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# Calcific Aortic Valve Disease

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## 1. Introduction

Aortic stenosis due to calcific aortic valve disease (CAVD) is currently the main indication for aortic valve replacement in developed countries (Lung et al, 2003). Due to an aging population and a decline in rheumatic heart disease, CAVD has become the most common heart valve disease in the Western countries, affecting approximately 25% of adults over 65 years, of which 2-3% has clinically significant aortic stenosis (Stewart et al, 1997). Even mild CAVD is associated with adverse outcomes, with a 50% increased risk of cardiovascular death (Lloyd-Jones et al, 2009). There are no known therapies that slow disease progression, and surgical valve replacement is the only effective treatment for aortic stenosis. More than 85,000 aortic valve replacement surgeries are done in the United States, and over 275,000 are performed worldwide. These numbers are expected to triple by 2050 (Takkenberg et al, 2008). These statistics emphasize the burden of aortic valve disease and the necessity of understanding its mechanisms, underscored recently by recommendations set out by The National Heart, Lung and Blood Institute Aortic Stenosis Working Group (<http://www.nhlbi.nih.gov/meetings/workshops/cas.htm>).

CAVD is a progressive disease that starts with initial changes in the cell biology of the valve leaflets, which develop into atherosclerotic-like lesions and aortic sclerosis, and eventually lead to calcification of the valve, causing left ventricular outflow tract obstruction (Rajamannan et al, 2007, Otto, 2008). Although CAVD progresses with age, it is not an inevitable consequence of aging. CAVD traditionally has been considered a degenerative phenomenon, in which years of mechanical stress on an otherwise normal valve, cause calcium to deposit on the surface of the aortic valve leaflets. The evolving concept, however, is that CAVD is an actively regulated process that cannot be characterized simply as "senile" or "degenerative". The progressive calcification process involves lipid accumulation, increasing angiotensin-converting enzyme activity, inflammation, neovascularization, and extracellular matrix degradation.

Furthermore, the risk factors for CAVD are similar to those for atherosclerosis: age, gender, hypercholesterolemia, diabetes, smoking, renal failure, and hypertension (Stewart et al, 1997). In addition, pathological studies of explanted human stenotic aortic valves have identified lesions similar to those in atherosclerotic plaques, which contain inflammatory cells and calcific deposits (Otto et al, 1994). The involvement of high cholesterol levels is corroborated by studies demonstrating that patients with familial hypercholesterolemia develop aortic valve lesions that calcify with age (Rajamannan et al, 2001). Furthermore, preclinical studies have demonstrated atherosclerotic-like lesions in aortic valve leaflets in

atherosclerosis in rabbits and mice. From the notion that CAVD and atherosclerosis might share a similar mechanism, statins (3-hydroxy-3-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitors) emerged as a potential therapy for treating CAVD. Indeed, retrospective studies have demonstrated a reduction in disease progression when patients were treated with statins (Aronow et al, 2001, Novaro et al, 2001, Bellamy et al, 2002). In addition, animal studies confirmed that statin treatment inhibits calcification (Rajamannan et al, 2005, Aikawa et al, 2007). Large prospective clinical trials, however have not shown slowed CAVD progression in patients treated with high doses of statins (Cowell et al, 2005, Rossebo et al, 2008). This may be due to the late implementation of the statins, after aortic valve calcification has progressed to the irreversible stage.

The aortic valve consists of endothelial cells and valvular interstitial cells that maintain the health of the valve and are important in valvular disease. Valvular interstitial cells likely mediate the progression of CAVD (Mohler et al, 1999). Signals in aortic valve biology that trigger activation, differentiation, or pathological change are unclear. However, we know that in CAVD, valvular interstitial cells differentiate to myofibroblasts and osteoblast-like cells, which are eventually responsible for calcium deposition (Mohler et al, 2001). Possible pathological triggers include hemodynamic shear stress, solid tissue stresses, reactive oxygen species (ROS), inflammatory cytokines and growth factors, and physiological imbalances such as the metabolic syndrome, diabetes mellitus, end-stage renal disease, and calcium or phosphate imbalance (Schoen, 2008, New & Aikawa, 2011, Miller et al, 2010). The cellular and molecular factors involved in the development of aortic valve stenosis, however, remain largely obscure. The poor prognosis and increased mortality after the onset of symptoms provide a rationale for the pursuit of a better understanding of the disease process, which can lead to effective therapeutic strategies to prevent CAVD. This chapter discusses our current understanding of the pathophysiology, risk factors, cellular mechanisms, diagnosis, and clinical management of CAVD, and describes areas of future research vital for diagnosing, treating, and potentially preventing this disease.

## **2. Normal aortic valve physiology and function**

Aortic heart valves maintain unidirectional blood flow throughout the cardiac cycle with minimal obstruction, without regurgitation. The aortic valve prevents retrograde flow back into the left ventricle during diastole. Heart valves open and close approximately 40 million times a year, and 3 billion times over an average lifetime. Mechanical forces exerted by the surrounding blood and heart drive the aortic valve's function. The dynamic structure and physiology of aortic heart valves enables them to avoid excess stress concentration and to withstand wear and tear over many years (Breuer et al, 2004). Aortic valve functionality must be seen in conjunction with the aortic root, and be viewed as one apparatus (Schoen, 2008). A variety of pathological processes can lead to aortic valve malfunction, with serious clinical consequences. This malfunction usually is associated with calcific changes of the valve connective tissue, and eventually causes aortic stenosis. Before we elaborate on the pathological mechanisms of CAVD, we will discuss normal aortic valve anatomy and physiology and their relationship to aortic valve function.

### **2.1 Aortic valve anatomy**

The aortic cusps are thin, flexible structures that come together to seal the valve orifice during diastole. The aortic valve is normally composed of three cusps or leaflets. The

individual cusps are attached to the aortic wall in a semilunar fashion, ascending to the commissures (where adjacent cusps come together at the aorta) and descending to the basal attachment of each cusp to the aortic wall; this anatomical structure is also called the aortic valve annulus. A portion of the annulus is attached to cardiac muscle, while the other half is continuous with the fibrous leaflets of the mitral valve. The functional unit of the aortic valve includes the cusps and their respective aortic sinus complexes, also called the aortic root. The aortic root is a bulb-shaped structure to which the aortic cusps are attached. Behind the aortic valve cusps are dilated pockets in the aortic root known as the *sinuses of Valsalva*, from which the coronary arteries originate. The nomenclature of the aortic valve cusps and their respective sinuses are based on the position of the coronary artery ostia – the left coronary cusp, the right coronary cusp, and the non-coronary cusp (and their associated sinuses).

In the middle of the free margin of each cusp on the ventricular surface is a pronounced thickening, known as the *nodule of Arantius*. Coaptation of these three nodules ensures complete closure of the valve during diastole. Along the ventricular surface of each cusp, between the free edge and the closing edge, is a crescent shaped region called a *lunula*. These thin areas of leaflet contact the corresponding regions of both adjacent cusps to ensure a competent seal. The remainder of the cusp (noncoapting portion) is known as the *belly*. Native human aortic valves are virtually avascular, and receive nutrients through hemodynamic convection and diffusion. In most cases, the dimensions of the three leaflets are slightly unequal (Roberts, 1970). The dynamic, complex three-dimensional anatomy of the aortic trileaflet cusps and the aortic root allows for stress sharing between leaflets, the sinuses of Valsalva, and the aortic wall (Thubrikar et al, 1986a, Katayama et al, 2008).

## 2.2 Aortic valve function

The aortic valve functions synergistically with the aortic root to maintain efficient cyclic opening and closing – opening when exposed to forward flow, and then rapidly and completely closing under minimal reverse pressure. Importantly, opening of the valve precedes, rather than responds to, the forward movement of blood from the ventricle (Thubrikar et al, 1979, Higashidate et al, 1995). This illustrates the complex function of the valve, and is one of the reasons for considering movement of the whole root – from the level of the annulus to the sinotubular junction – when describing aortic valve functionality.

As blood decelerates in the aorta at the end of systole, vortexes in the sinuses of Valsalva behind the AV cusps facilitate valve closure. These vortexes help funnel oxygenated blood into the coronary arteries and create a small pressure gradient across the leaflet, which helps bring the leaflets to a smooth and efficient closure.

The ability to prevent reverse flow of the aortic valve depends on the stretching and molding of the three cusps to fill the orifice during the closed phase of the cardiac cycle, during which back pressure from the blood is present in the aorta. Simultaneously, the valve annulus expands, pulling the cusps and thus preventing collapse (Thubrikar et al, 1980).

