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Calcific Aortic Stenosis: Lessons Learned from Experimental and Clinical Studies

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Abstract

Calcific aortic stenosis is the most common indication for surgical valve replacement in the United States. For years this disease has been described as a passive degenerative process during which serum calcium attaches to the valve surface and binds to the leaflet to form nodules. Therefore, surgical treatment of this disease has been the approach towards relieving outflow obstruction in these patients. Recent studies demonstrate an association between atherosclerosis and its risk factors for aortic valve disease. In 2008, there are increasing number of epidemiology and experimental studies to provide evidence that this disease process is not a passive phenomena. There is an active cellular process that develops within the valve leaflet and causes a regulated bone formation to develop. If the atherosclerotic hypothesis is important in the initiation of aortic stenosis than treatments used in slowing the progression of atherosclerosis may be effective in patients with aortic valve disease. This review will review the pathogenesis and the potential for medical therapy in the management of patients with calcific aortic stenosis by examining the lessons provided from the experimental research.

Keywords

Valvular Heart Disease; Lipids; Pathophysiology Atherosclerosis; Experimental Models

Introduction

Calcific aortic stenosis is the most common indication for surgical valve replacement in the United States[1]. With the decline of acute rheumatic fever, calcific aortic stenosis has become the most common indication for valvular disorder in the US. Landmark epidemiologic studies identified risk factors for the aortic valve which are similar to those of vascular atherosclerosis, such as smoking, male gender, hypertension, elevated cholesterol levels, and renal failure[2–4]. For years this disease process was thought to be due to build-up of nodules along the valve surface to induce a mechanical stenosis in the valve[5]. Furthermore, surgical therapy for severe symptomatic aortic stenosis is the only treatment option in 2008 as defined in a landmark study from 1968[5]. This study also defined the classic triad of symptoms which include chest pain, shortness of breath and lightheadedness. Finally, this research demonstrated that the life expectancy of this patient population is reduced significantly, if they do not have surgical valve replacement at the onset of symptoms[5]. Currently, it is a Class I indication for surgical valve replacement according to the American Heart Association and American College of Cardiology guidelines for valvular heart disease[6]. Over the past decade, there are a growing number of studies evaluating human disease tissues to define the cellular pathways important in this

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calcific aortic stenosis. This review will bring together the basic science and clinical science to develop a unified approach towards treating this disease.

Aortic Valve Calcification

The presence of calcification in the aortic valve is responsible for valve stenosis. Recent descriptive studies from patient specimens have demonstrated the hallmark features of aortic valve disease: including early atherosclerosis, cell proliferation and osteoblast expression[7–10]. To understand aortic valve disease, three interrelated events responsible for the development of valve calcification to consider: 1) classical cardiovascular risk factors, 2) genetic factors and 3) valve biology. The interrelationship of these events results in the final common pathway of this disease: a calcifying osteoblast phenotype. The evidence for these three pathways leading to the development of human aortic valve calcification can be found in the experimental and clinical studies outlined in this review.

Traditional Cardiovascular Atherosclerotic Risk Factors

In the past decade, landmark studies[2, 3], has described the risk factors for calcific aortic stenosis as identified by large epidemiologic cohort studies which include lipids, hypertension, male gender, renal failure, and diabetes. Many population science papers have subsequently confirmed these findings[2, 11, 12,13]. These studies have implicated the traditional risk factors for cardiovascular atherosclerosis important in the development of calcific aortic stenosis. The role of lipids as a risk factor for vascular atherosclerosis has been defined in the literature for years. Atherosclerosis is a complex multifactorial process which produces a lesion composed of lipids[14, 15], macrophages[16], and proliferating smooth muscle cells[17] and apoptosis[18] which is regulated by endothelial nitric oxide synthase[19–23] which over time causes occlusion of the vessel diameter. The understanding of these clinical risk factors are providing the foundation for the cellular studies and the potential for targeted medical therapies for this disease similar to vascular atherosclerosis.

Surgical pathological studies have demonstrated the presence of LDL and atherosclerosis in calcified valves, suggesting a common cellular mechanism[24, 25]. Furthermore, patients who have the diagnosis of familial hypercholesterolemia develop aggressive peripheral vascular disease, coronary artery disease, as well as aortic valve lesions which calcify with age. The first descriptions of atherosclerotic aortic valve disease have been in patients with Familial Hypercholesterolemia(FH) who have an early atherosclerotic lesion along the aortic surface of the valve leaflet[26–28,29]. The discovery of atherosclerosis in the aortic valve in the FH patient population provides the initial proof of principal for the potential treatment of lipids to slow the progression of aortic valve disease.

