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Animal Models of Calcific Aortic Valve Disease

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Abstract

Calcific aortic valve disease (CAVD) is a slow, progressive disorder that encompasses from "early sclerosis, characterized by leaflet thickening without left ventricular outflow obstruction, to late stenosis with stiffen leaflets, flow is obstructed, and compromised cardiac function". CAVD was historically accepted as passive "senile" or "degenerative" process affecting a normal trileaflet or congenital bicuspid valve. But recent scientific discoveries have also shown that it is an active and highly cell-mediated pathobiological process that shares many risk factors with atherosclerosis. Much evidence states that calcific aortic valve disease is not a predictable consequence of aging and may be associated with specific risk factors. However, no drug regimes currently exist to prevent or halt the progression of CAVD in a clinically significant way and the only effective therapy is a surgical valve replacement. Therefore, there is an unmet scientific need to determine pathobiological mechanisms of CAVD and to identify new approaches to treat CAVD. Animal models are emerging as vital tools to this end, facilitated by the advent of new models and improved understanding of the utility of existing models. In this review paper, we will describe the most widely used small and large animal models that have been used to study CAVD.

Keywords: Calcific aortic valve diseases; Aortic valve sclerosis; Calcification; Aortic valve stenosis; Atherosclerosis; Animal models

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Abbreviations: CAVD: Calcific Aortic Valve Disease; AS: Aortic Stenosis; AVSc: Aortic Valve Sclerosis; BAV: Bicuspid Aortic Valve; LDL-C: Low-Density Lipoprotein-Cholesterol; HDL-C: High-Density Lipoprotein-Cholesterol; Lp (a): Lipoprotein (a); Ox-LDL: Oxidized Low-Density Lipoprotein; α-SMA: α-Smooth Muscle Actin; TNF-α: Tumor Necrosis Factor-α; IL-2: Interleukin-2; MMP: Matrix Metalloproteinases; ACE: Angiotensin Converting Enzyme; ICAM-1: Intracellular Adhesion Molecule-1; VCAM-1: Vascular Cell Adhesion Molecule-1; VECs: Valvular Endothelial Cells; VICs: Valvular Interstitial Cells; ECM: Extracellular Matrix; ANP: Atrial Natriuretic Peptide; BNP: Brain Natriuretic Peptide; β-MCH: β-myosin Heavy Chain; OCN: Osteocalcin; OPN: Osteopontin; PTN: Pleiotrophin; Dlk1: Delta-like 1 Homolog; Runx2: Runt-related Transcription Factor 2; ChmI: Chondromodulin-I; eNOS: Endothelial Nitric Oxide Synthase; SRY: Sex Determining Region Y-Box 9; MSX2 Msh Homeobox 2; BMP-4: Bone Morphogenetic Proteins; OPG: Osteoprotegerin; CNP: C-type Natriuretic Peptide; VEGF-A: Vascular Endothelial Growth Factor-A; ALCAM: Activated Leukocyte Adhesion Molecule; TPM-1: Tropomyosin α-1 chain; LDHB: L-lactate Dehydrogenase B chain; hsCRP: Highly Sensitive C-Reactive Protein; EGFR: Epithelial Growth Factor Receptor; PCNA: Proliferation Cell Nuclear Antigen; HMG CoA: β-Hydroxy β-methylglutaryl-CoA

Introduction

Background

Calcific aortic valve disease (CAVD) is a major public health problem in the world, in 2017; there were an estimated 12.6 million cases of CAVD Worldwide, particularly among people 70 or older, the age group in which disease burden is largest. In 2017, there were an estimated 102,700 CAVD deaths Worldwide. CAVD mortality

rates were highest in regions with a high sociodemographic index (SDI), such as Western Europe, the US, Canada, Chile, Argentina, Australia, and New Zealand [1]. Studies have shown that CAVD is currently the leading cause of heart valve disease in industrialized and developing countries [2]. CAVD comprises primary sclerosis, characterized by thickening of the cusps without left ventricle outflow obstruction, to late stenosis with stiffened leaflets, flow is obstructed, and impaired heart function [3]. It was believed to be a passive "senile" or "degenerative" process affecting a normal trileaflet or congenital bicuspid valve, and it is the most common cause of Aortic stenosis (AS) in adults [4], but in the last decade, various studies have shown that several notable molecular processes are involved in the development of this disorder and recent scientific findings have also shown that it is an active and very high level cell-mediated pathobiological process [5]. The results of both are remarkable: sclerosis is associated with a 50% increased risk of cardiovascular death and myocardial infarction and the prognosis for patients with stenosis is very poor (20 – 60% mortality) [6]. CAV D is a part of valvular heart disease 50% and is counted as 3rd most common disease of the heart following coronary artery disease and hypertension [7], and the most common cause of surgical heart valve replacement or transcatheter heart valve replacement [8]. Risk factors for CAVD are akin to those for atherosclerosis and contain bicuspid aortic valve, older age, male sex, smoking, hypercholesterolemia, hypertension, kidney failure, and diabetes mellitus [7]. The available evidence shows that approximately 50% of patients with AVS do not have clinically significant atherosclerosis. Importantly, mechanical injury caused by hemodynamic stress during constant opening and closing of the leaflets is considered an important risk factor for AVS. The congenital bicuspid aortic valve, which contributes to a high mechanical stress, reportedly leads to a rapid progression of AVS in younger patients with low atherosclerotic risk. Furthermore, the non-coronary leaflet is more likely to be affected compared to other leaflets due to increased mechanical stress as a consequence of the absence of diastolic coronary flow [9]. Tissue prosthetic valves have also been observed to calcify early through a process that resembles the natural history of bicuspid aortic valves [10]. The prevalence of Aortic valve sclerosis (AVSc) presents in 25% to 30% of patients aged >65 years, 40% of those aged >75years [11,12] and in up to 75% of those aged >85 years, with severe AS reaching a 3% prevalence in the population over 75 years [13]. Evidence from other studies suggests that chronic inflammation is critical not only for atherosclerotic calcification but also for CAVD. "This is revealed in human disease by the existence of macrophages, T cells, sub endothelial oxidized low-density lipoprotein (LDL) deposits and -Smooth muscle actin (-SMA) positive cells, associated with late CAVD, these are found within primary lesions (~22%) infrequently" [6,14-16]; increased superoxide and hydrogen peroxide [17]; "decreased endothelial nitric oxide synthase levels and increased oxidative stress" [18]; activation of complement [19];"elevated expression of tumor necrosis factor-α(TNF-α),active mast cells, matrix metalloproteinases (MMP-1,-2,-3,-9)" [20], "interleukin-2 (IL-2), angiotensin converting enzyme (ACE), angiotensin II (AngII), angiotensin II type-1 receptor (AT1R) and chymase" [14]; "and VEC expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)" [15], and E-selectin [21]. Inflammatory processes are associated with, and may drive, the valve ECM abnormalities that are characteristic of CAVD, containing leaflet thickening, collagen turnover, and fibrosis [22]; accumulation of proteoglycans and hyaluronan, fragmentation of elastin [6]; and calcification [17,23]. Finally, it is a maladaptation of the valve ECM that causes valve stiffness and dysfunction. While many features of human CAVD are well described (particularly for late-stage human CAVD), there is little understanding of early sclerosis. The many reports explaining early human valvular lesions suggest similarities to early atherosclerotic lesions [24-27], but most of the patients more than 60% with CAVD do not have clinically significant atherosclerosis [28], suggesting divergent processes.

Biology of the normal heart valves

In most of the humans, the aortic valve is composed of three semilunar cusps (tricuspid aortic valve) and sits at the junction between the left ventricular outflow tract and the aortic root. It is a supple membrane that opens and closes with each heartbeat more than 100,000 times a day to maintain unidirectional blood flow from the left ventricle to the systemic and coronary circulations [29,30]. In healthy humans, the valve leaflets are less than a millimeter thick and are covered by an endothelial layer, which is comprised of valvular endothelial cells (VECs) on both sides (ventricularis and fibrosa). The interstitium of the valve consists of three distinct layers: the laminae fibrosa, spongiosa, and ventricularis. The main cell population found here is composed of valvular interstitial cells (VICs) [31]. To encounter their functioning requisites under these demanding circumstances, the thin, flexible leaflets is arranged into three distinct layers of extracellular matrix (ECM (1) the lamina fibrosa on the aortic side of the leaflet ,representing the largest part of the valve and the load-bearing structure, mostly comprises circumferentially aligned collagen fibres (type 1 collagen fibrils) that provide most of the mechanical strength of the leaflets;(2) the Spongiosa is found at the middle of the leaflets. It contains a loose matrix of mucopolysaccharides, serving as a cushion to resist compressive forces and facilitate movements between the fibrosa and ventricularis during leaflet motion; and (3) the ventricularis layer on the ventricular side, consists of collagen and radially aligned elastin and contributes to flexibility, allowing for changes in leaflet shape during opening and closing [29,32,33]. Isolated macrophages often can be found in the ventricularis and spongiosa of normal adult aortic valve leaflets but are not present in the normal fibrosa [27]. Under normal conditions, all three layers are avascular with no cellular infiltrates and are innervated by adrenergic and cholinergic neural networks. To remain pliable, the aortic valve must undergo continuous repair throughout life [34]. The cellular components of the aortic valve encompass a heterogeneous population of Valvular endothelial cells (VECs) and Valvular interstitial cells (VICs), which sustain valve homeostasis and structural leaflet integrity. VICs, the most plentiful cell type in the heart valve, play a key role in CAVD progression [35]. VICs can be divided into five forms: mesenchymal VICs, quiescent VICs, progenitor VICs, active VICs and osteoblast VICs. Mesenchymal VICs are generated during valve development from endothelial cells of the endocardial cushion via epithelial-mesenchymal transition [36]. Quiescent VICs (qVICs) are present in the normal

valve and maintain its normal structure and function [36]. Progenitor VICs (pVICs) are stem cell VICs that can proliferate in response to injury. The inflammatory response to a pathological stimulus such as mechanical stress or lipids transforms qVICs into aVICs. The aVICs have myofibroblast characteristics including contractility, stress fibers and display the striated-muscle isoform of myosin heavy chain and have a profibrotic function. aVICs can be further transformed into obVICs that promote calcification [36]. VECs cover the surface of the heart valve to form an endothelial monolayer, and are unique in that they can undergo endothelial-to-mesenchymal transformation (EndMT) a critical process in developmental valvulogenesis [35].

