CALCIFIC AORTIC VALVE DISEASE- A NARRATIVE REVIEW

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ABSTRACT: Calcific aortic valve disease is a slowly progressive disorder with a disease continuum that ranges from mild valve thickening without obstruction of blood flow, termed aortic sclerosis, to severe calcification with impaired leaflet motion, or aortic stenosis. Möenckeburg gave the first detailed description of CAVD in - calcium deposition on the valve cusps making them sclerotic. He also described two mechanisms to explain this phenomenon: degeneration within the layers of the valve leaflets nearest the sinuses of Valsalva that propagated toward the tips of the cusps. Sclerotic changes of the aorta that extended to involve the valve cusps. Typically, the leaflets are ≤1 mm thick and are comprised of an outer layer of valve endothelial cells (VECs) and 3 internal layers made up of valve interstitial cells (VICs). These layers are known as the fibrosa, spongiosa, and ventricularis to reflect their anatomic location, cellular and extracellular matrix composition, and biomechanical properties (Rajamannan et al., 2011). VICs are abundant in all layers of the heart valves, and are crucial to function. VICs synthesize ECM and MMPs. Adult heart valve VICs in situ have characteristics of resting fibroblasts - quiescent. VIC's are activated during intrauterine valvular maturation (becomes quiescent after birth), abrupt changes in the mechanical stress state of valves and in disease states. Once activated, VICs can differentiate into a variety of other cell types, including myofibroblasts and osteoblasts, although valve osteoblasts may respond to cellular signals differently than skeletal osteoblasts. VECs resemble endothelial cells with phenotypic differences. VECs interact with VICs to maintain the valve integrity differential transcription by VECs on the opposite (i.e., aortic and ventricular) faces. These may contribute to the typical localization of early pathological AV calcification. VECs are activated by abnormal hemodynamic forces (such as hypertension, elevated stretch, or shear stresses). Interaction with activated VIC. Pathophysiology include congenital heart defect, lambl's excrescences, papillary fibroelastoma, calcification of aortic valve (CAVD). Symptoms of CAVD are Chest pain (angina) or tightness, feeling faint or fainting with exertion, Shortness of breath, especially with exertion, Fatigue, especially during times of increased activity, Heart palpitations — sensations of a rapid, fluttering heartbeat, Heart murmur. Analysis of CAVD is done by two methods Echocardiographic Imaging for CAVD and Computed Tomography in Quantifying Calcification in CAVD. Cellular mechanisms of CAVD includes: Bone morphogenetic protein and Wnt signaling in valve interstitial cells (VICs), Endothelial-to-Mesenchymal Transition, Inflammation and Immune Response, Stem and Progenitor Cells. We also have few other processes associated with CAVD they are as follows: Matrix Vesicle Formation and Microcalcifications, Extracellular Matrix Remodeling, Angiogenesis and Neovascularization. Potential targets of CAVD are inflammation, sodium dependent phosphate transporter 1, oxidized LDL, osteoprotegerin, PPAR gamma, lipo protein (a), HDL cholesterol, apolipo protein A I mimetic peptide, angiotensin 2 type 1 receptor, hydroxytryptamine receptor 2B, cadherin-11, notch 1, P 2 Y purinoreceptor 2, cathepsin S. Evaluation of CAVD is done by few techniques to measure the aortic valve calcium content they are alizarin red, arsenazo 3, atomic absorption, energy dispersive X-ray spectroscopy, O- cresolphthalein complexone, raman spectroscopy, scanning electron microscopy, transmission electron microscopy, von kossa.

1.Introduction:

Calcific aortic valve disease is a slowly progressive disorder with a disease continuum that ranges from mild valve thickening without obstruction of blood flow, termed aortic sclerosis, to severe calcification with impaired leaflet motion, or aortic stenosis.

1.1. Historical events in relation to Calcified aortic valve disease:

The earliest description of Calcified aortic valve disease (CAVD) was given by a French physician, Lazare Rivière who, in 1663. He noted left ventricular enlargement and identified large caruncle-like masses obstructing the left ventricular outflow tract. These crucial findings by Lazare riviere were lost in a debate which supported endocarditis as a potential cause behind this abnormal pathophysiology. In 1904, Möenckeburg gave the first detailed description of CAVD in which he reported that this pathology likely occurred as a result of calcium deposition on the valve cusps making them sclerotic. These finding refuted the endocarditis hypothesis. He also described two mechanisms to explain this phenomenon:

1. degeneration within the layers of the valve leaflets nearest the sinuses of Valsalva that propagated toward the tips of the cusps
2. sclerotic changes of the aorta that extended to involve the valve cusps.

Within the past 20 years, there has been a tremendous resurgence in interest in advancing these early hypotheses to identify the cellular and molecular mechanisms that initiate CAVD owing to the high prevalence of this disease, its associated morbidity and mortality, and the emerging idea that therapies that target these processes could increase the durability of surgically implanted and transcatheter bioprosthetic valves.

1.2. Anatomy of Aortic valve:

The aortic valve is located between the left ventricular outflow tract and the ascending aorta. The aortic valve is the cardiac centerpiece. Relative to the aorta, the mitral valve is located posterior and to the left, the tricuspid valve is located inferiorly and to the right, and both valves abut on the posteroinferior margins of the aortic root, albeit with the atrioventricular separating structures interposing between the root and the tricuspid valve (Aziz and Baciewicz, 2007). In most cases, the orifices of the coronary arteries arise within the 2 anterior sinuses of Valsalva, usually positioned just below the Sino tubular junction (Reid, 1970). However, as shown in the fig 1, arteries can occasionally be positioned superior relative to the Sino tubular junction. As a result of the semilunar attachment of the aortic valvar leaflets, 3 triangular extensions of the left ventricular outflow tract reach the level of the Sino tubular junction (Sutton et al., 1995). These triangles are formed of thinned fibrous walls of the aorta between the expanded sinuses of Valsalva. Their most apical regions represent areas of potential communication with the pericardial space and, in the case of the triangle between the right and left coronary aortic leaflets, with the plane of tissue interposed between the aorta and anteriorly located sleeve-like sub pulmonary infundibulum. The 2 interleaflet triangles bordering the noncoronary leaflet are also in fibrous continuity with the fibrous trigones, the mitral valve, and the membranous septum.

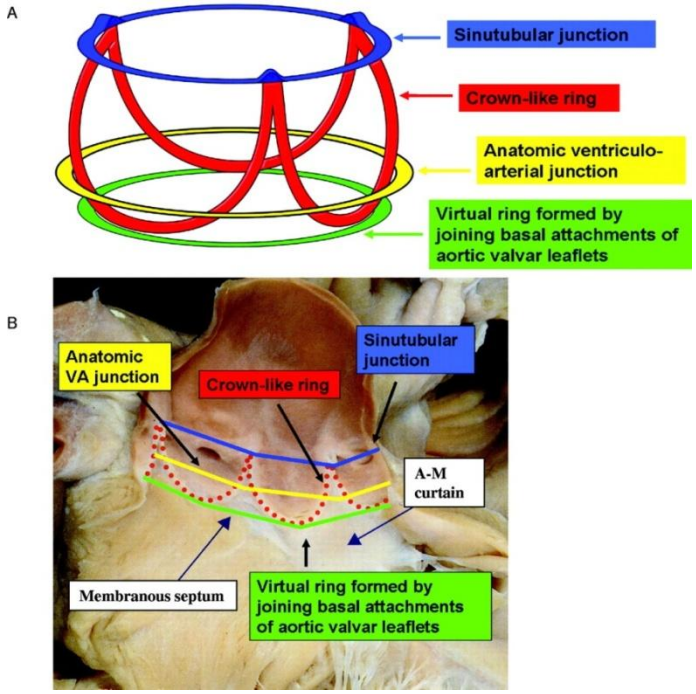


Fig 1: Gross anatomy of aortic valve. Where 'a' is the diagrammatic three-dimensional representation of the various parts of aortic valve and 'b' indicates the gross anatomy of dissected aortic valve.

Semilunar valve formation begins during the fourth week of gestation. At this time, opposing dextrosuperior and sinistroinferior endocardial cushions appear in the cephalad portion of the truncus arteriosus. Simultaneously, 2 additional intercalated endocardial cushions form, each located 90º from the aforementioned dextrosuperior and sinistroinferior endocardial cushions. The dextrosuperior and sinistroinferior cushions fuse and, in doing so, form the truncal septum. The truncal septum undergoes a complex process of differentiation, eventually forming the right and left aortic valve cusps and 2 leaflets of the pulmonic valve. Of the 2 intercalated endocardial cushions, the right cushion eventually forms the posterior aortic valve cusp, whereas the left forms the anterior pulmonic valve leaflet. This occurs during the counterclockwise rotation and caudal shift of the conotruncus. During this time, the endocardial cushions also undergo dedifferentiation from a myosin-heavy chain to an alpha-smooth muscle actin phenotype, resulting in mature arterial valvular leaflets. The improper fusion or the incomplete dedifferentiation of the previously mentioned endocardial cushions is thought to be responsible for the formation of anatomically and structurally congenitally abnormal aortic valves.

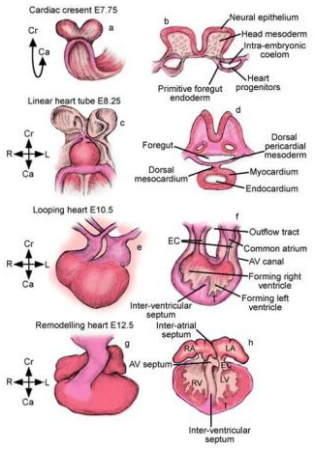


Fig 2: Embryological development of aortic valve

Gross anatomy of Aortic root:

The aortic root is the direct continuation of the left ventricular outflow tract. It is located to the right and posterior, relative to the sub pulmonary infundibulum, with its posterior margin wedged between the orifice of the mitral valve and the muscular ventricular septum extending from the basal attachment of the aortic valvar leaflets within the left ventricle to their peripheral attachment at the level of the Sino tubular junction (Anderson, 2000). Approximately two thirds of the circumference of the lower part of the aortic root is connected to the muscular ventricular septum, with the remaining one third in fibrous continuity with the aortic leaflet of the mitral valve. Its components include the sinuses of Valsalva, the fibrous interleaflet triangles, and the valvar leaflets themselves.

