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Pathological changes of Aortic Valve Calcification in Experimental Animal Models

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ABSTRACT

Calcific Aortic Valve Disease (CAVD) is a sluggish and progressive disease that comprises “early sclerosis, characterized by leaflet thickening without left ventricular outflow obstruction, to late stenosis with stiffened leaflets, obstructed flow and compromised cardiac function”. CAVD was formerly believed to afflict the tricuspid or congenitally bicuspid aortic valve and be a passive, senile, or degenerative disorder. However, recent investigations have demonstrated that this is a pathobiological activity that is active and heavily cell-mediated, which shares several risk factors with atherosclerosis. Numerous studies show that CAVD are not a normal aspect of aging and may be linked to certain risk factors. Nevertheless, no pharmacological therapy available to halt or arrest the development of CAVD in a clinically relevant way, and surgery is the only effective treatment option. As a result, there is an urgent scientific need to determine pathobiological mechanism of CAVD and to find new ways to treat CAVD. Animal models are developing as crucial instruments to this aim, assisted by the development of new models and greater knowledge of the efficacy of old models. In this review paper, we will present the most extensively utilized large and small animal models that were used to explore CAVD.

Keywords: Calcific aortic valve diseases; Aortic valve calcification; Aortic valve stenosis; Atherosclerosis; Experimental Animal Models

INTRODUCTION

CAVD is a serious global public health issue, in 2019; there were reportedly around 9,404,078 cases (male 5,027,261, female 4,376,817), of CAVD Globally, even with age standardization, global CAVD incidence, prevalence, and mortality increased 3.51-, 4.43-, and 1.38-fold from 1990 to 2019, respectively. In 2019, there were an estimated 126,827 patients (male 54,175 and female 72,652) died from CAVD globally. The highest rates of CAVD mortality were recorded in the USA, followed by Germany and Japan (248,256, 13,154, and 12,868, respectively), (Yi et al., 2021). According to studies, CAVD is presently the main reason for cardiac valve disease in both industrialized and developing nations. CAVD ranges from early sclerosis, which is described by thickening of the leaflets without obstructing the left ventricle's outflow, to late stenosis, which has rigid leaflets, obstructed flow, and compromised cardiac function (Gillis et al., 2017). CAVD is the most prevalent cause of aortic stenosis (AS) in adults and was previously thought to be a passive, senile, or degenerative process affecting a normal trileaflet or congenital bicuspid valve (Lilly & Braunwald, 2012), but over the past decade, a number of studies have revealed that a number of noteworthy molecular processes play a role in the emergence of this condition, and recent scientific findings have also shown that it is an active and highly cell-mediated pathobiological process (Le Quang et al., 2014). A 50% greater risk of cardiovascular mortality and myocardial infarction has been linked to sclerosis, whereas the prognosis for individuals with stenosis is quite dismal. (Sider et al., 2014). CAVD is the third most common cardiovascular disease in the western world after coronary artery disease and hypertension, accounting for half of all valvular heart disease (Scatena et al., 2018), and the main reason for transcatheter heart valve replacement or surgical heart valve replacement.(Hisamatsu et al., 2018). CAVD has similar risk factors to atherosclerosis: age, male gender, smoking, high cholesterol, hypertension, diabetes mellitus ,kidney failure, and bicuspid aortic valve (Scatena et al., 2018); see **Fig. 1**). According to current data, clinically significant atherosclerosis is not present in 50% of patients with CAVD. It is important to emphasize that mechanical damage induced by hemodynamic stress produced by continual leaflet opening and shutting is regarded to be a significant risk factor for aortic valve stenosis. The BCA, particularly adds to increased mechanical stress, has been observed to accelerate the course of AVS in younger individuals with a low risk of atherosclerosis. Additionally, the non-coronary leaflets is more susceptible to injury than other leaflets because to the greater mechanical stress caused by the lack of diastolic coronary flow. (Honda et al., 2014). Additionally, tissue prosthetic valves were seen to calcify prematurely, mimicking the natural course of bicuspid aortic valves. (Cohen et al., 2004). Aortic valve sclerosis (AVSc) affects 25 to 30 % of people over the age of 65, 40 % of patients over the age of 75 (Anselmo et al., 2018; Branchetti et al., 2013), and up to 75 % of patients over the age of 85. Severe AS affects the population over 75 years at a 3 % incidence rate (Parisi et al., 2015). Several research' data indicate that chronic inflammation is essential for both atherosclerotic calcification and CAVD. “This is demonstrated in human disease by the existence of macrophages ,T cells, sub endothelial oxidized low-density lipoprotein (LDL) deposits and α-Smooth muscle actin (α-SMA) positive cells, associated with late CAVD, these are found within primary lesions infrequently” (Alushi et al., 2020; Demer & Tintut, 2019; O’Brien, 2006; Sider et al., 2014);increased superoxide and hydrogen peroxide (Rajamannan et al., 2011); increased oxidative stress and reduced endothelial nitric oxide synthase(Miller et al., 2009); activation of complement (Helske et al., 2008); elevated expression of TNF-α, active mast cells, matrix metalloproteinases (MMP-1,-2,-3,-9) (Hakuno et al., 2010), interleukin-2 (IL-2), ACE, angiotensin II (Ang II), angiotensin II type-1 receptor (AT1R), and chymase (O'Brien, 2006), as well as valvular endothelial cells' (VEC's) expression of intracellular adhesion molecule-1

(ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) (Alushi et al., 2020), and E-selectin (Ghaisas et al., 2000). Inflammatory processes are related with, and may contribute to, the valve ECM changes associated with CAVD, including leaflet thickening, protein turnover, and fibrosis (Weisell, 2020); Proteoglycans and hyaluronan buildup, elastin fragmentation(Sider et al., 2014), and calcification (Rajamannan et al., 2011; Tanaka et al., 2005). Finally, valve stiffness or dysfunction are caused by an underdevelopment of the valve ECM, While many characteristics of human CAVD are clearly established (especially those of late-stage human CAVD), early sclerosis is little understood.

METHODS

Articles providing data on Experimental Animal Models of CAVD were found by searching PubMed with the following MESH terms for CAVD OR (Aortic Valve Sclerosis AND Aortic Valve Stenosis) AND Experimental Animal Models AND Atherosclerosis; added all terms with Boolean AND, OR operators. PubMed, Google Scholar, ScienceDirect.com, and the Cochrane Library databases were also searched for the above terms. Given the paucity of available updated material in the literature. The all-scientific information cited in this review were carefully selected, retrieved and reviewed.

