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Animal Model of Aortic Valve Calcification: Their Methodology Helps Us Understand Aortic Valve Calcification

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Abstract

Aortic valve calcification disease (CAVD) is the most prevalent degenerative valve disease in humans, leading to significant morbidity and mortality. Despite its common occurrence, our understanding of the underlying mechanisms remains incomplete, and available treatment options are limited and risky. A more comprehensive understanding of the biology of CAVD is essential to identify new therapeutic strategies. Animal models have played a crucial role in advancing our knowledge of CAVD and exploring potential treatments. However, these models have inherent limitations as they cannot fully replicate the complex physiological mechanisms of human CAVD. In this review, we examine various CAVD models ranging from pigs to mice, highlighting the unique characteristics of each model to enhance our understanding of CAVD. While these models offer valuable insights, they also have limitations and shortcomings. We propose that the guide wire model shows promise for future CAVD research, and streamlining the methodology could enhance our understanding and expand the research scope in this field.

Keywords

Animal Model, Aortic Valve Stenosis, Calcification, Cardiovascular

1. Introduction

Calcific aortic valve disease (CAVD) is the most prevalent degenerative valve condition in humans. As a result of societal advancements, an aging population, and a lack of preventive measures for the disease, there has been a significant

rise in the incidence of aortic disease, mortality rates, and medical interventions for this condition over the past two decades, starting from the late 20th century [1]. The prevalence of CAVD is more pronounced in developed nations, with an estimated 9.7 million cases reported globally in 2019 [2]. A registry in Australia revealed that moderate to severe aortic stenosis has resulted in a mortality rate exceeding 50% over the past 5 years [3]. Unfortunately, common risk factors such as hypertension, hyperlipidemia, and diabetes have been identified to significantly elevate the likelihood of CAVD occurrence [4] [5] [6]. Research indicates that CAVD shares similar risk factors with atherosclerosis, suggesting a common underlying physiological mechanism. Consequently, statins have been explored for their potential application in clinical settings. However, literature suggests that statins have limited efficacy in preventing or delaying aortic valve calcification [7].

Currently, the most effective treatment for aortic valve calcification is surgical treatment. Today, we have more mature aortic valve replacement (AVR) [8] or less invasive transcatheter aortic valve implantation (TAVI) [9]. After many rounds of innovation and reform, the clinical application of various artificial valves has greatly improved the quality of life of patients after operation [1]. However, the anticoagulation problem of mechanical valve or the dysfunction caused by structural valve disease of artificial biological valve [10], and the possibility of perivalvular leakage, conduction abnormalities, long-term durability, coronary recanalization and valve re intervention [11], still face great challenges. Therefore, to explore the physiological mechanism of CAVD, to study the therapeutic target of this disease, and to prevent or postpone the development of this disease is still a major problem for heart centers around the world. In general, although many features of human CAVD are well described (especially in advanced disease), the pathology of aortic valve calcification is complex and involves multiple disease effects, including lipoprotein deposition, elevated oxidized phospholipids, and the influence of various inflammatory factors and immune cell interactions [12] [13]. In addition, in the field of surgery, we can use AVR to prolong the life of patients and improve their quality of life, but we have not yet been able to prevent and delay the process of calcification at the level of mechanism. Therefore, it is an unslaked scientific demand to determine the pathophysiological mechanism of CAVD and to find original treatments for CAVD. Animal models are important tools for achieving this goal, which is promoted by the emergence of new models and a better comprehending of the utility of extant models. In this paper, we sum up and critically evaluate present small and large animal models of CAVD, and discuss the preferred uses of animal models in the field of CAVD research.

2. Animal Models of CAVD

Animal models such as mice are widely used in aortic valve calcification [13] [14] [15]. As an animal model, the physiological process of inducing CAVD should be similar to that of humans. Therefore, the known physiological processes of CAVD in humans studied in relevant literature also needs to appear in animal models to

a certain extent. Therefore, it is beneficial to create a simplified, stable and rapid model to more conveniently and effectively understand the occurrence and development of human diseases, both from the experimental cost and accurately observe the experimental results of the model. Under the background of known risk factors, the animal model to be introduced in this review is helpful to study the pathophysiological process of aortic valve calcification, as well as related delay and preventive treatment of the disease are studied. We sorted out the relevant literature and divided it into five categories: atherosclerosis related dietary induction model, gene knockout induction model, as well as mechanical injury model, warfarin induction model and CKD type dietary induction model developed in recent years. However, our study's findings demonstrate that many scientific studies now employ two or more modelling methods to expedite modelling time and increase success rates. Consequently, we cannot differentiate between other models in our classification. Therefore, we have established the following rules for classification: (1) If the model exhibits pathophysiological changes such as atherosclerosis and is fed with high-fat and high-sugar diets, in addition to lipid knockout genes like APOE, it is classified as an atherosclerosis-associated dietary-induced model [16]. (2) As warfarin causes an increase in the level of MGP mRNA expression in the aorta in animal models and accumulation of MGP antigens, we classify this model as an atherosclerosis-associated dietary-induced model. All models in this section are classified as warfarin-induced models due to the accumulation of MGP antigen. (3) The animal models were classified based on the presence of biochemical alterations such as BUN and PTH for CKD-induced models, artificial haemodynamic changes for guidewire models, and knockout of relevant genes without any other animal diets that could lead to calcification of the aortic valve for the final knockout animal models.

2.1. Atherosclerosis Related Dietary Induction Model

The animal model induced by high-fat and high-cholesterol feeding has been widely used in the past 20 years. Various high-fat and high-cholesterol feeding methods have been used to create animal models, including large animal pigs, medium-sized rabbits, and small rodents (**Table 1**).

2.1.1. Dietary Induction Model of Atherosclerosis in Pigs

The pig genome is similar in size, sequence and chromosome structure to that of humans, making it a particularly useful model for studying the aortic valve genome. In the high-fat and high-cholesterol feeding induction model, pigs were fed 12% fat and 1.5% cholesterol for 6 months. The initial pathological changes of the aortic valve were found to be on the aortic side, and there was no significant inflammatory response similar to human valve calcification [17] [18] [19]. With an increase in dietary induction time, hyperlipidemia caused extensive changes in atherosclerosis in swine [20] [21]. As some animal models develop atherosclerosis before valve calcification occurs [22], leading to their death, the experiment may become less efficient and modelling costs may increase.