Gross anatomy of Annulus

When defined literally, an “annulus” is no more than a little ring. The aortic valve annulus is a collagenous structure lying at the level of the junction of the aortic valve and the ventricular septum, usually a semilunar crownlike structure demarcated by the hinges of the leaflets. This serves to provide structural support to the aortic valve complex as it attaches to the aortic media distally and the membranous and muscular ventricular septum proximally and anteriorly. The valvar leaflets are attached throughout the length of the root. Seen in 3 dimensions, therefore, the leaflets take the form of a 3-pronged coronet, with the hinges from the supporting ventricular structures forming the crownlike ring (Hamdan et al., 2012). The base of the crown is a virtual ring, formed by joining the basal attachment points of the leaflets within the left ventricle. This plane represents the inlet from the left ventricular outflow tract into the aortic root. The top of the crown is a true ring, the Sino tubular junction, demarcated by the sinus ridge and the related sites of attachment of the peripheral zones of apposition between the aortic valve leaflets. It forms the outlet of the aortic root into the ascending aorta.

Gross anatomy of Fibrous trigones

The larger part of the noncoronary leaflet of the valve, along with part of the left coronary leaflet, is in fibrous continuity with the aortic or anterior leaflet of the mitral valve, with the ends of this area of fibrous continuity being thickened to form the so-called fibrous trigones. These trigones anchor the aortic-mitral valvar unit to the roof of the left ventricle.

The inter-leaflet triangle located between the right coronary and noncoronary aortic leaflets is confluent with the membranous septum (Maganti et al., 2010). Together, the membranous septum and the right fibrous trigone form the central fibrous body of the heart. This is the area within the heart where the membranous septum, the atrioventricular valves, and the aortic valve join in fibrous continuity. The hinge of the septal leaflet of the tricuspid valve separates the membranous septum into its atrioventricular and interventricular components. This relationship is key to understanding the relationship between the aortic valve and the conduction system.

Gross anatomy of Cusps

The normal aortic valve is trifoliate, and their semilunar attachments have already been described. The 3 aortic valve cusps are aptly named for the sinuses that they overlie. The right and left cusps are usually equal in size, with the posterior cusp being slightly larger in two thirds of individuals; however, this has no clinical significance. Each cusp has 2 free edges, both shared with the adjacent cusps (Iskandar and Thompson, 2013). At the center of each free edge is a small fibrous bulge named the nodule of Arantius. These nodules are located at the contact site of valve cusp closure. The rim of each valve cusp is slightly thicker than the cusp body and is known as the lunula. The lunulae of adjacent cusps slightly overlap each other at the time of valve closure, serving a role of increased valve support. The lunula can have fenestrations, most often located adjacent to the commissures; however, these are also not of clinical consequence.

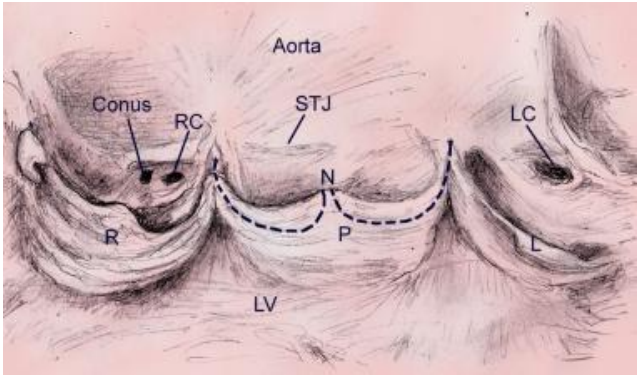


Fig 3: This image shows an opened aortic valve demonstrating the right (R), left (L), and posterior (P) cusps. The dashed line marks the closing edge of the posterior cusp. Two lunular areas, representing the surfaces of apposition between adjacent cusps during valve closure are located between the free and closing edges of each cusp. The commissures (*) attain the level of the aortic Sino tubular junction (STJ). Conus = conus coronary ostium; LC = left coronary ostium; LV = left ventricle; N = nodule of Arantius; RC= right coronary ostium.

Gross anatomy of Commissures

Each cusp is attached to the wall of the aorta by the outward edges of its semicircular border. The level at which this attachment occurs is known as the Sino tubular junction and is the functional level of the aortic valve orifice. A line of demarcation known as the supraaortic ridge identifies the Sino tubular junction. This "ridge" was originally described by Leonardo da Vinci and is essentially thickened aortic wall.

The small spaces between each cusp's attachment point are called the aortic valve commissures. The 3 commissures lie at the apex of the annulus and are equally spaced around the aortic trunk. The commissures are composed of collagenous fibers oriented in a radial fashion that penetrate into the aortic intima and are anchored in the media of the aorta. This microscopic configuration

provides optimal support of valvular structures, with stress on the valve cusps being transmitted into the aortic wall. The commissure between the left and posterior cusp is located at the right posterior aspect of the aortic root, whereas the commissure between the right and noncoronary cusp is located at the right anterior aspect of the aortic root.

1.3. Histology of normal Aortic valve:

Typically, the leaflets are ≤1 mm thick and are comprised of an outer layer of valve endothelial cells (VECs) and 3 internal layers made up of valve interstitial cells (VICs), <5% smooth muscle cells, and myofibroblasts. These layers are known as the fibrosa, spongiosa, and ventricularis to reflect their anatomic location, cellular and extracellular matrix composition, and biomechanical properties (Rajamannan et al., 2011).

The ventricularis, on the ventricular side of the leaflet, is composed of elastin-rich fibers that are aligned in a radial direction, perpendicular to the leaflet margin.

The fibrosa, on the aortic side of the leaflet, comprises primarily fibroblasts and collagen fibers arranged circumferentially, parallel to the leaflet margin.

The spongiosa is a layer of loose connective tissue at the base of the leaflet, between the fibrosa and ventricularis, composed of fibroblasts, mesenchymal cells, and a mucopolysaccharide-rich matrix. These layers work in concert to provide tensile strength and pliability for decades of repetitive motion.

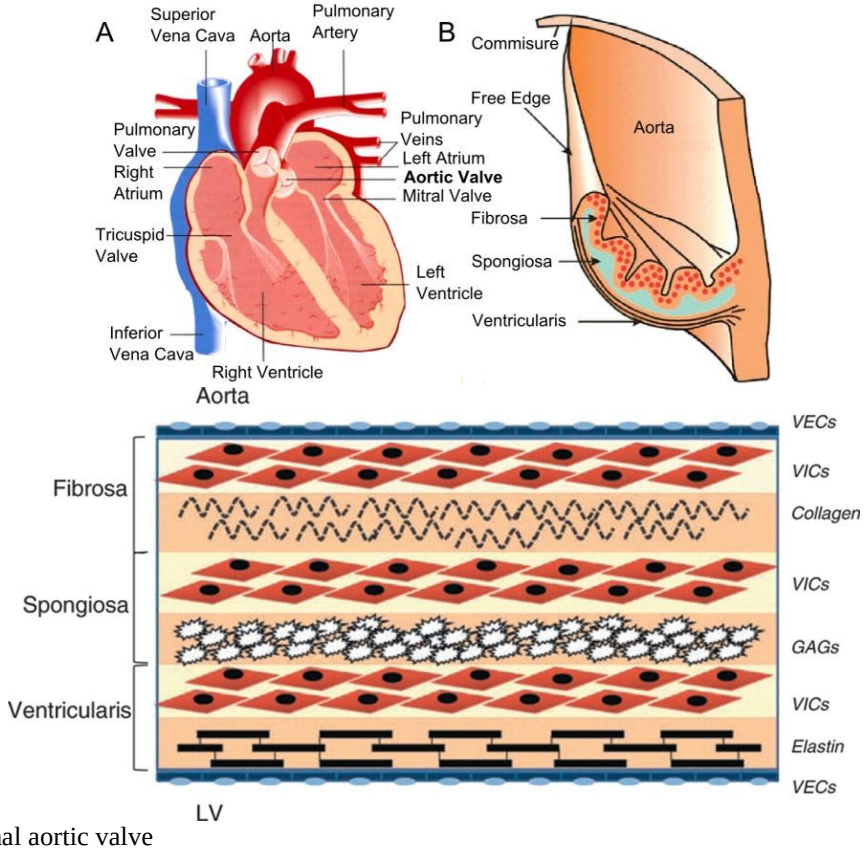


Fig 4: Histology of normal aortic valve

As shown in the fig 3 Valve endothelial cells (VECs) line the outer surface of the valve and function as a barrier to limit inflammatory cell infiltration and lipid accumulation. Valve interstitial cells (VICs) are a heterogeneous and dynamic population of specific cell types that have many unique characteristics. They are responsible for maintaining the extracellular scaffold that provides the mechanical characteristics vital for sustaining the unique dynamic behavior of the valve (Otto et al., 1994). These VICs express molecular markers similar to those of skeletal, cardiac and smooth muscle cells (SMCs) and in particular, many VICs express smooth muscle (SM) α-actin, a marker of myofibroblasts. In this respect, these cells can exhibit a profile unlike skin fibroblasts, which may allude to their role in valve function. Finally, The spongiosa contains glycosaminoglycans (GAGs) that lubricate the fibrosa and ventricularis layers as they shear and deform during the cardiac cycle (Chen and Simmons, 2011).

Cardiac Valve Cell Types:

Valvular Interstitial Cells

VICs are abundant in all layers of the heart valves, and are crucial to function. VICs synthesize VECM and express matrix-degrading enzymes (including matrix metalloproteinases and their inhibitors) that mediate and regulate remodeling of collagen and other matrix components. VICs comprise a diverse, dynamic, and highly plastic population of resident cells. They modulate function among phenotypes in response to changes in stimulation by the mechanical environment or by certain chemicals during valvular homeostasis, adaptation, and pathology. Adult heart valve VICs in situ have characteristics of resting fibroblasts; they are quiescent, without synthetic or destructive activity for extracellular matrix. VICs are activated during intrauterine valvular maturation, by abrupt changes in the mechanical stress state of valves, and in disease states, and VICs continuously repair a low level of injury to the VECM that occurs during physiological functional remodeling of AV tissue. Once activated, VICs can differentiate into a variety of other cell types,3 including myofibroblasts and osteoblasts, although valve osteoblasts may respond to cellular signals differently than skeletal osteoblasts.

Valvular Endothelial Cells

VECs resemble endothelial cells elsewhere in the circulation in some respects. However, they are phenotypically different from VECs in the adjacent aorta and elsewhere in the circulation.8 VECs probably interact with VICs to maintain the integrity of valve tissues and potentially mediate disease. Evidence indicates that different transcriptional profiles are expressed by VECs on the opposite (ie, aortic and ventricular) faces of a normal adult pig AV, and some investigators have hypothesized that these differences may contribute to the typical localization of early pathological AV calcification, predominantly near the outflow surface secondary to inhibitors along the inflow surface. Studies indicate that abnormal hemodynamic forces (such as hypertension, elevated stretch, or shear stresses) experienced by the valve leaflets can cause tissue remodeling and inflammation, which may lead to calcification, stenosis, and ultimate valve failure.