1. Biology of the normal Aortic valve

The majority of people have a tricuspid aortic valve, which has three semilunar cusps and is situated where the left ventricular outflow tract and the aortic root converge. In order to sustain bidirectional blood flow from the left side of the heart to the systemic and coronary circulations, it is a flexible membrane that opens and closes more than 100,000 times per day (Hulin & Oury, 2018; Lerman & Alotti, 2015). In healthy individuals, the valve cusps are less than a millimeter thick and are surrounded by an endothelial layer composed of valvular endothelial cells (VECs) on both sides. The valve's interstitium is composed of three separate layers: fibrosa, spongiosa, and ventricularis as shown in figure 2. The predominant cell type identified here is valvular interstitial cells (VICs) (Rutkovskiy et al., 2017). To meet their functional requirements under these adverse conditions, the thin, flexible leaflets are organized in three different layers of extracellular matrix (ECM). (1) The lamina fibrosa on the aortic side of the leaflet, which constitutes the majority of the valve and also the load-bearing structure, is composed primarily of circumferentially aligned collagen fibers (type 1 collagen fibrils) that contribute the majority of the leaflet's mechanical strength; (2) the Spongiosa is located in the center of the leaflet. It consists of a loose mucopolysaccharides matrix that promotes movement between the fibrosa and ventricularis during leaflet motion and serves as a cushion against compressive stresses; and (3) collagen and radially aligned elastin encompass the ventricularis layer on that ventricular side, which contributes to leaflet flexibility by permitting changes in leaflet shape during opening and shutting (Goody et al., 2020; Hulin et al., 2018; Sider & Simmons, 2011). Isolated macrophages are often seen in the ventricularis or spongiosa of mature human aortic valve cusps but not in the normal fibrosa (O'Brien et al., 1996), All three layers are avascular with no cellular infiltrates and are innervated by adrenergic and cholinergic neural networks in normal conditions. The aortic valve must be repaired on a regular basis throughout one's life in order to remain flexible (Xu, et al., 2010). Valvular endothelial cells (VECs) and valvular interstitial cells (VICs), which maintain valve homeostasis and structural leaflet integrity, are among the biological components of the aortic valve, and VICs, the most common cell type in the heart valve, are important in the evolution of CAVD (Hjortnaes et al., 2015). Mesenchymal VICs, quiescent VICs, progenitor VICs, active VICs, and osteoblast VICs are the five types of VICs. During valve development, epithelial-mesenchymal transition generates mesenchymal VICs from endothelial cells of the endocardial cushion (Liu, 2007). The normal

valve contains quiescent VICs (qVICs), which maintain its normal structure and function (Liu, 2007) ,VICs that can multiply in reply to injury are known as progenitor VICs (pVICs) and during the inflammatory reaction to pathogenic stimuli like mechanical stress or lipids, pVICs are transformed to aVICs. The aVICs function as profibrotic cells and have myofibroblast features such as contractility, stress fibers, and the striated-muscle isoform of myosin heavy chain. Further processing of aVICs results in obVICs, which accelerate calcification (Liu, 2007). VECs create an endothelial monolayer on the surface of the heart valve and are special because they may undergo endothelial-mesenchymal transformation, and an important phase in developing valvulogenesis. (Hjortnaes et al., 2015).

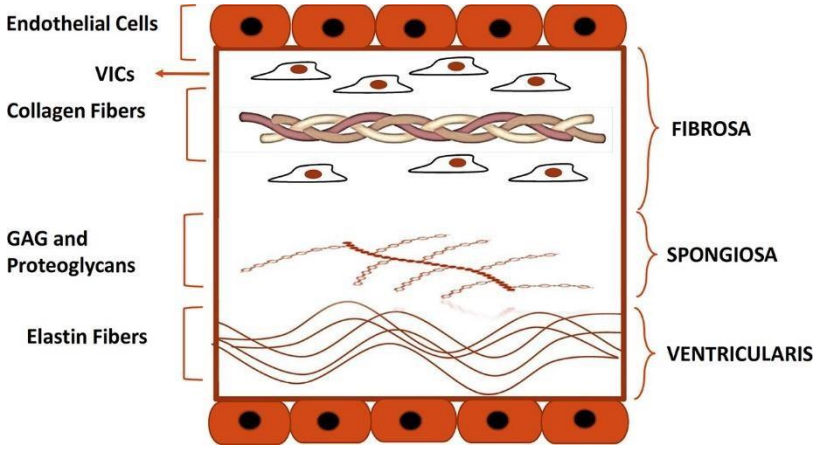

Fig. 1. Histological structure of the aortic valve. Depicted is the histological structure of the healthy aortic valve. Fibrosa, spongiosa, and, ventricularis are the three layers that make up the structure of a normal aortic valve. The Fibrosa layer is composed of type I and III collagen fibers and contains VICs. Spongiosa and ventricularis layers are respectively composed of GAG and proteoglycans and elastin fibers. Endothelial cells form a monolayer on each side of the cusp. GAG, glycosaminoglycans; VIC, valve interstitial cell (Alushi et al., 2020).

2. Pathobiology of CAVD

The initiation phase and the propagation phase are two separate stages in the pathobiology of CAVD. The propagation phase is characterized by fibrosis, calcification, and neoangiogenesis, while the early phase is characterized by endothelium damage, lipid accumulation, and inflammation. CAVD is carried on by endothelium dysfunction, which can be caused by turbulent flow with low shear stress (Dweck & Newby, 2012; Lindman & Mathieu, 2016; Ohukainen & Rysa, 2018). LDL, Lp (a), and proinflammatory cytokines such as monocytes and lymphocytes may enter the valve as a result of endothelial injury, when LDL and Lp (a) are oxidized and build up in the valve, inflammatory cells and valvular interstitial cells release inflammatory cytokines and chemokines. When macrophages ingest lipoproteins in the valve, foam cells are formed. The stimulation of valve interstitial cells (VICs) further mediates the fibro-calcific process, which is triggered by inflammation. Extracellular matrix remodeling is facilitated by activated VICs (aVICs), which increase collagen synthesis and disrupt the valve's natural structure. Osteoblast VICs (obVICs) are formed as the illness progresses, and they emit osteogenic markers. Proliferation occurs when calcification creates greater mechanical stress and injury that leads to even more calcification. This cycle repeats again.(Pawade & Dweck, 2015).