A further increase in the annular radius at end diastole and into the isovolumic contraction phase of the cardiac cycle pulls the cusps from their commissures such that a small stellate orifice results, even without the presence of transvalvular flow (Butcher et al, 2011). The valve orifice changes quickly from stellate, to triangular, and finally to a circular pattern, as blood is ejected from the ventricle. The aortic root adjusts from a conical to a cylindrical shape during ejection, providing optimal hemodynamics at the larger flow volume

(Thubrikar et al, 1979). The aortic valve closes much more gradually than it opens. The total surface area of the three cusps is approximately 40% larger than the cross-sectional area of the aortic root at the annular level. This allows each cusp to bow slightly towards the left ventricular outflow tract, which prevents the cusp from inverting during diastole (Ho, 2009). The normal aortic valve orifice area (AVA) is 2.6-3.5 cm<sup>2</sup>, however depends on the body mass area of the individual (Fowler, 1979).

### 2.3 Aortic valve structure

The structure of the aortic valve cusps is organized into three layers: (i) the *zona ventricularis*, closest to the left ventricle chamber and composed largely of elastin, which can extend in diastole and recoil in systole to minimize cusp area; (ii) the *zona fibrosa*, closest to the outflow surface, rich in densely packed collagen organized in radial and circumferential direction, which provides the strength and stiffness of the cusps and is mainly responsible for bearing diastolic stress; and (iii) the centrally located *zona spongiosa*, which consists mainly of glycosaminoglycans (GAGs) that accommodate shear forces of the cuspal layers, and absorbs shock during the valve cycle (Schoen, 2008). (Figure 1)

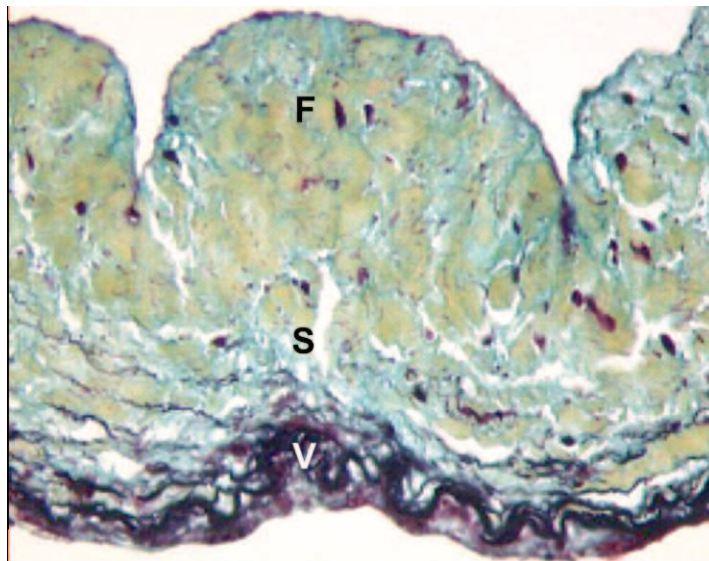


Fig. 1. Human normal aortic valve histopathology depicts a tri-layered structure (F) – fibrosa, (S) – spongiosa and (V) – ventricularis. Modified from Aikawa E. et al, *Circulation*, 2006

This organization of the aortic native heart valve allows for certain unique qualities. First, accordion-like folds called corrugations, present in the valve cusps, allow for the cuspal shape and dimensions to vary with the cardiac cycle. Second, microscopic collagen folding (also known as *crimp*) allows lengthening of the valve at minimal stress. Cusp tissue also displays anisotropy, the quality conferred by collagen architecture that permits differences in radial and circumferential extensibility. Finally, the macroscopic collagen alignment enables forces from the cusps to transfer to the aortic wall (De Hart et al, 2004). By employing these properties, the native heart valve avoids excess stress concentration on the cusps and supporting tissues and can withstand biomechanical loads caused by repetitive deformations. In addition, biomechanical stress may induce the remodelling and repair of connective tissue (Breuer et al, 2004). The aortic valve tissue possesses a cellular make-up that withstands a large amount of pressures and stresses.



## 2.4 Aortic valve biology

The native aortic heart valve consists of two types of cells: valvular interstitial cells (VICs) that permeate the entire valve tissue, and valvular endothelial cells (VECs) that cover the surface. The components of the extracellular matrix (ECM) are synthesized, degraded, and maintained by VICs, which seem to have adaptive characteristics. In the healthy aortic valve, they have primarily quiescent fibroblast-like properties, but they can change to an activated phenotype during valvular remodelling, response to injury, or pathology. VECs regulate immune and inflammation responses and provide the native heart valve with its nonthrombogenic properties (Butcher et al, 2004). Small populations of smooth muscle cells (Cimini et al, 2003) and nerve cells have also been described (Chester et al, 2008, Marron et al, 1996).

### 2.4.1 Valvular interstitial cells

VICs remodel ECM proteins in the aortic valve leaflet, and are of mesenchymal origin. Different phenotypes of VICs are present in the mature native valve. Five identifiable phenotypes of VICs have been described: embryonic mesenchymal cells, quiescent VICs (qVICs), activated VICs (aVICs), progenitor VICs (pVICs), and osteoblastic VICs (oVICs) (Yperman et al, 2004, Taylor et al, 2003, Liu et al, 2007, Rabkin-Aikawa et al, 2004, Rabkin et al, 2001). qVICs are at rest in the valvular interstitium and maintain normal valve physiology by becoming activated in response to injury or disease. This process leads to the differentiation of VICs into activated VICs, demonstrating a fibroblast-like phenotype. More specifically, aortic valve VICs secrete and turn over proteins at an increased rate compared to other cell types *in vivo*, which indicates that VICs continually repair mechanically induced tissue micro-damage to enable long-term durability. ECM remodelling results from the synthesis of ECM-degrading enzymes by VICs, such as matrix metalloproteinases (e.g., MMP-1, MMP-2, MMP-9, and MMP-13) and cathepsins (e.g., cathepsins S, K, and B), and tissue inhibitors (e.g., tissue inhibitors of metalloproteinases [TIMP] Rabkin et al, 2001, Schoen, 2008). The cellular and molecular mechanisms that control this phenotype differentiation also participate in aortic valve pathology. In particular, VICs become activated when stimulated by mechanical loading, and mediate connective tissue remodelling to restore a normal stress profile in the tissue (Aikawa et al, 2006).

The pressure load applied to the leaflet causes an increase in circumferential and radial leaflet length, which increases strain on the tissue (Butcher et al, 2008). Valve strain seems highest at areas where leaflets attach to the aortic wall; these locations are also where calcification begins in CAVD (Thubrikar et al, 1986b, Levitt et al, 1984). Mechanical forces acting on the valve translate into biological responses at the tissue level, which in turn lead to a VIC response at the cellular level – which causes constant synthesis and renewal of the ECM (Mulholland and Gotlieb, 1996). Intracellular signaling leads to changes that include increased VIC stiffness and increased ECM biosynthesis. Concurrently, the higher valvular pressure gradients on the left side of the heart lead to larger local tissue stress on VICs, which in turn lead to higher VIC stiffness and collagen biosynthesis in the left-sided valves (Merryman et al, 2006).

### 2.4.2 Endothelial cells

The surface of the aortic valve is lined with a single monolayer of VECs that maintain non-thrombogenicity. VECs are similar to arterial endothelial cells in the expression of von

Willebrand factor because they produce nitric oxide and have prostacyclin activity (Butcher et al, 2008). Cell junctions between VECs are also similar to those of arterial endothelial cells. VECs however seem to be phenotypically different from arterial endothelial cells (Jaffee, 1967), based on the observation that VECs originate developmentally from sources different from arterial endothelial cells. In addition, VECs are oriented circumferentially across leaflets, perpendicular to the direction of the blood flow, in contrast to vascular endothelium, which aligns parallel to flow (Deck, 1986; Schoen, 2008). Comparing valvular and vascular endothelium in a static culture has led to the identification of significantly different genes (Butcher et al, 2006). In addition, although VECs and arterial endothelial cells are both prone to calcification, different mechanisms lie at the heart of the process. VECs show an increased expression of genes involved in chondrogenic differentiation, while arterial endothelial cells more strongly express osteogenic genes (Butcher et al., 2011). Due to their location at the surface of the aortic valve, VECs are important in relation to the hemodynamic forces exerted on the heart valve. The ventricularis part of the valve (inflow) is exposed to rapid, pulsatile, unidirectional shear stress. In contrast, the fibrosa part of the valve (outflow) experiences a lower, almost oscillatory shear stress (Kilner et al, 2000). Interestingly, recent evidence suggests that different transcriptional profiles are expressed by the endothelium on the opposite faces (ventricularis vs. fibrosa) of a normal adult pig aortic valve. (Simmons et al, 2005) Studies have demonstrated that high pulsatile shear stress on the fibrosa side of the aortic valve increases inflammatory receptors and the expression of bone morphogenic protein (BMP) (Sucosky et al, 2009), but also decreases the expression of inhibitors of fibrosis and calcification, including osteoprotegerin (OPG), C-type natriuretic peptide (CNP), and chordin (Yip et al, 2009). In addition, *in vivo* studies have demonstrated that CAVD initiates on the fibrosa side of the valve (Mohler et al, 2001, Mohler, 2004). Investigators have hypothesized that these differences in endothelium between opposite sides of the aortic valve may contribute to the typical predominant localization of pathologic aortic valve calcification near the outflow surface (*zona fibrosa*) (Simmons et al, 2005). In addition, a recent study has demonstrated that mechanical stress may induce the osteogenic potential of VECs, providing evidence that the valvular endothelium harbours a reserve of progenitor cells that can repopulate the leaflet with osteogenic-like interstitial cells (New & Aikawa, 2011, Wylie-Sears et al, 2011). Future studies are needed to show whether this mechanism, in combination with pro-inflammatory factors, contributes to valve calcification.