1.4. Pathophysiologic Variants of Aortic Valve:

Unicuspid aortic valve:

Unicuspid aortic valve is a congenital valvular defect with an incidence of 0.02% in the general population. It is commonly associated with clinically significant aortic stenosis, usually manifesting during the third decade of life. All valves are unicommissural with the posterior commissural attachment. The free edge of the valve extends from the single commissure without further communication with the aorta. An estimated 50% of individuals with unicuspid aortic valve have associated ascending aortic dilatation. This is a rare cardiac anomaly but should be suspected in patients presenting at a young age with clinically significant aortic stenosis (Maganti et al., 2010), (Novaro et al., 2003).

Bicuspid aortic valve:

Bicuspid aortic valve is the most common congenital cardiac anomaly, occurring in 1-2% of the population, with a 2:1 male predominance. Evidence exists of familial clustering, with the incidence as high as 10% in some families. Bicuspid aortic valve may be clinically silent but can lead to early development of aortic stenosis or aortic insufficiency, most commonly in the fifth and sixth decades of life. Conditions associated with bicuspid aortic valve include patent ductus arteriosus, Williams syndrome, Turner syndrome, and coarctation of the aorta. Of clinical importance is the association of aortic root dilatation and ascending aortic aneurysm (Warnes, 2003) .

Quadricuspid aortic valve

Quadricuspid aortic valve (QAV), first described in 1862 by Balington, is a rare congenital valvular abnormality that affects both the pulmonic and aortic valves in a 10:1 ratio. The incidence of QAV is estimated at 0.0125–0.033% in the general population. It most commonly occurs as an isolated defect but has been associated with patent ductus arteriosus, Ehlers-Danlos syndrome, hypertrophic obstructive cardiomyopathy, and subaortic stenosis. Aortic valvular insufficiency is commonly observed in QAV. It occurs secondary to a central orifice formed from malcoaptation of the 4 valvular leaflets. In a small case series, 56% of subjects with QAV had significant valvular insufficiency, with a mean age at presentation of 46 years (Holt et al., 2007).

Lambl's excrescences:

Lambl's excrescences are fine filamentous lesions of valvular leaflets. The incidence increases with age, and it is considered as a degenerative change on the surface of leaflets due to mechanical wear and tear. Aortic valve is commonly involved. Fine strands have acellular connective tissue cores with some elastic fibers. Multiple adjacent excrescences may stick together and grow up to large, complex form called "giant Lambl's excrescence." Whether the excrescences may serve as a nidus for bacterial growth or cause a systemic embolism is controversial. In echocardiography, it appears as very thin, delicate, lintlike mobile threads arising from the free borders or ventricular surfaces of aortic leaflets. It may be multiple and several centimeters long. Improving image quality increases identification of this lesion. The echocardiographic significance of Lambl's excrescences lies in the differential diagnosis from the vegetation of infective endocarditis (Aziz and Baciewicz, 2007).

Papillary fibroelastoma:

Papillary fibroelastoma is a benign avascular tumor arising from the normal endocardium. It can occur anywhere in the heart, but most frequently arises from valvular endocardium. Most papillary fibroelastomas are found in elderly; it may be a hamartoma that develops in a degenerative wear-and-tear process. Characteristic numerous gelatinous papillary fronds of tumor surface consist of dense connective tissue core covered by endothelium. On echocardiography, a small mobile tumor with fine frond-like surface attaches to the downstream side of the valve by a small stalk. Surgical resection is needed because it may cause a systemic embolism (Holt et al., 2007) (Klarich et al., 1997).

Calcification of aortic valve:

Aortic valve calcification is a condition in which calcium deposits form on the aortic valve in the heart. These deposits can cause narrowing at the opening of the aortic valve. This narrowing can become severe enough to reduce blood flow through the aortic valve, a condition called aortic valve stenosis.

2. Definition of Calcified Aortic Valve Disease:

Calcific aortic valve disease is a slowly progressive disorder with a disease continuum that ranges from mild valve thickening without obstruction of blood flow, termed aortic sclerosis, to severe calcification with impaired leaflet motion, or aortic stenosis. In the past, this process was thought to be “degenerative” because of time-dependent wear-and-tear of the leaflets with passive calcium deposition. Now, there is compelling histopathologic and clinical data suggesting that calcific aortic valve disease is an active disease process akin to atherosclerosis with lipoprotein deposition, chronic inflammation, and active leaflet calcification. The overlap in the clinical factors associated with calcific aortic valve disease and atherosclerosis and the correlation between the severity of coronary artery and aortic valve calcification provide further support for a shared disease process.

3. Symptoms associated with CAVD:

Angina pectoris in patients with aortic stenosis is typically precipitated by exertion and relieved by rest. Thus, it may resemble the angina of heart disease. Heart failure symptoms (ie, paroxysmal nocturnal dyspnea, orthopnea, dyspnea on exertion, and shortness of breath) may be due to systolic dysfunction from afterload mismatch, ischemia, or a separate cardiomyopathic process. Alternatively, diastolic dysfunction from LV hypertrophy or ischemia may also result in heart failure symptoms. Syncope from

aortic stenosis often occurs upon exertion when systemic vasodilatation in the presence of a fixed forward stroke volume causes the arterial systolic blood pressure to decline. It also may be caused by atrial or ventricular tachyarrhythmias. Syncope at rest may be due to transient ventricular tachycardia, atrial fibrillation, or (if calcification of the valve extends into the conduction system) atrioventricular block. Another cause of syncope is abnormal vasodepressor reflexes due to increased LV intracavitary pressure (vasodepressor syncope). Syncope may be accompanied by convulsions (Rodrigues et al., 2010). Patients with aortic stenosis may have a higher incidence of nitroglycerin-induced syncope than does the general population. Always consider aortic stenosis as a possible etiology for a patient who demonstrates particular hemodynamic sensitivity to nitrates.

Gastrointestinal bleeding due to angiodysplasia (ie, Heyde syndrome) or other vascular malformations is present at a higher-than-expected frequency in patients with calcific aortic stenosis. These malformations usually resolve following aortic valve surgery. Patients may present with manifestations of infective endocarditis (ie, fever, fatigue, anorexia, back pain, and weight loss). The risk of infective endocarditis is higher in younger patients with mild valvular deformity than in older patients with degenerated calcified aortic valves, but it can occur in either population. It can occur in patients of any age with hospital-acquired Staphylococcus aureus bacteremia. Patients with bicuspid valve have an increased incidence of aortic root dilatation (25-40% of patients) and aortic dissection. Calcific aortic valve disease rarely may cause emboli of calcium to various organs, including the heart, kidney, and brain.

4. Physical Examination of CAVD:

In severe aortic stenosis, the carotid arterial pulse typically has a delayed and plateaued peak, decreased amplitude, and gradual down slope (pulsus parvus et tardus). However, in elderly individuals with rigid carotid vessels, this sign may not be present. A lag time may be present between the apical impulse and the carotid impulse. Systolic hypertension can coexist with aortic stenosis. However, a systolic blood pressure higher than 200 mm Hg is rare in patients with critical aortic stenosis.

Pulsus alternans can occur in the presence of LV systolic dysfunction. The jugular venous pulse may show prominent a waves reflecting reduced right ventricular compliance consequent to hypertrophy of the interventricular septum. A hyperdynamic LV is unusual and suggests concomitant aortic regurgitation or mitral regurgitation. S1 is usually normal or soft. The aortic component of the second heart sound, A2, is usually diminished or absent, because the aortic valve is calcified and immobile and/or the aortic ejection is prolonged and it is obscured by the prolonged systolic ejection murmur. The presence of a normal or accentuated A2 speaks against the presence of severe aortic stenosis.

Paradoxical splitting of the S2 also occurs, resulting from late closure of the aortic valve with delayed A2. P2 may also be accentuated in the presence of secondary pulmonary hypertension. An ejection click is common in children and young adults with congenital aortic stenosis and mobile valve leaflets, but it is rare in elderly individuals with acquired calcific aortic stenosis, in whom the cusps become immobile and severely calcified. This sound occurs approximately 40-60 milliseconds after the onset of S1 and is frequently heard best along the mid to lower left sternal border; it is often well transmitted to the apex and may be confused with a split S1. A prominent S4 can be present and is due to forceful atrial contraction into a hypertrophied left ventricle. The presence of an S4 in a young patient with aortic stenosis indicates significant aortic stenosis, but with aortic stenosis in an elderly person, this is not necessarily true.

The classic crescendo-decrescendo systolic murmur of aortic stenosis begins shortly after the first heart sound. The intensity increases toward midsystole, then decreases, and the murmur ends just before the second heart sound. It is generally a rough, low-pitched sound that is best heard at the second intercostal space in the right upper sternal border. It is harsh at the base and radiates to 1 or both carotid arteries. In elderly persons with calcific aortic stenosis, however, the murmur may be more prominent at the apex, because of radiation of its high-frequency components (Gallavardin phenomenon). This may lead to its misinterpretation as a murmur of mitral regurgitation. Accentuation of the aortic stenosis murmur following a long R-R interval (as in atrial fibrillation or following a premature beat) distinguishes it from the mitral regurgitation murmur, which usually does not change.

The intensity of the systolic murmur does not correspond to the severity of aortic stenosis; rather, the timing of the peak and the duration of the murmur corresponds to the severity of aortic stenosis. The more severe the stenosis, the longer the duration of the murmur and the more likely it peaks at late systole. The murmur of valvular aortic stenosis is augmented upon squatting or following a premature beat; the murmur intensity is reduced during Valsalva strain. This is contrary to what occurs with hypertrophic obstructive cardiomyopathy, in which a Valsalva maneuver increases the intensity of the murmur.

When the left ventricle fails and cardiac output falls, the aortic stenosis murmur becomes softer and may be barely audible. Atrial fibrillation with short R-R intervals can also decrease the murmur intensity or make it inaudible. A high-pitched, diastolic blowing murmur may be present if the patient has associated aortic regurgitation. Rarely, right ventricular failure with systemic venous congestion, hepatomegaly, and edema precedes LV failure. This is probably due to the bulging of the interventricular septum into the right ventricle, with impedance in filling, elevated jugular venous pressure, and a prominent venous "a" wave (Bernheim effect).