Pathobiology of CAVD

The pathobiology process of CAVD can be broadly split into two discrete phases, the initiation phase and the propagation phase. Initiation phase show endothelium injury, lipid accumulation and inflammation whereas the propagation phase covers fibrosis, calcification and neo angiogenesis. CAVD is initiated from endothelium dysfunction that can be caused by turbulent flow with low shear stress [37-39]. Endothelium injury permits LDL, Lp (a) and inflammatory cells such as monocytes and lymphocytes to enter the valve. LDL and Lp (a) become oxidized and to accumulate in the valve; this leads to marked secretion of inflammatory cytokines and chemokines from inflammatory cells and valvular interstitial cells. In the valve, monocytes differentiate into macrophages that engulf lipoproteins and form foam cells. Inflammation stimulates the activation of valve interstitial cells (VICs) that further mediate the fibro-calcific process. Activated VICs (aVICs) promote collagen production and a disorganization of the normal structure of the valve, leading to extracellular matrix remodeling. During disease progression, aVICs transform into osteoblast VICs (obVICs) that release osteogenic markers. The propagation phase is self-perpetuating as calcification causes even more mechanical stress and damage which further leads to more calcification [40].

CAVD animal models

Animal models are tools, which are valuable for understanding the initiation and progression of CAVD in vivo, as well as for judging the effects of various therapeutic interventions. To be most effective; models should mimic human disease and the conditions in which human CAVD develops. The most common species used to model CAVD are swine, rabbit, and mouse. Of these, only swine develops CAVD naturally with age, but this process is slow and is usually accelerated by diet-induced hypercholesterolemia, others, such as rabbits and mice; have not been shown to develop lesions naturally but are responsive to diet-induced hypercholesterolemia, and mice require a genetic predisposition to promote advanced disease [32]. “Although no model can entirely replicate the complexities seen in human pathologies, they are decisive in assessing mechanisms of disease, as well as evaluating novel diagnostic technologies, preventions and therapies” [41,42]. This article reviews swine, rabbit and mouse models of CAVD, together with their pros and cons of the most commonly utilized animal models of CAVD, which are summarized in **(Tables 1- 3).**

Small animal models

The pathogenesis of the disease of the small animals models are very interesting to investigate, as well as to test some treatment strategies. Models such as rats and mice are especially cost-effective and easy to manipulate due to their small size. Such small animals are increasingly used to investigate CAVD [46].

Mouse models: The main advantages of this species are the short gestation period and low cost of breeding and housing. The knowledge of its genome, the ability to modify it and the rapid data acquisition of genomic modification make attractive the use of mice for studying diverse mechanisms that are affected during the development of cardiovascular diseases [47,48].

Nutritionally and genetically-susceptible mouse models: Mice need to be genetically modified and sometimes require dietary intervention to induce advanced CAVD. Mice with LDL receptor deficiency (Ldlr−/−) are mostly used [46]. Drolet et al. studied early degenerative aortic valve stenosis (AS) in adult wild-type (WT) and in low-density lipoprotein receptor-deficient (LDLr−/−) mice that were fed with or without a high-fat/high-carbohydrate (HF/HC) diet for four months with a low cholesterol content; Wild-type mice on a HF/HC diet became mildly hypercholesterolemic, obese, and hyperglycemic(mild metabolic syndrome) compared to WT+ normal diet, and LDLr−/−mice on a HF/HC diet developed a severe metabolic syndrome with signs of early degenerative(AS) on echocardiography. Both WT and LDLr-/- mice revealed smaller valve areas and higher transvalvular velocities compared to WT+ normal diet. Aortic valve leaflets

Table 1 Advantage and disadvantage of swine model of CAVD [103,135].

Advantages	Similar haemodynamics and pathogenesis to humans: Lesion location, morphology and content
	Akin heart size and cardiovascular anatomy
	Alike lipid metabolism, except for Apo II deficiency in porcine
	Highly defined genotypes for genetic manipulation Minipig version offer option with lower cost
	Not like mouse and rabbit, it can spontaneously develop atherosclerosis with an accelerated rate when fed with atherogenic diet
	Simple to carry out imaging, e.g., Ultrasound, CT and MRI compared to smaller species
Disadvantages	Toxic diet required for induction of atherosclerosis
	Large in size, which limits its practical use
	High cost of purchase and maintenance [135]
	Difficulty in handling (except for minipig strains)
	Atheroma formation requires longer time than in other species [97]

Table 2 Advantage and disadvantage of Rabbit models of CAVD.

Advantages	Lipoprotein metabolism relatively akin to humans (except for hepatic lipase deficiency in rabbits)
	Easy to handle and maintain, no special requirements [97]
	Akin morphology of lesion development
	Low economical cost for maintenance due to its small size
	High availability
	Larger artery allow clinical evaluation: Ultrasound and MRI can be applied to determine plaque composition and its vulnerability
	Good response to dietary cholesterol
	Availability of hyperlipidemic mutant strains
	Large enough to permit physiological experiments [135]
Disadvantages	Highly abnormal diet required for the development of hypercholesterolemia and atherosclerosis
	Long-term high-cholesterol feeding induces massive inflammation and hepatic toxicity due to Low hepatic lipase activity [97]
	Does not always respond to dietary cholesterol
	Diverse cardiovascular physiology with human: HDL as the predominant plasma lipoprotein, absence of Apo AII, low hepatic lipase activity
	Plaque lesion dissimilar with human: foam cells with more fatty streak and macrophage rich, advanced lesion (e.g., Fibrosis and haemorrhage and ulceration) are not seen
	Diverse predilection site: Atherosclerotic plaque preferentially deposited in aorta, iliac arteries

Table 3 Advantage and disadvantage of Mice models of CAVD.

Advantages	Low price
	High accessibility
	simple to handle and maintain
	Manageable breeding
	Well-established protocols for directed genetic manipulation
	Well-defined genetics and availability of inbred strains
	Short generation time
Disadvantages	High resistance to atherosclerosis development in wild-type mice.
	Lack of plasma CETP activity
	Most cholesterol is transported through HDL particles
	The small size of mice limits frequent blood sampling and dissection of small arteries
	Requirement of genetically modified mice (e.g., apoE-deficient, LRLD-deficient)
	Plasma lipid profile markedly dissimilar to humans
	Alterations in the morphology of the arterial wall due to the small size of murine vessels (e.g., reduced thickness of the medial layer, lack of vasa vasorum)
	Lack of plaque rupture and luminal thrombosis in most vessels [97]

were more echogenic, thicker and infiltrated with lipids and macrophages in both HF/HC groups. This study confirmed that multiple atherogenic factors commonly found in humans (obesity, hyperglycaemia and mild dyslipidaemia) may play a significant role in the development of AVS, and therefore isolated hypercholesterolemia should not be the sole target of therapy [49]. Weiss et al., have evaluated a Low-density lipoprotein receptor-deficient apolipoprotein B-100-only (LDLr-/-ApoB100/100) hypercholesterolemic old mice that were fed normal chow. By 20 months, LDLr-/-ApoB100/100 mice exhibited functionally significant sever AS, with reduction in valve area (>50%) compared to controls, on both echocardiography and angiography. LDLr-/-ApoB100/100, mice were also developed left ventricular hyperthrophy and decreased ejection fraction due to severe hemodynamic effects compared with LDLr-/-ApoB100/100 without aortic stenosis. Von Kossa staining showed abundant mineralization in LDLr-/-ApoB100/100 mice. Furthermore, Superoxide was present more plentiful in valve tissue of mice with aortic stenosis indicative of the onset of oxidative stress which provides a well-recognized association between tissue oxidant stress and valve disease [50] recently, Quang et al. Have established the type 2 diabetes mellitus prone LDLr−/−/ApoB100/100/IGF-II mouse model of calcific aortic valve disease. In this model mice that were fed a high fat/sucrose/cholesterol (HFSC) diet for 6 months revealed significant AS, calcification, mineralization of the aortic leaflets and the presence of inflammatory infiltrates (mainly macrophages) compared to control. The were also showed upregulation of hypertrophic genes atrial natriuretic peptide, brain natriuretic peptide, β-myosin heavy chain (anp, bnp, β-mch) in myocardial tissues and of osteogenic genes (spp1, bglap, runx2) in aortic tissues of diabetic mice [5]. A particularly notable feature of this study was that 80% of diabetic LDLr−/−/ApoB100/100/IGF-II and 40% of nondiabetic LDLr−/−/ApoB100/100/) mice developed AS after 6 months of HFSC feeding. It is noteworthy that the proportion of non-diabetic LRKOB100 mice that developed AS in the present