#### 2.4.3 Cellular interaction

VICs and VECs exist in close communication, which indicates that cellular interaction is vital for leaflet biology. Studies have demonstrated that VECs interact with VICs in a complex hemodynamic and mechanical environment to maintain aortic valve cusp tissue integrity. Additionally, VECs regulate VIC function through paracrine signals, such as controlling VIC contractility and leaflet mechanics (El-Hamamsy et al, 2009). More specifically, valvular endothelial dysfunction has been implicated as the initiator of inflammatory reactions, blood clots, and even calcification (Butcher & Nerem, 2006). The interaction between vascular endothelial cells and vascular smooth muscle cells, and its importance to normal vessel function, has been well documented. Such interaction likely exists between valvular endothelium and VICs.

VIC-VEC co-culture studies have corroborated that VECs help maintain a qVIC phenotype (Butcher & Nerem, 2006). Moreover, when exposed to shear stress, the presence of endothelium stabilized VIC proliferation, increased the synthesis of ECM proteins by VICs, and decreased GAG loss (Butcher & Nerem, 2006). These results suggest that damage to or loss of valvular endothelium leads to VIC hyperplasia and myofibroblast activation. When the endothelial layer of aortic valve explants was removed, it promoted the formation of calcific nodules (Mohler et al, 1999). Studies also have demonstrated that neurogenic dilation of aortic valve cusps only occurred only when the endothelium was intact (Chester et al, 2008). The communication between VECs and VICs is fundamental in normal and pathological signalling, but more work is needed to further delineate these relationships.

### 3. Clinical aspects of calcific aortic valve disease

CAVD is a progressive disease that begins with initial changes in the cell biology of valve leaflets, develops into atherosclerotic lesions and aortic sclerosis, that eventually leads to calcification of the valve, which causes left ventricular outflow tract obstruction. This is known as calcific aortic stenosis, which is viewed as the critical end stage of this disease process, and is associated with poor outcomes.

#### 3.1 Epidemiology

CAVD is the most common cause of aortic stenosis in the developed world. Increased aortic valve cusp thickness due to fibrosis and lipid accumulation, but without left ventricular outflow tract obstruction, is known as *aortic valve sclerosis*. Aortic valve sclerosis and aortic stenosis are generally viewed as the early stage and late stage, respectively, of the CAVD pathological process. Aortic sclerosis is common in the elderly population; the prevalence in the general population is 29% (Stewart et al, 1997). A landmark study (Stewart et al, 1997) identified 26% of study population older than 65 years having aortic sclerosis, indicating that aortic sclerosis associates with age. In the same population, 2% had aortic stenosis. These numbers increase with age – those over 74 years old, 37% had aortic sclerosis, and almost 3% had aortic stenosis. Importantly, approximately 16% of patients with aortic sclerosis will develop aortic stenosis within 6 to 8 years (Cosmi et al, 2002). Other studies have reported that up to 33% of patients with aortic sclerosis developed aortic stenosis within 4 years of follow-up (Faggiano et al, 2003). No known therapies slow aortic valve disease progression, and surgical valve replacement currently is the only effective treatment for aortic stenosis. In addition, aortic valve sclerosis increases the risk of myocardial infarction or cardiovascular death by 50% (Lloyd-Jones, 2009). As such, aortic valve disease has a serious impact on general health.

#### 3.2 Risk factors

Calcific aortic stenosis shares nearly identical risk factors with atherosclerosis. Clinical risk factors for calcific aortic stenosis include age, male sex, hypertension, smoking, elevated serum levels of lipoprotein (A), and low-density lipoprotein (LDL) levels (Stewart et al, 1997). Other studies have demonstrated that traditional risk factors that are important in atherosclerosis, including the metabolic syndrome and renal failure, also associate with calcific aortic stenosis (Aronow et al, 1987, Katz et al, 2009, Otto et al, 1997, Fox et al, 2006). This overlapping of risk factors has led to the hypothesis that calcific aortic valve disease



and atherosclerosis have similar etiologies, but there are epidemiological discrepancies, as demonstrated by the inconsistency in coexisting prevalence between calcific aortic stenosis and coronary artery disease. Only 50% of patients with severe calcific aortic stenosis have significant coronary artery disease, and the majority of patients with coronary artery disease have no calcific aortic stenosis (Otto & O'Brien, 2001). Furthermore, metabolic diseases such as hyperparathyroidism (secondary to chronic renal failure, Paget's disease) have also associated with accelerated progression of aortic valve calcification and stenosis (Horl, 2004, Hultgren, 1998). Further studies are needed to understand the differences between the pathophysiologies of atherosclerosis and aortic valve disease.

### 3.3 Pathophysiology and diagnosis

A discrepancy exists between the onset of symptoms and the onset of disease. Symptoms usually do not occur until calcific aortic stenosis has developed. The symptoms and clinical signs of CAVD are better understood by discussing the physiological changes that occur. Calcific aortic stenosis leads to left ventricular outflow tract obstruction, which causes several physiological changes, best described in a left ventricular pressure-volume loop. Ventricular emptying is impaired by outflow tract resistance, which results from a reduced aortic valve orifice area during systole. This, in turn, causes a large pressure gradient over the aortic valve – which means that ventricular pressure needs to exceed the increased aortic pressure gradient, causing increased peak systolic pressure and subsequent aortic valve closure due to an increased end-systolic volume. Consequently, stroke volume decreases. Higher end-systolic volume raises the afterload, and thus the incoming venous return, leading to increased end-diastolic volume. This process activates the Frank-Starling mechanism, which increases contraction force and thus maintains a normal stroke volume when the aortic stenosis is mild. Stenosis severity correlates with the increase of left ventricular outflow tract obstruction and afterload. When the end-systolic volume increases more than the end-diastolic volume, stroke volume will decrease, leading to a reduction in arterial pressure. The cardiovascular system will strive to maintain arterial pressure, increasing peripheral vascular resistance. In addition, the left ventricular heart muscle will demonstrate hypertrophy to compensate for a chronic increase of afterload. Most cardiovascular systems can compensate for aortic stenosis until the orifice diameter is less than  $0.6 \text{ cm}^2/\text{m}^2$  ( $\sim 1.0 \text{ cm}^2$ ). The patient will remain relatively asymptomatic up to this point, due to adequate physiological compensatory mechanisms. Symptoms generally appear when the valve orifice is around  $1.0 \text{ cm}^2$ , and typically include shortness of breath, syncope, and chest pain. Left ventricular hypertrophy combined with a stenotic aortic valve may lead to impaired blood flow to the heart muscle, in turn causing increased oxygen demand by the heart muscle and leading to angina pectoris. Aortic stenosis can present itself clinically with a systolic ejection murmur at the right upper sternal border, often radiating to the neck. Peaking of the murmur late in systole, a palpable delay of the carotid upstroke, and a soft second heart sound can all point to aortic stenosis. Aortic stenosis is usually confirmed using ultrasound echocardiography (Nakamura et al, 1984, Braun & Comeau, 1951). The severity of aortic valve dysfunction is determined by the combination of the following hemodynamic indices: peak ejection velocity, effective orifice area, and mean transvalvular pressure gradient (Nakamura et al, 1984). As described earlier, CAVD progression involves the narrowing of the valve orifice and increased ventricular ejection velocities and pressure gradients. Mild aortic valve stenosis is generally defined by

restricted opening of the valve cusps, with a mean transvalvular pressure gradient of less than 25 mm Hg; moderate aortic valve stenosis by a mean gradient between 25 and 40 mm Hg; and severe aortic valve stenosis by a mean gradient of 40 mm Hg or more (Cosmi et al, 2002). Because direct imaging of the aortic valve still involves technical challenges, echocardiography remains the gold standard for assessing aortic valve dysfunction (Aikawa & Otto, 2011).

