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Dog models of human atherosclerotic cardiovascular diseases

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Abstract
Cardiovascular diseases (CVD) are one of the leading causes of death worldwide. Eighty-five percent of CVD-associated deaths are due to heart attacks and stroke. Atherosclerosis leads to heart attack and stroke through a slow progression of lesion formation and luminal narrowing of arteries. Dogs are similar to humans in terms of their cardiovascular physiology, size, and anatomy. Dog models have been developed to recapitulate the complex phenotype of human patients and understand the underlying mechanism of CVD. Different methods, including high-fat, high-cholesterol diet and genetic modification, have been used to generate dog models of human CVD. Remarkably, the location and severity of atherosclerotic lesions in the coronary arteries and branches of the carotid arteries of dog models closely resemble those of human CVD patients. Overt clinical manifestations such as stroke caused by plaque rupture and thrombosis were observed in dog models. Thus, dog models can help define the pathophysiological mechanisms of atherosclerosis and develop potential strategy for preventing and treating CVD. In this review, we summarize the progress in generating and characterizing canine models to investigate CVD and discuss the advantages and limitations of canine CVD models.

Introduction

The domestic dog (*Canis familiaris*) is a striking example of domestication, and this species consists of over 400 distinct breeds with extreme phenotypic diversification (Ostrander et al. 2017). Most breeds of dogs have undergone intense artificial selection derived from a few founders, which share morphologic and behavioral traits competitive for occupation and esthetics. Genome sequencing and comparative evolution studies have driven our understanding of dog domestication. In 2005, a joint group announced the first whole-genome sequence of a dog, the Boxer, thus, providing a new perspective for studying dog domestication (Lindblad-Toh et al. 2005). Shared living environments and food resources between dogs and humans drive parallel evolution in genes involved in digestion, metabolism, and neurological processes (Wang et al. 2013). Compared to wolves, dogs show a higher starch digestion ability and show an increased copy number of the *AMY2B* gene, which encodes the alpha amylase 2B (Axelsson et al. 2013). Domestic dogs have impressed humans because of their pro-social behaviors toward humans. For example, dogs show asymmetrical tail wagging in different social contexts (Ren et al. 2022). Domestic dogs are deeply involved in human society as pets, in addition to them being used for hunting, grazing, and police detection. For example, 54.29 million dogs were raised by the urban families of China in 2021 (https://m.thepaper.cn/baijiahao_16373326). Among the various breeds of dogs, Beagles are chosen for laboratory research and biomedical use because they are of medium size, can be easily tamed, and are emotionally kind and gentle (Ruple et al. 2022).

Dogs have made a great contribution to the history of biomedicine and the development of therapeutic drugs (Camacho et al. 2016). Dog models are used to study human diseases as dogs have a similar organ size, anatomy, physiological development, and pathological trajectory as humans (Gilmore and Greer 2015; Tsai et al. 2007). Approximately 450 hereditary diseases have been identified in dogs, and about half of these diseases have their counterparts in humans; thus, dogs are considered as a unique genetic animal model for human diseases (Switonski 2014; Tsai et al. 2007). An estimated number of 192.1 million animals were used for scientific purposes worldwide in 2015; among

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these animals, an estimated total of 207,724 dogs were used, mainly in the United States (61,101) and China (65,546) (Taylor and Alvarez 2019).

Cardiovascular diseases (CVD) are considered the main cause of death worldwide. The number of CVD-associated deaths increased from 12.1 million in 1990 to 18.6 million in 2019, representing 32% of all global deaths in 2019 (Afjeh-Dana et al. 2022; Roth et al. 2020). Of these CVD-associated deaths, 85% were due to heart attack and stroke (Afjeh-Dana et al. 2022; Liu et al. 2021). Atherosclerosis is the main pathological cause of CVD, which is related to long-term habits such as smoking, physical activity, and diet, and health factors such as blood cholesterol, blood pressure, and glucose control (Libby et al. 2011; Virani et al. 2021). The clinical manifestations of atherosclerosis are plaque rupture, thrombosis, vascular stenosis, and occlusion, which ultimately lead to organ ischemia and hypoxia infarction. Atherosclerosis is caused by chronic inflammatory state driven by endothelial dysfunction, lipid accumulation, and immune cell recruitment, leading to plaque formation within the intimal layer of the arterial wall (Libby et al. 2011). The treatment of CVD aims to reduce the size of atherosclerotic lesions, increase the stability of plaques by lowering blood lipids, and/or inhibit inflammation. Animal models with different risk factors are important for elucidating the pathogenesis of CVD and for developing drugs for CVD. The choice of the animal model needs to be consistent with the research purpose. The attempt of using dog models to conduct cardiovascular research has been a continuous effort and has shown unique advantages compared with rodent models. Dog models of CVD include spontaneous, induced, and genetically modified models, most of which show hypercholesterolemia and various stages of plaque development similar to human patients. These dog models are invaluable for the development and evaluation of new therapies, including drug development and intravascular surgery to ameliorate atherosclerosis-related diseases.