study (40%) was similar to that previously observed (33%) by the Weiss et al [50]. while, in this study, AS was induced in a much shorter period (6 versus 20 months), which can be explained by the use of a cholesterol-enriched diabetogenic diet (HFSC diet) versus a standard diet used by Weiss et al [50]. Scatena et al also investigated male LDLr-/-:ApoB100/100 mice. In this investigation, the were randomly assigned to two groups fed either a diabetogenic, procalcific diet (DB; Bio-Serv., 1.25% cholesterol, 57.5% kcal fat, 27.4% kcal carbohydrate) or normal chow (NC) as dietary control. By 14 months, LDLr-/-ApoB100/100 mice fed the DB diet revealed 77% hemodynamically significant AS, thickened leaflets and calcification. In comparison, normal chow (NC) fed LDLr-/-ApoB100/100 mice had 38% incidence of AS, thinner valve leaflets and very little valve calcification. LDLr-/-ApoB100/100 mice DB fed were also developed T2DM and metabolic syndrome when compared with normal chow fed LDLr-/-ApoB100/100 mice. Finally, mice fed the DB diet with AS revealed significantly reduced ejection fraction and fractional shortening enhanced valve interstitial cell (VIC) matrix calcium deposition [7]. In addition to LDLr-/- mice, other genetically modified and widely used model is the "endogenously hyperlipidemic" [51] ApoE-deficient (Apoe-/-) mouse [23,52,53], which allows receptor-mediated clearance of very-low density lipoprotein (VLDL) from the circulation. Previous research showed the effects of lipids in CAVD in a second common genetic model of Apolipoprotein E-deficient (Apoe-/-) mice. They achieved similar inflammatory changes as in humans, with, recurrent apoptotic cell death, αSMA, osteocalcin (OCN) and chemokine expression, macrophage and T-cell infiltration, nodular calcifications, mild regurgitation, and significant increases in transvalvular velocity in the aortic valve of (Apoe-/-) mice [23]. Zeadin et al.,have evaluated additional version of the Apoe-/- mice to test the effects of adipocytokine leptin on both valvular calcification and lesion size. However, leptin treated mice failed to exhibit hypercholesterolemia and atherosclerotic lesion size change. In addition, leptin treated mice also showed significantly increased valvular calcification and ALP- positive staining and associated with an increase expression of the osteoblast-specific markers (osteocalcin (OCN) and osteopontin (OPN) [53]. According to the investigation of Srivastava et al, "the effects of acrolein, a dietary aldehyde generated during inflammation and oxidative stress, on atherosclerosis". In this investigation, male Apoe-/- mice were fed (2.5 mg/kg/day) for 8 weeks. After 8 weeks mice were exposure to acrolein significantly developed hypercholesterolemia, lipid and macrophage infiltration, and significantly increased E-selectin and PAI-1 levels. These results suggest that exposure to acrolein causes platelet and endothelial activation in vivo [52]. Therapeutic like rosuvastatin and lithium chloride have been shown experimentally to have anti-inflammatory effects on the aortic valve of Apoe-/-mice fed high fat/cholesterol, as a significant decrease in macrophage infiltration and vascular cell adhesion molecule- 1 (VCAM-1) expression was detected [54,55]. In addition to nutritionally and genetically susceptible mouse models, Honda et al, established a novel model of mechanical wire injury, where a spring guidewire is inserted through the right common carotid artery of C57BL/6 mice into the left ventricle of the heart under echocardiographic guidance. The wire is then twisted to create endothelial damage. Induction of AVS can be detected by measuring the velocity of blood flow by echocardiography. Echocardiography showed increased aortic blood flow velocity, and decreased left ventricular fractioning shortening, compared with sham mice. Furthermore, increased production of reactive oxygen species, expression of inflammatory cytokines and osteochondrogenic factors and valvular calcification [9]. This approach has recently been corrected upon to produce effectively reliable results [56]. It is now possible to provoke mild, moderate, or severe stenosis by altering the wire type, tip angle, and the number of rotations [56]. Mouse aortic anatomy differs from humans in that they do not have a trilayer architecture [57]. It is, therefore, fundamentally necessary to reproduce any findings from mice in human valves or human cells. Fujisaka et al have found that administration of high-dose Ang II (1000 ng/kg/min) to Male ApoE-null mice for 4 weeks significantly revealed aortic valve thickening, endothelial disruption and increased myofibroblasts infiltration when compared with control group. Furthermore, these phenomena were inhibited by treatment with olmesartan, an Ang II type 1 receptor blocker. Olmesartan also suppressed aortic diameter dilation in ApoE-deficient mice [58]. Rattazzi et al Recently compared the effect of warfarin and rivaroxaban on the progression of aortic valve sclerosis in ApoE null mice. In this study the ApoE-/- mice were divided in three groups: 1) controls group receiving Western-Type Diet (WTD; 2) Warfarin group receiving 3mg warfarin; and 3) rivaroxaban group receiving 5 mg rivaroxaban/kg/day for 8 weeks. Histologic evaluation revealed mice treated with warfarin significantly developed calcium deposition on the aortic valve leaflets, with higher degenerative calcification of aortic valve as compared to mice treated with rivaroxaban. In summary, this novel study showed a safer profile of rivaroxaban on the progression aortic valve calcification risk [59].

Congenitally-susceptible and developmental mouse models: Multiple studies have confirmed that "a congenital bicuspid aortic valve is associated with considerably increased risk of CAVD" [60]. The Notch pathway has been concerned to cause BAV and CAVD development in human, and a number of mouse studies have been undertaken to explore the roles of Notch and Notch effectors in embryonic development of the aortic valve. "The normally developing mouse valve shows higher Notch1 levels than during postnatal growth" [61]. "Notch1-null mice are embryonically lethal due to vascular defects, however mice heterozygous for Notch1 (Notch1+/-) fed a Western diet with 0.2% cholesterol for 10 months exhibit five-fold greater aortic valve calcification than WT controls, but do not exhibit bicuspid valves" [62]. The occurrence of BAVs in Notch1+/- mice is rare, some studies have reported no occurrences [62], while others have reported incidence rates of up to 6%, but without demonstration of statistical significance vs. WT incidence rates [63]. In mice with VEC-specific homozygous deletion of Notch1 (post-endo-MT, using Nfatc1enCre mice), BAVs have an incidence of ~30%, with LV hypertrophy and impaired LV function, functionally-significant aortic stenosis, and thickened, fibrotic, and proteoglycaneous aortic valve leaflets [64]. Periostin is highly expressed in the endocardial cushions during embryogenesis, and its absence leads to ectopic expression of the proosteogenic

growth factor pleiotrophin (Ptn) and overexpression of delta-like 1 homolog (Dlk1), a negative regulator of Notch1. This resulted in suppression of Notch1 signaling, strong induction of the central transcriptional regulator of osteoblast cell fate Runx2, upregulation of osteopontin and osteocalcin expression, and subsequent calcification of the aortic valve [65]. At 10 months of age, the aortic valve of Postn-/- mice exhibited a severely deformed bicuspid-like morphology showing expression of Runx2, osteopontin (OPN), and osteocalcin (OCN), along with significant valvular calcification (von Kossa) [65]."Paradoxically, when Postn−/− mice are fed a high-fat diet for four months, they show decreased valve thickness, macrophage infiltration, myofibroblast differentiation, annular fibrosis, and MMP-2/13 expression levels when compared to the same regimen as WT mice, perhaps reflecting a reduced ability of myofibroblasts and macrophages to adhere to and infiltrate the ECM" [20]. Periostin expression is mutually exclusive to that of chondromodulin-I (ChmI), an antiangiogenic factor. Aged ChmI−/− mice exhibit increases in valve thickness, lipid accumulation, calcification, VEGF-A, and angiogenesis [66]. In humans, expression of eNOS in the valvular endothelium is considerably reduced in bicuspid valves [67], and approximately (~27- 42%) of mice reported that are deficient in endothelial nitric oxide synthase (eNOS−/−) are born with RC/NC bicuspid aortic valves [68], which are believed to occur as a result of impaired, shear-stress- and nitric oxide (NO)-dependent epithelial-to-mesenchymal transformation and reduced invasion of the endocardial cushion by mesenchymal cells [69]. BAVs were not reported in heterozygous Nos3+/- mice. Nos3-/- mice with BAVs develop fibrosis and leaflet calcification by 6 months of age, but even by 18 months, Nos3-/- mice with normal tricuspid aortic valves (TAVs) were only fibrotic, not calcified and function was not impaired [70,71]. Interestingly, "Nos3-/- mice do not develop atherosclerosis when fed a high-cholesterol atherogenic diet" [72], "a phenomenon which may be the result of reductions in eNOS-driven LDL oxidation in the vasculature" [73]. A study have shown that targeted deletion of Gata5 in mice resulted in BAV and hypoplastic hearts formation. In humans, most BAVs result from fusion of either the right-coronary and left-coronary leaflets (R-L) or the right-coronary and noncoronary leaflet (R-N). In all cases, "the observed BAVs resulted from fusion of the right-coronary and noncoronary leaflets, the subtype associated with the more severe valve dysfunction in humans" [74]. Gata5 regulates eNOS and Notch signaling: *Gata5-/-* mice have significantly downregulated transcription of *Nos3* and the Notch ligand *Jag1*, and the murine *Nos3* promoter has multiple GATA binding sites. Adult *Gata5-/-* mice display mild LV hypertrophy and significantly impaired aortic valve function [74]. It should be noted that previous studies have indicated that epithelial growth factor receptor (EGFR) Signaling pathways regulate embryonic formation of the aortic valve in mice [75], and putatively in humans [76], Mice heterozygous for a dominant loss-of-function mutation in epithelial growth factor receptor, which are EgfrVel/+ (Velvet) mice exhibit anomalous aortic valves, valve dysfunction, and valvular cardiomyopathy [77]. By 2.5 to 4 months of age mice, microscopy revealed gross congenital anomalies in 79% of EgfrVel/+ aortic valves, which similar to human unicuspid aortic valves. By12 months of age, histologic structure was grossly distorted in EgfrVel/+ aortic valves. Echocardiography analyses noticed moderate or severe aortic regurgitation, or aortic stenosis was present in 38% of EgfrVel/+ mice at 2.5 months of age and in 74% by 8 months of age when compared with control mice [77].