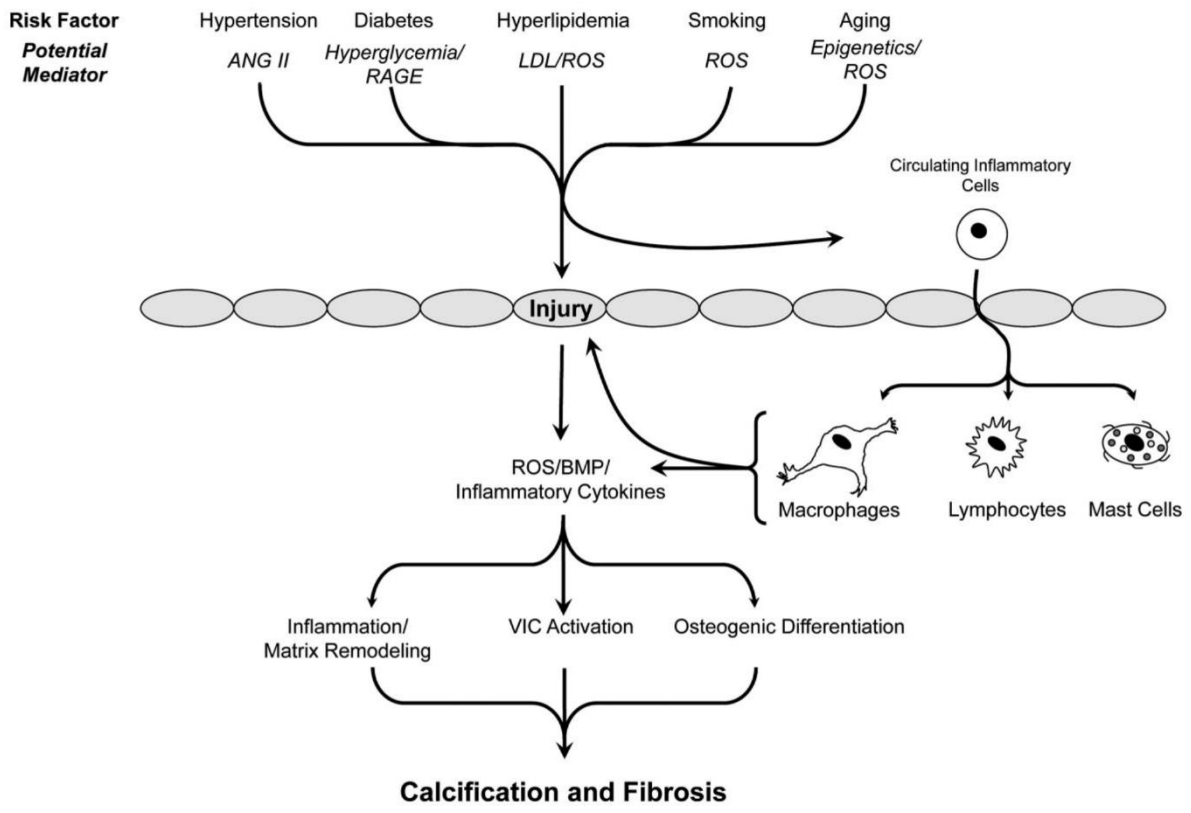

Fig. 2. Overview of risk factors and potential mechanisms that contribute to Calcification and Fibrosis of the aortic valve (Miller & Heistad, 2011).

3. CAVD Animal Models

There are several advantages of using animal models to study CAVD in vivo as well as assessing the outcomes of various therapy approaches. To be most successful, models should closely resemble real diseases and the circumstances under which genuine CAVD develops. The three animals that are most frequently used to model CAVD are swine, rabbits, and mice. Only swine have been proven to naturally acquire lesions with age, although this is a gradual process that is typically sped up by hypercholesterolemia brought on by diet. Some animals, including mice and rabbits, have not been found to naturally progress lesions but are reactive to diet- suggested hypercholesterolemia. Mice also need a genetic propensity to stimulate severe illness (Sider et al., 2011). Although no model can entirely replicate the intricacies present in human pathologies, they are crucial in analyzing disease mechanisms as well as novel diagnostic tools, preventions, and therapies (Chorro & López-Merino, 2009; Walters et al., 2012). This paper will reviews swine, rabbit and mouse models of CAVD, together with their advantages and drawbacks of the most usually used animal models of CAVD, which are summarized in **(Table 1-3).**

Table 1. Advantages and drawbacks of Mice models of CAVD

Advantages	Disadvantages
Low price and short generation time	Wild-type mice have a high resistance to the development of atherosclerosis
Extremely accessible	CETP activity in the plasma is absent
Easy to use and maintain	The vast majority of cholesterol is transmitted by HDL particles
Ridable reproduction	Because of their small size, collecting blood from mice is challenging due to the cutting up of tiny arteries
Standardized procedures for targeted genetic manipulation	It is required to use genetically manipulated mice (e.g., apoE-deficient, LRLD-deficient)
Availability of inbred strains and clearly defined genetics	In most vessels, there is no plaque rupture or luminal thrombosis.

(Fuster & Andrés, 2012)

Table 2. Advantages and drawbacks of the swine CAVD model

Advantages	Disadvantages
Lesion location, morphology and content are similar to human haemodynamics and pathogenesis	Its large size restricts its practical application
Cardiovascular anatomy and heart size are similar to human	To induce atherosclerosis, a toxic diet is required
Similar lipid metabolism, except for Apo II deficiency in swine	Purchase and maintenance costs are both high
For genetic manipulation, highly specified genotypes are required. The minipig variant is a less expensive option	Handling difficulty (except for minipig strains)
When fed an atherogenic diet, it can spontaneously acquire atherosclerosis at a faster rate than mice and rabbits	Atheroma development takes longer time in humans than in other animals
In comparison to smaller species, imaging techniques such as ultrasound, CT, and MRI are quite simple.	

(Lee et al., 2017; Leong & Jaarin, 2015; Leong et al., 2015; Fuster et al., 2012)

Table 3. Advantages and drawbacks of Rabbit models of CAVD

Advantage	Disadvantage
Lipoprotein metabolism is similar to that of humans (except for hepatic lipase deficiency in rabbits)	The development of hypercholesterolemia and atherosclerosis requires a highly abnormal diet
No particular needs, easy to manage and maintain	Long-term high-cholesterol eating causes massive inflammation and damage in the liver du low hepatic lipase activity
Similar lesion development morphology	Does not always respond to cholesterol in the diet
Because of its small size, it has a low maintenance cost and high availability	Human cardiovascular physiology: HDL being the major plasma lipoprotein, lack of Apo AII, decreased liver lipase activity
Clinical assessment is possible with larger arteries: Ultrasound and MRI can be used to identify plaque composition and vulnerability	Plaque lesion that is not human-like: Advanced lesion (e.g., fibrosis, haemorrhage, and ulceration) with increased fatty streak and macrophage rich foam cells are not visible
Response to dietary cholesterol is favorable Hyperlipidemic mutant strains are available Large enough to accommodate physiological research	Site of diverse predilection: Atherosclerotic plaque deposits preferentially in the aorta and iliac arteries

(Leong et al., 2015; Fuster et al., 2012)

3.1. Small Animal Models

Both the pathophysiology of the illness in small animal models and the evaluation of therapeutic approaches are quite interesting to investigate further. Rats and mice are very practical and manageable owing to their small size. Small animals are becoming more the focus of CAVD research (Roosens et al., 2013).