5. Imaging techniques used for analysis of CAVD:

5.1. Echocardiographic Imaging for CAVD:

Because of its ability to detect and quantify valve-related hemodynamic obstruction, echocardiography long has been recognized as a useful clinical tool for monitoring aortic stenosis (AS), the later, obstructive stage of CAVD. Echocardiography can reliably visualize aortic valve anatomy, although once severe calcification is present, distinguishing a bicuspid from a trileaflet valve can be difficult. Echocardiographic measures of AS severity have been well validated in numerous studies and now are the clinical standard for patient management (Miller et al., 1999). Guidelines recommend measurement of aortic jet velocity, mean pressure gradient, and continuity equation valve area. Although clinically robust, these measures are subject to several sources of error, including physiological changes, recording technique, and measurement variability. In addition, there is marked variability between patients in the rate of hemodynamic progression and the degree of stenosis that results in clinical symptoms. Aortic sclerosis is defined on echocardiography as focal areas of leaflet thickening without significant obstruction to left ventricular outflow, with an aortic velocity <2.6 m/s (Nightingale and Horowitz, 2005). Echocardiographic measures of aortic jet velocity and leaflet

calcification have been shown to be robust predictors of clinical outcome. In the design of clinical trials, we will need to consider the effects of the variability of echocardiographic data on sample-size calculations and define the imaging standards and protocols for the use of this tool to quantify noninvasively the level of disease in the patients.

5.2. Computed Tomography in Quantifying Calcification in CAVD

The early stage of CAVD, aortic sclerosis, is characterized by aortic valve calcium (AVC) accumulation, but not hemodynamic obstruction. Because echocardiography does not have the resolution for quantifying AVC, it is less useful for monitoring early-stage CAVD. In contrast, computed tomography (CT) is a relatively sensitive and precise tool for quantifying AVC. Thus, CT has emerged as a useful tool for studying aortic sclerosis, complementing the utility of echocardiography in studying AS. Moreover, because the aortic valve leaflets lie in the same anatomic plane as the coronary arteries, AVC can be quantified by any CT scan obtained for the purpose of quantifying coronary artery calcium (Cartlidge et al., 2016). Taking advantage of these issues, investigators have used CT to study traditional and novel risk associations for AVC in the Multiethnic Study of Atherosclerosis (MESA), a 6780-participant study of risk factors for subclinical coronary artery disease. In MESA, the metabolic syndrome is a strong risk factor for prevalent and early-stage disease. Thus, metabolic syndrome appears to be an adverse risk factor in all stages of CAVD.

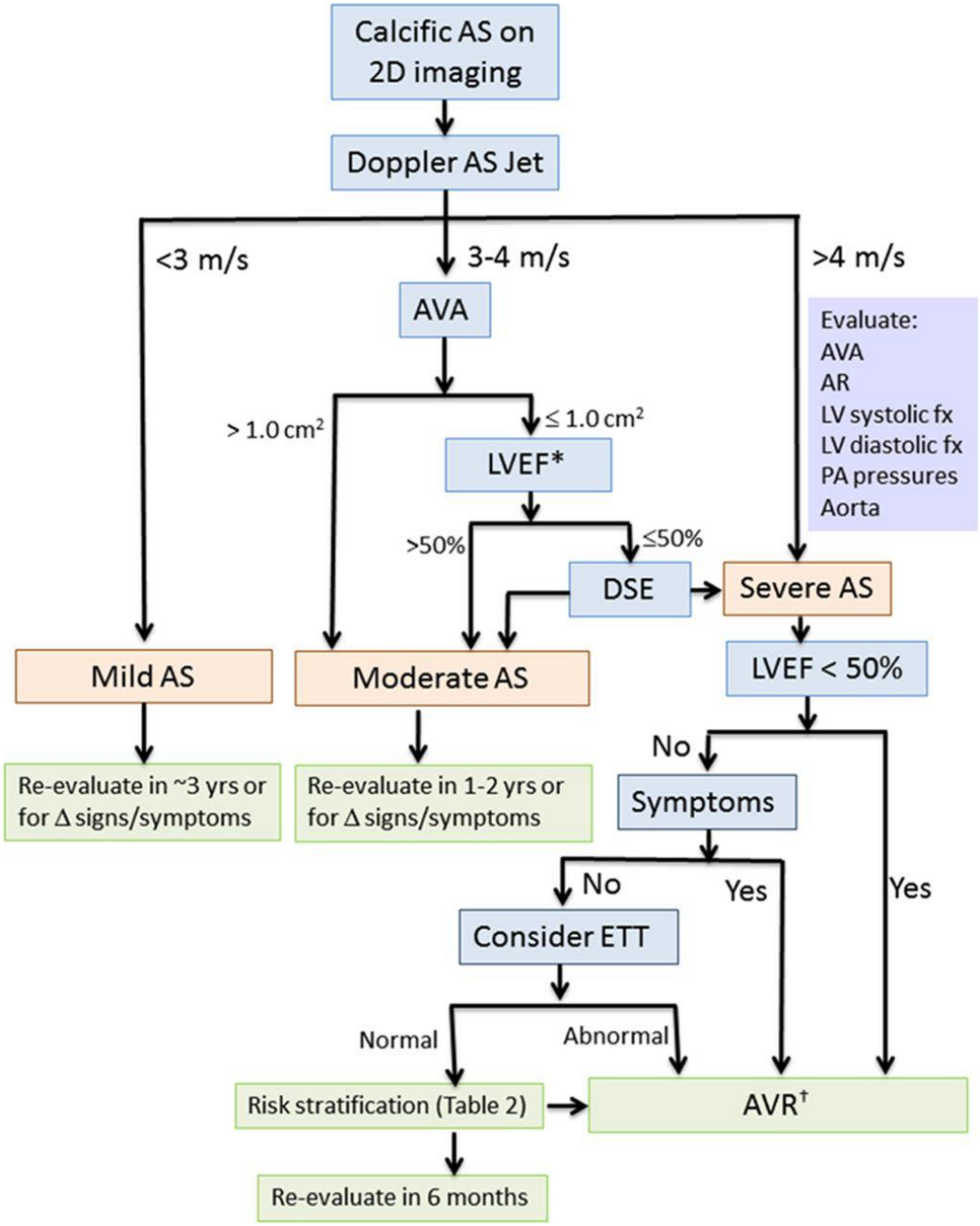


Fig: Flow chat for systematic diagnosis of CAVD by 2D Echocardiography analysis

6. Prevalence, Genetics, and Cardiovascular Risk Factors associated with CAVD

Calcific aortic valve disease (CAVD) affects 25% of people over 65, and the late-stage stenotic state can only be treated with total valve replacement, requiring 85,000 surgeries annually in the US alone. The presence or progression of CAVD has been associated with several clinical, genetic, and anatomic factors. Bicuspid AV valve disease is the most common congenital heart abnormality. A congenitally bicuspid AV is present in >50% of adults undergoing valve replacement for severe CAVD, and nearly all patients with a bicuspid valve will eventually need valve surgery, either for regurgitation in young adulthood or for stenosis in the fifth or

sixth decade of life. Bicuspid valve disease appears to be inherited in an autosomal dominant pattern in some families, and a mutation in the NOTCH1 gene segregates with both bicuspid valve anatomy and premature valve calcification and complex congenital heart defects. Calcification of trileaflet AVs also may be affected by genetic factors, based on population studies and case-control comparisons for specific polymorphisms, including the Vitamin D receptor, estrogen receptor, apolipoprotein E4, and interleukin 10 alleles. Mild CAVD, called aortic sclerosis, is present in ≈25% of adults >65 years of age, and is associated with adverse cardiovascular outcomes with about a 50% increased risk of cardiovascular events over 5 years. Similar to the cardiovascular risk factors defined by the Framingham study for vascular atherosclerosis, clinical factors associated with the presence of CAVD in the Cardiovascular Health Study included older age, male sex, serum lipoprotein(a) and LDL levels, height, hypertension, metabolic syndrome, and smoking. The association with elevated LDL is relatively weak in those >65 years old, the group at greatest risk of progressing to aortic stenosis.

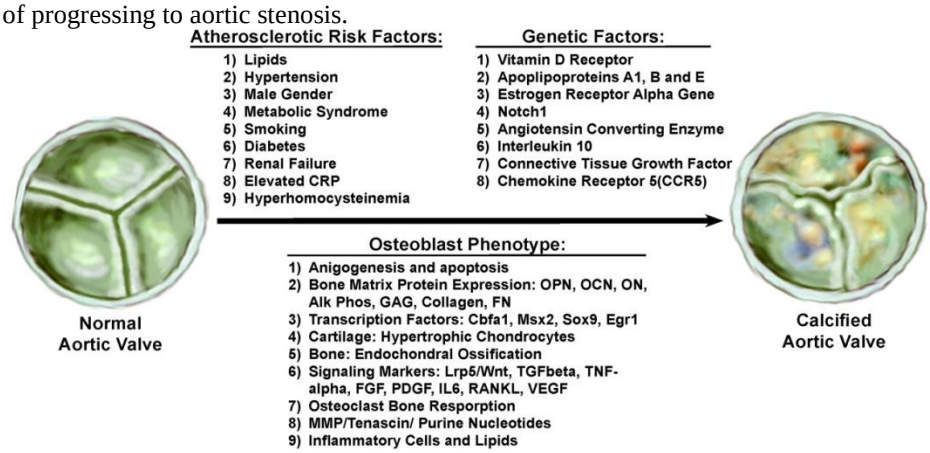
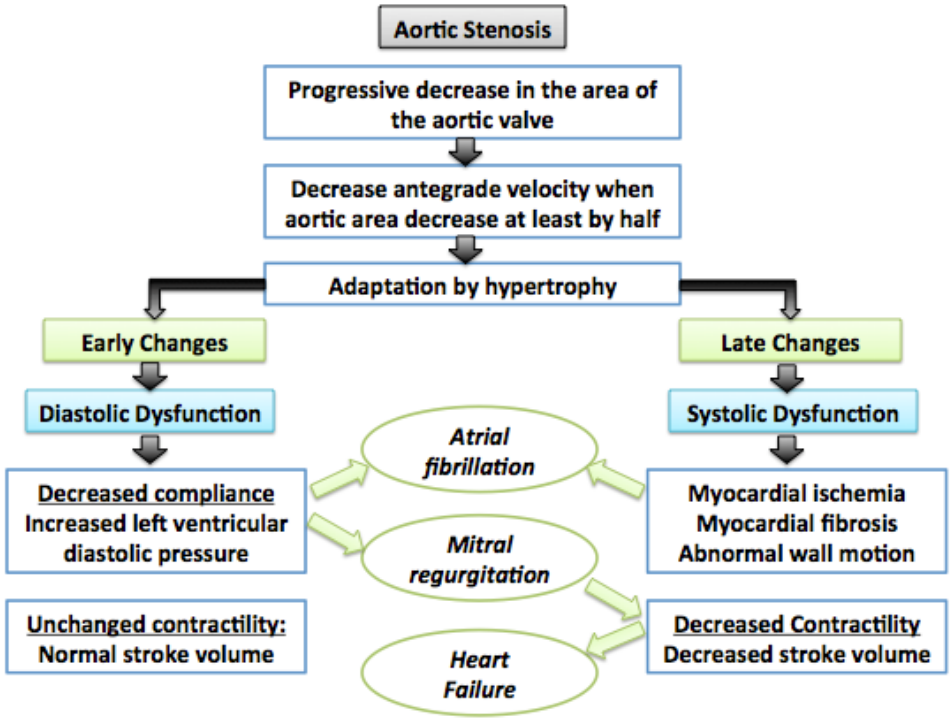


Fig: Risk Factors associated with CAVD.