Table 1. Summary of atherosclerosis induced animal models used to study aortic valve disease.

animal model	Gene and inciting factors	Feeding patterns	Time	age/weight
Mice				
C57Bl/6J mice [41]	LDLr-/-	HF/HC (59% of calories are high in palmitic acid, no cholesterol)	2 - 4 months	20 to 25 g
C57BL/6 background mice [39]	LDLr-/-ApoB100/100	normal chow	mean 20.1 months	17 to 22 months
C57BL/6 background mice [135]	LDLr (-/-)/ApoB (100/100)/IGF-II	diet high in fat (55%), sucrose (28%) and cholesterol (0.2%)	6 months	12 weeks
C57BL/6Jbackground mice [38]	ApoE-/-; age	normal chow	≥43 weeks	≥43 weeks
C57BL/6 background mice [136]	ApoE-/-	0.2% high cholesterol diet	24 weeks	6 - 8 weeks
C57BL/6Jbackground mice [137]	apoE-/-	atherogenic diet (42% milk fat,0.2% total cholesterol)	20 weeks	30 weeks
C57BL/6Jbackground mice [138]	apoE-/-	an atherogenic diet (15% fat,1.25% cholesterol)	8 weeks	8 weeks
C57BL/6Jbackground mice [139]	Lrp5-/-/ApoE-/-	a 0.2% cholesterol (w/w) diet	23 weeks	6 - 8 weeks
Adult waved-2 mice [140]	C57BL/6JEgfrWa2/Wa2	65.7% lipids, 19.5% carbohydrate, and 4.5% cholesterol, Subcutaneous injection three times weekly 16,000 IU/g vita-minD3	6 or 9 months	1.5 months of age
Rabbit				
Normal New Zealand White rabbits [141]	no	2% cholesterol (w/w) added to soybean oil (10% w/w)	29 days	2.5 kg, 8 weeks old
Male New Zealand White rabbits [31]	no	diet supplemented with 1.0% (wt/wt) cholesterol	8 weeks	2.5 to 3.0 kg
male New Zealand White rabbits [49]	no	fed with 0.5% cholesterol enriched chow plus 50,000 IU/day vitamin D2 in drinking water.	12 weeks	2 to 2.5 kg
male New Zealand white rabbits [142]	no	fed 0.5% cholesterol-enriched chow plus 25,000 IU/day vitamin D2	12 weeks	2.5 to 3.0 kg
male New Zealand White rabbits [143]	no	Place on vitamin D-enriched atherogenic diet (purified rabbit chow supple-mented with 0.5% cholesterol and 10,000 IU/day vitamin D)	3 weeks + 4 weeks	3 months/3.7 ± 0.2 kg
Swine				
male swine [17]	no	an isocaloric diet high in fat (12%) and cholesterol (1.5%)	2 week or 6 months	6 months
Gene + HCVD diet				
Notch1 and RBPJk targeted mutant mice [144]	Notch1 and RBPJk targeted mutant	The HCVD diet	16 weeks	8 - 10 weeks

Apo = apolipoprotein; eNOS = endothelial nitric oxide synthase; LDLr = low-density lipoprotein receptor. HCVD = 18% lactalbumin, 7% saturated fat, 1.25%cholesterol and 0.5 sodium cholate supplemented with 5 IU of vitamin D3 (cholecalciferol).

2.1.2. Dietary Induction Model of Atherosclerosis in Rabbits

Due to the advantages of rabbits, such as low cost, short gestation (relative to large animals) and large body size relative to rodents (preclinical studies of some surgical experiments can be carried out) [23], coupled with its sensitivity to high cholesterol diet, as well as its unique lipid metabolic system and human-like atherosclerotic lesions [24], therefore, it has become the second most commonly used animal model in atherosclerotic diet induction.

In **Table 1**, we introduced five atherosclerotic diet induced rabbit models. The rabbit model did not involve gene knockout, and the atherosclerotic diet was dominated by high cholesterol and Vitamin D. According to different research purposes, high cholesterol feeding induction concentrations are generally selected from 0.5% to 2%. While it has been pointed out in the literature that high dietary cholesterol concentration will lead to liver function damage in rabbits [25] [26]. Supplementation with Vitamin D accelerates atherosclerosis and calcium deposition at low concentrations of high cholesterol [27] [28], shorten induction time—vitamin D supplementation at 0.5% cholesterol levels ranges from 10,000 to 50,000 IU/day.

In the simple high cholesterol model, the induction time of 2% cholesterol and 10% soybean oil in the rabbit model was only 29 days, which was significantly shorter than that of other groups. However, only liposomes were formed in the valve in terms of histological changes, and there was no obvious leaf thickening. Two-dimensional echocardiography did not detect significant valvular calcification. However, atherosclerotic lesions and elevated blood cholesterol levels were observed in rabbits that were fed a high-cholesterol diet for 12 weeks. Following dietary induction with Vitamin D, rabbits developed varying levels of calcium deposition and aortic valve stenosis at approximately 10 weeks. Echocardiography showed that the model successfully induced pathological changes similar to early human CAVD, such as osteopontin expression [29] [30] [31]. When the induction time is less than 10 weeks, the model only has histological changes were observed. On the contrary, there are hemodynamic changes such as decreased AVA.

Through the rabbit atherosclerosis induction model, drugs such as pioglitazone, eglitaxel, and Triptolide, which are DPP-4 inhibitors, can reduce the pathophysiological process of valve calcification caused by hypercholesterolemia to varying degrees [30] [32] [33]. These experimental model results offer new insights into delaying or preventing aortic valve calcification.

2.1.3. Dietary Induction Model of Atherosclerosis in Mice

The atherosclerotic diet-induced mice model is commonly used to study aortic valve calcification. In 1992, two independent laboratories reported the creation of ApoE-deficient mice, which are widely used in research [34] [35], the main characteristic is susceptibility to severe atherosclerosis. Valve and vascular calcification occur due to high cholesterol and a high-fat diet. The two most commonly used mouse models lack the ApoE and LDLR genes [36]. Compared to

the latter, the former is more likely to spontaneously develop arteriosclerosis and valvular lesions, regardless of a high cholesterol/high fat diet or an ordinary diet. Additionally, a high cholesterol/high fat diet can accelerate the development of lesions [37] [38] [39]. Both transgenic mice fed with normal diet for a long time will have outflow tract stenosis caused by aortic valve calcification and significant hemodynamic gradient, which proves that aging is closely related to aortic valve calcification in humans.

Aortic valve calcification is similar to atherosclerosis in terms of clinical risk factors, pathogenesis, and suggestive stages, particularly in the ossification and proinflammatory mechanisms of arterial and valve calcification [5] [6] [40]. According to the above findings, several high-fat/high-cholesterol diets were used to hasten the advancement of arterial and valve calcification in mice (see **Table 1**). Under the 0.2% cholesterol diet, ApoE-/- or LDLR-/- mice can exhibit pathological changes, including valve calcification, endothelial activation, inflammation (macrophage accumulation), lipid deposition, osteoblast ossification, and extracellular matrix increase of VIC. Additionally, most models showed hemodynamic changes, such as decreasing AVA and increasing transvalvular flow velocity. Of the various types of feeds, including those high in fat and cholesterol, mice models fed a "North American diet" consisting of high non-cholesterol carbohydrates and fats are more appropriate for studying CAVD in humans. This model replicates the early stages of CAVD, as well as hemodynamic changes, and demonstrates that severe hypercholesterolemia is not necessary to induce aortic valve disease in these mice. Compared to the high-fat and high-cholesterol model, this diet is similar to the human diet and will not cause blood lipid concentrations that cannot be reached by humans. Its significance lies in providing new insights into the best treatment methods for patients with aortic valve diseases in lipid metabolism [41].