3.1.1. Mouse Models

The key benefits of this species are its short gestation and affordable housing and breeding costs. Due to their awareness of their genomes, ability to edit them, and capacity for rapid data collecting of genomic modification, mice are an interesting model system for studying multiple processes that are impacted during the development of cardiovascular illnesses. (Bostick & Duan, 2011; Doevendans, 1995).

3.1.1.1. Nutritionally and Genetically-Susceptible Mouse Models

To produce advanced CAVD, mice must be genetically manipulated and sometimes-nutritional intervention is required. The majority of the mice utilized are LDL receptor deficient (Ldlr/) mice (Roosens et al., 2013). Drolet et al. studied the effects of a four-month high-fat/high-carbohydrate (HF/HC) diet with low cholesterol on the development of early degenerative aortic valve stenosis (AS) in adult wild-type (WT) and low-density lipoprotein receptor-deficient (LDLr/) mice. Wild-type mice on an HF/HC diet developed mild metabolic syndrome (hypercholesterolemia, obesity, and hyperglycemia).This study suggests that various atherogenic factors, including obesity, hyperglycemia, and mild dyslipidaemia, may significantly contribute to the development of AVS and that treating isolated hypercholesterolemia alone is not the most effective strategy. (Drolet et al., 2006). Weiss et al. examined low-density lipoprotein receptor-deficient apolipoprotein B-100-only (LDLr-/-

ApoB100/100) elderly mice with hypercholesterolemia who were fed standard chow. At 20 months, LDLr-/-ApoB100/100 mice showed functionally significant severe AS on echocardiography, with a decrease in valve area (>50%) compared to controls. Additionally, animals with aortic stenosis had increased quantities of superoxide in their valve tissue, indicating the beginning of oxidative stress. This finding supports the previously established relationship between tissue oxidative stress and valve disease. (Weiss et al., 2006) see **Fig. 3**. Le et al. have established the type 2 diabetes mellitus susceptible LDLr/ApoB100/100/IGF-II mice model of CAVD. Mice fed a high fat/sucrose/cholesterol (HFSC) diet for six months developed severe AS, calcification and the development of inflammatory infiltrates as compared to control mice (mostly macrophages). Additionally, diabetic mice's aortic tissues showed overexpression of osteogenic genes like spp1, bglap, and runx2, as well as myocardial tissues demonstrated upregulation of hypertrophic genes like atrial natriuretic peptide, brain natriuretic peptide, and myosin heavy chain (anp, bnp, and mch) (Le Quang et al., 2014). A noteworthy finding of this study was the development of AS in 40% of nondiabetic LDLr/ApoB100/100/) mice and 80 % of diabetic LDLr/ApoB100/100/IGF-II mice after 6 months of the HFSC diet. It is significant to note that the proportion of LRKOB100 mice in the current study (40%) that developed AS was comparable to that seen in Weiss et al. (2017). (33 %) (Weiss et al., 2006). Although AS developed in this research in a significantly shorter amount of time (6 versus 20 months), this can be attributed to the use of a cholesterol-enriched diabetogenic diet (HFSC diet) as opposed to the conventional diet recommended by (Weiss et al., 2006), also explored male LDLr-/-:ApoB100/100 mice. In this study, the rats were randomly allocated to either the usual chow or the diabetogenic, procalcific diet group (NC). After 14 months on the diabetogenic diet, LDLr-/-ApoB100/100 mice exhibited calcification, thickened leaflets, and 77 % hemodynamically significant AS. Those fed normal chow (NC) revealed a 38 % incidence of AS, and very tiny valve calcification in contrast to LDLr-/-ApoB100/100 mice. T2DM and metabolic syndrome were also developed in diabetogenic, procalcific diet fed LDLr-/-ApoB100/100 animals compared to normal chow fed mice (Scatena et al., 2018). The "endogenously hyperlipidemic" ApoE-deficient (Apoe-/-) mouse is another genetically engineered and extensively used model, which facilitates receptor-mediated clearance of very-low density lipoprotein (VLDL) from the circulation. Previous study in Apolipoprotein E-deficient (Apoe-/-) mice revealed the effects of lipids on CAVD. They observed inflammatory responses that were similar to those seen in humans, with repeated apoptotic cell death, α-SMA, osteocalcin and chemokine expression, macrophage and T-cell accumulation, nodular calcifications, mild regurgitation, and significant rises of transvalvular velocity in the aortic valve of (Apoe-/-) mice (Tanaka et al., 2005). Zeadin et al. (2015) tested the special effects of the adipocytokine leptin on valvular calcification and lesion size in a new version of the Apoe-/-mice. Leptin-treated mice did not develop hypercholesterolemia or a change in the size of atherosclerotic lesions. Furthermore, leptin-treated animals had considerably enhanced valvular calcification and ALP-positive staining, which was linked to an increase in the expression of osteoblast-specific markers (osteocalcin (OCN) and osteopontin (OPN) (Zeadin et al., 2009). Srivastava et al. (2011) revealed "the effects of acrolein, a dietary aldehyde formed during inflammation and oxidative stress, on atherosclerosis." In this study, male Apoe-/-mice were fed (2.5 mg/kg/day) for 8 weeks in this study. Mice exposed to acrolein exhibited hypercholesterolemia, lipid and macrophage infiltration, and dramatically elevated E-selectin and PAI-1 levels after 8 weeks. These findings imply that acrolein stimulation of platelets and endothelial cells occurs in vivo (Srivastava et al., 2011). Medications such as rosuvastatin and lithium chloride have been demonstrated to have anti-inflammatory effects on the aortic valve of Apoe-/-mice fed high fat/cholesterol diets, as evidenced by a considerable reduction in