Experimental Models of Valvular Heart Disease

If atherosclerotic risk factors are important in the development of valvular heart disease, than experimental models of atherosclerosis are important in the understanding this disease process. Studies in mice and rabbits have confirmed that experimental hypercholesterolemia causes both atherosclerosis and calcification in the aortic valves[10, 30–34]. The experimental hypercholesterolemia diet has been used for over one hundred years for to evaluate the mechanisms of vascular disease. Table 1 demonstrates the *in vivo* rabbit and mouse models of valvular heart disease. The first study to describe early endothelial abnormalities in the aortic valves was in experimental hypercholesterolemia rabbits[35–38]. This hypothesis has been further developed in the rabbit model to test for multiple markers of the atherosclerotic process within the valve which are critical steps towards the development of valvular calcification process. The initial disease markers described in valve atherosclerosis include cell proliferation and apoptosis[30]. The early valve lesion of aortic valve sclerosis was also shown in a rabbit

model of hypercholesterolemia[39]. This model was extended using atorvastatin to modify gene markers to define the bone and atherosclerotic pathways and to demonstrate attenuation with a statin[10]. Atorvastatin attenuated the early gene markers of bone formation and macrophage infiltration along the aortic surface of the experimental hypercholesterolemia aortic valve[10]. The rabbit model of cholesterol with and without atorvastatin was extended to three and six months duration, to determine the timing of calcification in the valves. Three months of cholesterol induced the early mineralization and eNOS modification in the valve as shown by MicroCT and standard eNOS assays[21].

The next experimental proof of principle in the rabbit model, was to demonstrate complex calcification with chronic duration of cholesterol diet to prove the bone differentiation mechanism. 6 months of cholesterol diet treatment induced marked thickening and complex calcification in the aortic valve leaflets with pharmacologic attenuation with atorvastatin by MicroCT analysis[40]. The effects of statins were also confirmed by testing *in vitro* for the inhibition of extracellular matrix [41, 42]. Finally, the most recent experimental model tested the effect of an angiotensin receptor blocker(ARB) on the inhibition of atherosclerotic pathways in the rabbit model of hypercholesterolemia[43]. These findings demonstrate experimentally the beneficial effects of HMG CoA Reductase Agents and ARB's *in vivo* and *in vitro*.

Hemodynamic studies have evolved to study the noninvasive evidence for the development of aortic stenosis[31], in rabbits treated with cholesterol + vitamin D model. These studies demonstrate the presence of early stenosis with an increase in the pulse wave Doppler velocity across the aortic valve leaflet. This was further confirmed in the $LDLR^{-/-}$ mouse model of hypercholesterolemia[34]. The next hemodynamic study demonstrated severe stenosis and calcification with chronic cholesterol treatment for 24 months of cholesterol.[44] The study tested the genetic knock-out mouse which lacks the receptor for the Low-density lipoprotein receptor and expresses only the receptor for the human apoB100 ($LDLr^{-/-}apoB^{100/100}$) in an aging genetic mouse model which developed mineralization in the valve. The imaging experiments have been taken one step further to measure the development of the osteoblast phenotype in the aortic valve by using multimodality imaging[33]. The authors hypothesize that the flexion area of the aortic leaflets near the attachment of the aortic root (commissure) encounters the highest mechanical forces, which might induce endothelial cell activation/injury and expression of adhesion molecules such as VCAM-1, intracellular adhesion molecules-1, and E-selectin. Molecular imaging of the earliest stagesof calcification may identify high-risk valves while disease is silent and may enable the monitoring of valvular osteogenic activity during therapeutic interventions such as lipid lowering.

An important mechanistic study, demonstrated in native aortic valves in hypercholesterolemic mice that ten percent of cells are bone marrow derived cells within the atherosclerotic lesion[45]. These investigators hypothesize that likely both altered lipid metabolism and aging are essential for the development of murine aortic sclerosis, which potentially causes functional stenosis and regurgitation. Their findings suggest that some of the smooth muscle-like and osteoblast-like cells in degenerative valves might derive from bone marrow. It is likely that growth factors expressed in the endothelium with abnormal oxidative stress may play a role, at least in part, in the recruitment and homing of bone-marrow-derived cells to the site of valvular remodeling. Future studies evaluating mechanisms of the myofibroblast differentiation process, stem cell homing experiments, other medical therapies will provide further proof of the mechanisms for valvular heart disease and further understanding of the osteoblast differentiation cascade.