2.2. CKD Type Dietary Induction Model

The disturbance of mineral metabolism and the development of heart valve calcification have been widely assessed in patients with chronic kidney disease [42] [43]. These metabolism are regulated by hormones such as 1,25-dihydroxyvitamin D and PTH [44]. Their disorder leads to diffuse calcification and the development of hydroxyapatite crystals in tissues, including aortic valves [45]. A simple animal model based on the risk factor of renal failure for CAVD, which is a multifactorial disease (**Table 2**), can be established by altering the amount of Vitamin D, PTH, and other hormones in the diet.

In the atherosclerotic induced model, we have studied that the development of CAVD can be induced within 12 weeks by simultaneous supplementation of vitamin D2 and cholesterol in rabbit diet [46] [47] [48] [49]. As the studies did not evaluate the effect of vitamin D2 alone in inducing aortic valve calcification, it is unclear whether the model induction reflects additive or synergistic effects between vitamin D2 and cholesterol. In their study, Cardiology Unit et

al administered rabbits with vitamin D2 (25,000 IU, 4 days per week) for 8 weeks, which resulted in a significant reduction in aortic valve area. However, the transvalvular velocity and transvalvular pressure gradient increased slightly [47]. This is similar to the calcification of valves in early humans [50]. Furthermore, alterations in blood biochemistry were observed, such as elevated serum cholesterol and creatinine levels [51]. The mice were administered high-dose vitamin D for three days in addition to continuous daily vitamin D intake, followed by a standard diet for six weeks. This resulted in calcification of valves and medial vessels, as well as metabolic disorders in liver and kidney function [52]. Furthermore, studies have shown that adenine metabolic disorder can result in gradual renal injury and cardiovascular disease [53] [54]. Jia Gu and colleagues induced an aortic calcification model in a short period of time by administering an adenine diet for three weeks and intraperitoneal injection of vitamin D (8.75 mg/kg/day) for ten days, simulating the formation of aortic valve calcification in human CKD. In this model, serum urea nitrogen (BUN) and creatine (SCR) significantly increased, along with a moderate increase in serum phosphorus. This finding provides a useful research tool for further study of calcific aortic valve disease (CAVD) caused by calcium and phosphate disorders [55].

In addition to Vitamin D, hyperphosphatemia is also associated with arterial wall and valve calcification in CKD. This has been shown to be a direct stimulator of vascular calcification in many studies [56] [57] [58]. Relevant animal studies have shown that calcium deposition in the valve also increases with an increase in phosphate feed [59]. Two hyperphosphate induction models were introduced (see **Table 2**). Rats were subjected to 0.75% adenine induction and 5/6 nephrectomy while on a high phosphate diet (≥ 1.5%) for a period of more than 7 weeks. The resulting histological changes included valve calcium deposition, as well as increases in blood biochemistry markers such as SCR, BUN, and PTH. Notably, 5/6 of the nephrectomy rats also experienced goiter and other cardiovascular changes.

High fat/high cholesterol, high phosphate and vitamin D all reflected their induction value in the diet of the model. Alexander Assmann and his colleagues combined high cholesterol [60], Vitamin D and high phosphate diets of different dietary schemes in rats, and measured the data of rats at 4, 8 and 12 weeks. The results showed that the concomitant use of the three high-dose diets led to the most rapid development of aortic valve and aorta calcification, as well as a significant increase in heart mass and hemodynamic changes. However, the low-dose model exhibited only mild pathological changes in the cardiovascular system. Additionally, biochemical indexes such as serum calcium and blood cholesterol showed only slight increases, which reduced the body injury and weight loss caused by prolonged induction time. Therefore, this model appears to be more suitable for studying the chronic evolution process of CAVD [58].

Table 2. Summary of CKD induced animal models used to study aortic valve disease.

Animal	Weight/age	Induction mode	Induction time	Related pathological changes	biochemistry
Rabbit and mice					
male rabbits [47]	2 - 2.5 kg	vitD2 (25,000 IU/4 days per week)	8weeks	Fibrosis/calcification, inflammatory activation, atherosclerotic changes	Calcium phosphate product, total cholesterol and SCR↑
male C57BL/6J mice [52]	16 weeks age	injection of 100 μL VD3 (5.5×10^5 U/kg) once daily for three consecutive times	3 days + 6 Weeks	medial arterial calcification	VD3↑↑↑, BUN and ALT↑, Tissue metabolism disorder
male C57BL/6 mice [55]	6 weeks age	0.2% adenine in chow+intraperitoneally injected VitD (8.75 mg/kg/day)	adenine 3 weeks, vitD 10 day	calcification	BUN, SCR and Ca2+ ↑↑↑, P↑↑
Rat					
male rats [145]	8 weeks old/250 g	fed high-adenine (0.75%) + high phosphate diet (1.5%) + normal rat chow	7 weeks + 2 weeks	calcification, ossification, inflammation	SCR and P↑↑↑, PTH, Hyperparathyroidism
male rats [146]	8 weeks old/250 g	5/6Nx + HP (P = 2.0%)	8, 12, 16 weeks	calcification（$[Ca_5(PO_4)_3(OH)]$）、glycosylation	SCR, BUN and 24 hour urine protein↑, PTH↑↑↑, P↑↑
Male Wistar rats [147]	200 - 250 g	vitD: 300,000 IU/kg, CH: 2%, PH: 1.5%	4, 8, 12 weeks	Ca, TC, Lipid vacuoles (most in 2 groups), Ca deposition in aortic valve lobules	LDL/HDL↑, Hypertrophic neointima
		vitD: 150,000 IU/kg, CH: 1%, PH: 0.75%			LDL/HDL↑, Maximum heart mass, maximum AVPG
		vitD: 300,000 IU/kg, PH: 1.5%			rigid spine
		vitD: 300,000 IU/kg, CH: 2%			rigid spine , Hypertrophic neointima

increases: ↑; increased moderately: ↑↑; significantly increased: ↑↑↑; BUN = Blood urea nitrogen; P = serum phosphorus; SCR = Serum creatinine; TC = Total serum cholesterol; PH = Primary Hyperparathyroidism; PTH = parathyroid hormone; AVPG = aortic valve pressure gradients; 5/6Nx = 5/6 nephrectomy; HP = high phosphate diet (P = 2.0%); $[Ca_5(PO_4)_3(OH)]$ = Hydroxyapatite.