macrophage infiltration and expression of vascular cell adhesion molecule-1 (VCAM-1). (Choi et al., 2010; Monetti et al., 2007). In addition to nutritionally and genetically vulnerable mouse models, Honda et al. developed a unique mechanical wire injury model in which a spring guidewire is placed into the left ventricle of the heart under echocardiographic guidance through the right common carotid artery of C57BL/6 mice. After that, the wire is twisted to cause endothelium injury. The velocity of blood flow can be measu6red by echocardiography to indicate AVS development. When compared to sham mice, echocardiography revealed enhanced aortic blood flow velocity. Furthermore, valvular calcification, increased formation of reactive oxygen species, expression of inflammatory cytokines and osteochondrogenic factors, and increased expression of inflammatory cytokines (Honda et al., 2014). By changing the wire type, tip angle, and number of turnings, mild, moderate, or severe stenosis can now be induced (Niepmann et al., 2019). The aorta anatomy of mice differs from that of humans in that they lack a trilayer structure (Hinton Jr et al., 2008). As a result, it is important to replicate any findings from mice in human valves or human cells. When compared to a control group, Fujisaka et al. discovered that giving Male ApoE-null mice high-dose Ang II (1000 ng/kg/min) for 4 weeks resulted in aortic valve thickness, endothelial disruption, and enhanced myofibroblast infiltration. Furthermore, management with olmesartan, an Ang II type 1 receptor blocker, prevented these effects. In ApoE-deficient mice, olmesartan also reduced aortic diameter dilation.(Fujisaka et al., 2013). Rattazzi et al. (2018) examined the impact of warfarin and rivaroxaban on the emergence of aortic valve calcification in ApoE deficient mice. In this study, they were split into three groups as follows: Western-Type Diet (WTD) group, 3 mg of warfarin for the warfarin group, and 5 mg of rivaroxaban for the rivaroxaban group over an 8-week period. Animals given warfarin experienced greater aortic valve degeneration and calcification as compared to mice given rivaroxaban treatment. These changes included calcium deposition on the aortic valve leaflets. According to the results of this ground-breaking research, rivaroxaban had a decreased risk of aortic valve calcification development.(Rattazzi et al., 2018).

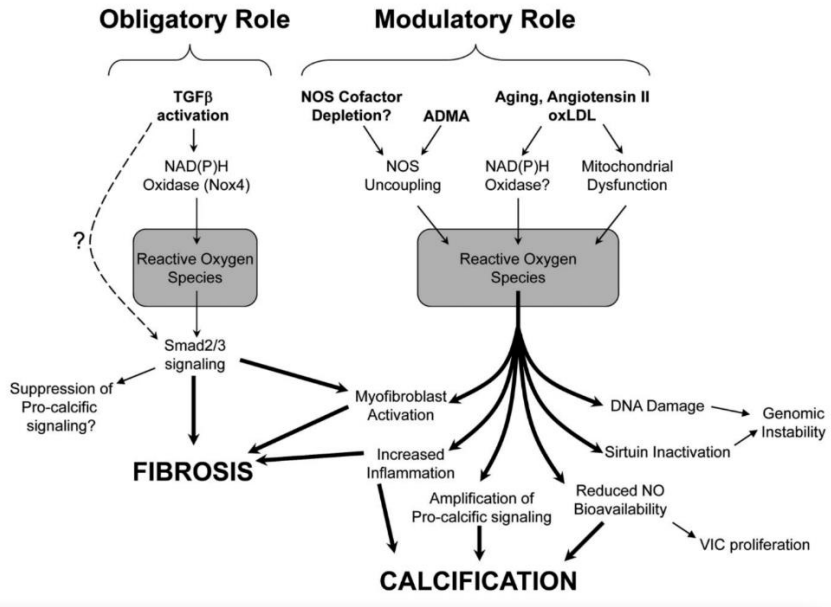

Fig. 3. Mechanisms whereby reactive oxygen species (ROS) may modulate pro-calcific and profibrotic signaling in calcific aortic valve stenosis (Miller et al., 2011).

3.1.1.2. Congenitally-Susceptible Mouse Models

According to various studies, "a congenital bicuspid aortic valve is associated with a significantly elevated risk of CAVD " (Hoffman & Kaplan, 2002).The Notch pathway has been linked to the formation of BAV and CAVD in humans, and a number of mouse studies have been conducted to investigate the functions of Notch and Notch effectors in aortic valve embryonic progress."Notch1 levels are greater in the developing mouse valve than throughout postnatal growth " (Garg et al., 2005). Notch1-null mice are embryonically fatal due to vascular abnormalities; however, mice that are Notch1 heterozygous (Notch1+/-) have five times the amount of calcium in their aortic valves compared to WT controls, but they do not have bicuspid valves (Nigam & Srivastava, 2009). In some investigations, no BAVs were observed in Notch1+/- mice(Nigam & Srivastava, 2009), while others showed rates as high as 6%, all without statistical significance when compared to WT incidence rates (Nus et al., 2011). Mice with VEC-specific homozygous deletion of Notch1 (post-endo-MT, utilizing Nfatc1enCre mice) showed aortic valve thickening, fibrosis, and proteoglycaneous aortic valve leaflet, with a 30% occurrence (Wang et al., 2017). Pleiotrophin (Ptn) and delta-like 1 homolog (Dlk1), a powerful Notch1 inhibitor, are ectopically generated in the endocardial cushions when periostin is missing throughout development. This resulted in a reduction in Notch1 signaling, a significant rise in Runx2, the main transcriptional regulator of osteoblast cellular proliferation, and aortic valve calcification (Tkatchenko et al., 2009). At 10 months of age, Postn-/- mice had an abnormal aortic morphology with Runt-related transcription factor 2 (runx2), osteopontin (OPN), and osteocalcin (OCN) expression, as well as significant valvular calcium deposition (Yoshioka et al., 2006). When compared to WT mice fed the same diet, Postn/ mice with a high-fat diet have reduced valve thickness, macrophage infiltration, myogenic differentiation, circumferential fibrosis, and MMP-2/3 increased expression, possibly reflecting a reduced ability of myofibroblasts and monocytes to adhere to and infiltrate the ECM (Hakuno et al., 2010).There is no correlation between the expression of periostin and chondromodulin-I (ChmI). Increases in valve thickness, lipid buildup, calcification, vascular endothelial growth factor-A (VEGF-A), and angiogenesis are all seen in older ChmI/ mice (Yoshioka et al., 2006). Human bicuspid valves have reduced expression of eNOS in the valvular endothelium (Aicher et al., 2007), and approximately (27-42%) of mice that are defective in endothelial nitric oxide synthase have RC/NC bicuspid aortic valves, which are thought to result from impaired, shear-stress and nitric oxide (NO)-dependent epithelial to mesenchymal transformation and reduction invasion of the endothelial cushion by mesenteric mesenchym (Fernández et al., 2009). In heterozygous Nos3+/- mice, no BAVs were found. By 6 months of age, Nos3-/- mice with BAVs develop fibrosis and leaflet calcification, but even by 18 months, Nos3-/- mice with normal tricuspid aortic valves (TAVs) were just fibrotic, not calcified, and function was unaffected (El Accaoui et al., 2014). A targeted deletion of Gata5 in mice led to BAV and hypoplastic heart development, according to a study. The majority of BAVs in humans are caused by the fusion of the right-coronary and left-coronary leaflets (R-L) or the right-coronary and noncoronary leaflets (R-N) (R-N)."The detected BAVs caused by fusion of the right-coronary and noncoronary leaflets, the subtype associated with more severe valve malfunction in people," the researchers concluded (Laforest et al., 2011). Gata5 controls eNOS and Notch signaling; Gata5-/-mice had substantially lower Nos3 and Notch ligand Jag1 transcription, and the mouse Nos3 promoter has several GATA binding sites. Adult Gata5-/-mice have considerably compromised. aortic valve function (Laforest et al., 2011). Epithelial growth factor receptor (EGFR) signaling pathways are known to control the development of the embryonic aortic valve in mice (Chen et al., 2000), and possibly humans (McBride et al., 2011).