### **3.4 Treatment**

#### **3.4.1 Surgical treatment**

Unless discovered during monitoring for other conditions, patients rarely exhibit detectable symptoms of aortic valve disease until after it has already progressed to an advanced stage (Rosenhek et al, 2000). Patients with severe aortic stenosis have a life expectancy of less than 10 years if untreated. Of these patients with concomitant heart failure, 50% will die within a year (Carabello & Paulus, 2009). Aortic valve replacement is the only effective treatment, but optimal timing for surgery in asymptomatic patients remains unclear (Stout & Otto, 2007). Asymptomatic patients have good survival without surgery, and combined with the surgical risk for operative mortality and post-operative complications, surgeons are reluctant to perform valve replacement in these patients (Owen & Henein, 2011). Replacement valves, be they mechanical or bioprosthetic, have several shortcomings. The body recognizes a mechanical valve as foreign material, giving rise to thromboembolic complications that require lifelong anticoagulation therapy. (Hammermeister et al, 2000). Bioprosthetic valves are prone to reduced durability (~20 years) because of structural dysfunction resulting from progressive leaflet deterioration and calcification, eventually requiring reoperation (Hammermeister et al, 2000, Bloomfield et al, 1991). Bioprosthetic valves are the conduits of choice for patients over 60–65 years who are relatively physically inactive, and when there is a contraindication for anticoagulation therapy. Mechanical valves are chosen for active patients under 60 years, who can tolerate anticoagulation therapy.

#### **3.4.2 Pharmacological treatment**

The need for alternatives to surgery is emphasized by the increasing age of the general population and the rising prevalence of CAVD. Therapeutic strategies to restrict disease progression are needed to delay and possibly avoid surgical valve replacement. Because CAVD and atherosclerosis have similar disease progression and risk factors, pharmacological treatment of CAVD mostly has been focused on lipid-lowering agents (statins) and angiotensin-converting enzyme (ACE) inhibitors (Stewart et al, 1997, O'Brien et al, 2002). LDLs are present in human aortic valve lesions. In addition, studies have demonstrated the presence of oxidized lipids in calcifying areas of aortic valves. (Olsson et al, 1999) Statins inhibit the pathway for synthesizing cholesterol in the liver and lower plasma cholesterol levels. Animal studies and retrospective clinical studies have demonstrated that statins could potentially slow CAVD progression (Rosenhek et al, 2004, Novaro et al, 2001, Shavelle et al, 2002, Moura et al, 2007). However, large clinical trials have demonstrated that statins do not affect CAVD in general valve disease population (Rosenhek et al, 2004, Novaro et al, 2001, Shavelle et al, 2002, Moura et al, 2007; Holme et al, 2010; Chan et al, 2010; Cowell et al, 2005). ACE, angiotensin II, and angiotensin II type 1 receptors have also been identified in aortic sclerotic lesions (O'Brien et al, 2002, Helske et al, 2004). Retrospective studies associate ACE inhibitors with a lower rate of aortic valve

calcification, but ACE inhibitors do not inhibit CAVD progression (Rosenhek et al, 2004). Interestingly, angiotensin II type 1 antagonists have prevented aortic valve lesion formation in hypercholesterolemic rabbits (Arishiro et al, 2007). Trials are ongoing to elucidate further the effect of ACE inhibitors on the progression of CAVD. As we gain more information about the specific mechanisms of CAVD, different potential pharmaceutical targets surface. The valve endothelium has received a great deal of attention and could potentially be a drug delivery platform. A recently developed method tests for the presence or absence of diseased aortic valves in atherosclerotic mice using an anti-VCAM-1 peptide to target early-stage aortic valve disease endothelium (Aikawa et al, 2007a). We still generally lack clinically significant pharmacological therapies for CAVD, which indicates the importance of additional research to elucidate mechanisms of CAVD progression.

## 4. Pathology of calcific aortic valve disease

### 4.1 Pathology

CAVD is a progressive disorder that ranges from mild valve thickening to severe calcification with impaired leaflet motion or aortic valve stenosis (Freeman & Otto, 2005). Though CAVD traditionally was viewed as a passive degenerative disease resulting from years of stress, we now recognize it as an actively regulated disease, with evidence suggesting that it follows a mechanism akin to bone formation (Mohler et al, 2001). Pathologically, stenotic valves are characterized by the presence of chronic inflammatory cellular infiltrates such as macrophages and T-lymphocytes, by the accumulation of lipids, and by thickening of the *fibrosa* and mineralization (Otto et al, 1994). Early CAVD lesions are similar to atherosclerosis lesions, consisting of prominent LDL, lipoprotein (a), and apolipoproteins (O'Brien et al, 1996). Inflammatory cells and lipids in stenotic valve leaflets co-localize near the surface of CAVD lesions, supporting the notion that CAVD is an active inflammatory disease process.

The extent to which the mechanism of CAVD is similar to that of atherosclerosis remains unclear (Mohler, 2004, Rajamannan et al, 2007). Both CAVD and atherosclerosis seem to be initiated by endothelial dysfunction involving differentiation of underlying interstitial cells. In both diseases, endothelial dysfunction relates to disturbed blood flow, to bifurcations and sinuses in larger arteries, and to the outflow (*fibrosa*) side of the aortic valve (Porat et al, 2004). Both CAVD lesions and atherosclerotic lesions contain inflammatory cells, with advanced lesions containing calcium deposits (Hjortnaes et al, 2010). In addition, pathological studies of human stenotic valves have identified lesions similar to those in atherosclerotic plaques (Otto et al, 1994, Olsson et al, 1999), and similar lesions have been described in aortic valve leaflets of atherosclerosis in rabbits and mice (Tanaka et al, 2005, Aikawa et al, 2007b).

But differences exist between the pathologies of CAVD and atherosclerosis. First, CAVD can be present in younger patients – such as patients with bicuspid aortic valves, which usually are heavily calcified shortly after birth (Sabet et al, 1999). Second, discrepancies may exist between the pathological compositions of CAVD lesions and atherosclerosis lesions. Aortic plaques at the late stage of atherosclerosis contain lipid accumulations and fibrous tissue, and large calcific nodules mainly formed through apoptosis or matrix vesicles formation. Aortic valve lesions, in contrast, are pathologically composed of large, bone-like matrix nodules formed by osteoblast-like cells. In addition, calcified lesions in end-stage aortic valve disease progressively stiffen the valve leaflets, causing motion impairment. End-stage

atherosclerotic lesions, however, demonstrate plaque ruptures and thrombotic occlusions. These differences support the notion that CAVD has characteristics that are different from those of atherosclerosis, which indicates the presence of potentially different and complex mechanisms.

The current pathological concept of CAVD is that mechanical stress, together with atherosclerotic risk factors, leads to valvular endothelial dysfunction, followed by the deposition of LDL particles and other compounds that trigger inflammation. The inflammatory state can lead to the activation of inflammatory signalling pathways, macrophage infiltration, and T-lymphocyte activation. Activation of inflammatory pathways contributes to the disease process, which in turn activates VICs to express osteoblastic phenotypes and cause calcium deposition. Our current understanding of the role of VECs and VICs in the disease process will be discussed later in this chapter.

#### 4.2 Endothelial dysfunction

As described earlier, the surface of the aortic valve is lined with an endothelial monolayer. Although the exact initiating factors for the inflammatory process in CAVD are unclear, studies have demonstrated that the endothelium plays an important role. Because VECs are located at the surface of the aortic valve, the endothelium is subjected to hemodynamic forces. Different shear stresses are exerted on each side of the aortic valve. *In vivo* studies have demonstrated that early CAVD lesions initiate on the fibrosa (outflow) side of the valve (Sucosky et al, 2009). Endothelium subjected to abnormal blood flow seems more susceptible to inflammatory cytokines (Aikawa et al, 2006, Sacks & Yoganathan, 2007). Research has shown that high pulsatile shear stress on the *fibrosa* side of the aortic valve induces upregulation of inflammatory receptors by VECs for circulating cytokines and inflammatory monocytes, leukocytes and T-lymphocytes (Muller et al, 2000, Shavelle et al, 2008). In addition, elevated stretch loading on the aortic valve induced pro-inflammatory cytokine (bone morphogenetic protein [BMP2/4]) expression on the fibrosa part of the aortic valve. These results indicate the potential role of BMPs in early CAVD lesions {Balachandran et al, 2010}. Another mechanism proposed as an initiating factor in CAVD, but also in atherosclerosis, is the expression of pro-oxidant phenotypes (ROS) in VECs (Butcher et al, 2006, Sucosky et al, 2009, Sorescu et al, 2004). ROS, including oxidized lipids, can cause endothelial cell injury, which can lead to a loss of endothelial alignment and an increase the upregulation of cell adhesion molecules – permitting increased inflammatory infiltration (Mirzaie et al, 2003). These events may together or individually initiate and/or sustain chronic inflammation, and lead to the development of CAVD (Mohler, 2004). The expression of endothelial nitric oxide synthase (eNOS) – a vasodilator that protects blood vessels against atherosclerosis – is reduced in conditions with oxidative stress, such as disturbed blood flow, but is elevated by antioxidant signalling. Although its role in CAVD is less clear, eNOS expression is reduced on the VECs of the fibrosa side compared to the ventricularis side of the aortic valve. This reduction indicates that eNOS might be involved in the *fibrosa* susceptibility in CAVD pathogenesis (Butcher et al, 2006). Moreover, inhibition of eNOS expression results in increased cusp stiffness of the aortic valve (El-Hamamsy et al, 2009). Statins decreased the amount of calcification and increased VEC eNOS in rabbits, which suggests that eNOS may protect against CAVD. Our own studies, however, demonstrated an induction of eNOS expression on the fibrosa vs ventricularis sides during fetal valve development (Figure 2). Therefore, further studies are warranted to investigate these mechanisms.