2.3. Non-Modified/Modified Guide Wire Mouse Model (Table 3(a), Table 3(b))

Mechanical injury-induced hemodynamic changes are a significant risk factor in aortic valve calcification [61] [62]. Additionally, non-coronary valve damage is more severe [63]. Using ultrasound guidance, Honda and his colleagues inserted a spring wire into the left ventricle through the mice's right carotid artery. They then scratched the lobule with a steel wire 20 times, correctly positioned the tip of the steel wire on the left ventricular side of the valve, and rotated it 50 times to achieve the desired moulding [64] (**Table**

3(a)). After 1 week of postoperative injury, the trans-valvular velocity of the aortic valve was significantly increased, and the valve area was significantly reduced. After four weeks of operation, the valvular lobules showed marked thickening and progressive osteochondrosis under the microscope. Three important osteochondrogenic signals, BMP-2, Sox9, and Runx2, were expressed. Fibroproliferative changes and aggregation of inflammatory cells were observed. Histologically, significant calcium deposits were observed in the damaged valve twelve weeks after surgery.

Table 3. (a) Summary of non-modified guide wire mouse model used to study aortic valve disease; (b) Summary of modified guide wire mouse model used to study aortic valve disease.

(a)

basic operation	Age	concrete operations	Time	characteristic	Histological change
Under echocardiographic guidance, a spring guidewire was implanted into the left ventricle of mice through the right common carotid artery, resulting in aortic valve injury.	8 - 10 weeks age	The spring-loaded wire (0.36 mm diameter) is inserted into the artery by bending it at an angle of 15˚, with the tip of the wire on the left ventricular side of the valve. The valve is incised 20 times and rotated 50 times with the body of the wire.	4, 8, 12, 16 weeks	significant hemodynamic stenosis and heart failure The mortality of mice with aortic valve injury within 4 weeks ≈ 20%	Ostochondroid changes, calcification, collagen deposition, neovascularization

(b)

basic operation	concrete operations	Aortic valve blood flow peak velocity	Death rate caused by AI	Calcium deposits	Histological change
Mild and moderate injuries - straight guidewire; severe injuries - conventional guidewire with 15˚ angled tip; echocardiographically guided guidewire insertion into the left ventricle Advance the guidewire into the left ventricular apex and withdraw it into the left ventricular outlet just below the level of the aortic valve. (4 - 5 mm amplitude). The wire then rotates across the valve at a rate of two rotations per second.	(1) Mild (tip guide wire): pushed back and forth 20 times, rotate 50 times	No significant change	Not know	No	Not know
	(2) Medium (tip guide wire): push back and forth 50 times, rotate 100 times	increased after 1 week, stabilized after 4 weeks	11.25% mild	No	Valve thickening, inflammation, fibrosis, no obvious calcification
	(3) Severe (wire with 15˚ angled tip): pushed back and forth 20 times, rotate 200 times	increased after 1 week, continued to increase after 4 weeks	50%, 18.75% moderate	After 8 weeks	Valve thickening, inflammation, fibrosis and calcification were obvious

Animal: male C57/BL6 mice; Age of improved guide wire model: 10 - 12 weeks age; AI = aortic valve insufficiency; Non-modified guide wire mouse model [64]; modified guide wire mouse model [65].

In conclusion, the pathological changes in the valve are similar to those in humans. Based on this, Sven Thomas Niepmann and his colleagues modified and expanded the moulding technology and operation [65]. This included changes to wire type, tip angle, and quantity (**Table 3(a)**), resulting in a model with the characteristics of light, medium, and severe wire injury through graded injury [65]. In echocardiography, there was a positive correlation between the incidence of aortic regurgitation and the severity of aortic valve injury in mice. Four weeks after surgery, mice with severe valve injury not only had an expanded left ventricular diameter, but also a decreased left ventricular ejection fraction and an increased left ventricular posterior wall. The mild and moderate injury models did not show these changes. Histological analysis revealed a significant increase in aortic valve area and thickness in moderate and severe injury models compared to sham-operated models. Immunofluorescence demonstrated a significant increase in inflammation and fibrosis levels, while calcification was only evident in the severely injured model 8 weeks after surgery.

The injury model does not involve any related metabolic disorder, only hemodynamic changes. This model can be used to exclude the influence of factors that induce aortic valve calcification in experimental studies, thus making the experiment reproducible. For instance, the aope-/- mice model fed with high fat and cholesterol and the guidewire injury mice model were used, with simultaneous knockout of the ChemR23 gene. The results showed the same pathological and hemodynamic changes. Thus, it is confirmed that the blood lipid is normal and there is no atherosclerosis. Additionally, the reproducibility of the beneficial effect on aortic valve disease mediated by ChemR23 signaling is also confirmed [66].

2.4. Warfarin Induction Models (Table 4)

Epidemiological evidence suggests that vitamin K supplementation can reduce vascular calcification in rats with vitamin K deficiency and CKD [67]. The use of warfarin, a Vitamin K antagonist, can systematically increase calcium deposition in the cardiovascular system [68] [69]. The lack of vitamin K causes cardiac valve and vascular calcification due to the suppression of vitamin K-dependent calcium-matrix Gla protein (MGP), which serves as a calcification inhibitor in vivo [70] [71]. The function of MGP was investigated by targeting gene deletions in mice. MGP knockout mice showed obvious calcification of the aortic wall, coronary artery, and elastic plate of the aortic valve at 2 weeks old, with continuous calcified spots. After 3 weeks, signs of bone retardation and bone mineral density reduction began to appear. The growth of MGP knockout mice was significantly slower than that of wild-type mice, and their lifespan was also much lower [72]. Based on the characteristics outlined above, researchers investigated the impact of vitamin K inhibitors on soft tissue calcification by administering warfarin to rodents (**Table 4**). However, it should be noted that warfarin also inhibits coagulation function, resulting in almost all animal models dying from internal bleeding within one month of birth. Fortunately, studies of vitamin K-dependent osteocalcin [73] [74] have revealed a basic dichotomy in the ability

of vitamin K to counteract the effects of warfarin in different tissues. Warfarin inhibits MGP while maintaining normal clotting time [75].

Table 4. Summary of warfarin induction animal models used to study aortic valve disease.