Large animal models: Animal model "are used predominantly to improve human health, and to enable translatable scientific discoveries with practical applications. Large animals can facilitate in these goals, as they exhibit disease characteristics similar to humans, giving mechanistic insight into the biological and pathological processes" [78]. In contrast to the mouse, larger animals are more expensive to purchase, feed, and maintain in conditions appropriate to modern animal husbandry. Additionally, the development of complex atherosclerotic lesions usually involves a longer period of than in the mouse. Nevertheless, some large animal models such as swine and, rabbits are valuable models to investigate CAVD and have a better translational bridgen between preclinical and clinical studies because of their anatomical (trilayer valve) and physiological similarities to humans [79-82].

Swine models: Swine are excellent models for atherosclerosis studies as they: 1) share similar systemic hemodynamic variables and heart anatomy (including trilayered aortic valve leaflets), lipid profile, lipoprotein metabolism, and have a similar genome in size and chromosomal structure to humans, making porcine models attractive for genomic studies; and 2) produce human-type atherosclerotic lesions on high-fat/high-cholesterol diets and develops naturally with age, though their high-density lipoprotein (HDL) level does rise with hypercholesterolemic diets. These attributes highlight the pig have recently been used as an ideal model to study CAVD and display the potential for developing valvular lesions [6,83-85]. Nonetheless, the large size of pigs limits their widespread use. Recently, genetically engineered mini-pigs in which hyperlipidemia and consequently atherosclerosis were successfully induced became available; they are cheaper to maintain compared to full-sized pigs. A close examination of its pathophysiological mechanisms revealed similarities with human atherosclerosis, as in the full-sized pigs, that are not observed in mouse models [86,87].

Nutritionally-susceptible porcine models: Although porcine models have been used primarily in atherosclerosis research, they have recently been used to study CAVD [32]. "There is some evidence that diets started before sexual maturity may be more effective in producing advanced disease" [85]. Sider et al investigated a porcine model of early aortic valve sclerosis [6] In this investigation, pigs were fed either a standard or high fat/cholesterol (HF/HC) diet for 2-5 months. Swine fed on the HF/HC diet developed significantly thicker lesions on the aortic side of coronary aortic valve leaflets, with histologically opaque regions consisting of proteoglycans, collagen and elastin, within the fibrosa layer as similarly observed in early human CAVD [6]. Increased expression of osteochondrogenic markers including SRY (sex determining region Y)-box 9 (SOX9) and Msh Homeobox 2 (MSX2) has been observed in dense proteoglycan-rich lesion onlays with the HF/HC diet. Go et al recently developed a model associated with Mechanical and structural degenerative changes of the Aortic Valve in Female Yorkshire domestic pigs [88]. In

this model, the animals were fed either a normal or high-fat/high-cholesterol (HF/HC) diet for 16 weeks. The control group were fed a normal diet consisting of a standard swine feed containing 14.5% protein and 3% fat with 3.3 Kcal/g of feed. The experimental group were fed a high-fat and high-fructose feed containing 17% protein and 20% fat with 4.1 Kcal/g of feed. After 16 weeks, the HF diet group had increased weight, total cholesterol, systolic and diastolic pressure. The extracellular matrix of the aortic valve showed loss of elastin fibers and increased collagen deposition (types 1& 3). The initial stages of microcalcification were observed, and the aortic cusps revealed that the HF diet group expressed a decrease in the tensile strength and elastic modulus compared to the control diet group. It was also observed that in the HF diet group, the presence of proteins: phospholipase A2 associated with lipoproteins, expression of proteins derived from osteoblasts, osteocalcin (OCN) and osteopontin (OPN) were concentrated along the aortic side of the valve leaflet [88]. The above study has demonstrated that experimental metabolic syndrome (MetS) in pigs induced by a high fat (HF) diet for16 weeks resulted in mechanical and structural aortic valve degeneration and calcification [88]. By means of dietary intervention a significant association between endothelial phenotypical heterogeneity, local hemodynamics and susceptibility to regional CAVD was previously reported in normal and hypercholesterolemic swine. Report suggested that aortic valve sclerosis preferably develops on the aortic side of the valve leaflets, and is associated with a side-specific endothelial upregulation of eNOS and activated leukocyte adhesion molecule (ALCAM) [89-91]. For example, aortic and ventricular side aortic Valve surface endothelial cells (VECs) from adult male pigs have been compared, using microarray and quantitative Real time polymerase chain reaction (qRT-PCR) to measure gene expression. In this study, side-specific expression differences were found between the aortic and ventricular VECs [91]. Interestingly, higher expression was noted in the aortic side of the valve of genes associated with vascular calcification and skeletal development, such as bone morphogenetic proteins (BMP-4). Lower expression of factors shown to inhibit ectopic calcification was also observed in the aortic side VECs, including osteoprotegerin (OPG), C-type natriuretic peptide (CNP) and chordin (an inhibitor of the osteoinductive activity of BMPs) [91]. In addition to this, "greater expression of antioxidative genes and an absence of differential expression of pro-inflammatory factors on the aortic side suggests potential protection in the normal valve against lesion development and inflammation "[91,92]. Like in other animal models, atherosclerotic plaque development in swine can be accelerated by combining high-cholesterol diet with locally produced vascular injury inflicted by different means, including guide-wire-induced injury, [93] endovascular balloon inflation with or without stent deployment [94-96] partial vessel ligation, [97] and balloon angioplasty followed 2 weeks later by percutaneous intramural injection of a mixture of cholesteryl esters and human oxLDL [98,99]. Atherogenic diets plus vascular injury protocols not only reduce the difficulties in care and high maintenance cost associated with the use of swine models by reducing the duration of the study but are also highly relevant models for translational research in the field of percutaneous interventions and cardiovascular imaging animal models [45].

Genetically-susceptible porcine models: Naturally, occurring mutations have also been exploited in swine to develop models of non-diet-induced hypercholesterolemia for CAVD. These models have mutations in the LDLR and/or apolipoprotein genes. Some common models include (1) familial hypercholesterolemia due to an LDLR mutation with altered lipid profiles [100,101]; A recently study of Familial hypercholesterolemia (FH) have evaluated that a total of 21 female animals were included: 4 juvenile (0.25-year-old) wild type (WT) swine, 3 one-year-old (1 yrs) WT swine, 3 juvenile (0.25 yrs) Rapacz FH (RFH) swine, 4 two-year-old (2 yrs) adult RFH swine, and 5 three-year-old (3 yrs) RFH swine. The animal were fed on a standard swine diet (75.8%, 14.7%, and 9.4% of daily calories from carbohydrates, protein, and fat, respectively) throughout the duration of the study. Adult RFH exhibited early features of CAVD. A significant thickening of the leaflets, accompanied by extensive remodeling of the extracellular matrix, including enrichment of proteoglycans, disruption of collagen and elastin fragmentation. Increased lipid oxidation and macrophage infiltration. Echocardiography revealed mild aortic valve sclerosis, but intact valve function. Analysis of valve microarrays from adult and juvenile RFH animals revealed significant upregulation of genes related to inflammation, as well as several commonalities with atherosclerosis and overlap with human CAVD [102]. (2) familial hypercholesterolemia due to mutations relating to ApoB and LDLR [103]. Rapacz pig is a wild-type model with a natural mutation in ApoB and LDLR genes, which were produced by selective breeding of pigs with high cholesterol [104]. Within 2-4 years on a normal diet, these pigs developed increased hypercholesterolemia, with LDL as the main circulating lipoprotein, associated with the development of coronary atherosclerosis [43]. their use is limited due to the long periods of time that are required to develop complex atherosclerotic lesions even when challenged with atherogenic diets (2–3 years) and the large size and weight they reach (>200 kg). These difficulties in care and high maintenance cost are reduced with the use of smaller swine strains, such as the Yucatan miniature pig, which also develop humanoid complicated lesions with abundant necrosis and cholesterol deposits and extensive calcification [95,105-109]. Very recently, Thim et al reported the generation of a downsized hypercholesterolemic pig strain named FBM that was produced by crossing the Rapacz familial hypercholesterolemic pig bearing the R84C LDLR mutation with a smaller pig (Chinese Meishan) and then crossing the offspring with an even smaller minipig from Brentocelles, France. FBM pigs breed like normal pigs, develop atherosclerotic lesions on standard diet, and disease progression is aggravated by atherogenic diet feeding, whereby plasma total cholesterol rose to (>800 mg/dl) and plaques mirrored humanlike features, including a large necrotic core covered by a thin and inflamed fibrous cap, neovascularization, intraplaque hemorrhage, and expansive remodeling [96].

Rabbit models: The first evidence that atherosclerosis can be induced in laboratory animals was provided in 1908 by Ignatowski, He fed rabbits a protein-rich diet (mainly meat, milk, and egg yolk), which led to the formation of atherosclerotic lesions in the aortic wall [110]. Since then, a number of species, such as rabbits, mice, rats, guinea pigs, hamsters, birds, swine,

dogs and non-human primates, have been developed [110].