Genetics of Aortic Valve Disease

A growing number of studies are providing further evidence towards the genetic predisposition for aortic valve disease. Two of the studies have correlated genetic lipoprotein abnormalities in patients predisposed to the development of calcific aortic stenosis[46, 47]. The initial genetic study demonstrated an association of the vitamin D receptor polymorphism with calcific aortic valve stenosis. These investigators found that the B allele of the vitamin D receptor is more common in patients with calcific aortic valve stenosis[48]. In this study, the investigators found an association between the B allele predisposes the carriers to a decrease in calcium absorption and therefore an increase in bone loss. The discovery of this polymorphism further confirms the potential abnormal bone signaling pathways important in the development of this disease.

The next landmark discovery is the loss of function mutation in the Notch1 receptor in patients with calcific aortic stenosis[49]. These patients were identified in the Texas Heart Study as having valvular heart disease and accelerated calcification. It is important to note that the kindred of patients also had associated congenital heart abnormalities present in individual family members. Thus, implicating Notch1 in the development of congenital heart abnormalities as well as accelerated valve calcification. Another study demonstrated that the Pvull polymorphisms in the estrogen receptor alpha gene is related to both the presence of aortic stenosis in postmenpausal women and to lipid levels in adolescent females, suggesting that this polymorphism may influence the risk of aortic stenosis by affecting gender and lipid levels[50]. An interesting study[51], demonstrated a familial aggregation for calcific aortic valve disease in the western part of France. These investigators found that the geographic distribution of calcific aortic valve disease is highly heterogeneous, with an average frequency of operated calcific aortic valve disease of 1.13 per 1000 inhabitants but up to 9.38 per 1000 in specific parishes. These genetic and familial studies show that lipids, Vitamin D, Estrogen receptor and Notch1 signaling in addition to a familial aggregation have important implications in the development of aortic stenosis and that an early atherosclerotic lesion secondary to genetic lipid abnormalities are important in the early initiation of this disease. Future genetic testing in the development of calcification in patients without traditional risk factors may play an important role in the treatment of this disease.

Osteoblast Phenotype is the Final Common Pathway for Aortic Valve Calcification

The presence of calcification in the aortic valve is responsible for hemodynamic progression of aortic valve stenosis. Recent descriptive studies from patient specimens have demonstrated the critical features of aortic valve calcification: including osteoblast expression, cell proliferation and atherosclerosis[7–10]. These studies define the biochemical and histological characterization of these valve lesions. Furthermore, these studies have also shown that specific bone cell phenotypes are present in calcifying valve tissue from human specimens[52, 53]. The vascular biologists have performed the initial studies[54,55] which demonstrate the ability of calcifying vascular cells have the multipotential ability to differentiate to calcifying phenotypes. Calcification in the aortic valve is the final common pathway that leads to aortic valve stenosis. This was confirmed in a landmark echocardiographic study[56], demonstrating severe aortic stenosis and severe calcification have a worse prognosis than patients with mild calcification and severe aortic stenosis. The data further corroborates the evidence that calcification is the defining feature clinically for prognostic future prognostic implications for this patients population.

Studies have shown that cardiovascular calcification is composed of hydroxyapatite deposited on a bone-like matrix of collagen, osteopontin (OP), and other minor bone matrix proteins [8, 57, 58]. This was confirmed histologically with the presence of osteoblast bone formation in

calcified aortic valves removed from surgical valve replacement[8, 9]. In addition, osteopontin expression has been demonstrated in the mineralization zones of heavily calcified aortic valves obtained at autopsy and surgery[7, 8]. This discovery has been extended in a study which shows by RTPCR analysis, histomorphometry and microCT that an osteoblast-like cellular phenotype is present in calcified aortic valves removed at the time of surgical valve replacement[9]. The increased gene expression of osteopontin, bone sialoprotein, and Cbfa1 (the osteoblast specific transcription factor for bone formation) were increased in the calcified aortic valves as compared to the control valves from heart transplantation. This is the first to provide the evidence for the gene differentiation pathway in this calcifying tissue and an upregulation of the osteoblast gene program. These discoveries are the foundation for the hypothesis that the cells residing in the aortic valve have the potentiality to differentiate into a bone forming cell which over time mineralizes and expresses an ossification phenotype.