3.2. Large Animal Models

The primary aims of using animal models are to advance human health and to make scientific discoveries that can be translated into practical applications. As they reveal disease characteristics similar to those in humans and provide mechanistic understanding into the biological and pathological processes, large animal models can help accomplish these objectives (Tsang et al., 2016). Larger animal models, as opposed to mice, are more costly to buy, feed, and care for under contemporary animal husbandry circumstances. Complex atherosclerotic lesions, on the other hand, take a longer time to form in mice than they do in humans. CAVD may be studied in large animals such as pigs and rabbits because of their structural and functional parallels to humans, making them useful models for preclinical and clinical investigations (Brousseau & Hoeg, 1999; Cimini et al., 2005; Fernández-Jiménez et al., 2015; Swinkels & Demacker, 1988).

3.2.1. Swine Models

In studies of atherosclerosis, swine are excellent models because they: 1) have comparable systemic hemodynamic factors and cardiac structure, serum cholesterol, lipid metabolism, and a akin genome in size and chromosomal structure to living beings, making swine models attractive for genomic studies; and 2) develop human atherosclerotic lesions on high-fat/high-cholesterol diet and develops naturally with high-fat diets to study CAVD. These characteristics emphasize the swine as an attractive model for studying CAVD and demonstrating the propensity for developing valvular lesions (Dixon et al., 1999; Gerrity et al., 2001; Sider et al., 2014; Skold & Ramsey, 1966). On the other hand, Pigs are not often used because of their large size. Mini pigs that have been genetically modified to have hyperlipidemia and atherosclerosis have just come on the market; they are less expensive to maintain than full-sized pigs. Its pathophysiological processes were examined in detail and found to be identical to human atherosclerosis, which is not the observed in mouse models. (Agarwala et al., 2013; Davis et al., 2014).

3.2.1.1. Nutritionally-Susceptible Swine Models

Research on CAVD has recently begun using Swine models, which have traditionally been employed in studies on atherosclerosis (Sider et al., 2011). According to certain studies, starting a diet early in life may be more successful in creating advanced illness (Gerrity et al., 2001). A swine model of early aortic valve sclerosis was studied by Sider et al, in this study, swine were fed either a typical diet or a high fat/cholesterol (HF/HC) diet for 2–5 months. The coronary aortic valve leaflets of swine given the HF/HC diet established thicker lesions on the aortic side and histologically opaque regions of proteoglycans, collagen, and elastin within the fibrosa layer, comparable to early human CAVD.(Sider et al., 2014). Recently, Go et al. investigated a model for morphological and mechanical changes in the aortic valve in female Yorkshire domestic pigs (Go et al., 2018). The animals were nourished a normal or a high-fat/high-cholesterol (HF/HC) diet for 16 weeks in this experiment. The control group was administered a normal swine feed with 14.5 % protein, 3% fat, and 3.3 Kcal/g of feed. The experimental group was given a high fat, high-fructose diet with 17 % protein, 20% fat, and 4.1 Kcal/g of feed. Aortic valve degradation and calcification were seen in pigs fed a high-fat diet for 16 weeks in this study, which is consistent with the findings of the previous research (Go et al., 2018). Through dietary intervention, a substantial link between endothelial phenotypic heterogeneity, regional CAVD susceptibility, and local hemodynamics was previously discovered in normal and hypercholesterolemic pigs.

The results show that aortic valve calcification largely affects the aortic side of the valve leaflets and is related to a side-specific elevation of eNOS and activated leukocyte adhesion molecule in endothelial cells (ALCAM)

(Guerraty et al., 2010 ; Simmons et al., 2005). Microarray and quantitative Real time polymerase chain reaction (qRT-PCR) were used to compare gene expression in aortic and ventricular side aortic Valve surface endothelial cells (VECs) from adult male swine. The aortic and ventricular VECs were revealed to have side-specific expression differences in this investigation (Simmons et al., 2005). Genes linked with vascular calcification and skeletal development, such as bone morphogenetic proteins, were shown to have increased expression on the aortic side of the valve (BMP-4). In the aortic side VECs, lower expression of proteins known to suppress ectopic calcification, such as osteoprotegerin (OPG), C-type natriuretic peptide (CNP), and chordin, was also found (an inhibitor of the osteoinductive activity of BMPs) (Simmons et al., 2005). Furthermore, "higher expression of antioxidative genes on the aortic side and the absence of differential expression of pro-inflammatory proteins on the aortic side suggests potential protection in the normal valve against lesion development and inflammation," according to the researchers (Butcher et al., 2011; Simmons et al., 2005). In swine, atherosclerotic plaque progress can be enhanced by combining a high-cholesterol diet with nearby formed vascular injury forced by a variety of methods, such as guide-wire-induced injury (De Smet et al., 1998; Granada et al., 2009), endovascular balloon inflation with or without stent deployment, (Thim et al., 2010), partial vessel ligation,(Ishii et al., 2006) in addition to percutaneous intramural injection of cholesteryl esters and human oxLDL after a 2-week balloon angioplasty (Granada et al., 2005; Granada et al., 2007). In addition to reducing the problems in care and high preservation costs linked with the use of swine models by shortening the study's duration, atherogenic diets and vascular injury protocols are also highly relevant models for translational research in the field of percutaneous interventions and cardiovascular imaging animal models (Fuster et al., 2012).