Nutritionally-susceptible rabbit models: Rabbits have also been used to study early to advanced aortic valve disease. In general, a hypercholesterolemic diet is administered to promote pathogenesis [111]. Mourino-Alvarez et al. studied a rabbits model of calcific aortic stenosis. In this study, the Male New Zealand white rabbits weighing 2–2.5 kg were divided into two groups: animals in the control group were fed with normal rabbit chow; animals in the pathological group were fed with 1% cholesterol-enriched chow plus 50,000 IU/kg vitamin D2 for 12 weeks [112,113]. Echocardiographic evaluations revealed higher peak gradient and thickened AVs from the pathological group, which was concomitant with increase in cholesterol (total, LDL, HDL and non-HDL), characteristics that have been previously described in patients with CAS [113-115]. Histological analysis revealed the presence of moderate calcium deposits, abundant infiltration of macrophages (RAM11-positive cells) and high expression of α-actin, which is characteristic of smooth muscle cells and myofibroblasts. Protein analysis revealed three proteins were significantly altered: tropomyosin α-1 chain (TPM-1) and L-lactate dehydrogenase B chain (LDHB) followed the same trend in plasma and tissue, whereas transitional endoplasmic reticulum ATPase (TERA) was upregulated in tissue and downregulated in both rabbit and human plasma [113]. In 2003, Drolet et al. studied the effects of a hypercholesterolemic diet with vitamin D supplements on the development of the aortic valve calcification. In this study the Male New Zealand White rabbits were divided in three groups: 1) controls receiving normal rabbit chow without any dietary supplement; 2) animals fed with 0.5% cholesterol-enriched chow; and 3) animals fed with 0.5% cholesterol-enriched chow plus 50,000 IU/day vitamin D2 [116]. After 12 weeks, no change was observed in group 1 but groups 2 and 3 showed high levels of total cholesterol. Interestingly, vitamin D2 caused an additional increase in serum cholesterol despite similar cholesterol intake. Calcium levels were slightly more elevated in-group 3. Echocardiography revealed decreased Aortic valve area (AVA) by 36%, the maximal gradient increased by 300%, and the mean gradient increased by 107% (all $p < 0.05$) [116]. Malergue et al demonstrated that “plasma levels of vitamin D3 were directly correlated with the development of AVS in a group of patients with chronic renal failure” [117]. “Elevated calcium-phosphate product in patients with normal renal function was also associated with the severity of AVS after adjusting for age, gender, and creatinine clearance” [118]. Ngo et al have reported the effects of 8 weeks' treatment with vitamin D2 alone at 25,000 IU/4 days weekly on aortic valve stenosis (AVS) in male New Zealand white rabbits. Vitamin D2 treated rabbits developed AVS with increased aortic valve backscatter (AVBS), increased transvalvular velocity and pressure gradient compared to the control group. There was associated valve calcification, lipid deposition and macrophage infiltration. Endothelial function was markedly impaired, and intravalvular thioredoxin-interacting protein (TXNIP) concentration increased. Histological features were similar to those of early AVS in humans and associated endothelial dysfunction/redox stress. AVS development may result from the loss of nitric oxide suppression of TXNIP expression [119]. Rabbit models have also proven useful in studying the effect of potential therapies on AVS. Rajamannan and colleagues found whether hypercholesterolemia causes an atherosclerotic proliferative valve lesion associated with an increase in cholesterol, highly sensitive C-reactive protein (hsCRP) [120], proliferation cell nuclear antigen (PCNA), macrophage (RAM 11), and osteopontin and osteoblast gene markers (alkaline phosphatase, osteopontin, and osteoblast lineage-specific transcription factor (Cbfa-1) in the cholesterol-fed rabbits compared with control rabbits. All markers except hsCRP were reduced by HMG CoA reductase inhibitors treatment [121,122]. Hamilton et al assessed the effect of dietary modification and/or statin treatment on established aortic valve disease in a rabbit model of Aortic valve sclerosis (AVS) to examine the tissue response to therapy. In this evaluation Male New Zealand White rabbits were fed on a 0.25% cholesterol supplemented diet for 6 months, and then tittered (0.125–0.25%) to maintain cholesterol levels ca. 500 mg/dl. Six control rabbits were fed on normal chow. By 15 months, five cholesterol-fed rabbit cusps exhibited thickening due to lipid deposition, macrophage infiltration colocalized in the fibrosal layer, and osteopontin expression. The remaining cholesterol-fed rabbits were divided in four groups. Cholesterol-fed rabbits were fed 0.125% cholesterol-supplemented chow, dietary treatment-only rabbits were fed normal chow, statin treatment-only rabbits were fed 2.5 mg/kg per day of atorvastatin calcium [121,123-125] in 0.125% cholesterol-supplemented chow, and statin and dietary treatment rabbits were fed 2.5 mg/kg per day of atorvastatin calcium in normal chow for an additional 15 months. By 30 months on the atherogenic diet alone, rabbit cusps displayed significant increase collagen deposition, lipid, macrophage infiltrate, and osteopontin expression, and increases in CD3+ lymphocyte invasion and calcification were also observed. With statin treatment, however, the valve cusps showed significant diminished immune cell infiltration and osteopontin expression. Unfortunately, lipid was retained and calcification persisted in all treated valves. Finally we say that the cellular response to statin therapy does not result in full regression of the sclerotic process in established AVS [125]. Arishiro et al have found that the Atherosclerotic changes in the aortic valves of rabbits fed with 1% cholesterol diet for 8 weeks with ARB (olmesartan, 1 mg/kg/day) for the last 4 weeks. Treatment with olmesartan was resulted with significantly decreased Lipid deposition, macrophage accumulation, osteopontin expression, Angiotensin-converting enzyme and alpha-smooth muscle actin-positive myofibroblasts, decrease amounts of messenger ribonucleic acid for osteoblast-specific transcription factor core binding factor alpha-1 (Cbfa-1 mRNA) expression and increased eNOS expression. preserved endothelial integrity on the lesion-prone aortic side of the valve and transdifferentiation of valvular fibroblasts into myofibroblasts and/or osteoblasts in valve leaflets was inhibited [126].

Genetically-susceptible rabbit models

However long-term high-fat diet feeding of rabbits is discouraging, because it is frequently accompanied by noxious side effects and increased mortality owing to hepatic toxicity [45], genetically modified rabbits have been developed to produce spontaneous atherosclerotic lesions. For example, (1) Watanabe heritable hypercholesterolemic rabbit (WHHL), a LDLR-deficient model, [124,127,128]; (2) St. Thomas Hospital rabbits, which

acquire hypertriglyceridemia as well as hypercholesterolemia [129]; (3) rabbits with altered lipid profiles, such as induced human ApoB100 [129] or Apo(a) [130]. Of these, The most widely used is the Watanabe heritable hyperlipidemic (WHHL) rabbit [131], has been used in a study of CAVD to show that Hypercholesterolemia-induced AV calcification is attenuated by atorvastatin and is mediated in part by the Lrp5/ β-catenin pathway. This developmental pathway may be important in the signaling pathway of this disease [124]. Recently Hara et al. identified a myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbit, an animal model of familial hypercholesterolemia and atherosclerosis [132]. He demonstrated that (WHHLMI) rabbits with normal chow or without a high-cholesterol diet or vitamin D supplement showed age-dependent progression of aortic valve sclerosis. In this investigation WHHLMI rabbits [132,133], aged 20 or 30 months, and control Japanese White rabbits, aged 30 months, were evaluated. The lipid profile were similar between 20 and 30 months old WHHLMI rabbits. Thirty-month-old WHHLMI rabbits exhibited significantly smaller aortic valve area and higher maximal transvalvular pressure gradient than 20-month-old rabbits. Macroscopic examination revealed thickened and degenerated valve leaflets at 30 months. Histological evaluation confirmed thickened leaflets with calcified nodules at 30 months. Real-time polymerase chain reaction (PCR) of resected aortic valve also showed increased expression level of calcification-related molecules including Osteopontin (OPN), Sox9 [134], Bmp2, receptor activator of nuclear factor kappa B ligand (RANKL), Osteoprotegerin (OPG), and transcription factor for osteoblast differentiation (Runx2) [135] in 30-month-old rabbits. WHHLMI rabbits may be useful models of early-stage AS in vivo [132].

Conclusion

Animal models that are chosen and then implemented properly are valuable resources for investigating pathobiological mechanisms of CAVD and potential therapeutic interventions. Several animal models have been shown to recapitulate important aspects of human CAVD pathobiology, thus enabling detailed investigations that are otherwise unfeasible or impossible to conduct in humans. The development of new models and our improved understanding of the utility of existing models have quickly advanced the impact that animal model-based studies are making within this field. Various animal species have been used as experimental models of this disease. While most animal models are still being used to a greater or lesser extent, none of them can be considered an ideal model of the human pathology. CAVD in swine closely recapitulates the main morphological and biochemical characteristics of human CAVD, therefore the results obtained in large animal models are more easily extrapolated to humans in translational research studies, such as the development of imaging techniques and interventional devices and the assessment of novel therapeutic strategies. However, research with large animal species encounters a number of important obstacles, such as difficulties in handling, the high economical cost of maintenance. With the advancement in genetic technology, the development of mini-pigs is a favourable trade-off between human-like physiology compared to non-human primate; and ease of handling compared with small animal, with high resemblance to human cardiac anatomy, physiology, lipid metabolism and atherosclerotic pathophysiology. In contrast, small animals, such as mice, rats, and rabbits, have many advantages for experimental studies (e.g., easy handling, low cost), but do not typically develop the advanced vulnerable lesion that are characteristic of human individuals suffering severe CAVD. The uses of all animal models available nowadays will undoubtedly continue to permit major advances in CAVD research that should translate into improved treatment, prevention, and diagnosis of CAVD.

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Disclosures

None declared.

References

1 Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, et al. (2012) Heart disease and stroke statistics-2012 update: a report from the American Heart Association. Circulation 125: e2-e220.