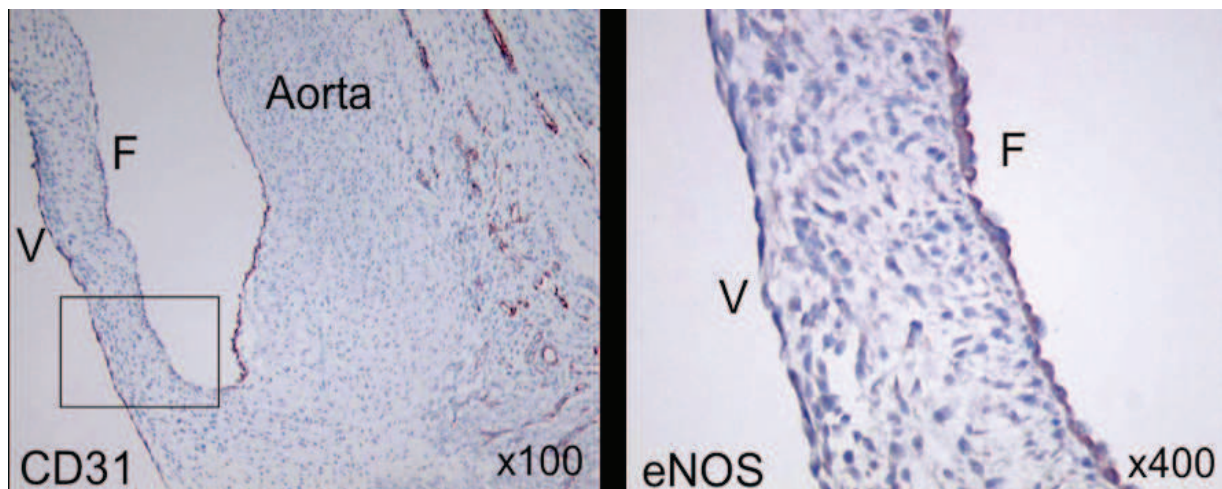


Fig. 2. Human fetal aortic valve section immunostained with anti-CD31 and anti-eNOS reveals side-specific expression of eNOS. (F) – fibrosa, (V) - ventricularis

#### 4.3 Valvular interstitial cell remodelling and the role of the extracellular matrix

VICs are responsible for the physiological remodelling that maintains integrity and pliability in the aortic valve. In terms of CAVD, the general hypothesis is that VICs undergo myofibroblast differentiation to become osteoblast-like cells, which in turn deposit calcium in the aortic valve. This myofibroblast activation seems to be activated by invading inflammatory cells and activated endothelial cells that produce cytokines such as TGF- $\beta$ 1, BMP2/4, IL-1, IL-6 and TNF- $\alpha$ . Increased  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and smooth muscle myosin (SMM), decreased vimentin, increased migration, and increased proliferation characterize myofibroblast activation of VICs. (Taylor et al, 2003, Yip et al, 2009, Jian et al, 2003, Kaden et al, 2005b) Furthermore, studies have demonstrated that in CAVD, inflammatory cells and VICs secrete MMPs and cathepsins, which are involved in extracellular remodelling (Aikawa et al, 2009, Rabkin et al, 2001, Soini et al, 2001, Fondard et al, 2005). The exact role of the inhibitors of these proteins, such as TIMPs and cystatin C, which are also expressed by VICs in CAVD, remains to be established. In CAVD, the *zona fibrosa* is especially prone to this remodelling process, characterized by disruption and disorganization of collagen bundles, fragmentation of elastin, and an increase in proteoglycan deposition. The disruption of the ECM contributes to the release of growth factors such as TGF- $\beta$ 1, which consequently influences VIC differentiation (as described earlier). The osteoblast-like cell differentiation of VICs is subject to a number of mechanisms, which are described in the next section.

#### 5. Cellular and molecular mechanisms of CAVD

CAVD is an active disease process characterized by progressive calcification of the valve leaflet by VICs and inflammatory cells. The cellular and molecular mechanisms are complex and involve growth factors, lipoproteins, and biomechanical factors. The molecular pathways that may participate in CAVD include Wnt, the angiotensin/kinin system, and the OPG/RANKL/RANK signalling pathways. Growth factors such as TGF- $\beta$  (Jian et al, 2003) and cytokines (IL-6 and TNF- $\alpha$ ) (O'Brien et al, 2002, Kaden et al, 2005b) also seem to affect the osteoblastic differentiation of VICs. Specific bone-cell phenotypes are present in calcifying human valves (Rajamannan et al, 2003). Increased

levels of specific bone formation markers have been identified in CAVD. These include the non-collagenous bone matrix proteins such as osteopontin, osteocalcin, and bone sialoprotein (Kaden et al, 2004b, Mohler et al, 1997). The osteoblast transcription factor *runx2 / cbfa1* has also been identified in calcified aortic valves (Caira et al, 2006). The LDL-receptor-related protein 5/wingless-type MMTc integration site family member 3 (*Lrp5/Wnt3*) signalling pathway regulates osteoblast differentiation in the aortic valve (Johnson & Rajamannan, 2006). Neoangiogenesis also has been implicated in the development of CAVD lesions (Chalajour et al, 2004). The exact cellular and molecular mechanisms of CAVD, however, remain scant.

### 5.1 Biomechanics

The roles of mechanical and hemodynamic factors have been well studied, considering the context of designing and analyzing prosthetic valves. CAVD associates with mechanical and hemodynamic factors, as demonstrated by the correlation of CAVD lesions with regions of disturbed blood flow. Mechanical forces are vital in valve homeostasis, but deviations from normal mechanical stress patterns can exacerbate pathological differentiation, as seen in CAVD. Contrary to the traditional belief that mechanical forces contribute to valve disease in a wear-and-tear fashion, we now see a direct relationship between mechanics and the active regulation of the aortic valve cell phenotype. This relationship is corroborated by studies that describe a site-specific pathological susceptibility of CAVD, demonstrating that the *zona fibrosa* especially suffers from mechanically induced pathological development. This relationship also reflects the difference in cellular level strains in valve tissue layers when subjected to mechanical forces. Mechanical forces and hemodynamic changes associated with CAVD exert their influence on the cells with which they are in direct contact, namely endothelial cells. The exposure of shear stress to valve endothelial cells leads to the downregulation of genes associated with the activation and osteogenic differentiation of VICs (Butcher et al, 2006, Sucosky et al, 2009). VICs exposed to pathological cyclic strain demonstrated increased expression of  $\alpha$ -SMA, BMP2/4, MMP, and cathepsin activity (Balachandran et al, 2010). Similarly, hypertensive pressure showed increased VCAM-1 expression and decreased osteopontin (Warnock et al, 2006). Myofibroblast activation is also observed in autologous replacement valves implanted in an increased pressure environment, such as pulmonary autografts (Ross procedure) or tissue-engineered heart valves (Rabkin-Aikawa et al, 2004a, Rabkin et al, 2002). Moreover, increased mechanical stiffness of the ECM occurs due to changes resulting from CAVD, (Weinberg et al, 2009) and VICs cultured on stiff substrates demonstrate increased myofibroblast differentiation and calcification (Yip et al, 2009).

All in all, studies have demonstrated that changes in the mechanical environment and hemodynamic forces are important in the pathological process and changes occurring in CAVD. More specifically, biomechanics in the context of CAVD can catalyze the disease process, but are not an independent factor of osteoblastic differentiation.