7. Cellular Mechanisms of CAVD

7.1. Major Mechanisms:

In Situ Transition of VICs to Osteoblast-Like Bone-Forming Cells:

One mechanism implicated in the pathogenesis of CAVD posits that normally quiescent VICs become activated and undergo a phenotype transition to become osteoblast-like bone-forming cells. These activated VICs are responsive to typical osteogenic mediators such as BMPs. BMPs are members of the transforming growth factor-β superfamily, stimulate osteoblasts to initiate skeletal bone formation, and have been implicated in vascular calcification (reviewed in Bostrom et at).

It is likely that BMPs play an important role in the pathogenesis of CAVD . In experimental models, activated endothelial cells have been shown to secrete BMP-2 and BMP-4 in response to changes in laminar flow patterns and BMP-2 has been detected in VICs isolated from the aortic valve of aged rats BMPs stimulate calcification by activating Smad and Wnt/β-catenin signaling as well as upregulating expression of the osteochondrogenic transcription factor Msx2. These signaling pathways converge to induce expression of the master osteoblast transcription factor Runx2. Once Runx2 is expressed, cells are committed to an osteoblast lineage, upregulate expression of calcification-related proteins, including osteopontin, bone sialoprotein II, and osteocalcin, and undergo calcification.

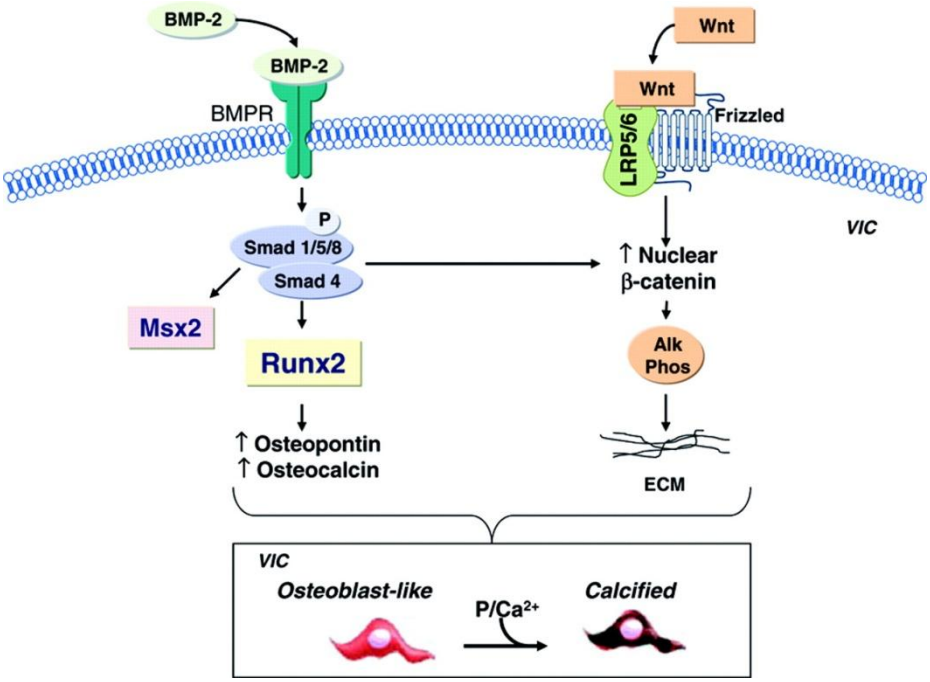


Fig: Bone morphogenetic protein and Wnt signaling in valve interstitial cells (VICs).

Bone morphogenetic proteins (BMPs) bind to the bone morphogenetic protein receptor (BMPR) to phosphorylate (P) and activate Smad signaling. Smad signaling increases transcription of the osteoblast transcription factor Runx2, which leads to upregulation of Runx2-dependent calcification proteins. Smad signaling also increases expression of Msx2 and participates in β-catenin-mediated gene transcription. BMPs also promote Wnt signaling. Wnt ligands bind to receptor complexes of frizzled protein/lipoprotein receptor-related protein (LRP) 5 or 6 to activate β-catenin signaling and upregulate expression of alkaline phosphatase. Together these signaling pathways promote transition of VICs to osteoblast-like cells that are able to calcify in the presence of phosphate and calcium. There is evidence to indicate that BMP signaling is activated in calcified valves. Smad 1/5/8 and Runx2 have been detected in calcified human aortic valves and levels of these markers have been shown to increase before there is evidence of valve leaflet calcification. These findings correlate well with the observation that there is a significant increase in the Runx2-dependent calcification-related proteins osteopontin (7.4-fold) and bone sialoprotein II (5.8-fold) in calcified human valves.

BMPs also activate the Wnt/β-catenin signaling pathway to increase the expression of alkaline phosphatase, which is also necessary to facilitate calcification. Wnt proteins belong to a family of secreted lipid-modified polypeptide ligands that bind to receptor complexes of frizzled protein/lipoprotein receptor-related protein 5 or 6 leading to an accumulation of β-catenin in the nucleus. Activation of this signaling pathway in experimental models of CAVD and explanted human valves has been confirmed by demonstrating the expression of the Wnt ligand Wnt3a, the coreceptor lipoprotein receptor-related protein 5, and nuclear β-catenin in calcified valve tissue.

BMPs also increase the expression of the osteochondrogenic transcription factor Msx2 that is important for intramembranous bone formation. Msx2 has been identified in valves from experimental models of CAVD where it is localized to areas of calcification. Because Msx2-positive cells secrete Wnt ligands such as Wnt3a, these cells may upregulate Runx2 expression in adjacent VICs. Downstream of the BMP-Smad and BMP-Wnt/β-catenin signaling pathways are the transcription factors osterix and NFATc1. These transcription factors have also been shown to be necessary for bone formation and have been detected in activated VICs and inflammatory cells in calcified human aortic valves. Taken together, these studies indicate that BMPs are capable of driving VICs to an osteoblast-like phenotype in which these cells express all of the markers of functional osteoblasts, elaborate bone matrix proteins, and mineralize to form calcific nodules typical of CAVD.

Endothelial-to-Mesenchymal Transition

The primary function of VECs is to maintain valve homeostasis; however, valve endothelium may also undergo differentiation to osteoblast-like cells as a result of endothelial-to-mesenchymal-transition (EnMT). During this process, VECs lose their endothelial cell properties, no longer express endothelial-specific markers such as vascular endothelial cadherin, acquire phenotypic characteristics of mesenchymal cells or myofibroblasts, and express α-smooth muscle actin, Type I collagen, and vimentin. These "transformed" VECs also exhibit increased motility and may migrate into surrounding tissues. This phenomenon may be stimulated by transforming growth factor-β, Endothelial Growth factor, JAG1-NOTCH signaling, BMP 7 mediated SMAD signaling. All of which are present in calcifying aortic valves. Once EnMT occurs, VECs may participate in pathological fibrosis of the valve and/or undergo osteogenic differentiation through the same mechanisms as VICs exposed to BMPs, calcify, and contribute to CAVD.

Although EnMT is known to play a prominent role in the endocardial cushion during valve formation, much of the data to implicate EnMT in valve calcification have been derived from preclinical studies of the mitral valve. Experimental models using ovine mitral VEC clones have found that select clones underwent EnMT when stimulated with transforming growth factor-β and that these cells were capable of osteogenic and chondrogenic differentiation. Similarly, evidence of EnMT and bone-related proteins was observed in mitral leaflets after high levels of mechanical stretch. Further studies are required to determine if this mechanism of calcification is operative in human CAVD.