animal	Age/weight	Inciting factors	Induced time	The histologic	Special change
Male Sprague-Dawley rats [76]	6 weeks	inject 15 mg/100g warfarin twice a day + 1.5 mg vitamin K1/100 g per day or one half of this dose.	1, 2, 3, 4, 5 weeks	Calcification of the elastic plate layer (Coronary, aortic valves, hilus arteriesa, pulmonary arteries), Mineralization of abdominal aorta, carotid artery and aorta	Tissue calcium and MGP mRNA↑, serum calcium, phosphorus levels, bone growth constant
male Sprague-Dawley rats [148]	8 weeks, weight 250 - 280 g	treated with warfarin (20 mg/kg/d) and subcutaneous injection of vitamin K (15 mg/kg/d) on days 1, 3, 5, 7, 14, 21, 28.	4 weeks	Aortic root and aortic valve mineralization	heart rate↓, hemodynamic changes
Male Wistar rats [149]	weight 200g			Mineralization(Aortic root and apex, aortic wall and heart apex), Apoptotic cells in apex of aorta↑	hemodynamic changes, The main thoracic calcification PMV↑↑, TPTVV↑
male C57/Bl6 mice [86]	8 weeks	250 mg/kg/d warfarin and 30 mg/kg/d vitamin K1	4 weeks	Obvious calcium deposition (aortic valve)	Not know
DBA/2Nchr mice [87]	after birth	1) 0.03 mg/g warfarin + 1.5 mg/g vitaminK1 2) 0.3 mg/g warfarin + 1.5 mg/g vitamin K1 3) 3 mg/g warfarin + 1.5 mg/g vitaminK1	1, 4, 7 weeks	"3 mg" group: most obvious calcification(myocardial tissue, aortic tissue), MGP mRNA is the lowest, osteopontin is the highest	SBP, Ca, P, CRP and BUN no change, PWV, TPTVV, AVPG↑ (0.3 mg most), t-ucMGP 0.3 mg (the highest)
ApoE-/- mice [83]	10 weeks	WTD (0.25% cholesterol and 15% cocoa butter), WTD + VitK1 (1.5 mg/g food) + warfarin (3.0 mg/g food)	12 weeks + 1, 4 weeks	Thoracic aorta calcium levels, Endometrial calcification plaque and Calcified nodule area↑	PC, Ca, P and weight no change, carboxylated MGP↓↓, uncarboxylated MGP↑, plaque apoptosis↑, VSMC loss
ApoE-/- mice [84]	8weeks	WTD + vitK1 (1.5 mg/g food), 3 mg VKA warfarin (V/K1 diet)	8 weeks + 8 weeks	Calcium accumulation in the aortic valve↑↑	weight, plasma lipid, Ca, P (no significant differences), Atherosclerosis and ALP activity was similar.

increases: ↑; increased moderately: ↑↑; decrease: ↓; decreased significantly: ↓↓; PMV = pulse wave velocity; CRP = C-reactive protein; BUN = Blood urea nitrogen; AVPG = Aortic valve peak gradient; SBP = systolic blood pressure; TPTVV = the peak transaortic valve velocity; PC = Plasma cholesterol; Ca = calcium; P = phosphorus; VSMC = vascular smooth muscle cells; t-ucMGP = serum levels of total uncarboxylated matrix Gla protein.

In 1998, Paul A. Price and his colleagues (see **Table 4**) discovered that subcutaneously injecting male Sprague-Dawley rats with 15mg/100g warfarin twice a day and 1.5mg/100g vitamin K1 once a day may cause calcification of the elastic plate of the coronary artery and may be accompanied by calcification of the cardiac aortic valve. Over time, the number of calcified plaques gradually increased. However, unlike the MGP knockout mice model, calcification in this model is focal and progresses slowly. Phenotypes such as reduced bone growth and osteomalacia are not readily apparent [76]. The CAVD model can be established in rats by administering warfarin (20 mg/kg/day) orally and vitamin K (15 mg/kg/day) via subcutaneous injection for four weeks (see **Table 4**). This model not only induces aortic valve and aortic mineralization, but also causes hemodynamic changes. The induction time in mice is similar to that in rats, and as time progresses and the warfarin dosage increases, the changes in aortic calcium deposition and hemodynamics become more pronounced. As previously stated, the animal model of atherosclerosis exhibits pathological processes of ossification and cartilaginization that contribute to calcium deposition. [77] [78] [79]. MGP is an effective calcification inhibitor that can prevent vascular calcification induced by bone morphogenetic proteins 2 and 4 (BMP-2 and -4) during vitamin K carboxylation [80] [81] [82]. The correlation between the two can be extended by demonstrating that warfarin significantly increases vascular calcification in hyperlipidemic mice. This effect was observed in a hyperlipidemic model of atherosclerotic calcification [83] [84]. The model involved inducing high cholesterol/high-fat diet for 8 to 12 weeks, followed by the addition of vitamin K1 (1.5 mg/g comestible) and warfarin (3.0mg/g comestible) to the atherosclerotic diet for 4 to 8 weeks. The model involved inducing high cholesterol/high-fat diet for 8 to 12 weeks, followed by the addition of vitamin K1 (1.5 mg/g comestible) and warfarin (3.0 mg/g comestible) to the atherosclerotic diet for 4 to 8 weeks. Calcium deposits are present in the aortic valve. It is noteworthy that there was no significant difference in serum calcium, phosphorus, BUN, and other biochemical markers between the mice model and the control group. Additionally, there were no significant changes in growth and development.

The rat model of calcific aortic valve disease (CAVD) was established by administeringwarfarin in combination with vitamin K. This model has provided new insights into the pathways and signaling molecules that play a crucial role in the process of vascular and valve calcification, offering potential therapeutic targets for the treatment of aortic valve disease. For instance, when compared to warfarin, which induces valve calcification in APOE-/- mice, rivaroxaban does not significantly delay or hinder valve calcification in APOE-/- mice. However, it also does not negatively regulate MGP metabolism and has certain anti-inflammatory effects on VIC activation [82]. Therefore, warfarin use may raise the risk factors of acute coronary events in the formation of coronary atherosclerotic plaque [83]. In conclusion, the choice of anticoagulant drugs may affect a certain prognosis in cardiovascular diseases. In addition, our known studies have found that 10 mm warfa-

rin and 1.6 mm inorganic phosphate can accelerate the calcification of pavic (porcine aortic valve stromal cells) [85]. When compared to C57/B16 mice that were injected with warfarin and vitamin K1 to establish an in vivo animal model, it was discovered that EGb761 (Ginkgo biloba extract), which has a protective effect on cardiovascular disease, significantly inhibited the BMP2-mediated Smad1/5/Runx2 signal pathway and improved warfarin-induced aortic valve calcification. This suggests that the drug has great potential for use in clinical medicine in the future [86]. Thilo Kr ü GER and his colleagues described the model of extensive cardiovascular injury induced by warfarin in wild-type DBA/2 mice for the first time. They are also deciphering the mechanism of vascular and valve calcification, researching and developing new treatment strategies and providing new methods [87].