### 5.2 Transcription growth factor- $\beta$

Transcription growth factor- $\beta$  (TGF- $\beta$ ) regulates biological functions in various systems. In the aortic valve, TGF- $\beta$  affects VIC differentiation, increasing the expression of  $\alpha$ -SMA, smooth muscle myosin, and calponin (ten Dijke and Hill, 2004). TGF- $\beta$  binds to its receptors (TGF- $\beta$  receptors I and II), which initiates signalling through Smad proteins, which in turn

interact with the transcription factors FoxH1, c-Jun, c-fos, and Gli-3. TGF- $\beta$  can also initiate mitogen-activated protein kinase (MAPK) pathways. Both signalling pathways regulate cell cycle, proliferation, migration, cytokine secretion, and ECM synthesis and degradation – all of which are important in normal valvular biology (Walker et al, 2004). The ECM components heparin and fibronectin participate in the effect of TGF- $\beta$  on VICs (Taipale et al, 1996). By binding TGF- $\beta$  to the pericellular environment, heparin induces  $\alpha$ -SMA expression in VICs. Heparin also induces new TGF- $\beta$  synthesis by VICs. VICs express fibronectin, a major component of the ECM that can sequester TGF- $\beta$  to activate VICs (Cushing et al, 2005). In CAVD, elevated VIC activation by TGF- $\beta$  causes pathological remodelling of the ECM. More specifically, increased TGF- $\beta$ 1 expression increases collagen, glycosaminoglycan, and hyaluronic acid syntheses. In addition, studies of calcified valves have indicated increased expression of TGF- $\beta$  in ECM. *In vitro* studies demonstrate that TGF- $\beta$  promotes migration, aggregation, and formation of apoptotic alkaline phosphatase nodules. Anti-apoptotic agents prevent apoptosis and calcification in TGF- $\beta$ -treated VICs. This indicates that TGF- $\beta$  also promotes CAVD through a process involving apoptosis similar to atherosclerotic plaques (Jian et al, 2003).

### 5.3 Lipoproteins and the Wnt signalling pathway

Landmark studies of patient aortic valve specimens with CAVD demonstrated the presence of LDLs in the valvular tissue. It became clear that lipids participate in the calcification of aortic valves relate to events leading to the eventual osteoblast-like differentiation of VICs. Lipids induce oxidative stress in the endothelium. Similar to vascular disease, endothelial dysfunction predisposes LDL migration through the endothelium. The accumulation of LDL can recruit inflammatory cells, subsequently leading to the release of inflammatory cytokines, which can activate VICs to differentiate into osteoblast-like cells.

The active role of lipids in the calcification process is confirmed in humans and in animals, which show a higher rate and severity of CAVD (Aikawa et al, 2007b). Interestingly, studies have demonstrated a relation between the bone formation signalling pathway and the LDL metabolism pathway. More specifically, the regulation of the LRP5/Wnt signalling has been implicated in cardiovascular calcification (Caira et al, 2006, Rajamannan et al, 2005, Shao et al, 2005). LDL receptor-related protein (LRP) signalling in bone is regulated through the canonical Wnt pathway. Wnt, a growth factor involved in bone and heart development (Clevers, 2006, Chakraborty et al, 2008), binds to receptors composed of frizzled protein and either of LRP5 or LRP6. This inhibits  $\beta$ -catenin degradation and leads to its accumulation and subsequent entry into the cellular nucleus. Calcified valves express elevated LRP5 and  $\beta$ -catenin, compared to healthy controls (Caira et al, 2006, Rajamannan et al, 2005).  $\beta$ -catenin modulates the expression of several target genes, including cyclin D, Runx2/Cbfa1, and Sox9 (Shao et al, 2005). These transcription factors are crucial for myofibroblast differentiation to osteoblasts. The exact role of lipids in CAVD has yet to be elucidated. The recent failure in lipid-lowering pharmaceutical trials indicates that treating hyperlipidemia alone may not affect CAVD.

### 5.4 The OPG/RANKL/RANK signalling pathway

Calcified valves demonstrate the expression of RANKL (ligand of receptor activator of nuclear factor  $\kappa$  B, RANK) and osteoprotegerin (OPG), which also are parts of a cytokine system that regulates bone turnover (Kaden et al, 2004a, Kaden et al, 2004b). RANKL, a

member of the TNF- $\alpha$  superfamily, is a transmembrane protein located on the surface of osteoblasts, stromal cells, T cells, and endothelial cells (Hofbauer & Schoppet, 2004). RANKL interacts with RANK, a transmembrane protein located on osteoclast precursors or mature osteoclasts, and induces osteoclastogenesis via NF $\kappa$ B. The interaction of RANKL with RANK also increases the binding of osteoblast transcription factor runx2/cbfa-1, which is essential for osteoblast differentiation (Kaden et al, 2004b). This interaction can be blocked by OPG, subsequently limiting the activation of RANK and thus preventing osteoclast differentiation. This pathway is critical in cardiovascular calcification, and provides a possible link between cardiovascular calcification and bone metabolism (Hjortnaes et al, 2010), but determining the exact mechanism will require further investigations. Studies have shown that RANK/RANKL are highly increased in stenotic valves (Steinmetz et al, 2008). In VICs, RANKL treatment induces an osteoblast-like phenotype, that favours bone formation, increased nodule formation, and increased alkaline phosphatase activity, along with elevated synthesis of matrix elements and enhanced DNA binding of Runx2/Cbfa-1 (Kaden et al, 2005a). Treatment of VICs with TNF- $\alpha$  causes similar effects (Kaden et al, 2005b). Furthermore, mice deficient in OPG show calcification of large-sized and medium-sized vessels (Nanes, 2003), which supports the protective role of OPG against calcification. Interestingly, OPG has the opposite effect on skeletal bone, and inhibits bone resorption. Our recent study demonstrated that cardiovascular calcification inversely correlates with low bone mineral density of long bones in an animal model of CAVD (Hjortnaes et al, 2010). These results led to the hypothesis that calcium metabolites in the valve may originate from bone, and are mediated through inflammatory signalling. Further studies are needed to evaluate this hypothesis.

### 5.5 The renin-angiotensin system

Emerging evidence suggests that the renin-angiotensin system (RAAS) and the kallikrein-kinin system (KKS) are important in the regulation of heart valve homeostasis. In terms of CAVD, the RAAS/KKS balance seems to shift toward pro-fibrotic. ACE is a potent pro-fibrotic protein, capable of forming the equally pro-fibrotic angiotensin II (ATII) (Mehta & Griendling, 2007). ACE is produced by monocytes/macrophages and binds to circulating LDL particles. ACE can inactivate the anti-fibrotic enzyme bradykinin (BK), which is generated by the KKS (Helske et al, 2004). Studies have demonstrated the presence of ATII receptors on VICs in CAVD; the density of these receptors is significantly higher in CAVD than in healthy aortic valves. The importance of the RAAS is demonstrated in studies where the ATII type 1 antagonist olmesartan had similar protective effects as atorvastatin in CAVD in rabbits (Arishiro et al, 2007). Retrospective studies with ACE inhibitors also seem to slow calcification (Rosenhek et al, 2004), but randomized clinical trials are warranted.

### 5.6 Neoangiogenesis

Healthy human aortic valves are avascular and receive their nutrients through diffusion. In CAVD, however, neo-vessel formation or angiogenesis occurs, especially around calcified nodules. Histopathological studies have demonstrated the expression of vascular endothelial growth factor in calcified valves (Soini et al, 2003). Furthermore, endothelial progenitor cells localize in the *zona fibrosa* of calcified native and bioprosthetic valves, indicating that cells of extra-valvular origin contribute to CAVD (Skowasch et al, 2005). Whether VIC differentiation or inflammatory cells are involved in the neovascularization is



unclear. Interestingly, the aortic valve also expresses anti-angiogenic factors, such as chondromodulin-1 and endostatin (Chalajour et al, 2004). This observation has led to the hypothesis that the aortic valve possesses mechanisms to inhibit neovascularization in the healthy native valve, but in CAVD these mechanisms are disrupted, adding to its pathological process. Further studies are needed to investigate the precise role of angiogenesis in CAVD.

### 5.7 Genetics

The potential involvement of genes in CAVD has received much attention. Lessons learned from studies of bicuspid aortic valves, which associate with relatively quick calcific deterioration, demonstrate the potential role of a genetic component – namely, NOTCH1 and eNOS. For instance, 40% of adult mice that lack eNOS develop bicuspid aortic valves (Lee et al, 2000). NOTCH1 is a receptor-based transcriptional activator and has been shown to promote endothelial-to-mesenchymal transformation (EMT) and valve formation during valvular development. NOTCH1 normally inhibits calcification by inducing the expression of its target genes Hey1 and Hey2, which in turn interact with and repress the activity of Runx2/Cbfa1. Inactivation or mutations of NOTCH1 catalyze the progression of Runx2/Cbfa1-mediated calcification (Rusanescu et al, 2008). Much research is still needed to elucidate the role of a genetic component in CAVD, but evidence indicates that its development could be multifactorial.