2 Iung B, Baron G, Butchart EG, Delahaye F, Gohlke-Bärwolf C, et al. (2003) A prospective survey of patients with valvular heart disease in Europe: The Euro Heart Survey on Valvular Heart Disease. Eur Heart J 24: 1231-1243.

3 Gillis K, Roosens B, Bala G, Remory I, Hernot S, et al. (2017) Interaction of renal failure and dyslipidaemia in the development of calcific aortic valve disease in rats. Acta Cardiol 72: 537-546.

4 Lilly LS, Zipes D (2018) Braunwald's heart disease: a textbook of cardiovascular medicine, 2-Volume Set. Elsevier Health Sciences.

5 Le Quang K, Bouchareb R, Lachance D, Laplante MA, El Husseini D, et al. (2014) Early development of calcific aortic valve disease and left ventricular hypertrophy in a mouse model of combined dyslipidemia and type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol 34: 2283-2291.

6 Sider KL, Zhu C, Kwong AV, Mirzaei Z, de Langé CFM, et al. (2014) Evaluation of a porcine model of early aortic valve sclerosis. Cardiovasc Pathol 23: 289-297.

7 Scatena M, Jackson MF, Speer MY, Leaf EM, Wallingford MC,et al.

(2018) Increased Calcific Aortic Valve Disease in response to a diabetogenic, procalcific diet in the LDLr-/-ApoB100/100 mouse model. Cardiovasc Pathol 34: 28-37.

8 Hisamatsu T, Miura K, Fujiyoshi A, Kadota A, Miyagawa N, et al. (2018) Serum magnesium, phosphorus, and calcium levels and subclinical calcific aortic valve disease: A population-based study. Atherosclerosis 273: 145-152.

9 Honda S, Miyamoto T, Watanabe T, Narumi T, Kadowaki S, et al. (2014) A novel mouse model of aortic valve stenosis induced by direct wire injury. Arterioscler Thromb Vasc Biol 34: 270-278.

10 Cohen DJ, Malave D, Ghidoni JJ, Iakovidis P, Everett MM, et al. (2004) Role of oral bacterial flora in calcific aortic stenosis: an animal model. Ann Thorac Surg 77: 537-543.

11 Anselmo W, Branchetti E, Grau JB, Li G, Ayoub S, et al. (2018) Porphyrin-Based SOD Mimic MnTnBu OE-2-PyP5+ Inhibits Mechanisms of Aortic Valve Remodeling in Human and Murine Models of Aortic Valve Sclerosis. J Am Heart Assoc 7: e007861.

12 Branchetti E, Sainger R, Poggio P, Grau JB, Patterson-Fortin J, et al. (2013) Antioxidant enzymes reduce DNA damage and early activation of valvular interstitial cells in aortic valve sclerosis. Arterioscler Thromb Vasc Biol 33: e66-e74.

13 Parisi V, Leosco D, Ferro G, Bevilacqua A, Pagano G, et al. (2015) The lipid theory in the pathogenesis of calcific aortic stenosis. Nutr Metab Cardiovasc Dis 25: 519-525.

14 O'Brien KD (2006) Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more). Arterioscler Thromb Vasc Biol 26: 1721-1728.

15 Alushi B, Curini L, Christopher MR, Grubitzch H, Landmesser U, et al. (2020) Calcific aortic valve disease-natural history and future therapeutic strategies. Frontiers Pharmacol 11: 685.

16 Demer LL, Tintut Y (2019) Heart valve calcification, in Principles of Heart Valve Engineering. Elsevier pp: 307-319.

17 Rajamannan NM, Evans FJ, Aikawa E, Grande-Allen KJ, Demer LL, et al. (2011) Calcific aortic valve disease: Not simply a degenerative process a review and agenda for research from the National Heart and Lung and Blood Institute Aortic Stenosis Working Group. Circulation 124: 1783.

18 Miller JD, Weiss RM, Serrano KM, BrooksII RM, Berry CJ, et al. (2009) Lowering plasma cholesterol levels halts progression of aortic valve disease in mice. Circulation 119: 2693.

19 Helske S, Oksjoki R, Lindstedt KA, Lommi J, Turto H, et al. (2008) Complement system is activated in stenotic aortic valves. Atherosclerosis 196: 190-200.

20 Hakuno D, Kimura N, Yoshioka M, Mukai M, Kimura T, et al. (2010) Periostin advances atherosclerotic and rheumatic cardiac valve degeneration by inducing angiogenesis and MMP production in humans and rodents. J Clin Invest 120: 2292-2306.

21 21. Ghaisas NK, Foley JB, O'Briain DS, Crean P, Kelleher D, et al. (2000) Adhesion molecules in nonrheumatic aortic valve disease: endothelial expression, serum levels and effects of valve replacement. J Am Coll Cardiol 36: 2257-2262.

22 Jonna W (2020) Studies on calcific aortic valve disease: from experimental models to human disease. University of Eastern Finland.

23 Tanaka K, Sata M, Fukuda D, Suematsu Y, Motomura N, et al. (2005) Age-associated aortic stenosis in apolipoprotein E-deficient mice. J Am Coll Cardiol 46: 134-141.

24 Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD (1994) Characterization of the early lesion of'degenerative'valvular aortic stenosis. Histological and immunohistochemical studies. Circulation 90: 844-853.

25 Kuusisto J, Räsänen K, Särkioja T, Alarakkola E, Kosma VM(2005) Atherosclerosis-like lesions of the aortic valve are common in adults of all ages: a necropsy study. Heart 91: 576-582.

26 26. Walton K, Williamson N, Johnson AG (1970) The pathogenesis of atherosclerosis of the mitral and aortic valves. J Pathol 101: 205-220.

27 27.O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, et al. (1996) Apolipoproteins B,(a), and E accumulate in the morphologically early lesion of 'degenerative'valvular aortic stenosis. Arterioscler Thromb Vasc Biol 16: 523-532.

28 Rapp AH, Hillis LD, Lange RA, Cigarroa JE (2001) Prevalence of coronary artery disease in patients with aortic stenosis with and without angina pectoris. Am J Cardiol 87: 1216-1217.

29 Hulin A, Hego A, Lancellotti P, Oury C (2018) Advances in pathophysiology of calcific aortic valve disease propose novel molecular therapeutic targets. Front Cardiovasc Med 5: 21.

30 Lerman DA, Prasad S, Alotti N (2015) Calcific aortic valve disease: molecular mechanisms and therapeutic approaches. Eur Cardiol 10: 108.

31 Rutkovskiy A, Malashicheva A,Sullivan G, Bogdanova M, Kostareva A, et al. (2017) Valve interstitial cells: the key to understanding the pathophysiology of heart valve calcification. J Am Heart Assoc 6: e006339.

32 Sider KL, Blaser MC, Simmons CA (2011) Animal models of calcific aortic valve disease. Int J Inflam 2011: 364310.

33 Goody PR, et al. (2020) Aortic valve stenosis: from basic mechanisms to novel therapeutic targets. Arterioscler Thromb Vasc Biol 40: 885-900.

34 Xu S, Liu AC, Gotlieb AI (2010) Common pathogenic features of atherosclerosis and calcific aortic stenosis: role of transforming growth factor-β. Cardiovasc Pathol 19: 236-247.

35 Hjortnae J, Shapero K, Goettsch C, Hutcheson JD, Keegan J, et al. (2015) Valvular interstitial cells suppress calcification of valvular endothelial cells. Atherosclerosis 242: 251-260.

36 Liu A, Joag VR, Gotlieb AI (2007) The emerging role of valve interstitial cell phenotypes in regulating heart valve pathobiology. Am J Pathol 171: 1407-1418.

37 Dweck MR, Boon NA, Newby DE (2012) Calcific aortic stenosis: a disease of the valve and the myocardium. J Am Coll Cardiol 60: 1854-1863.

38 Lindman BR, Clavel MA, Mathieu P, lung B, Lancellotti P, et al. (2016) Calcific aortic stenosis. Nat Rev Dis Primers 3: 16006.

39 Ohukainen P, Ruskoaho H, Rysa J (2018) Cellular mechanisms of valvular thickening in early and intermediate calcific aortic valve disease. Curr Cardiol Rev 14: 264-271.

40 Pawade TA, Newby DE, Dweck MR (2015) Calcification in aortic stenosis: the skeleton key. J Am Coll Cardiol 66: 561-577.

41 Walters EM, Wolf E, Whyte JJ, Mao J, Renner S, et al. (2012) Completion of the swine genome will simplify the production of swine as a large animal biomedical model. BMC Med Genomics 5: 1-11.

42 Chorro FJ, Such-Belenguer L, López-Merino V (2009) Animal models of cardiovascular disease. Revista Española de Cardiología 62: 69-84.

43 Lee YT, Laxton V, Lin HY, Chan YWF, Fitzgerald-Smith S, et al. (2017) Animal models of atherosclerosis. Biomed Rep 6: 259-266.

44 Leong XF, Ng CY, Jaarin K (2015) Animal models in cardiovascular research: hypertension and atherosclerosis. Biomed Res Int 2015: 528757.

45 Fuster JJ, Castillo AI, Zaragoza C, Ibáñez B, Andrés V (2012) Animal models of atherosclerosis. Prog Mol Biol Transl Sci 105: 1-23.

46 Roosens B, Bala G, Droogmans S, Camp GV, Breyne J, et al. (2013) Animal models of organic heart valve disease. Int J Cardiol 165: 398-409.

47 Bostick B, Yue Y, Duan D (2011) Phenotyping cardiac gene therapy in mice. Methods Mol Biol 709: 91-104.

48 Doevendans PA (1995) Strategies for studying cardiovascular diseases in transgenic and gene-trageted mice. Strategies in Transgenic Animal Science pp: 107-144.