2.5. Gene Knockout Model (Table 5)

With the development of gene transfer technology, researchers have created a variety of CAVD transgenic mice models. The most representative of these is the Notch1 gene. The heterozygous mutation of this gene is the unique known genetic cause of human CAVD. Notch1 is involved in the development of early embryos, including the development of aortic and pulmonary valves [88] [89]. Its mutation leads to the up regulation of the activation of Runx2, the downstream central regulator, resulting in the activation of osteopontin and osteocalcin transcription in the early stage of the valve and the final calcification of the aortic valve [90]. BAV (Bicuspid aortic valve) is an ordinary congenital heart defect. Patients develop significant CAVD in adulthood. Notch 1 mutation is one of the causes of human non syndromic BAV [91]. Christina v. theodoris and his colleagues hybridized Notch1-/- C57BL/6 mice with mice lacking telomerase RNA component TERC (MTR) and analyzed several generations of telomere shortened N1 haploid deficient mice (N1 +/− mtrg2). It was found that aortic valve calcification in the second and third generations of hybrid mice was more serious than that in the first generation. The study found that by down regulating osteoclast and cell adhesion related genes, Promote the migration of fibrogenic cells to atherosclerotic lesions and valve osteogenesis and calcification [92]. In addition, no plays an important role in cardiovascular homeostasis. Similar to Notch1, eNOS deficient mice also displayed a high incidence of BAV (in eNOS deficient aortic valves, fibrosis and calcification showed significantly different time patterns and progression rates: fibrosis began in youth, while calcification was dominant in older mitral valves) [93]. Vidu Garg, M.D., and colleagues established a brand-new model of aortic valve disease in hyperpermeable mice: NotCH1 and NOS3 composite mutant mice (Notch1þ/-; Nos3-/-), which greatly increases the probability of valve thickening and is accompanied by hemodynamic changes [94]. In addition, cardiac valve antiangiogenic factor ChM-I and Smad6 gene, which plays a special role in the progress and homeostasis of cardiovascular system, are widely used in the animal model of CAVD [95] [96].

Table 5. Summary of gene knockout animal models used to study aortic valve disease.

animal	Gene knockout	Age/weight	BAV	Significant hemodynamic stenosis?	Histopathological changes of AV
C57BL/6J background mice [95]	Chm-I	8 and 20 weeks of age	Not known	Not known	Calcification, Neoangiogenesis, Lipid deposition
in C57BL/6J but not 129S1/SvImJ mice [150]	EGFRWa2/Wa2	≤15 months	Not known	Yes, but background strain dependent	Cellular proliferation, ectopic cartilage for mation , extensive calcification, inflammatory infiltrate, Fibrosis
mixed 129/SvEv×BALB/cBy background mice [96]	Madh6-/-	4 - 6 months	Not known	Yes, may affect resistance vessels	Calcification, ossification, Excessive proliferation of mesenchymal cells
C57BL/6 background mice [72]	MGPm1/MGPm1 (-/-)	After birth	Not known	Not known	Calcification
C57BL/6J background mice [151]	eNOS-/-	25 to 30 g/embryos at day 13.5 of gestation	Bicuspid aortic valves in0~40% of mice	Not known	Bicuspid aortic valves in0~40% of mice
mice [152]	NOS3-/-; Notch1+/-	6 - 8 weeks	Aortic valve malformations occur in nearly 100% of cases, the most common being BAV	highly penetrate BAV and develop hemodynamically significant aortic valve stenosis and regurgitation	Calcification, aortic valve malformations
C57BL/6 background mice [153]	Notch1+/-	10 months of age	No	No	Calcification
$NI^{+/-}$ mTR^{WT} mice (generation 1-3) [92]	Notch+/-mTRG2	≥1.5 months	Not known	No	Calcification
mice [91]	Notch1 (mutation)	After birth	Relevant, specific data cannot be speculated	Not known	Calcification, Bicuspid aortic valves
C57BL/6 background mice [90]	Postn-/-	6 or 10 months	Not known	Not known	Calcification, Fibrosis
C57BL/6 background mice **[154]**	Postn-/-	12 weeks	Not known	No	Reduced valve thickening, Calcification, fibrosis

BAV = Bicuspid aortic valves; AV=aortic valve.

3. Discussion

Animal models are a significant platform for studying the occurrence and development of CAVD *in vivo* and evaluating the effects of therapeutic interventions. To achieve the most effective results, the animal model should simulate human diseases or at least important aspects and conditions of human CAVD development. This section will discuss the advantages and disadvantages of five models to assist researchers in selecting animal models for CAVD research.

Pigs are commonly used in the study of atherosclerosis due to their ability to spontaneously develop atherosclerotic changes in blood vessels and valves in the traditional atherosclerosis-related feeding induction model [97]. However, it is important to note that during the process of high-fat and high-cholesterol feeding, the valve has not been calcified, and the coronary artery has been narrowed due to atherosclerosis, which can result in the death of pigs. The use of this model is more efficient and cost-effective than previous methods. In recent years, porcine aortic valve stromal cells (pAVIC) have been increasingly used by researchers to study the mechanism and pathological process of aortic valve calcification [33] [98] [99] [100]. Additionally, the pAVIC model is used as a supplement to in vivo animal experiments (such as the warfarin and Vitamin D induction model) in the study of new therapeutic drugs related to delaying or inhibiting valve calcification [85] [86] [101]. The model improves not only the tracking time but also the experimental process, while excluding the influence of other factors in the body to enhance the study's accuracy.

During the experiment, induction of a high-fat and high-cholesterol diet in an animal model of CAVD can often result in blood lipid/cholesterol concentrations that cannot be achieved in humans. Such high concentrations may have adverse effects on mice and increase the error of experimental simulation [102]. Therefore, it is important to reduce the limitations of relevant experiments. Researchers have gradually explored the correlation between vitamin D and CAVD with the development of an atherosclerotic diet-induced model [101] [103] [104]. The use of rabbits induced by a vitamin D and high cholesterol diet has become a popular choice for experimental models. This model is preferred over the simple high cholesterol model as it avoids the hepatotoxicity and fat accumulation associated with high cholesterol in rabbits [105]. It shortens the time of valve calcification induced by low concentration cholesterol and reduces the cost of the experiment. However, inducing the rabbit CAVD model with vitamin D alone resulted not only in histological changes similar to human CAVD, such as fibrosis and inflammation, but also hemodynamic changes [33]. The successful induction of this model prompted researchers to re-examine the fundamental role of high fat/high cholesterol in aortic sclerosis. However, high-dose vitamin D affects not only the cardiovascular system, causing biochemical abnormalities such as a rise in blood cholesterol, but also results in physical damage such as kidney injury and weight loss [106]. It limits the process of continuous observation of chronic AVS. And in clinical medication, the treatment of human body with vitamin D does not necessarily affect the blood cholesterol level [107]. When studying drugs for the treatment of CAVD, it

is important to exclude any potential effects. Table 1 shows that when the dietary cholesterol concentration remains constant, and the concentration of vitamin D is changed within a certain range, there is no significant difference in induction time, and the animal model exhibits similar histological and hemodynamic changes. It may be necessary for researchers to re-examine whether varying concentrations of vitamin D result in statistical changes in the model.

Compared to pigs and rabbits, dealing with large and medium-sized animals such as rats is less expensive and more convenient. Additionally, rats offer the same affordability and ease of treatment as mice. Furthermore, the corresponding histological changes of blood vessels and heart are easier to obtain through laboratory examination than in mice. However, it is important to note that rats are not prone to atherosclerosis and there are few transgenic rats available [108]. The CAVD model in rats is typically induced by non-transgenic and non-atherosclerotic diets, such as warfarin and high phosphate diets.