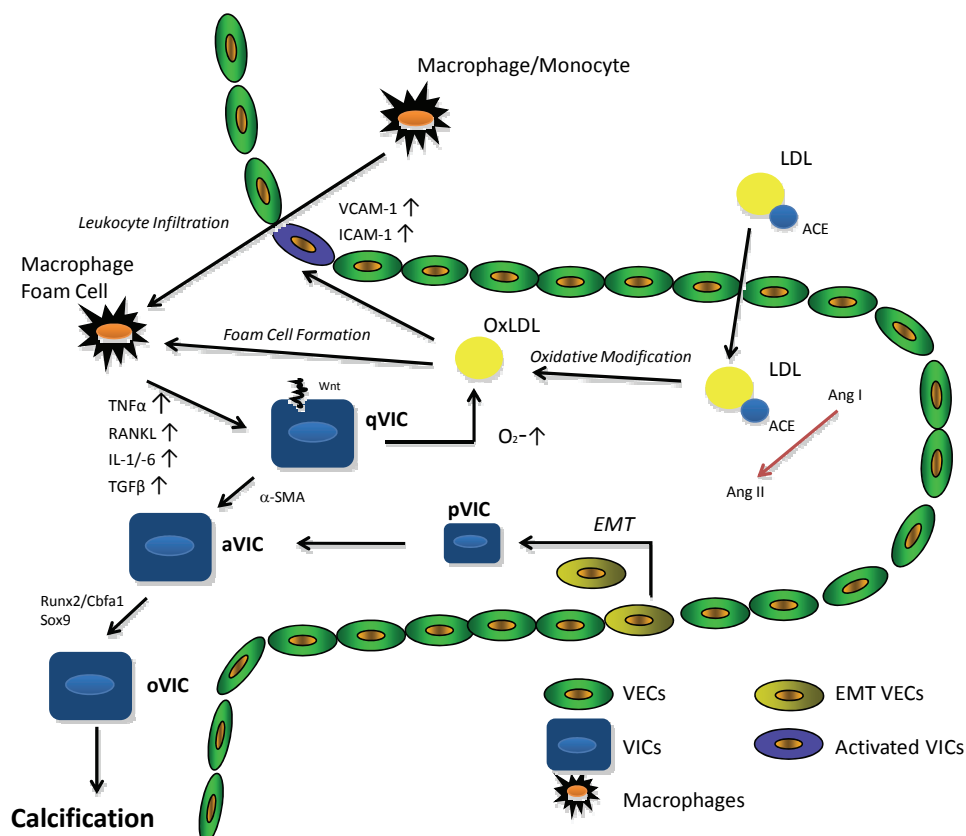


Fig. 3. Schematic depiction of cellular and molecular mechanisms in CAVD. qVIC – quiescent VICs, aVIC – activated VICs, pVIC – progenitor VICs, oVIC – osteoblastic VICs

### 5.8 Final common pathway

In summary, the previously discussed mechanisms lead to a final common pathway of CAVD – the active mineralization of valvular matrix by activated VICs that have differentiated into osteoblast-like cells (Figure 3). The calcification process initiates mainly in the *zona fibrosa*. The osteogenic differentiation of VICs is characterized by the presence of osteoblast-related genes such as osteocalcin, osteonectin, and the transcription factors *runx2/cbfa1*. The RANKL/RANK/OPG and Lrp5/Wnt signalling pathways can lead to the stimulation of *runx2/cbfa1*-mediated calcification of the aortic valve. Excess of circulatory LDL, accompanied by ACE, acts through the Lrp5/Wnt signalling pathway and induces mineralization of the valve stroma. Inflammation also stimulates VIC differentiation into osteoblast-like cells (Aikawa et al, 2007). Inflammatory cells produce cytokines that stimulate the RANKL/RANK/OPG pathway; ACE that acts through Wnt; and enzymes that degrade the ECM (e.g., MMPs, cathepsins). The inflammatory state and the degradation of ECM upregulates TGF $\beta$ , which stimulates the myofibroblast differentiation of VICs and further degrades ECM. Biomechanical forces, in which increased mechanical pressure environments can stimulate myofibroblast differentiation of VICs, also influence ECM homeostasis. CAVD should be considered a multifactorial disease for which the onset and progression to aortic valve stenosis mandate further investigations.

## 6. Animal models

Animal models are important in studying CAVD *in vivo* and in evaluating the effects of new therapies. The perfect animal model should be able to simulate the human disease. Murine, rabbit and porcine models are most commonly used in CAVD research. The main difference between these models is that mice require genetic manipulation to create disease, while rabbits and pigs, naturally susceptible to cardiovascular calcification, often suffice with diet-induced hyperlipidemia.

### 6.1 Murine models

Transgenic mouse models have proved very effective in recreating human disease. Apolipoprotein E (ApoE) knockout and low-density lipoprotein receptor (LDLr) knockout mice are the most commonly used animal models. ApoE is a protein that allows for receptor-mediated removal of very low-density lipoprotein (VLDL) from the circulation. Spontaneous hypercholesterolemia occurs in ApoE<sup>-/-</sup> mice, and as they age, they demonstrate increased transvalvular velocity, aortic regurgitation, and nodular calcification (Tanaka et al, 2005). When subjected to a hypercholesterolemic diet, accelerated early disease is observed, characterized by thickened leaflets, activated endothelial cells, and subendothelial lesions rich in macrophages, which co-localize with MMPs, cathepsins,  $\alpha$ -SMA, ALP, Runx2/Cbfa1, and osteocalcin expression (Aikawa et al, 2007a, Aikawa et al, 2007b). Moreover, studies have shown that ApoE<sup>-/-</sup> mice display aortic valve sclerosis similar to that in humans. By knocking out the LDL receptor, the removal of circulatory LDL is inhibited, also leading to a hypercholesterolemic state. Fed a high-cholesterol diet, these mice develop extreme hyperlipidemia, hyperglycemia, and mineral deposition at 16 weeks of age. They also show increases in valve thickness, macrophage accumulation, activated myofibroblasts and osteoblasts, and ectopic mineralization (Matsumoto et al, 2010). An expansion of this model, the Reversa mouse, is achieved by inhibiting apolipoprotein B (ApoB) 100 and incorporating a conditional knockout of the microsomal triglyceride transfer

protein (Mttp) under the control of an inducible Mx1-Cre<sup>+/+</sup> gene (LDLr<sup>-/-</sup>, ApoB100/100, Mttp fl/fl, Mx1-Cre<sup>+/+</sup>) (Miller et al, 2009). Fed a high-cholesterol diet, these mice develop calcific aortic stenosis in 6 months; profibrotic signalling, myofibroblast activation, and procalcific signalling are also observed (Miller et al, 2009, Miller et al, 2010). Our recent studies have induced chronic renal disease (CRD) in ApoE<sup>-/-</sup> mice, to cause accelerated ectopic calcification (Aikawa et al, 2009, Hjortnaes et al, 2010).

## 6.2 Rabbit models

Rabbits are used in CAVD research because they have tri-layer aortic valve morphology, respond to a high-cholesterol diet, and show similarities to human lipoprotein metabolism. They also are susceptible to accelerated calcification with vitamin D2 and are available as transgenic strains (Otto et al, 1999, Fan & Watanabe, 2000). Their disadvantages, however, include the requirement of very high cholesterol levels to form atherosclerotic lesions, and vitamin D2 admission to cause accelerated calcification. Rabbits also seem to demonstrate atherosclerotic lesions different from those in humans. The standard rabbit model is the New Zealand white rabbit (NZWR), subjected to a high-cholesterol diet, a vitamin D2-supplemented diet or both. After 1 week, the subendothelial region of the fibrosa shows the accumulation of macrophages, collagen fibers, elastin fragmentation, and proteoglycan accumulation (Filip et al, 1987). After 8 weeks, myofibroblast proliferation, ACE, osteopontin, and osteoblast gene expression are increased (Rajamannan et al, 2002b). Calcification is present after 12 weeks (Rajamannan et al, 2005). Similar to murine models, the LDLr and apolipoproteins can be altered in rabbits, resulting in hypercholesterolemia. One such rabbit model used in CAVD research is the Watanabe heritable hyperlipidemic (WHHL) rabbit, characterized by a spontaneous LDLr mutation (Fan & Watanabe, 2000).