To test the osteoblast hypothesis further, evidence for signaling pathways are important in the development of this disease. The Lrp5 pathway was discovered to regulate bone formation in different diseases of the bone[59, 60]. There are three studies which have confirmed the regulation of the Lrp5/Wnt pathway in cardiovascular calcification in experimental models of calcification[40,52,61]. The Lrp5 pathway is one of many signaling pathways important in the development of bone formation. The Lrp5 receptor and other signaling pathways are important in the development of calcific aortic stenosis. Many of these signaling factors are similar to those found in vascular atherosclerosis and bone formation. Matrix MetalloProteinases (MMP)[53, 62], Interleukin 1[63], Transforming growth factor-beta(TGF-beta)[64], purine nucleotides[42, 65], RANK[66], osteoprotegrin(OPG)[66], and TNF alpha[67], have all been identified as signaling pathways important in the development of this disease process. Evidence, for the angiotensin converting enzyme pathway expressed and colocalize with LDL in calcified aortic valves also plays a role in future potential medical therapy[68]. Recent studies have shown that an increased expression of elastolytic cathepsins S, K, and V and their inhibitor Cystatin C in stenotic aortic valves[69]. These signaling studies from *ex vivo* human calcified aortic valves are the critical links between the experimental and translational implications for the future treatment of valvular heart disease. Figure 1, demonstrates the signaling pathways and cellular events important in the development of this disease. In the presence of lipids, the aortic valve endothelium is activated and abnormal oxidation state develops. The myofibroblast cells then begin to proliferate and synthesize extracellular bone matrix proteins with the upregulation of the various signaling pathways outlined in this review. These proteins overtime mineralize and calcify. ACE inhibitors and statins have the potential to modify this disease process and slow the progression of this disease.

Summary of In vivo, In vitro and Ex vivo Models of Aortic Valve Disease

These models have provided the clues for the development of therapeutic approaches for this disease. Understanding these models and genetics will help our future understanding of this complex disease process. First, the initiating events in vascular disease and valvular disease may be similar, but the outcomes and the understanding of treatment of this disease are different because of the different biologic endpoints for vascular disease as compared to valvular heart disease. Figure 2, shows the interrelated events important in the development of aortic valve disease which is important in the understanding of the complexity for developing clinical trials in this field. Future clinical trials will need to include the atherosclerotic hypothesis in addition to the potential of genetics affecting the development of this disease. Finally, understanding the signaling pathways involved in the development of cell proliferation and osteoblast bone formation will allow for the medical therapy for these patients.

Discussion

Recent epidemiological studies have revealed that the risk factors for arterial atherosclerosis, male gender, smoking, and elevated serum cholesterol, are similar to the risk factors associated with development of aortic valve stenosis. The risk factors, growing number of models of experimental hypercholesterolemia which produce atherosclerosis in the aortic valve are similar to the early stages of vascular atherosclerotic lesion formation. The interplay of genetics, environmental risk factors and cellular biology play a critical role towards the underlying mechanism of this disease process. In summary, these findings suggest that medical therapies may have a potential role in patients in the early stages of this disease process to prolong the time to severe aortic stenosis and to delay the timing of surgery.