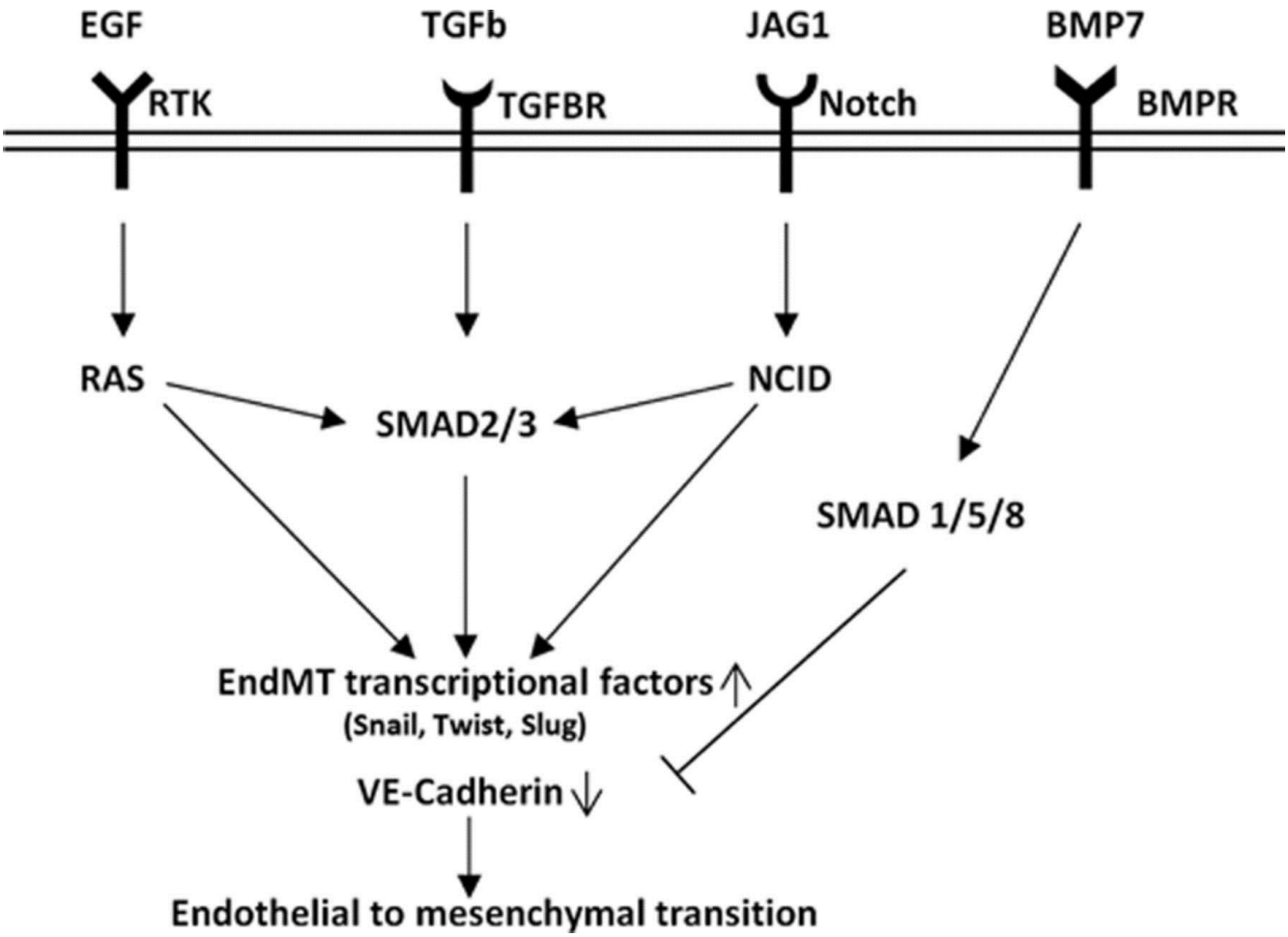


Fig: Mechanism of Endothelial to mesenchymal transition

Inflammation and Immune Response

Similar to atherosclerosis, CAVD may also be considered an immune-inflammatory disease process. This is supported by the observation that explanted normal human aortic valves contain relatively few macrophages whereas there is abundant leukocyte and macrophage infiltration seen in explanted calcified human aortic valves. Valve macrophage and inflammatory cell infiltration is typically seen at sites where VECs are activated and express adhesion molecules that facilitate the recruitment and trans endothelial migration of monocytes and macrophages into the valve. Using molecular imaging, macrophage infiltration was identified as an early event in the development of CAVD in atherosclerosis-prone mouse models and macrophage burden was shown to correlate with the degree of valve calcification. In explanted human congenital bicuspid aortic valves, which are predisposed to early calcification, the density of infiltrating valve macrophages was greater than that observed for tricuspid valves. These areas of inflammatory cell infiltration were associated with the expression of proinflammatory cytokines that have been implicated in CAVD, including tumor necrosis factor-α, interleukin-1β, and receptor activator of nuclear factor κ-B ligand. Once present, macrophages also release matrix metalloproteases and cysteine endoproteases that lead to degradation of collagen and elastin in the valve matrix to disrupt the normal valve architecture.

There is increasing evidence to suggest a role for immune modulatory cells in CAVD as well. Calcified aortic valves have been shown to contain expanded populations of T cell clones that differed from peripheral CD8 or CD4 T cell subsets. A number of the T cell clones were of CD8 lineage (cytotoxic T cells or natural killer cells), suggesting that these T cells likely participated in the pathogenesis of CAVD and were not merely a secondary response. Other studies using flow cytometry similarly identified increased levels of activated CD8 cells and memory effector cells in the peripheral blood of patients with calcified valves, indicating a systemic adaptive immune response may be associated with CAVD. Whether this adaptive immune response modulates other cellular processes involved in CAVD has not yet been determined.

Stem and Progenitor Cells

In addition to VECs and VICs, bone marrow-derived cells have been shown to populate normal aortic valves and it has been suggested that these progenitor cells may participate in CAVD. For example, mesenchymal progenitor cells, which have been identified in porcine aortic valves, have the capacity to undergo differentiation to osteoblast-like cells. Using clonal analyses, a high frequency of these progenitor cells were shown to possess the capacity for self-renewal, undergo osteogenic differentiation, and elaborate bone matrix in the valve. Other studies have determined that the local environment drives osteogenic differentiation of mesenchymal progenitor cells. When these cells were cocultured with explanted calcified valves, they differentiated toward an osteoblastic lineage. Thus, under conditions that favor calcification, subpopulations of mesenchymal progenitors within the valve are capable of contributing to valve calcification.

Endothelial progenitor cells have also been identified in diseased aortic valves. It is now believed that at sites of endothelial injury, the function of endothelial progenitor cells is to participate in the repair process by transiently establishing residence and secreting factors that facilitate proliferation and migration of resident endothelial cells. In CAVD, there is evidence to demonstrate that endothelial progenitor cells are dysfunctional and the repair process is ineffective. Individuals with calcified aortic valves were found to have a significant reduction in endothelial progenitor cell number and ex vivo functional capacity as compared with control subjects. Moreover, endothelial progenitor cells isolated from patients with CAVD had increased proapoptotic caspase-3 activity and decreased telomere-repeating factor-2 expression indicating progenitor cell senescence. Thus, the combined effect of mesenchymal progenitor cell transition to osteoblast-like cells and endothelial progenitor cell dysfunction represents another cellular mechanism to promote CAVD.

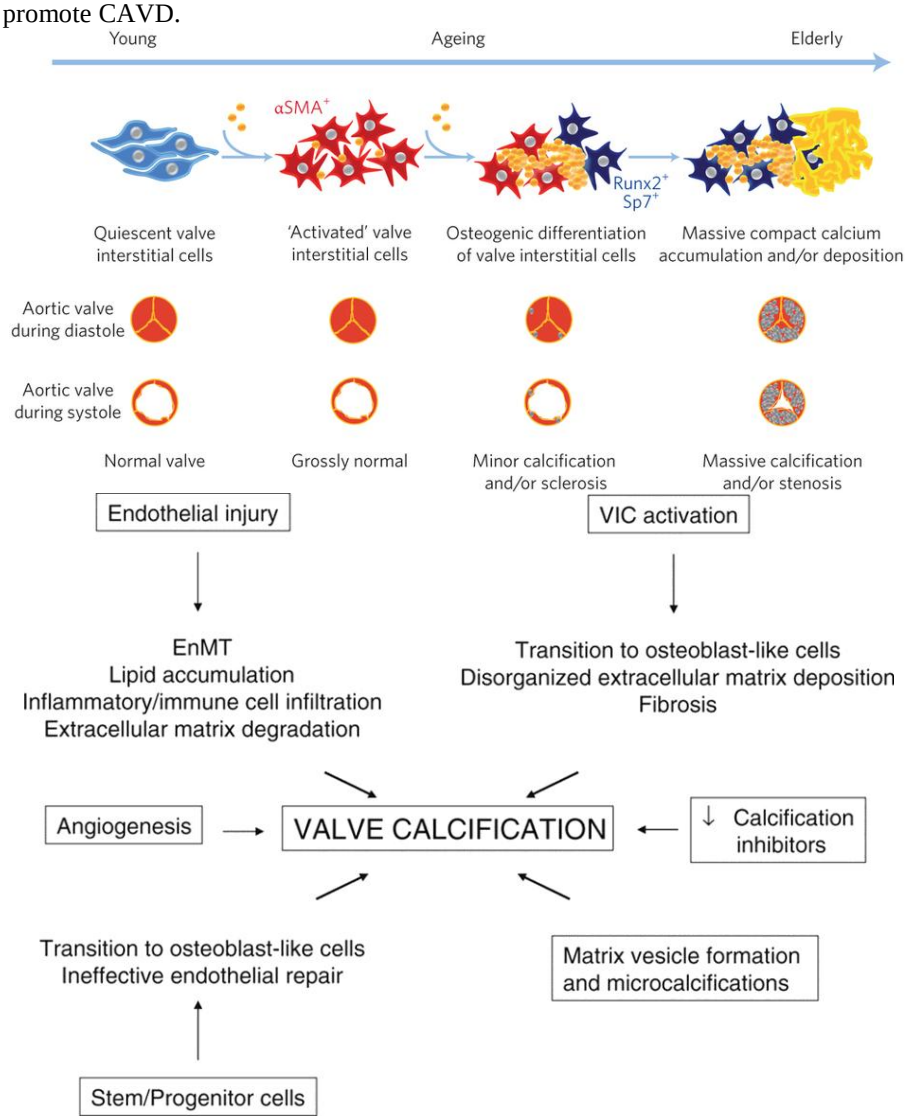


Fig: Summary of the molecular Mechanisms behind calcific aortic valve disease.

7.2. Other Processes Associated With AVC

Matrix Vesicle Formation and Microcalcifications

AVC has also been associated with the formation and release of matrix vesicles that serve as a nidus for calcium deposition. Matrix vesicles are important for bone and cartilage mineralization and have been implicated in ectopic vascular calcification. In the presence of high levels of calcium and/or phosphate, smooth muscle cells have been shown to release vesicles of 100 to 300 nm that are derived from the plasma membrane. These vesicles may be retained by the cells and attract calcium leading to areas of microcalcification. Although these vesicles have been shown to contain the calcification inhibitors MGP, fetuin-A, and osteoprotegerin, it is likely that encapsulation of these inhibitors in the vesicles serves as a mechanism to sequester them and render them inactive. Microcalcifications have been shown to occur under inflammatory conditions suggesting that inflammation plays a role in their genesis.

Other studies using electron microscopy have suggested that some of the 10 to 500 nm size particles present in calcified valves are actually derived from a type of bacteria referred to as nanobacteria. These particles stain positive for calcium–phosphate in a heterogeneous pattern, contain DNA, and appear to contain cell walls indicating a bacterial origin. Nanobacteria particles have been isolated from calcified aortic valves and one study found that 48 of 75 explanted calcified human valves contained calcifying nanoparticles consistent with nanobacteria. Despite these findings, the existence of nanobacteria has been challenged and it has been suggested that nanobacteria are actually calcium–phosphate nanoparticles containing fetuin-A, albumin, or apolipoproteins and bind ions, nucleic acids, lipids, and carbohydrates. These calcifying nanoparticles nucleate, crystalize, and ultimately increase in size and become insoluble to function as a nidus for matrix calcification.