49 Drolet MC, Roussel E, Deshaies Y, Couet J, Arsenault M (2006) A high fat/high carbohydrate diet induces aortic valve disease in C57BL/6J mice. J Am Coll Cardiol 47: 850-855.

50 Weiss RM, Ohashi M, Miller JD, Young SG, Heistad DD (2006) Calcific aortic valve stenosis in old hypercholesterolemic mice. Circulation 114: 2065-2069.

51 Daugherty A, Rateri DL (2005) Development of experimental designs for atherosclerosis studies in mice. Methods 36: 129-138.

52 Srivastava S, Sithu SD, Vladykovskaya E, Haberzettl P, Hoetker DJ, et al. (2011) Oral exposure to acrolein exacerbates atherosclerosis in apoE-null mice. Atherosclerosis 215: 301-308.

53 Zeadin M, Butcher M, Werstuck G, Khan M, Yee CK, et al. (2009) Effect of leptin on vascular calcification in apolipoprotein E–deficient mice. Arterioscler Thromb Vasc Biol 29: 2069-2075.

54 Monetti M, Canavesi M, Camera M, Parente R, Paoletti R, et al. (2007) Rosuvastatin displays anti-atherothrombotic and anti-inflammatory properties in apoE-deficient mice. Pharmacol Res 55: 441-449.

55 Choi SE, Jang HJ, Kang Y, Jung JG, Han SJ, et al. (2010) Atherosclerosis induced by a high-fat diet is alleviated by lithium chloride via reduction of VCAM expression in ApoE-deficient mice. Vascul Pharmacol 53: 264-272.

56 Niepmann ST, Steffen E, Zietzer A, Adam M, Nordsiek J, et al. (2019) Graded murine wire-induced aortic valve stenosis model mimics human functional and morphological disease phenotype. Clin Res Cardiol 108: 847-856.

57 Hinton Jr RB, Alfieri CM, Witt SA, Glascock BJ, Khoury PR, et al. (2008) Mouse heart valve structure and function: echocardiographic and morphometric analyses from the fetus through the aged adult. Am J Physiol Heart Circ Physiol 294: H2480-H2488.

58 Fujisaka T, Hoshiga M, Hotchi J, Takeda Y, Jin D, et al. (2013) Angiotensin II promotes aortic valve thickening independent of elevated blood pressure in apolipoprotein-E deficient mice. Atherosclerosis 226: 82-87.

59 59. Rattazzi M, Faggin E, Bertacco E, Nardin C, Pagliani L, et al. (2018) Warfarin, but not rivaroxaban, promotes the calcification of the aortic valve in ApoE−/− mice. Cardiovasc Ther 36: e12438.

60 Hoffman JI, Kaplan S (2002) The incidence of congenital heart disease. J Am Coll Cardiol 39: 1890-1900.

61 Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, et al. (2005) Mutations in NOTCH1 cause aortic valve disease. Nature 437: 270-274.

62 Nigam V, Srivastava D (2009) Notch1 represses osteogenic pathways in aortic valve cells. J Mol Cell Cardiol 47: 828-834.

63 Nus M, MacGrogan D, Martínez-Poveda B, Benito Y, Casanova JC, et al. (2011) Diet-induced aortic valve disease in mice haploinsufficient for the Notch pathway effector RBPJK/CSL. Arterioscler Thromb Vasc Biol 31: 1580-1588.

64 Wang Y, Wu B, Farrar E, Lui W, Lu P, et al. (2017) Notch-Tnf signalling is required for development and homeostasis of arterial valves. Eur Heart J 38: 675-686.

65 Tkatchenko TV, Moreno-Rodriguez RA, Conway SJ, Molkentin JD, Markwald RR, et al. (2009) Lack of periostin leads to suppression of Notch1 signaling and calcific aortic valve disease. Physiol Genomics 39: 160-168.

66 Yoshioka M, Yuasa S, Matsumura K, Kimura K, Shiomi T, et al. (2006) Chondromodulin-I maintains cardiac valvular function by preventing angiogenesis. Nat Med 12: 1151-1159.

67 Aicher D, Urbich C, Zeiher A, Dimmeler S, Schäfers HJ (2007) Endothelial nitric oxide synthase in bicuspid aortic valve disease. Ann Thorac Surg 83: 1290-1294.

68 Lee TC, Zhao YD, Courtman DW, Stewart DJ (2000) Abnormal aortic valve development in mice lacking endothelial nitric oxide synthase. Circulation 101: 2345-2348.

69 Fernández B, Durán AC, Fernández-Gallego T, Fernández MC, Such M, et al. (2009) Bicuspid aortic valves with different spatial orientations of the leaflets are distinct etiological entities. J Am Coll Cardiol 54: 2312-2318.

70 El Accaoui RN, Gould ST, Hajj GP, Chu Y, Davis MK, et al. (2014) Aortic valve sclerosis in mice deficient in endothelial nitric oxide synthase. Am J Physiol Heart Circ 306: H1302-H1313.

71 Weiss RM, Miller JD, Heistad DD (2013) Fibrocalcific aortic valve disease: opportunity to understand disease mechanisms using mouse models. Circ Res 113: 209-222.

72 Shi W, Wang X, Shih DM, Laubach VE, Navab M, et al. (2002) Paradoxical reduction of fatty streak formation in mice lacking endothelial nitric oxide synthase. Circulation 105: 2078-2082.

73 Rajamannan NM (2011) Bicuspid aortic valve disease: the role of oxidative stress in Lrp5 bone formation. Cardiovasc Pathol 20: 168-176.

74 Laforest B, Andelfinger G, Nemer M (2011) Loss of Gata5 in mice leads to bicuspid aortic valve. J Clin Invest 121: 2876-2887.

75 Chen B, Bronson RT, Klaman LD, Hampton TG, Wang JF, et al. (2000) Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nat Genet 24: 296-299.

76 McBride KL, Zender GA, Fitzgerald-Butt SM, Seagraves NJ, Fernbach SD, et al. (2011) Association of common variants in ERBB4 with congenital left ventricular outflow tract obstruction defects. Birth Defects Res A Clin Mol Teratol 91: 162-168.

77 Weiss RM, Chu Y, Brooks RM, Lund DD, Cheng J, et al. (2018) Discovery of an experimental model of unicuspid aortic valve. J Am Heart Assoc 7: e006908.

78 Tsang HG, Rashdan NA, Whitelaw BAC, Corcoran BM, Summers KM, et al. (2016) Large animal models of cardiovascular disease. Cell Biochem Funct 34: 113-132.

79 Cimini M, Boughner DR, Ronald JA, Aldington L, Rogers KA (2005) Development of aortic valve sclerosis in a rabbit model of atherosclerosis: an immunohistochemical and histological study. J Heart Valve Dis 14: 365-375.

80 80. Brousseau ME, Hoeg JM (1999) Transgenic rabbits as models for atherosclerosis research. J Lipid Res 40: 365-375.

81 Swinkels DW, Demacker PN (1988) Comparative studies on the low density lipoprotein subfractions from pig and man. Comp Biochem Physiol B 90: 297-300.

82 Fernández-Jiménez R, García-Prieto J, Sánchez-González J, Agüero J, López-Martín GJ, et al. (2015) Pathophysiology underlying the bimodal edema phenomenon after myocardial ischemia/reperfusion. J Am Coll Cardiol 66: 816-828.

83 83. Skold B, Getty R, Ramsey FK (1966) Spontaneous atherosclerosis in the arterial system of aging swine. Am J Vet Res 27: 257-273.

84 Dixon JL, Stoops JD, Parker JL, Laughlin MH, Weisman GA, et al. (1999) Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. Arterioscler Thromb Vasc Biol 19: 2981-2992.

85 Gerrity RG, Natarajan R, Nadler JL, Kimsey T (2001) Diabetes-induced accelerated atherosclerosis in swine. Diabetes 50: 1654-1665.

86 Agarwala A, Billheimer J, Rader DJ (2013) Mighty minipig in fight against cardiovascular disease. Sci Transl Med 5: 166fs1-166fs1.

87 Davis BT, Wang XJ, Rohret JA, Struzynski JT, Merricks EP, et al. (2014) Targeted disruption of LDLR causes hypercholesterolemia and atherosclerosis in Yucatan miniature pigs. PloS one 9: e93457.

88 Go JL, Prem K, Al-Hijji MA, Qin Q, Noble C, et al. (2018) Experimental metabolic syndrome model associated with mechanical and structural degenerative changes of the aortic valve. Sci Rep 8: 1-11.

89 Guerraty MA, Grant GR, Karanian JW, Chiesa OA, Pritchard WF, et al. (2010) Hypercholesterolemia Induces Side-Specific Phenotypic Changes and Peroxisome Proliferator–Activated Receptor-γ Pathway Activation in Swine Aortic Valve Endothelium. Arterioscler Thromb Vasc Biol 30: 225-231.

90 Guerraty MA, Grant GR, Karanian JW, Chiesa OA, Pritchard WF, et al. (2011) Side-specific expression of activated leukocyte adhesion molecule (ALCAM; CD166) in pathosusceptible regions of swine aortic valve endothelium. J Heart Valve Dis 20: 165-167.

91 Simmons CA, Grant GR, Manduchi E, Davies PF (2005) Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circ Res 96: 792-799.

92 Butcher JT, Mahler GJ, Hockaday LA (2011) Aortic valve disease and treatment: the need for naturally engineered solutions. Adv Drug Deliv Rev 63: 242-268.

93 Mihaylov D, van Luyn MJ, Rakhorst G (2000) Development of an animal model of selective coronary atherosclerosis. Coron Artery Dis 11: 145-149.