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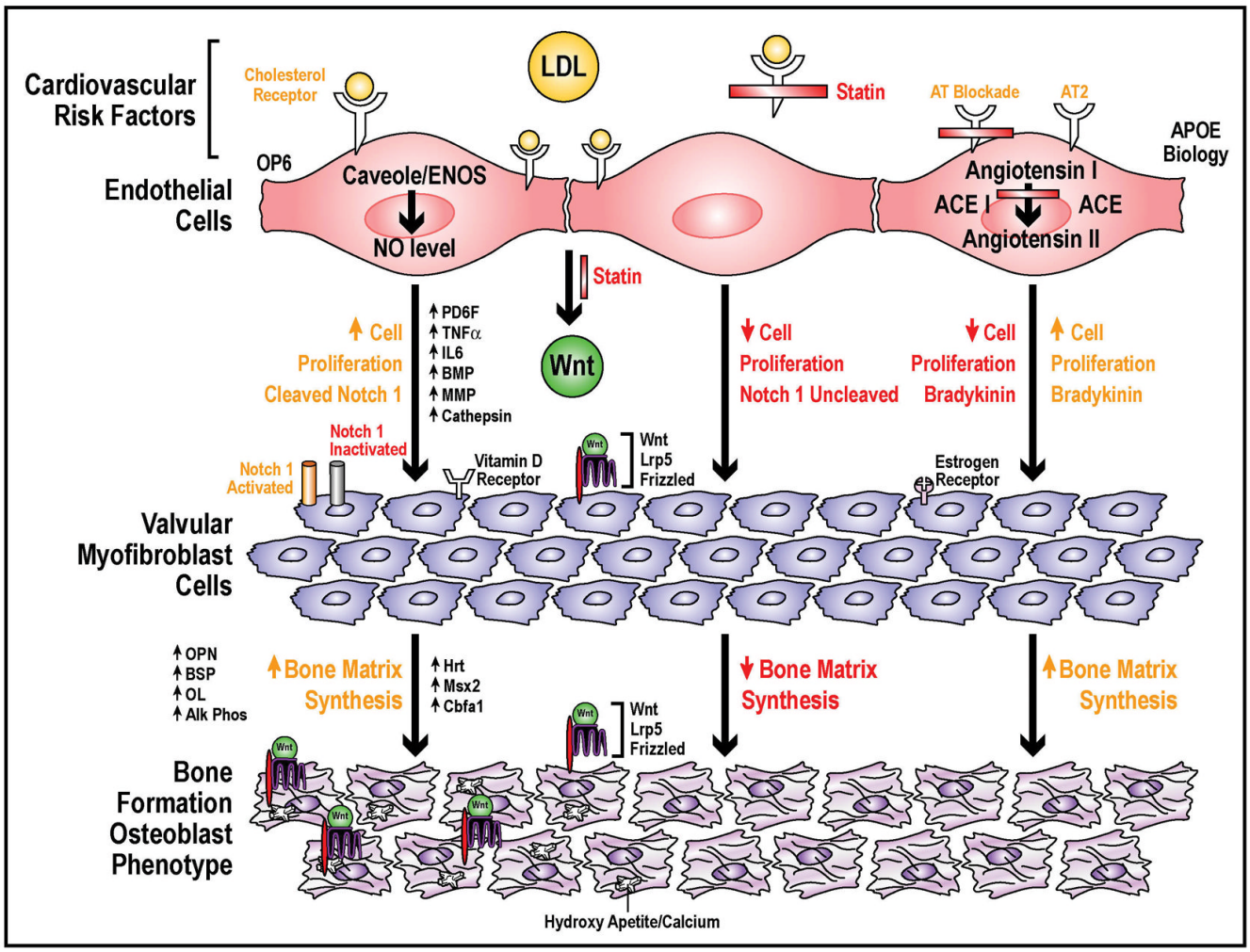

Figure 1. Cellular, Molecular and Genetic Mechanisms of Calific Aortic Stenosis

Model Implicating Lipids in the Development of Calcific Aortic Stenosis and the potential for Future Medical Therapies Targeting this disease at the cellular level in the Aortic Valve.