We have determined that there is a clear consistency between the progression of CKD in animals and humans, characterized by an increase in blood urea nitrogen and plasma creatinine, hyperparathyroidism, and hyperphosphatemia. A model of chronic kidney disease (CKD) was induced in rats by feeding them a high purine and high phosphate diet, resulting in acute renal injury and vascular calcification [109]. This model is unique in its ability to induce valve calcification in a short amount of time, reverse pathological calcification, and restore the valve to its original state prior to induction, all without the need for transgenic animals [110]. It mimics the expression of osteogenic transformation and ossification-specific proteins found in human valve calcification. The model is simple to operate. It enables the continuous evaluation of the dynamic and reversible processes of calcific aortic valve disease (CAVD) and the development of new therapeutic options at critical stages of valve calcification. The other model is nephrectomy + high phosphoric acid diet feeding model. Compared with the former, the process is relatively long, and the operation requirements are higher. After 16 weeks, most of the rats died of CKD-related cardiac injury, which is not suitable for long-term evaluation of the pathological process of CAVD [111] [112]. In addition, this model can only study the heart damage caused by renal failure or CKD, and the research scope is narrow. But it is most suitable for simulating the condition of human cardiovascular system after CKD. The researcher can adjust the model scheme through the research content and accurately experiment process.

Studies have shown that dietary supplementation with high cholesterol and vitamin D can cause vascular calcification in rats [113]. In rat models of CKD, vitamin D (either calcitriol or its analogue) generally accelerates vascular calcification, with calcium levels in the aorta at least doubling [114]. Assmann *et al.* (year) evaluated the effect of simultaneous supplementation of high cholesterol, vitamin D, and phosphate on valvular deformations. The timing of valvular calcification varied among rats administered different doses of the three supplements (Table 2). In general, a high-dose diet of cholesterol, vitamin D, and phosphate accele-

rated valve calcification and reduced modeling time in rats. However, hemodynamic changes were more pronounced in the low-dose group than in the high-dose group, which is advantageous for observing the continuous process of valve calcification, despite the longer induction time. The required levels of vitamin D were higher than those in the model of rabbits induced with vitamin D and high cholesterol [47]. The hemodynamic performance is more noticeable. The model was compared with the histological appearance of human disease, and no obvious inflammation was associated with aortic valve tissue. In general, this *in vivo* model promotes valvular degeneration, which further expands the stenosis of the aortic flap. Therefore, extensive research on the prevention and hospitalization strategy of cardiovascular calcification is necessary.

Although the structure of the aortic valve in mice differs significantly from that of humans [115], valve calcification can still be induced through dietary or other interventions [59] [97] [116]. But compared with large animals, it has the favors of low outlay, convenient management, uncomplicated breeding and high efficiency. In addition, mice genetics has been extensively described and its full genome sequence is available. It is possible to study the molecular mediators of CAVD due to the ease of genetic manipulation and availability of cloned samples [116] [117].

It has been nearly half a century since the discovery of APOE. Mice models that create atherosclerosis have been in nearly 25 years. And this model has been widely used in the study of cardiovascular diseases. However, APOE deficiency is a utmost condition and barely occurs in humans [118]. And the most universal cause of type III hyperlipidemia is the existence of defective forms of APOE receptor binding, like apoE2 [119]. Therefore, the limitation of APOE deficiency as a model for inducing aortic calcification is that type III hyperlipidemia is an extreme and rare condition in humans. Of course, a large number of studies have applied this model to the physiological process of arterial plaque formation and aortic calcification disease [120], and achieved more results, so we can ignore the limitations of this rare situation.

In addition, our analysis revealed that diets with higher percentages of cholesterol led to significantly shorter induction times. However, this was accompanied by the serious issue of severe coronary artery stenosis, ultimately resulting in model complications and death. For instance, low-density lipoprotein receptor-deficient mice serve as a model for familial hypercholesterolemia. Under normal dietary conditions, these mice primarily develop moderate hypercholesterolemia due to the accumulation of low-density lipoprotein cholesterol, resulting in cholesterol levels around 250 mg/dl. In contrast, humans lacking LDL receptors can have plasma LDL levels as high as 1,000 mg/dL. This disparity may be attributed to differences in the rate of LDL production between mice and humans [121] [122]. In addition, LDL-receptor-deficient mice did not develop notable atherosclerotic lesions on a normal diet [123]. However, these mice were highly sensitive to diet-induced hypercholesterolemia. When fed a Western diet, LDL-receptor-deficient mice developed severe hypercholesterolemia. The entire aorta tree showed a sig-

nificant atherosclerotic lesion and calcium deposits in the aortic valve [39] [124]. When exposed to a diet high in cholesterol and cholic acid, cholesterol levels increased to over 1500 mg/dl, leading to the rapid development of numerous xanthomatosis and severe atherosclerotic lesions [125]. Transgenic mice with dual loss of APOE and LDLr were fed an athero-sclerotic diet, resulting in severe hyperlipidemia, coronary artery stenosis, and myocardial infarction. This model led to increased complications, decreased life expectancy, reduced sample size, and higher experimental and management costs [126]. Additionally, a mice model of aortic valve calcification induced by a high fat/high carbohydrate "North American diet" was described, successfully mimicking metabolic abnormalities, valvular degeneration, and hemodynamic changes seen in early stages of aortic valve calcification in humans with mild hypercholesterolemia. This model closely mirrors the physiological progression of the disease in humans, prompting a reevaluation of the role of high cholesterol in the development of CAVD [127]. Atherosclerosis models are widely utilized in cardiovascular research, and researchers should focus on optimizing the simulation process to replicate early human disease-related metabolic environments, considering various risk factors. It is important to shorten the induction period and minimize potential side effects of the diet to prevent reductions in sample size during induction.

Adenine and vitamin D models in mice are commonly utilized due to their ease of design and promising outcomes. Unlike the rat model involving nephrectomy, this model does not necessitate surgery, exhibits a higher survival rate, and has a relatively short induction time, making it suitable for rapid pre-experimentation. However, a notable limitation of the rat vitamin D and adenine induced models is the occurrence of weight loss in the rats. Some studies have reported that rats fed 0.75% adenine experienced a 50% reduction in body weight within 5 weeks [128] [129] [130]. In mice, feeding 0.2% adenine did not result in significant weight loss, but did lead to effective aortic valve calcification in the short term, resembling the progression of cardiovascular disease in CKD patients. However, it is important to note that the renal failure induced by adenine feeding is only suitable for studying vascular calcification in CKD patients, and may not encompass all risk factors for valve calcification. Previous studies have indicated that the induction factors of this model are linked to biological metabolic processes, while the guidewire injury model is based on hemodynamic changes resulting from mechanical injury. Research has suggested that high mechanical stress is more likely to impact non-atherosclerotic leaflets [63] [131]. The guidewire-induced model exhibited pronounced hemodynamic disorders and heart failure in comparison to other models, such as the high-fat/high-cholesterol model which did not consistently display hemodynamic changes. Additionally, the induction time for this model is shorter than that of most CAVD models. However, the complexity of the surgical procedure during induction poses high demands on researchers, resulting in challenges to establish consistent and reproducible models. This difficulty may contribute to an approximate 20% mortality rate in mice within a 4-week period [64]. The experiment involved reducing the sample

