptor: a review of available data from in vitro studies and experimental models in rodents. *Exp Physiol*. 2008;93(5):557-563. doi:10.1113/expphysiol.2007.040030.
67. Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand LA. Valvular myofibroblast activation by transforming growth factor-beta: implications for pathological extracellular matrix remodeling in heart valve disease. *Circ Res*. 2004;95(3):253-260. doi:10.1161/01.RES.0000136520.07995.aa.
68. Leask A. The role of endothelin-1 signaling in the fibrosis observed in systemic sclerosis. *Pharmacol Res*. 2011;63(6):502-503. doi:10.1016/j.phrs.2011.01.011.
69. Rodríguez-Pascual F, Busnadiego O, González-Santamaría J. The profibrotic role of endothelin-1: is the door still open for the treatment of fibrotic diseases? *Life Sci*. 2014;118(2):156-164. doi:10.1016/j.lfs.2013.12.024.
70. Rodriguez-Pascual F, Busnadiego O, Lagares D, Lamas S. Role of endothelin in the cardiovascular system. *Pharmacol Res*. 2011. doi:10.1016/j.phrs.2011.01.014.
71. Peltonen T, Taskinen P, Napankangas J, et al. Increase in tissue endothelin-1 and ETA receptor levels in human aortic valve stenosis. *Eur Heart J*. 2009;30(2):242-249. doi:10.1093/eurheartj/ehn482.
72. Horio T, Tokudome T, Maki T, et al. Gene Expression, Secretion, and Autocrine Action of C-Type Natriuretic Peptide in Cultured Adult Rat Cardiac Fibroblasts. *Endocrinology*. 2003;144(6):2279-2284. doi:10.1210/en.2003-0128.
73. Soeki T, Kishimoto I, Okumura H, et al. C-type natriuretic peptide, a novel antifibrotic and antihypertrophic agent, prevents cardiac remodeling after myocardial infarction. *J Am Coll Cardiol*. 2005;45(4):608-616. doi:10.1016/j.jacc.2004.10.067.
74. Peltonen TO, Taskinen P, Soini Y, et al. Distinct downregulation of C-type natriuretic peptide system in human aortic valve stenosis. *Circulation*. 2007;116(11):1283-1289. doi:10.1161/CIRCULATIONAHA.106.685743.
75. Dollery CM, McEwan JR, Henney AM. Matrix Metalloproteinases and Cardiovascular Disease. *Circ Res*. 1995;77(5):863-868. doi:10.1161/01.RES.77.5.863.