Spontaneous and surgery-induced CVD in dogs

During 30,000 years of domestication, dogs have shared human living environments and eating habits (Vonholdt et al. 2010). Therefore, dogs and humans have an apparent convergent evolution in digestion and metabolism, nervous system, and other anatomical structures, and physiological functions. This convergent evolution supports that dogs are unique model animals for CVD. One obstacle in modeling CVD is the chronic progression of atherosclerotic lesions, resulting in a rarity of overt clinical manifestations until many years later (Daugherty et al. 2017). Beagle dogs are gentle and easy to train, having a lifespan of over 15 years; thus, various examinations such as ultrasonography analysis of plaque development and blood lipid profiling can be performed on conscious, unrestrained subjects in a long time window. The cardiac anatomy of dogs is similar to that of humans, accounting for a large proportion of body weight (0.8% of body weight) (Tsai et al. 2007). Due to these features, dogs have been used to mimic chronic hypertension, stroke, and myocardial ischemia with surgical methods (Reimer et al. 1977; Tian et al. 2013; Zu et al. 2013).

Chronic hypertension with bilateral renal artery constriction by clip induced carotid proliferative plaques in Beagle dogs (Tian et al. 2013). Pathological analysis revealed that the hypertension-induced carotid plaques were mainly composed of smooth muscle cells, collagen fibers, and proteoglycans (Tian et al. 2013). Historically, dog models have been developed to study myocardial ischemia and arrhythmia by using acute surgical methods such as coronary artery occlusion in previously healthy tissue (Reimer et al. 1977). Dog hearts have an electrophysiological system prone to develop atrial fibrillation, similar to that in humans (Brundel et al. 2005; Huang et al. 2020). At the protein level, dogs have a heart (Kooij et al. 2014) and brain (Hong et al. 2022) proteome similar to that in humans. Dogs have an extensive epicardial coronary collateral circulation (Schaper 1995). The collateral circulation in the dog heart is similar to that in an aged human heart with ischemic heart disease, which promotes collateral artery growth (Camacho et al. 2016). Dilated cardiomyopathy is characterized by progressive ventricular dilation and functional impairment. Domesticated dogs frequently develop dilated cardiomyopathy but barely show myocardial infarction (Camacho et al. 2016). One canine breed with familial dilated cardiomyopathy is Doberman Pinscher, which develops this disorder due to a mutation in the *PDK4* gene that functions in mitochondria (Meurs et al. 2012).

Diet-induced atherosclerosis in dogs

High-fat and high-cholesterol diet (HFHC) is a commonly used method to induce atherosclerotic lesions in multiple species of animals (Daugherty et al. 2017). Indeed, in the several decades of research conducted on this topic, atherosclerosis has been induced in animals ranging from mice to monkeys. Different formulations of diet have been investigated for the induction of canine atherosclerosis, mainly in the 1970s. Dogs are considered resistant to naturally occurring and experimentally induced atherosclerosis (Mahley et al. 1976). However, dogs fed commercial dog food supplemented with corn oil, butter, cholesterol, and 2-thiouracil (an anti-thyroid drug) for 4 months developed hyperlipoproteinemia and atherosclerotic fatty plaques and streaks (Geer 1965). Another research group induced atherosclerosis and

thrombosis in hypothyroid dogs on diets containing beef tallow and lard or coconut oil, but not in euthyroid dogs (Mahley et al. 1974, 1976). Considerable evidence suggests that hypothyroidism could promote atherosclerosis and ischemic heart disease, and this could be ascribed to atherogenic lipid profile and impaired endothelial function (Ichiki 2010).