3.2.1.2. Genetically-Susceptible Porcine Models

In swine, naturally, occurring mutations have been used to establish non-diet-induced hypercholesterolemia models for CAVD. The LDLR and/or apolipoprotein genes exhibit mutations in these models. Among the most popular models are (Hasler-Rapacz et al., 1996; Hasler-Rapacz et al., 1998):(1) familial hypercholesterolemia with altered lipid profiles due to an LDLR mutation; In a recent investigation of Familial hypercholesterolemia (FH), a total of 21 female animals were evaluated:4 wild type (WT) swine, 3 one-year-old (1 yo) WT swine, 3 Rapacz FH (RFH) swine, 4 two-year-old (2 yo) adult RFH swine, and 5 three-year-old (3 yo) RFH swine Throughout the trial, the animals were fed a conventional swine diet (consisting of 75.8%, 14.7 percent, and 9.4% of daily calories from carbohydrates, protein, and fat, respectively). Adult RFH showed early signs of CAVD.A substantial thickening of the cusps as well as considerable extracellular matrix alteration, including proteoglycan enrichment, collagen disruption, and elastin fragmentation, lipid oxidation and macrophage infiltration have both increased. Mild aortic valve sclerosis was revealed on echocardiography. Valve microarrays from adult and juvenile RFH mice indicated considerable activation of inflammation-related genes, as well as various similarities and overlaps with atherosclerosis and human CAVD (Porras et al., 2015). (2) LDLR and ApoB mutations cause a second kind of familial hypercholesterolemia. (Grunwald et al., 1999). The Rapacz pig is a wild-type animal with a natural mutation in the ApoB and LDLR genes that was developed through selective breeding of high-cholesterol pigs (Davis et al., 1984). These pigs developed significant hypercholesterolemia, with LDL as the major circulating lipoprotein, within 2-4 years on a normal diet, which has been linked to the development of coronary atherosclerosis (Lee et al., 2017). Their use is restricted because of the considerable time it takes to develop complex atherosclerotic lesions, even when fed atherogenic diets (2–3 years), as well as their large size and weight (> 200 kg). The practice of smaller swine strains, such as the Yucatan mini pig, which progress

humanoid complicated lesions with profuse necrosis, lipid deposits, and significant calcification, reduces these issues in treatment and expensive maintenance costs (Barbeau et al., 1997; De Smet et al., 1998; Gal et al., 1990; Holvoet et al., 1998; Panepinto & Phillips, 1986; Reitman et al., 1982).

3.2.2. Rabbit Models

In 1908, Ignatowski wrote about the first evidence that experimental animals may produce atherosclerosis. He gave rabbits a high-protein diet that included meat, milk, and egg yolk, which caused atherosclerotic lesions to form on the aortic wall (Ignatowski, 1908). Animals including rabbits, mice, rats, guinea pigs, hamsters, birds, swine, dogs, and nonhuman primates have all been produced since then (Ignatowski, 1908).

3.2.2.1. Nutritionally-Susceptible Rabbit Models

Rabbits have also been used to investigate aortic valve disease in its various stages, from early to advance. A hypercholesterolemic diet is commonly used to induce pathogenesis. (Guerraty & Mohler, 2007) Capillary stenosis in rabbits was explored by Mourino-Alvarez and colleagues. Animals considering 2–2.5 kg were split into two separate groups: the control group received standard rabbit chow, while the pathological group received chow enhanced with 1 percent cholesterol and 50,000 IU/kg vitamin D2. The experiments lasted 12 weeks in each group. (Drolet, Couët, & Arsenault, 2008; Mourino-Alvarez et al., 2018) There was an increase in cholesterol in the diseased group, as well as thicker AVs on echocardiography. These features have been seen before in individuals with CAS (Akat et al., 2010; Kamath & Pai, 2008; Mourino-Alvarez et al., 2018).

Hematology showed considerable calcium deposits, a profusion of macrophages (RAM11-positive cells), and a profusion of -actin, a marker for smooth muscle cells including myofibroblasts-lactate dehydrogenase B chain (LDHB) and tropomyosin -1 chain (TPM-1) both revealed the similar pattern in plasma and tissue, whereas transitional endoplasmic reticulum ATPase (TERA) was upregulated in tissue and downregulated in both rabbit and human plasma (Mourino-Alvarez et al., 2018). The effect of a high-fat, high-cholesterol diet supplemented with vitamin D on the growth and development of heart valves as far back as 2003. The male New Zealand White rabbits utilized in this experiment were divided into the following groups: a control group fed regular food with no dietary supplements; b) animals fed food enriched with 0.5 percent cholesterol plus 50,000 IU of vitamin D2 per day; c) animals fed food enriched with 0.5 percent cholesterol plus 50,000 IU of vitamin D2 per day; and d) animals fed food enriched with 0.5 percent cholesterol plus 50,000 IU of vitamin D2 per day (Drolet et al., 2003), Group 1 exhibited no change in cholesterol levels after 12 weeks; however groups 2 and 3 had significantly higher levels. Vitamin D2 had a surprising effect on cholesterol levels, despite the fact that both cholesterol consumption and vitamin D2 levels were the same. In comparison to groups 1 and 2, calcium levels were somewhat higher in group 3. According to echocardiography, the Aortic valve area (AVA) decreased by 36%, the maximal gradient increased by 300%, and the mean gradient increased by 107% (all p 0.05) (Drolet et al., 2003).There was a clear correlation between vitamin D3 levels and the progress of AVS in a sample of chronic renal failure patients, as revealed by (Malergue et al., 997). After controlling for age, gender, overall creatinine clearance, raised calcium-phosphate product in individuals with adequate renal function was likewise linked with the severity of AVS (Mills et al., 2004). Eight weeks of treatment with vitamin D2 alone at 25,000 IU/4 days per week enhanced the amount of aortic valve stenosis (AVS) in male New Zealand white rabbits. An enhanced aortic valve backscatter (AVBS), enhanced transvalvular velocity, and higher pressure gradient were seen in rabbits treated with Vitamin D2. The calcification, lipid accumulation, and macrophage infiltration of the valves were all present. An increase in intravalvular thioredoxin-interacting protein (TXNIP) content was found in the endothelium. According to