size and increasing the cost. Niepmann *et al.* enhanced the guide wire induction model to improve consistency and stability by utilizing specific tools (see **Table 3**) to establish various levels of CAVD. This led to more severe hemodynamic disorders and heart failure. The modified CAVD model demonstrated shorter induction times and more efficient and stable operations. Different stages of CAVD in clinical settings can be investigated using various CAVD models, offering insights into each disease stage and broadening research possibilities. However, severe aortic regurgitation could elevate mortality rates in mice. Overall, the guidewire model successfully replicated key features of human aortic disease (such as aortic apex thickening, fibrosis, macrophage infiltration, and calcium deposition) using hemodynamics as the sole risk factor. This model significantly contributes to CAVD research. Nevertheless, its intricate operation, lack of consistency, and poor stability may explain why the guide wire model is not widely adopted. It primarily serves as a comparative model for atherosclerosis to eliminate the impact of relevant drugs on atherosclerosis [66] [99].

The warfarin induction model was utilized to assess the impact of warfarin and Vitamin K on vascular and valve calcification in humans. This model has a short induction time, simple operation, and extensive valve tissue mineralization. It effectively demonstrates the stability of the internal environment, noticeable hemodynamic changes, and serves as a key model for studying vitamin K in the cardiovascular system. However, it is important to note that while the model primarily shows elastic lamellar calcification, it does not exhibit other significant blood biochemical or heart organic changes. In contrast, human valves display a wider range of mineralization patterns, including valve interstitial cell mineralization, lipid infiltration and oxidation, tissue remodeling, and angiogenesis induced by inflammation [51]. Therefore, this model is suitable for studying vitamin K and related drugs in calcification of the cardiovascular system. In other words, the study radius is narrow. In addition, diets with calcium and phosphate ratios of different proportions may lead to accelerated calcification of the kidneys and other soft tissues in warfarin-treated rats [132] [133] [134]. It will lead to various complications and affect the experimental results. So researchers need to carefully adjust the amount of calcium and phosphate in the diet to eliminate these problems.

This text offers a concise overview of five models and their characteristics, providing recommendations for researchers. When simulating aortic valve calcification due to internal environmental disorders in humans, focusing on the pathophysiological process of its development, researchers may find the atherosclerosis-induced model or the CKD-induced model more suitable. Additionally, researchers can consider including atherosclerosis or nephrogenic heart disease based on the pathogenesis. In our cardiac research center, we utilize the guidewire induction model to stabilize modeling, accelerate valvular calcification, and shorten research cycles. To enhance the model, incorporating mice, such as knockout mice, to mimic the internal human environment and eliminate errors is recommended. Studies have not conclusively determined whether warfarin

promotes or inhibits aortic valve calcification in animals, limiting its use as a pharmacological tool for studying warfarin. Further research is needed to elucidate mechanisms and strategies for limiting congenital aortic valve calcification, with relevant knockout models being most suitable for this purpose (Table 6).

Table 6. Advantages and disadvantages of the 5 models.

Animal model	Dominance	Inferior
Atherosclerosis Related Dietary Induction Model	1) Cell isolation experiments can be performed 2) The long induction period allows careful study of the pathophysiological changes at each stage of valve calcification.	1) The lipids and cholesterol given to the animals are too high to mimic the human high-fat, high-cholesterol dietary dose. 2) Susceptible to other diseases such as atherosclerosis or fatty liver that can lead to premature death of the model. 3) Induction time is long 4) Not operable in rats
CKD Type Dietary Induction Model	1) Induction of valve calcification in a short period of time. 2) Reverses pathological calcification and restores the valve to its original state before induction. 3) Continuous assessment of dynamic and reversible processes in CAVDs. 4) Simple and complex models are available for use in different research centres.	1) The model valve has no significant inflammatory response. 2) Models prone to end-stage renal disease and other electrolyte disturbances leading to premature death. 3) Most models are only suitable for studying vascular calcification in patients with CKD.
Non-modified/modified guide wire mouse model	1) Has continuous haemodynamic changes in the natural state 2) It can be combined with various models to simulate the dynamic balance of the internal environment of the human body. 3) Short induction time, efficient and stable operation. 4) Reproduces most pathophysiological changes in the human aortic valve.	1) Complicated surgical procedures can easily cause death in mice, with the biggest cause of death coming from severe regurgitation of the aortic valve caused by the operation. 2) Single animal model, only in mice with relevant literature. 3) Unable to simulate human internal environmental disturbances.
Warfarin induction models	1) Short induction time and simple operation. 2) Extensive mineralisation of the valve tissue.	1) No other significant blood biochemical or cardiac organic changes were demonstrated. 2. Narrow scope of the study. 3) Relevant studies have shown that warfarin can protect the cardiovascular in certain circumstances, the mechanism is not clear.

Continued

Gene knockout model	1. Ideal for studying congenital aortic valve calcification. 2) Can be combined with other diet-induced models to accelerate valve calcification.	1) Narrow scope of the study. 2) Knockout mice are more expensive and do not have a high cut survival rate.

4. Conclusion

The induction of an atherosclerosis diet-related animal model can be time-consuming and may impact experimental outcomes due to aortic calcification, which is often an initial step in atherosclerosis development. Despite not perfectly mimicking the targeted disease, this model has been utilized as a supplementary tool. Animal models induced by chronic kidney disease (CKD) may be more susceptible to aortic calcification, but can also influence experimental results due to associated renal damage. Genotype-induced models are specific to human bicuspid aortic valve conditions and are commonly employed in congenital aortic valve calcification research. The use of a guidewire-induced model can artificially modify hemodynamic responses, allowing for the study of disease physiology and potential therapeutic strategies at various disease stages. Combining this model with others can help exclude confounding factors like metabolic disorders, enabling a clearer understanding of the pharmacological and medical effects involved. By elucidating the pharmacological mechanisms of disorders through modeling, researchers can gain valuable insights. However, the complexity of the procedures involved has deterred some researchers. Simplifying the modeling process could make it a crucial tool in advancing our comprehension of atherosclerosis pathology.

Abbreviations and Acronyms

Aortic valve calcification disease = CAVD
Aortic valve replacement = AVR
Transcatheter aortic valve implantation = TAVI
Chronic kidney disease = CKD
High-fat = HF
High cholesterol = HC
High cholesterol/VitD3 diet = HCVD
Parathyroid hormone = PTH
Left ventricular ejection fraction = LVEF
Matrix Gla protein = MGP
Bone morphogenetic proteins = BMP
Bicuspid aortic valve = BAV
Porcine aortic valve stromal cells = pAVIC

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Conflicts of Interest

The authors declare no conflicts of interest.

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