## 6.3 Porcine models

Pigs demonstrate many similarities to humans, such as hemodynamic environment, heart anatomy, tri-layered aortic valve leaflets, similar lipid profiles, and lipoprotein metabolism (Dixon et al, 1999, Gerrity et al, 2001). They are popular mostly in atherosclerosis research. Yorkshire swine and miniature pig breeds fed with high-cholesterol diets have been used in CAVD research. After 6 months, these pigs demonstrate small calcific nodules histologically, and subendothelial lipid infiltration in the *zona fibrosa* (Simmons et al, 2005). Atherosclerotic pigs demonstrate a similar inflammatory state, but this is observed only in vascular walls, not in valves (Gerrity et al, 2001). Mutations in LDLr and apolipoprotein genes have also yielded porcine models suitable for atherosclerosis and CAVD research, achieving complex lesions at 2 years old without a high-cholesterol diet (Prescott et al, 1991). Size is the main limitation of porcine models, requiring relatively high expenditures in animal care and maintenance.

## 7. “The point of no return”

An important discussion in the field of CAVD research involves whether the disease is reversible, whether there is a point of no return in the disease process, and where such a point may lie. Answers to these questions would have vital implications for therapeutic intervention, including determining the optimal timing of surgery. We would need to develop imaging tools that can evaluate the disease process in patients with risk factors. Traditional imaging modalities such as echocardiography and computerized tomography,

albeit very suitable in identifying and quantifying calcification, are limited because they are unable to detect early CAVD lesions. Molecular imaging has emerged in the search for new technologies to allow early detection and offer insight into the mechanism of CAVD, as a successful tool that can detect pathobiological processes associated with inflammation and early stages of calcification *in vivo* at the cellular level (Nahrendorf et al, 2008, Aikawa et al, 2007b, Jaffer et al, 2007, Hjortnaes et al, 2010). Research into the mechanisms of CAVD has enabled the development of molecular imaging agents that can visualize key cellular and molecular processes. Studies have successfully detected pro-inflammatory, pro-osteogenic, and proteolytic activity in cardiovascular calcification *in vivo* (Aikawa et al, 2009, Aikawa et al, 2007b, Aikawa et al, 2006, Deguchi et al, 2006). Imaging agents use molecular processes to generate image contrast using high-resolution imaging technology. This approach has led to the discovery of imaging agents that chemically attach to an affinity ligand, such as a fluorochrome or magnetic compound (e.g., biphosphonate-conjugated fluorescent agents, cross-linked iron oxide fluorescent nanoparticles to detect macrophages). We can visualize enzyme activity in CAVD by employing molecular imaging agents to interact with enzymes that, when active, undergo a chemical change leading to signal amplification (Deguchi et al, 2006, Aikawa et al, 2007b). Currently, only optical imaging modalities can be used to detect calcification and early stages of the disease, due to limited signal detection by conventional imaging techniques such as CT and MRI. Visualizing pathways involved in early stages of CAVD is warranted in the pursuit of new therapeutic targets. Studies have successfully utilized multimodal molecular imaging to detect and monitor over time the dynamic changes in inflammation and ectopic calcification in mouse models of cardiovascular calcification (Aikawa et al, 2009, Aikawa et al, 2007b, Hjortnaes et al, 2010) (Figure 4). These changes are undetectable by conventional imaging techniques. In addition, molecular imaging provides the opportunity to effectively visualize biological processes simultaneously using different imaging agents. This research demonstrates the importance of developing imaging techniques able to detect early calcification before a “point of no return,” and to establish the reversibility potential of CAVD.

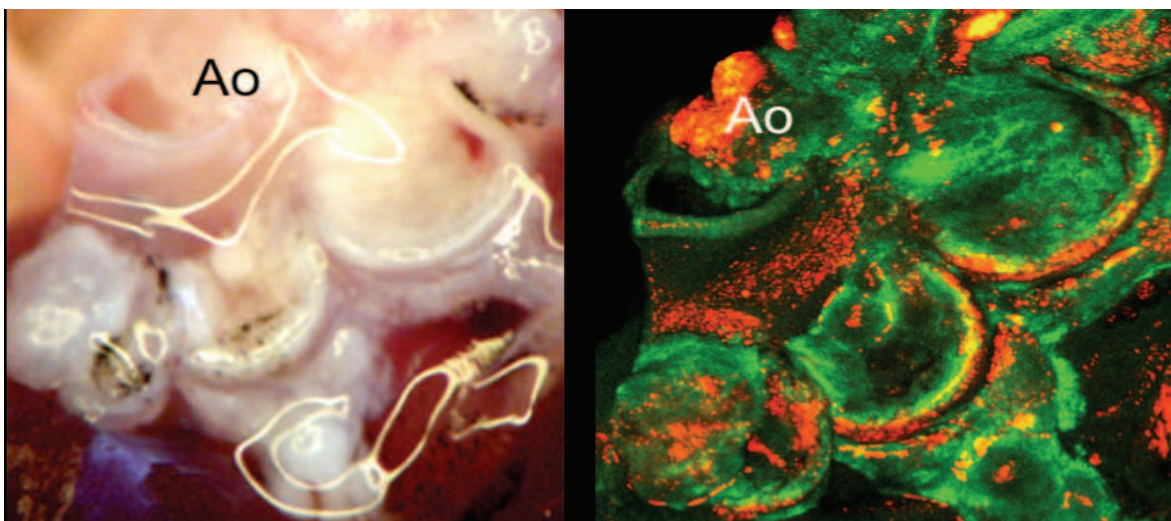


Fig. 4. Aortic valves of apoE<sup>-/-</sup> mice had characteristics of CAVD. Gross morphology of calcified aortic valve (left). Fluorescence microscopy (image stacks; right) visualized osteogenic activity (OsteoSense-680, red) in the areas of leaflet attachment to the aortic wall in inflamed valve (CLIO-750, green). Modified from Aikawa et al, *Circulation*, 2009



## 8. Conclusion

CAVD is a growing burden in the Western world. It is a progressive disease ranging from mild valve thickening to severe calcification with aortic valve stenosis. Despite its increasing prevalence and clinical significance, the mechanisms of CAVD are unclear. Fuelled by the absence of effective therapies other than aortic valve replacement, studies are needed to achieve a better understanding of CAVD – a complex disease in which multiple cellular and molecular mechanisms have been identified. The presence of additional comorbidities and clinical risk factors indicates a multifactorial pathogenesis.

The National Heart, Lung and Blood Institute Aortic Stenosis Working Group recently set out recommendations for CAVD research: 1) identification of genetic, anatomic, and clinical risk factors for the distinct phases of initiation and progression of CAVD; 2) development of high-resolution and high-sensitivity imaging modalities that can identify early and subclinical CAVD, including molecular imaging and other innovative imaging approaches; 3) understanding the basic science of CAVD, including signalling pathways and the roles of valve interstitial cells and endothelial cells, autocrine and paracrine signaling, the extracellular matrix and its stiffness, interacting mechanisms of calcification, biomechanics, and hemodynamics; 4) development of suitable multi-scale *in vitro*, *ex vivo*, and animal models; 5) identification of the relationship between CAVD and bone metabolism; 6) creation of tissue banks from valve tissue acquired from surgery, pathology, and autopsy, with and without CAVD; and 7) establishing clinical studies of CAVD to determine the feasibility of pharmacological intervention and optimal timing of surgical valve replacement.

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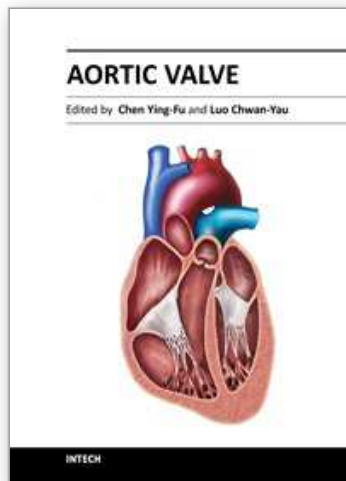


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Much has evolved in the field of aortic valve disease because of the increase in knowledge in the last decade, especially in the area of its management. This book "Aortic Valve" is comprised of 18 chapters covering basic science, general consideration of aortic valve disease, infective endocarditis, aortic sclerosis and aortic stenosis, bioprosthetic valve, transcatheter aortic valve implantation and a special section on congenital anomalies of the aortic valve. We hope this book will be particularly useful to cardiologists and cardiovascular surgeons and trainees. We also believe that this book will be a valuable resource for radiologists, pathologists, cardiovascular anesthesiologists, and other healthcare professionals who have a special interest in treating patients with aortic valve disease. We are certain that information in this book will help to provide virtually most new areas of aortic valve disease that will be employed in the current era.

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