histology results, early AVS in humans is associated with endothelial dysfunction and redox stress. Loss of nitric oxide regulation of TXNIP expression may lead to the development of AVS (Ngo et al., 2008). AVS therapy may also be tested on rabbits, which have shown to be a valuable tool. According to Rajamannan and colleagues (Galante et al., 2001), osteopontin and osteoblast gene markers (alkaline phosphatase, osteopontin, and osteoblast lineage-specific transcription factor (Cbfa-1) in the cholesterol-fed rabbits compared to the control rabbits were associated with an atherosclerosis proliferative valve lesion. Treatment with HMG CoA reductase inhibitors lowered the levels of all indicators except hsCRP (Jialal et al., 2001; Rajamannan et al., 2002). An animal model of Aortic valve sclerosis was used to test the effects of dietary modification and statin medication on the tissue response to therapy. Male New Zealanders were assessed in this study. White rabbits were fed a 0.25 % cholesterol-supplemented diet for six months in order to maintain cholesterol levels of 500 mg/dl, and they were then tittered (0.125–0.25 %). Six rabbits were fed as a control group. By 15 months, the cusps of five cholesterol-fed rabbits had thickened due to lipid deposition, macrophage infiltration, and osteopontin expression. The remaining cholesterol-fed rabbits were divided into four groups. Rabbits were fed food enriched with 0.125 % cholesterol. Standard chow was given to rabbits receiving only nutritional treatment, whereas rabbits receiving only statin medication got a pill containing atorvastatin 2.5 mg/kg every day (Hamilton et al., 2011; Rajamannan et al., 2005; Rajamannan et al., 2002; Stock, et al., 2005). Statin and dietary treatment rabbits received 2.5 mg/kg per day of atorvastatin calcium in regular chow for an additional 15 months, along with 0.125 % cholesterol-supplemented chow. Rabbit cusps showed a substantial rise in osteopontin expression, collagen deposition, lipid, macrophage infiltration, and osteopontin expression by 30 months on the atherogenic diet alone. Increases in CD3+ lymphocyte invasion and calcification were also noted. However, after statin therapy, osteopontin expression and immune cell infiltration significantly decreased in the valve cusps. Unfortunately, calcification and lipid retention persisted in all of the treated valves. We conclude that the cellular response to statin medication does not fully reverse the sclerotic process in established AVSc (Hamilton et al., 2011). Arishiro et al. discovered that taking ARB (olmesartan, 1 mg/kg/day) for the previous four weeks caused atherosclerotic changes in the aortic valves of rabbits fed a 1% cholesterol diet for eight weeks. Olmesartan treatment dramatically lowered lipid deposition, macrophage accumulation, osteopontin expression, angiotensin-converting enzyme, and alpha-smooth muscle actin-positive myofibroblasts, enhanced eNOS expression, and decreased messenger ribonucleic acid for Cbfa-1 mRNA production. Endothelial integrity was maintained and trans differentiation of valvular fibroblasts into myofibroblasts and/or osteoblasts in the valve leaflets was avoided on the lesion-prone aortic side of the valve (Arishiro et al., 2007).

3.2.2.2. Genetically-Susceptible Rabbit Models

Despite the fact that feeding rabbits a high-fat diet for the long term has unfavorable side effects and increases mortality owing to liver toxicity (Fuster et al., 2012), genetically engineered rabbits that induce spontaneous atherosclerotic lesions have been established. For instance, (1) An LDLR-deficient model is the Watanabe heritable hypercholesterolemic rabbit (WHHL) (Burnstock & Aliev, 1998; Rajamannan et al., 2005; Shiomi & Ito, 2009);(2) Rabbits from St. Thomas Hospital that have high cholesterol and triglycerides (Beaty et al., 1992); (3) rabbits with changed lipid profiles, such as induced human ApoB100 (Beaty et al., 1992) or Apo(a).(Fan et al., 2001). The Watanabe heritable hyperlipidemic (WHHL) rabbit (Watanabe, 1980), is the most widely used , and it was utilized in a CAVD investigation to show that atorvastatin reduces hypercholesterolemia-induced AV calcification, which is mediated in part via the Lrp5/-catenin pathway. This developmental mechanism could play

a role in the disease's signaling pathway (Caira, et al., 2005). Recently found a rabbit model of familial hypercholesterolemia and atherosclerosis, the Watanabe heritable hyperlipidemic (WHHLMI) rabbit, which is prone to myocardial infarction (Hara et al., 2018). The research demonstrated age-dependent progression of aortic valve sclerosis in (WHHLMI) rabbits fed normal chow or without a high-cholesterol diet or vitamin D supplement. This study used WHHLMI rabbits (Hara et al., 2018; Shiomi et al., 2003), aged 20 or 30 months, as well as control Japanese White rabbits, were assessed. WHHLMI rabbits that were 20 and 30 months old had comparable lipid profiles. In comparison to twenty-month-old WHHLMI rabbits, the aortic valve area and maximum transvalvular pressure gradient were much smaller in the thirty-month-old WHHLMI rabbits. A macroscopic study at 30 months revealed thickened and deteriorated valve leaflets. Histological analysis at 30 months showed thicker leaflets with calcified nodules. Real-time polymerase chain reaction (PCR) analysis of 30-month-old rabbits revealed elevated expression of molecules involved in calcification, including osteopontin (OPN), Sox9 (Caira et al., 2006), Bmp2, receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), and transcription factor for osteoblast differentiation (Runx2) (Wirrig & Yutzey, 2011). WHHLMI rabbits may serve as useful in vivo models of early AS (Hara et al., 2018).

CONCLUSION

It is crucial to employ carefully chosen and well-given animal models in order to better understand the pathophysiology of CAVD. In order to better comprehend the pathophysiology of CAVD, it is crucial to use animal models that are well chosen and adequately administered. Human CAVD pathophysiology has been replicated in many animal models, allowing researchers to undertake studies that would otherwise be hard or impractical to carry out on patients themselves. New models and better knowledge of the value of current models have pushed animal model-based research to new heights in this discipline. Experimental models for this illness have been found in a wide range of animal species. In spite of the fact that several animal models are being employed, none of them should be called an excellent model of human illness. Translational research studies can be more readily applied to people since CAVD in swine carefully resembles the key morphological and biochemical aspects of human CAVD, hence findings from large animal models can be more simply extrapolated to humans. Researchers working with huge animals face a variety of significant challenges, including problems in handling and the high costs of maintaining them. Genetic advances have made it possible to develop mini pigs with human-like physiology and ease of handling that are more human-like than non-human primates, and that have anatomical and physiological characteristics that are strikingly similar to those of humans, including lipoprotein metabolism and atherosclerotic pathophysiology. There are several benefits to using small animals for experiments (e.g. simple handling and inexpensive cost), but they don't often acquire the advanced susceptible lesion that is typical of human patients with severe CAVD. The utilization of all currently available animal models will definitely continue to allow for significant advancements in CAVD research, which should lead to better CAVD treatment, prevention, and diagnosis.

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Conflict of Interest

The authors have declared no conflict of interests exist regarding publication of this paper.

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