Microcalcifications have also been shown to occur at sites of cell death and some amorphous calcium deposits isolated from calcified valves have a crystalline ultrastructure and do not contain live cells. This may result from either cell necrosis or apoptosis. In this manner, the cytoskeletal remains of apoptotic or necrotic cells may allow for calcium deposition, nodule formation, and expansion although the relative contribution of this process to AVC is not yet well understood. Importantly, valve calcification through these mechanisms (vesicle formation, nanobacteria-derived microparticles, and acellular calcium deposition) or through transition of valve cells to an osteoblast-like phenotype is not a mutually exclusive process and it is plausible that these osteoblast-like cells may actively generate vesicles or undergo apoptosis to accumulate calcium and form microcalcifications.

Extracellular Matrix Remodeling

One of the hallmarks of AVC is abnormal extracellular matrix remodeling. This occurs as a result of increased expression of matrix metalloproteinases-1, -2, -3, and -9 and cathepsins (S, K, V, and G) by inflammatory cells and VICs (reviewed in Chen and Simmons). Once activated, matrix metalloproteases and cathepsins degrade collagen and elastin to form proinflammatory peptides; this process compromises valve integrity, augments the inflammatory response, and allows for the expansion of calcified nodules.

In addition to extracellular matrix degradation, there is also evidence of aberrant matrix deposition and valve fibrosis, which is extensive in AVC and contributes to the calcification of valves. Activated VICs secrete collagen, hyaluronan, and other extracellular matrix components; however, the deposition of these matrix proteins is often disorganized and alters the biomechanical properties of the valve. These changes in valve stiffness have also been shown to modulate the transition of VICs to osteoblast-like cells.

The profibrotic signaling molecule transforming growth factor-β is increased in experimental models of AVC and human valve tissue consistent with the degree of fibrosis seen in AVC. Similar to what has been observed during myocardial repair processes in which there is a return to the fetal gene program, expression of Twist1, which is important for endocardial cushion development and valve remodeling, has been identified in AVC in areas adjacent to calcium nodules. When overexpressed in mouse models, Twist1 leads to increased valve hypercellularity and cusp fibrosis. There is also evidence of increased thrombospondin-2, a matricellular protein that regulates extracellular matrix remodeling and fibrosis in human fibrocalcific aortic valve disease as well as the profibrotic enzyme neutral endopeptidase.

Angiogenesis and Neovascularization

Another feature of AVC is angiogenesis and neovascularization of the valve. Healthy human aortic valves are avascular structures; however, in calcified valves, there is evidence of angiogenesis near calcified nodules, under the leaflet border, and in areas infiltrated with inflammatory cells. In fact, calcified valves have been shown to contain small- and medium-sized microvessels as well as organized arterioles. Histological analysis of calcified aortic valves has identified a subpopulation of cells that express the endothelial markers Tie-2 and vascular endothelial growth factor receptor 2 as well as smooth muscle α-actin. These cells migrate and form capillary-like tubes in vitro and may represent a population of either activated VECs or VECs that have undergone EnMT.

Active calcification is prominent early in the disease process and is a major factor in the leaflet stiffness of severe stenosis. With aortic sclerosis, microscopic areas of calcification colocalize in areas of lipoprotein accumulation and inflammatory cell infiltration. Oxidized LDL stimulates valvular fibroblasts to release matrix vesicles, a nidus for early calcification. It has been shown that macrophages express osteopontin, a protein needed in bone formation, with the degree of mRNA expression of osteopontin corresponding to the degree and location of valvular calcification. A subset of valvular myofibroblasts are an osteoblast phenotype and have been associated with development of calcific nodules.18,19 An increased rate of calcific nodule formation by these myofibroblasts has been shown in vitro by exposure to oxidized lipids and transforming growth factor-β1.

As the disease progresses, active bone formation is seen. In an evaluation of 347 human aortic valves removed for aortic valve replacement, the majority (83%) had evidence of dystrophic calcification, and up to 13% contained lamellar or endochondral bone tissue with hematopoietic marrow and evidence of remodeling. Within the specimens that contained bone tissue, there was expression of factors that promote osteogenesis, including bone morphogenic protein-2 and -4. The importance of tissue calcification in the disease process is highlighted by the observation that subsets of patients with altered mineral metabolism have a higher prevalence of calcific aortic valve disease and more rapid disease progression. Anecdotally, it has been observed that in patients with osteoporosis or increased bone demineralization, the prevalence of any valvular calcification is higher, possibly related to increased body mineral turnover or ectopic calcification; however, this hypothesis has been examined in only a few published studies, with inconsistent results. Whether this association represents a true causal relationship or is just an incidental association due to the high prevalence of both disorders in the elderly is not evident at this point.

Genetic factors may be important in the development of valve leaflet calcification. In a recent case-control study of 100 patients with aortic stenosis matched for age, gender, and coronary artery disease compared with those without aortic stenosis, there was a significant difference in vitamin D receptor genotypes. In addition, other genetic polymorphisms of interleukin-10, connective tissue growth factor, and chemokine receptor-5 appear to influence the degree of valvular calcification. Other studies of apolipoprotein polymorphisms provide further support for a possible genetic component to valvular calcification and stenosis. In addition to native aortic valves, calcific changes in bioprosthetic valves are a prominent feature of primary valve failure; however, the prevalence of calcification and bioprosthetic valve failure appears to decrease with age in contrast to native valves. In a study of 196 patients receiving a bioprosthetic aortic valve, 18 of 20 cases of primary valve failure occurred in those <65 years old. Similarly, in another study of 653 patients who underwent aortic valve replacement, younger age was the only predictor of valve failure and need for reoperation. This paradox suggests that the calcific process of bioprosthetic valves is different from the process observed in native valves.

8. Potential therapeutic targets for CAVD

Inflammation: inhibit inflammation to reduce remodeling and release of cytokines involved in early CAVD. Might be valid only if targeted at initiating CAVD events.(Aikawa et al., 2007),
Sodium-dependent phosphate transporter 1: mediates calcium phosphate mineral deposition.(Crouthamel et al., 2013)

Oxidized LDL has been shown to induce osteogenic signaling via activation of this transporter; therefore, **inhibition** might reverse these effects.(Nadlonek et al., 2013)
Osteoprotegerin, an osteoclastogenesis inhibitory factor (OCIF), or tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) inhibits aortic valve calcification and preserves valve function in hypercholesterolemic mice.(Weiss et al., 2013)
PPAR γ : pioglitazone, a PPAR γ agonist, has shown potential in mitigating lipid-deposition and CAVD in hypercholesterolaemic mice.93 Action might be valve specific.(Chu et al., 2013)
Lipoprotein(a): a single nucleotide polymorphism in the gene encoding lipoprotein(a) is associated with CAVD. The level of lipoprotein(a) cannot be modulated with statins, but lowering by other means might be beneficial.(Yeang et al., 2016)
HDL cholesterol: a high level of HDL cholesterol is associated with improved valvular outcomes.(Olgun Küçük et al., 2015)
An **apolipoprotein A I mimetic peptide** might reverse CAVD-associated leaflet remodeling.(Weiss et al., 2006)
Angiotensin II type 1 receptor: block this receptor to inhibit TGFβ 1 expression and proteolytic enzymes involved in matrix remodeling. Might have to be combined with antifibrotic strategies.(O'Brien et al., 2005)
Hydroxytryptamine receptor 2B: inhibit TGF-β 1-induced activation of VICs and subsequent fibrotic remodeling. Might function in combination with angiotensin II receptors to modulate homeostasis.(Hutcheson et al., 2011)
Cadherin -11: target cadherin - 11 to inhibit myofibroblast differentiation and calcific nodule morphogenesis of VICs. Might be involved in both calcific and fibrotic cellular responses.(Sung et al., 2016)
Notch 1: activation of notch 1 signaling might inhibit both TGF-β 1 (fibrotic) and bone morphogenetic protein 2 (calcific) responses, Might also have a role in cellular mechanotransduction.(Acharya et al., 2011)
P2Y purinoreceptor 2: ATP promotes VIC survival via signaling at the P2Y Agonism of this receptor might provide a strategy to prevent apoptotic-driven dystrophic calcification.(Côté et al., 2012; Fish, 2014)
Cathepsin S: target cathepsin S to inhibit elastin degradation that induces both myofibroblastic and osteogenic reprogramming of VICs. Might be involved in early remodeling or inflammatory processes.(Aikawa et al., 2009)
Abbreviations: CAVD, calcific aortic valve disease; PPAR γ, peroxisome proliferator-activated receptor γ; TGF – β 1, transforming growth factor β 1; VIC, valve interstitial cell

9. Clinical Trials: 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Pathway

The first randomized, prospective study testing the effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in AV disease was published in 2005.In this double-blind, placebo-controlled trial, patients with calcific AS were randomly assigned to receive either 80 mg of atorvastatin daily or a matched placebo. Aortic valve stenosis and calcification were assessed with the use of Doppler echocardiography and helical CT, respectively. The Scottish Aortic Stenosis Lipid Lowering Therapy Impact on Regression (SALTIRE) investigators demonstrated a trend in slowing the progression of the AV stenosis, but it was not a statistically significant study for primary end points(Cowell et al., 2005). The SALTIRE investigators concluded that intensive lipid-lowering therapy does not halt the progression of calcific AS or induce its regression, and the reason for this negative trial might be the timing of therapy(Rajamannan et al., 2015).

In the Rosuvastatin Affecting Aortic Valve Endothelium (RAAVE) trial,(Rajamannan et al., 2015) performed a prospective trial of AS with Rosuvastatin targeting serum LDL, slowed progression of echo hemodynamic measurements, providing the first clinical evidence for targeted therapy using an 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in patients with asymptomatic moderate AS in a hypothesis-driven open-label study. These results are small and controversial, but test the lipid hypothesis. The next clinical trial, Simvastatin and Ezetimibe in Aortic Stenosis (SEAS), examined intensive lipid-lowering with Simvastatin and Ezetimibe in Aortic Stenosis. This trial was a randomized, double-blind trial involving 1873 patients with mild-to-moderate, asymptomatic AS (Freeman and Otto, 2005). Again, the investigators concluded that the medication did not reduce the composite outcome of combined AV events in patients with AS, including echo progression and vascular end points (Helske and Otto, 2009). Finally, the most recent trial, Aortic Stenosis Progression Observation Measuring Effects on Rosuvastatin (ASTRONOMER), randomly assigned patients to Rosuvastatin versus placebo in patients with moderate AV disease and bicuspid AV disease (Jassal et al., 2008). This study also did not demonstrate slowing of the progression of this disease. These 4 clinical trials have different results, which may be due to a number of reasons, including differences in trial designs, differences in enrollment criteria, differences in statin medication, or timing of therapy (Capoulade et al., 2013). Although the 3 randomized trials did not demonstrate slowing of the progression of AS, the largest trial, SEAS, did demonstrate improvement in primary end points of ischemic vascular disease. The future of clinical valve trials may need further analysis of the trial design, the type of medications, and the duration of the trials, but for now there is no primary indication for statin therapy in patients with valvular heart disease to slow progression of this disease (Capoulade et al., 2013). However, treatment of all cardiovascular patients with risk factors remains appropriate according to the guidelines as described by the American Heart Association and American College of Cardiology.