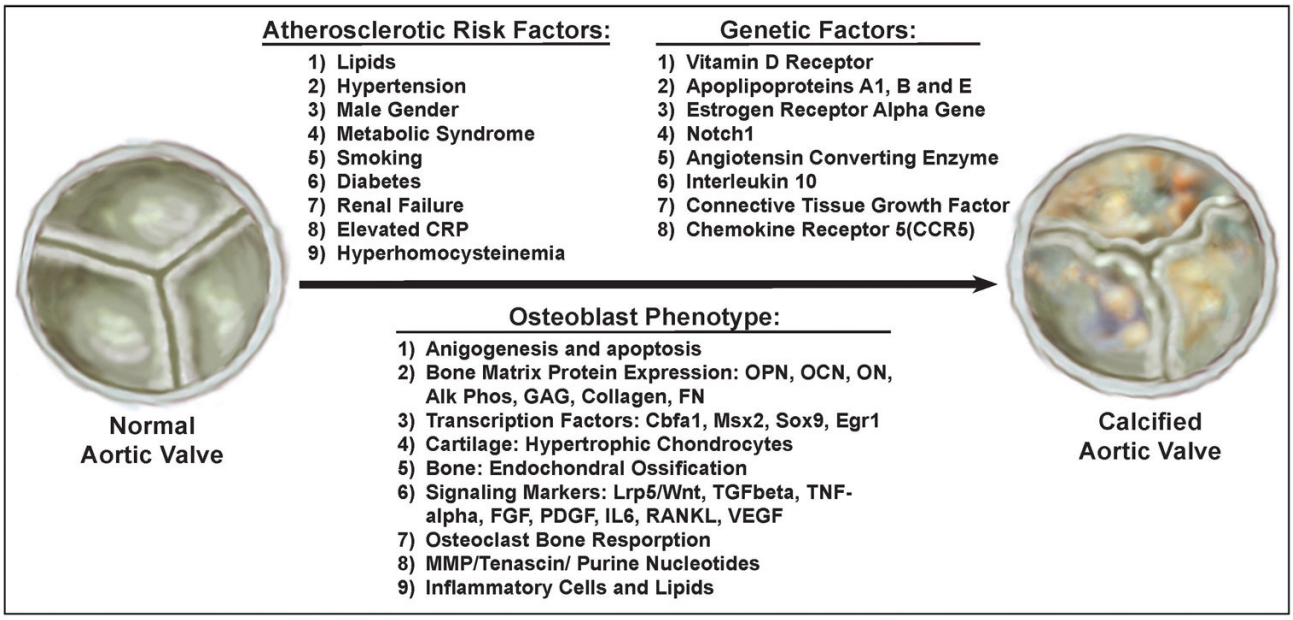

Figure 2.
Three Factors Responsible for the Development of Calcific Aortic Stenosis: Cardiovascular Risk Factors, Genetic Factors and Osteoblast Regulatory Pathways.

Table 1

Experimental Animal Models of Valvular Heart Disease

List of Experimental Models of Aortic Valve Disease.

Author	Year	Model	Diet	Medical Therapy	Experimental Results
Rabbit					
Sarphie([38])	1985	New Zealand White Rabbit	Cholesterol	None	Endothelial structral changes along aortic surface of the aortic valve
Sarphie([37])	1985	New Zealand White Rabbit	Cholesterol	None	Anioic Surface Properties of the Endothelium
Sarphie([36])	1986	New Zealand White Rabbit	Cholesterol	None	Cytochemical Surface Properties
Sarphie([35])	1987	New Zealand White Rabbit	Cholesterol	None	Interactions of IgG and beta VLDL
Rajamannan([30])	2001	New Zealand White Rabbit	Cholesterol	None	Apoptosis
Rajamannan([10])	2002	New Zealand White Rabbit	Cholesterol	Atorvastatin	Cell Proliferation, Extracellular Matrix production, Foam Cell Formation, Cbfal expression, Statin Inhibits Cell Proliferation and Matrix
Rajamannan([21])	2005	New Zealand White Rabbit	Cholesterol	Atorvastatin	eNOS expression and Regulation in the Aortic Valve, Calcification
Rajamannan([40])	2005	Watanabe Rabbit/LDLR$^{-/-}$	Cholesterol	Atorvastatin	Lrp5/Wnt/Cbfa1 regulation in severely calcified aortic valves and regulation of cell proliferation in myofibroblast cell culture
Drolet{[31])	2003	New Zealand White Rabbit	Cholesterol and Vitamin D	None	Hemodynamic Evidence of Early Stenosis
Cimini([39])	2005	New Zealand White Rabbit	Cholesterol	None	Early Aortic Valve Sclerosis and Atherosclerosis
Arishiro([43])	2007	Japanese Rabbit	Cholesterol	Olmesartan	ARB1 inhibits endothelial disurption and atherosclerosis
Mouse Species					
Shao([61])	2005	LDLR$^{-/-}$	Cholesterol	None	Valvular Fibrosis via Wnt-Msx2 Regulation
Drolet([34])	2006	LDLR$^{-/-}$	Cholesterol	None	Hemodynmic Evidence of Early Stenosis and Metabolic Syndorme
Weiss ([32])	2006	LDLR$^{-/-}$Apob$^{100/100}$	Cholesterol	None	Hemodynamic Evidence of Severe Stenosis, Mineralization and Abnormal Oxidative stress
Aikawa([33])	2007	ApoE$^{-/-}$	Cholesterol	None	Multiomodality imaging in Atherosclerotic Aortic Valves- Measuring Osteogenic Activites
Tanaka ([45])	2007	ApoE$^{-/-}$	Cholesterol	None	Mechanistic study for Aortic valve Disease