76. Liu P, Sun M, Sader S. Matrix metalloproteinases in cardiovascular disease. *Can J Cardiol*. 2006;22 Suppl B:25B-30B. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2780831&tool=pmcentrez&rendertype=abstract. Accessed November 27, 2015.
77. Edep ME, Shirani J, Wolf P, Brown DL. Matrix metalloproteinase expression in nonrheumatic aortic stenosis. *Cardiovasc Pathol*. 2000;9(5):281-286. http://www.ncbi.nlm.nih.gov/pubmed/11064275. Accessed November 27, 2015.
78. Satta J, Oiva J, Salo T, et al. Evidence for an altered balance between matrix metalloproteinase-9 and its inhibitors in calcific aortic stenosis. *Ann Thorac Surg*. 2003;76(3):681-8; discussion 688.
79. Fondard O, Detaint D, Iung B, et al. Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors. *Eur Heart J*. 2005;26(13):1333-1341. doi:10.1093/eurheartj/ehi248.
80. Knox JB, Sukhova GK, Whittemore AD, Libby P. Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. *Circulation*. 1997;95(1):205-212. http://www.ncbi.nlm.nih.gov/pubmed/8994438. Accessed November 27, 2015.
81. Newby AC. Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability. *Arterioscler Thromb Vasc Biol*. 2008;28(12):2108-2114. doi:10.1161/ATVBAHA.108.173898.
82. Liu J, Sukhova GK, Sun J-S, Xu W-H, Libby P, Shi G-P. Lysosomal cysteine proteases in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2004;24(8):1359-1366. doi:10.1161/01.ATV.0000134530.27208.41.
83. Lutgens SPM, Cleutjens KBJM, Daemen MJAP, Heeneman S. Cathepsin cysteine proteases in cardiovascular disease. *FASEB J*. 2007;21(12):3029-3041. doi:10.1096/fj.06-7924com.
84. Cheng XW, Shi G-P, Kuzuya M, Sasaki T, Okumura K, Murohara T. Role for cysteine protease cathepsins in heart disease: focus on biology and mechanisms with clinical implication. *Circulation*. 2012;125(12):1551-1562. doi:10.1161/CIRCULATIONAHA.111.066712.
85. Helske S, Syvaranta S, Lindstedt KA, et al. Increased expression of elastolytic cathepsins S, K, and V and their inhibitor cystatin C in stenotic aortic valves. *Arterioscler Thromb Vasc Biol*. 2006;26(8):1791-1798. doi:10.1161/01.ATV.0000228824.01604.63.
86. Gotoh T, Kuroda T, Yamasawa M, et al. Correlation between lipoprotein(a) and aortic valve sclerosis assessed by echocardiography (the JMS Cardiac Echo and Cohort Study). *Am J Cardiol*. 1995;76(12):928-932. http://www.ncbi.nlm.nih.gov/pubmed/7484833. Accessed August 22, 2015.
87. Glader CA, Birgander LS, Söderberg S, et al. Lipoprotein(a), Chlamydia pneumoniae, leptin and tissue plasminogen activator as risk markers for valvular aortic stenosis. *Eur Heart J*. 2003;24(2):198-208. http://www.ncbi.nlm.nih.gov/pubmed/12573277. Accessed August 22, 2015.
88. Bozbas H, Yildirir A, Atar I, et al. Effects of serum levels of novel atherosclerotic risk factors on aortic valve calcification. *J Heart Valve Dis*. 2007;16(4):387-393. http://www.ncbi.nlm.nih.gov/pubmed/17702363. Accessed August 22, 2015.
89. Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol*.

1997;29(3):630-634. doi:S0735109796005633 [pii].
90. Thanassoulis G, Campbell CY, Owens DS, et al. Genetic associations with valvular calcification and aortic stenosis. *N Engl J Med*. 2013;368(6):503-512. doi:10.1056/NEJMoa1109034; 10.1056/NEJMoa1109034.
91. Capoulade R, Chan KL, Yeang C, et al. Oxidized Phospholipids, Lipoprotein(a), and Progression of Calcific Aortic Valve Stenosis. *J Am Coll Cardiol*. 2015;66(11):1236-1246. doi:10.1016/j.jacc.2015.07.020.
92. Blencowe C, Hermetter A, Kostner GM, Deigner HP. Enhanced association of platelet-activating factor acetylhydrolase with lipoprotein (a) in comparison with low density lipoprotein. *J Biol Chem*. 1995;270(52):31151-31157. http://www.ncbi.nlm.nih.gov/pubmed/8537378. Accessed November 30, 2017.
93. Mahmut A, Boulanger M-C, El Husseini D, et al. Elevated Expression of Lipoprotein-Associated Phospholipase A2 in Calcific Aortic Valve Disease. *J Am Coll Cardiol*. 2014;63(5):460-469. doi:10.1016/j.jacc.2013.05.105.
94. Bouchareb R, Mahmut A, Nsaibia MJ, et al. Autotaxin Derived From Lipoprotein(a) and Valve Interstitial Cells Promotes Inflammation and Mineralization of the Aortic Valve. *Circulation*. 2015;132(8):677-690. doi:10.1161/CIRCULATIONAHA.115.016757.

Main findings

- The early stages of calcific aortic valve disease and coronary atherosclerosis are very similar.
- The main pathological contributors are inflammation, extracellular matrix remodeling, activation of local renin-angiotensin system, and lipoprotein (a) mediated processes.
- These initial stages lead to irreversible calcification, highlighting the importance of early prevention.