10. In Vivo Models of CAVD

Studies in the field of vascular calcification have set the stage for the experimental studies in valvular heart disease. Elevated LDL and its oxidative modification represent one of the major factors of CAVD. Therefore, addressing the mechanisms of CAVD in hypercholesterolemic animal models is a reasonable and essential approach. Development of CAVD has been shown in both apoE and LDL receptor-deficient mice. Aortic valves in hypercholesterolemic mice and rabbits, characterized by thickened leaflets with macrophage-rich subendothelial lesions in early stages and the formation of calcific deposits on the aortic site of the valve in late stages, reproduce key pathological features found in human valve disease. In addition, clinicopathological studies of stenotic AVs in humans identified lesions similar to those in inflamed atherosclerotic plaques. Cholesterol lowering in such models improves various features associated with atherogenesis and AV disease. These animal models are important and need to be characterized

further with regard to CAVD. However, these models also have limitations in that no one model recapitulates the human disease process completely, but each published model to date provides incremental mechanistic insight into the human disease process. The evidence that compares the osteogenic process in the valve with the bone is the most compelling to dissect the molecular mechanism and to demonstrate the foundation for both of these cellular processes and the potential for medical therapy.

11. **Invitro Models for CAVD**:

The ideal *in vitro* model would use primary human AVICs, but availability is the chief limiter of using human-derived samples. The next best cell would retain all characteristics of the human cells important to CAVD. Since it is believed that the important mediators of calcification are AVICs, we can narrow our search to finding a species with AVICs comparable to human AVICs.

Non-human primates are a logical choice because of their genetic similarity. However, maintenance of these organisms requires more space, time, money, and permissions than other organisms. Likely for these reasons, non-human primate AVICs have not been isolated, though *Macaca nemestrina* aortic smooth muscle cells have been isolated to investigate proteoglycan expression(Chang et al., 2000). Porcine hearts are both anatomically and physiologically similar to human hearts. The growth of the heart in swine from birth to four months is analogous to that in humans from birth to mid-teens and remodeling in atherosclerosis of micropigs closely resembles human pathology. Interestingly, their valves contain the same αSMA-positive population of cells in the ventricularis. Swine can also develop spontaneous valvular atherosclerotic lesions, a precursor to calcification(Simmons et al., 2005). The first isolation of porcine AVICs noted that they appear more homogenous than murine or leporine VICs and had a high recovery rate after being frozen, leading to the extensive use of porcine AVICs in in vitro studies (Johnson et al., 1987). Though these cells are widely used and multiple research groups have reported calcification and mineralization, Cloyd et al. reported that porcine AVICs cultured in osteogenic media with TGF-β1 (which should activate both dystrophic and osteogenic pathways) did not form mineral deposits. They used Raman spectroscopy to show that even Alizarin Red-positive nodules did not exhibit mineralization (Cloyd et al., 2012). While pig anatomy is highly similar to human anatomy, porcine AVICs in vitro is still a limited model. One important limitation specific to in vitro cell culture systems is the age of the cells. In 20% of long-term cell culture, AVICs become contact-inhibited monolayers and behave unstably. Also, metabolic activity of porcine AVICs was found to be passage number dependent. Late-stage cultured AVICs demonstrated higher numbers of myofibroblasts. Thus, porcine AVICs are generally used no later than passage 7. Though porcine AVICs have limitations, they are the best available model.

Ovine AVICs have been shown to form CNs when treated with TGF-β1 within 72 hours, and to calcify, assayed via Alizarin Red staining, within two weeks (Clark-Greuel et al., 2007). Canine AVICs was also considered early in the development of CAVD research Specifically, beagles demonstrate age-related changes to aortic valves, including calcification; changes were especially apparent in the fibroblasts, suggesting a similar mechanism to human calcification. In vitro, canine AVICs spontaneously formed CNs containing hydroxyapatite over two to three weeks, compared to human AVICs developing nodules in about six weeks under the same conditions. Also, while an imperfect model, many similarities exist between canine and human myxomatous mitral valve disease, reinforcing the likeness between human and canine valves. While canine AVICs were deemed very similar to humans', they are not often used, likely as a function of convenience – dogs have longer life spans than small animal models and are not maintained at a large scale for another purpose, as pigs are for food. Rabbits are used for in vivo studies, but not as often in vitro, likely because they require high cholesterol diets to develop calcification (Aupperle and Disatian, 2012), (Rajamannan et al., 2001).

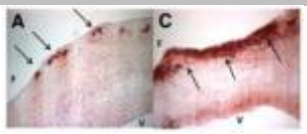
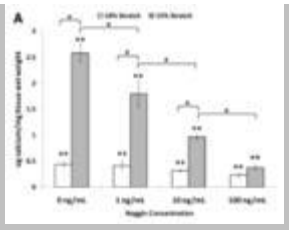
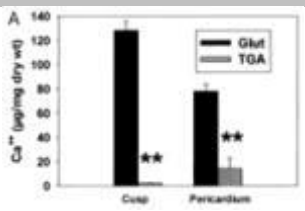
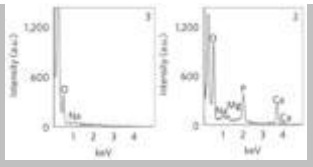
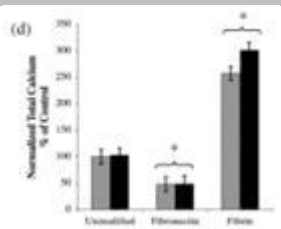
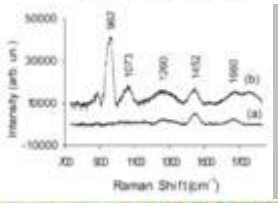
Mice are another popular model organism, perhaps because of their low cost, easy management, short life spans, and availability of genetic mutants. Murine cell lines can be easily immortalized, allowing for near indefinite expansion and use without regard for passage limitations. AVICs could be harvested from a variety of genetically-altered models such as ApoE−/−, Notch1+/−, and LDLr−/− (Nus et al., 2011; Swiatek et al., 1994) (Hjortnaes et al., 2010). Though some of these models are the only ones to exhibit the hemodynamic effects of aortic valve stenosis, murine valvular structure is significantly different from human. Specifically, human valves have trilaminar structure, but murine valves only have a fibrosa and spongiosa. While non-ideal, murine AVICs would provide a convenient model that facilitates genetic manipulation allowing for further exploration of CAVD mechanisms. A summary of the advantages and limitations of the AVICs derived from each model organism can be found in Table no 2.

Organism	**How are its AVICs useful?**	**Why are they imperfect?**
Human	Most appropriate	Difficult to obtain
Porcine	Similar anatomy to human; easy to obtain; swine spontaneously develop calcification precursors	More homogenous than human
Ovine	CNs develop more quickly than human	More difficult to obtain than porcine
Canine	CNs develop more quickly than human; pathology naturally occurs	Difficult to obtain; require ageing
Leporine	Many osteogenic markers upregulated; easy to obtain	Require high cholesterol diets over time

Table No 2: Advantages and disadvantages with various in models of CAVD.

12. **Evaluation of the calcification**:

Techniques for Evaluation of Calcification

Technique	Advantages	Limitations	Normal/Pathological Results	Notes on Images
Alizarin Red	Easy to stain; relatively easy to quantify with large range; inexpensive	Other elements, like magnesium, iron, and manganese also stain red		Tissue sections from porcine aortic valves; F=fibrosa; V=ventricularis; (Balachandran et al., 2011)
Arsenazo III	No interference from cations commonly found in plasma; easy to quantify; more stable and accurate than o-cresolphthalein complexone	Cannot differentiate between intracellular and extracellular calcium		Porcine aortic valves; 10% strain is physiologic; 15% is pathologic; (Balachandran et al., 2010)
Atomic Absorption	Gold standard to determine sample composition	Requires vaporization of sample; expensive		Calcium in porcine cusp or bovine pericardium after glutaraldehyde or triglycidylamine crosslinking in transplant rat model; (Connolly et al., 2005)
Energy-Dispersive X-ray Spectroscopy	Easily quantifiable; can perform during SEM or ESEM; ESEM yields more authentic data (no coating interference)	Expensive		Human aortic valves; region with and without calcific lesions; (Bertazzo et al., 2013)
O-Cresolphthalein Complexone	Easily quantifiable	Not as stable and accurate as Arsenazo III		Porcine AVICs on various coated tissue culture polystyrene; with TGF-β1 is pathologic (black); (Benton et al., 2008)
Raman Spectroscopy	Can be performed on live cells; algorithms can use data to accurately diagnose valve calcification	Expensive		Human aortic valves; a is physiologic; b is pathologic; (Otero et al., 2004)
Scanning Electron Microscopy	Topographical and compositional information; resolution ~nm; can be performed on hydrated samples (ESEM)	Difficult to quantify without EDS; expensive		Human aortic valves; scale bar is 3μm; green to orange represents increasing intensity; (Bertazzo et al., 2013)
Transmission Electron Microscopy	Chemical composition information; resolution ~pm	Expensive; difficult to perform on hydrated tissue		Human aortic valves; scale bar is 2μm; S=spherical particles; OM=organic matter; Pt=platinum; (Bertazzo et al., 2013)

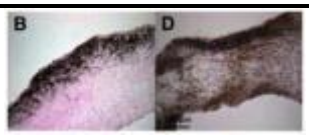
von Kossa	Easy to stain; inexpensive	Melanocytes in valves of a black or brown mouse will appear as false positive stain; not specific for calcium phosphate		Tissue sections from porcine aortic valves; black is calcification; (Balachandran et al., 2010)

Table No 3: Modalities for measurement of Aortic Valve calcium content.

13. Conclusion:

Calcific Aortic Valve Disease is mostly found in children and adults. Hence, serious consideration and attention is required to focus on the disease to avoid the increase of deaths and improve the quality of life.

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