94 Granada JF, Kaluza GL, Wilensky RL, Biedermann BC, Schwartz RS, et al. (2009) Porcine models of coronary atherosclerosis and vulnerable plaque for imaging and interventional research. EuroIntervention 5: 140-148.

95 De Smet BJGL, van der Zande J, van der Helm YJM, Kuntz RE, Borst C, et al. (1998) The atherosclerotic Yucatan animal model to study the arterial response after balloon angioplasty: the natural history of remodeling. Cardiovasc Res 39: 224-232.

96 Thim T, Hagensen MK, Drouet L, Sollier CBT, Michel Bonneau M, et al. (2010) Familial hypercholesterolaemic downsized pig with human-like coronary atherosclerosis: a model for preclinical studies. EuroIntervention 6: 261-268.

97 97. Ishii A, Viñuela F, Murayama Y, Yuki I, Nien YL, et al. (2006) Swine model of carotid artery atherosclerosis: experimental induction by surgical partial ligation and dietary hypercholesterolemia. Am J Neuroradiol 27: 1893-1899.

98 Granada JF, Moreno PR, Burke AP, Schulz DG, Raizner AE, et al. (2005) Endovascular needle injection of cholesteryl linoleate into the arterial wall produces complex vascular lesions identifiable by intravascular ultrasound: early development in a porcine model of vulnerable plaque. Coron Artery Dis 16: 217-224.

99 Granada JF, Wallace-Bradley D, Win HK, Alviar CL, Builes A, et al. (2007) In vivo plaque characterization using intravascular ultrasound–virtual histology in a porcine model of complex coronary lesions. Arterioscler Thromb Vasc Biol 27: 387-393.

100 Hasler-Rapacz J, Ellegren H, Fridolfsson AK, Kirkpatrick B, Kirk S, et al. (1998) Identification of a mutation in the low density lipoprotein receptor gene associated with recessive familial hypercholesterolemia in swine. Am J Med Genet 76: 379-386.

101 Hasler-Rapacz J, Kempen HJ, Princen HM, Kudchodkar BJ, Lacko A, et al. (1996) Effects of simvastatin on plasma lipids and apolipoproteins in familial hypercholesterolemic swine. Arterioscler Thromb Vasc Biol 16: 137-143.

102 102. Porras AM, Shanmuganayagam D, Meudt JJ, Krueger CG, Hacker TA, et al. (2015) Development of aortic valve disease in familial hypercholesterolemic swine: implications for elucidating disease etiology. J Am Heart Assoc 4: e002254.

103 Grunwald KA, Schueler K, Uelmen PJ, Lipton BA, Kaiser M et al. (1999) Identification of a novel Arg→ Cys mutation in the LDL receptor that contributes to spontaneous hypercholesterolemia in pigs. J Lipid Res 40: 475-485.

104 Davis HR, Vesselinovitch D, Wissler RW (1984) Reticuloendothelial system response to hyperlipidemia in rhesus and cynomolgus monkeys. J Leukoc Biol 36: 63-80.

105 Panepinto LM, Phillips RW (1986) The Yucatan miniature pig: characterization and utilization in biomedical research. Lab Anim Sci 36: 344-347.

106 Barbeau ML, Klemp KF, Guyton JR, Rogers KA (1997) Dietary fish oil: influence on lesion regression in the porcine model of atherosclerosis. Arterioscler Thromb Vasc Biol 17: 688-694.

107 Gal D, Rongione AJ, Slovenkai GA, DeJesus ST, Lucas A, et al. (1990) Atherosclerotic Yucatan microswine: An animalmodel with high-grade, fibrocalcific, nonfatty lesions suitable for testing catheter-based interventions. Am Heart J 119: 291-300.

108 Reitman JS, Mahley RW, Fry DL (1982) Yucatan miniature swine as a model for diet-induced atherosclerosis. Atherosclerosis 43: 119-132.

109 Holvoet P, Theilmeier G, Shivalkar B, Flameng W, Collen D (1998) LDL hypercholesterolemia is associated with accumulation of oxidized LDL, atherosclerotic plaque growth, and compensatory vessel enlargement in coronary arteries of miniature pigs. Arterioscler Thromb Vasc Biol 18: 415-422.

110 Ignatowski AC (2007) Influence of animal food on the organsim of rabbits. Izvest Imper Voennomed Akad St Petersburg 16: 154-173.

111 Guerraty M, Mohler ER (2007) Models of aortic valve calcification. J Investig Med 55: 278-283.

112 Drolet MC, Couët J, Arsenault M (2008) Development of aortic valve sclerosis or stenosis in rabbits: role of cholesterol and calcium. J Heart Valve Dis 17: 381-387.

113 Mourino-Alvarez L, Baldan-Martin M, Sastre-Oliva T, Martin-Lorenzo M, Sanz Maroto A, et al. (2018) A comprehensive study of calcific aortic stenosis: from rabbit to human samples. Dis Model Mech 11.

114 Kamath AR, Pai RG (2008) Risk factors for progression of calcific aortic stenosis and potential therapeutic targets. Int J Angiol 17: 63-70.

115 Akat K, Kaden JJ, Schmitz F, Ewering S, Anton A, et al. (2010) Calcium metabolism in adults with severe aortic valve stenosis and preserved renal function. Am J Cardiol 105: 862-864.

116 Drolet MC, Arsenault M, Couet J (2003) Experimental aortic valve stenosis in rabbits. J Am Coll Cardiol 41: 1211-1217.

117 Malergue M, Urena P, Prieur P, Guédon-Rapoud C, Pétrover M (1997) Incidence and development of aortic stenosis in chronic hemodialysis. An ultrasonographic and biological study of 112 patients. Arch Mal Coeur Vaiss 90: 1595-1601.

118 Mills WR, Einstadter D, Finkelhor RS (2004) Relation of calcium-phosphorus product to the severity of aortic stenosis in patients with normal renal function. Am J Cardiol 94: 1196-1198.

119 Ngo DT, Stafford I, Kelly DJ, Sverdlov AL, Wuttke RD, et al. (2008) Vitamin D2 supplementation induces the development of aortic stenosis in rabbits: Interactions with endothelial function and thioredoxin-interacting protein. Eur J Pharmacol 590: 290-296.

120 Galante A, Pietroiusti A, Vellini M, Piccolo P, Possati G, et al. (2001) C-reactive protein is increased in patients with degenerative aortic valvular stenosis. J Am Coll Cardiol 38: 1078-1082.

121 Rajamannan NM, Subramaniam M, Springett M, Sebo TC, Niekrasz M, et al. (2002) Atorvastatin inhibits hypercholesterolemia-induced cellular proliferation and bone matrix production in the rabbit aortic valve. Circulation 105: 2660-2665.

122 Jialal I, Stein D, Balis D, Grundy SM, Adams-Huet B, et al. (2001) Effect of hydroxymethyl glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels. Circulation 103: 1933-1935.

123 Rajamannan NM, Subramaniam M, Stock SR, Stone NJ, Springett M, et al. (2005) Atorvastatin inhibits calcification and enhances nitric oxide synthase production in the hypercholesterolaemic aortic valve. Heart 91: 806-810.

124 Rajamannan NM, Subramaniam M, Caira F, Stock SR, Spelsberg TC (2005) Atorvastatin inhibits hypercholesterolemia-induced calcification in the aortic valves via the Lrp5 receptor pathway. Circulation 112: I-229-I-234.

125 Hamilton AM, Boughner DR, Drangova M, Rogers KA (2011) Statin treatment of hypercholesterolemic-induced aortic valve sclerosis. Cardiovasc Pathol 20: 84-92.

126 Arishiro K, Hoshiga M, Negoro N, Jin D, Takai S, et al. (2007) Angiotensin receptor-1 blocker inhibits atherosclerotic changes and endothelial disruption of the aortic valve in hypercholesterolemic rabbits. J Am Coll Cardiol 49: 1482-1489.

127 Shiomi M, Ito T (2009) The Watanabe heritable hyperlipidemic (WHHL) rabbit, its characteristics and history of development: a tribute to the late Dr. Yoshio Watanabe. Atherosclerosis 207: 1-7.

128 Aliev G, Burnstock G (1998) Watanabe rabbits with heritable hypercholesterolaemia: a model of atherosclerosis. Histol Histopathol 13: 797-817.

129 Beaty T, Prenger VL, Virgil DG, Lewis B, Kwiterovich PO, et al. (1992) A genetic model for control of hypertriglyceridemia and apolipoprotein B levels in the Johns Hopkins colony of St. Thomas Hospital rabbits. Genetics 132: 1095-1104.

130 Fan J, Shimoyamada H, Sun H, Marcovina S, Honda K, et al. (2001) Transgenic rabbits expressing human apolipoprotein (a) develop more extensive atherosclerotic lesions in response to a cholesterol-rich diet. Arterioscler Thromb Vasc Biol 21: 88-94.

131 Watanabe Y (1980) Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit): incidence and development of atherosclerosis and xanthoma. Atherosclerosis 36: 261-268.

132 Hara T, Tsukada N, Okano M, Ishida T, Hirata KI, et al. (2018) Progression of calcific aortic valve sclerosis in WHHLMI rabbits. Atherosclerosis 273: 8-14.

133 Shiomi M, Ito T, Yamada S, Kawashima S, Fan J (2003) Development of an animal model for spontaneous myocardial infarction (WHHLMI rabbit). Arterioscler Thromb Vasc Biol 23: 1239-1244.

134 Caira FC, Stock SR, Gleason TG, McGee EC, Huang J, et al. (2006) Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. J Am Coll Cardiol 47: 1707-1712.

135 Wirrig EE, Yutzey KE (2011) Transcriptional regulation of heart valve development and disease. Cardiovasc Pathol 20: 162-167.