HFHC-induced atherosclerosis in dogs results in advanced lesions and thrombosis in coronary arteries, iliofemoral artery, cerebral artery, and internal carotid artery (Mahley et al. 1976). The location and severity of advanced atherosclerosis in these dogs are similar to that in human patients (Bentzon and Falk 2010; Stary et al. 1995). Therefore, the histological classification of dog atherosclerotic lesions can follow human standards (Stary et al. 1995). Quantitative analysis of atherosclerotic lesion size and plaque occlusion was performed to evaluate the progress of atherosclerosis in large- and medium-sized arteries in animal models (Zhang et al. 2021). In summary, experimental maneuvers such as induction of hypothyroidism, administration of HFHC diet, induction of hypertension, and mechanical trauma of the arteries are effective methods to induce easily detectable arterial lesions in dogs (Mahley et al. 1974, 1976; Stary et al. 1995).

Maintaining a high-plasma lipid level for several months is required for successful induction of atherosclerosis. The major lipoprotein components including major plasma lipid fractions, and fatty acid compositions within lipid fractions are conserved between humans and dogs (Yin et al. 2012). Blood apolipoproteins (Apo) can be determined by SDS-PAGE after sequential ultracentrifugation (Niimi et al. 2021), while plasma lipids and lipoprotein profiles can be analyzed by agarose gel electrophoresis and high-performance liquid chromatography (HPLC) (Niimi et al. 2016). Lipidomic profiling provides more detailed information on all lipid components. However, differences in lipid profiles exist between dogs and human. The majority of blood cholesterol in dog is high-density lipoprotein (HDL) particles, which is similar to rodents, while human is majority low-density lipoprotein (LDL) particles (Yin et al. 2012). The reason for this difference is that dogs have no or low level of cholesteryl ester transport protein (CETP), a key enzyme involved in plasma cholesterol transport from HDL to LDL (Yin et al. 2012). Statin treatment in dogs decreased fatty acid composition of cholesteryl esters, which is similar to that observed in dyslipidemic patients and different from that reported in rodents (Yin et al. 2012). Thus, dogs are also a useful model for developing LDL cholesterol-lowering drugs for the control of hypercholesterolemia and atherosclerosis.

Thus far, HFHC diet-induced CVD models in dog have not been widely used, probably due to the long and complex induction process and ethical constrains. However, genetically modified dog models can better mimic the natural process of human atherosclerosis, which show advanced pathological and clinical manifestations of human patients (see below).

Genetic modification in dogs

Dogs carrying mutations in specific genes can be generated using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene-editing system (Zhao et al. 2019). The first proof-of-concept *Myostatin* knockout (KO) dogs were successfully generated in 2015 by microinjecting CRISPR/Cas9 components into zygotes (Zou et al. 2015). The successful rate of generating mutant dogs depends on the targeting efficiency of sgRNAs (validated in fetal fibroblast cells in advance, multiple sgRNAs are recommended) and the accurate evaluation of ovulation time in female dogs (Zhao et al. 2019).

Dogs carrying mutations responsible for atherosclerosis and CVD serve as useful disease models for pathology studies and drug discovery. Apo E recognizes and binds to LDL and very-low-density lipoprotein (VLDL)/intermediate-density lipoprotein (IDL) with relevant receptors. In 1-month-old *ApoE* bi-allelic mutant dogs, the clearance of LDL and VLDL/IDL was found to be delayed, leading to hyperlipidemia occurrence (Feng et al. 2018). Hyperlipidemia induces oxidative modification of lipoproteins and promotes the formation of atherosclerotic lesions. We observed that *ApoE* bi-allelic and homozygous mutant dogs at age 2 years showed hypercholesterolemia and atherosclerosis with severe clinical consequences (Zhao et al. 2022). *ApoE* F0 KO dogs showed extensive and severe atherosclerotic plaques, arterial stenosis and occlusion, thrombosis, which finally led to stroke and gangrene at 2 years old (equivalent to 40 years of age in humans; Zhao et al. 2022). Among the 4 founder F0 KO mutants, one developed stroke, hemiplegia, and signs of myocardial ischemia; the other three developed hind limb gangrene. Gangrene was also present in 2-year-old F2 homozygous mutants (Zhao et al. 2022). Postmortem analysis of F0 and F2 KO mutants revealed unevenly bulged and yellowish arteries in brain and heart at different degrees, similar to pathological phenotypes in human patients (Zhao et al. 2022). We and others detected atherosclerotic lesions in the carotid artery, basilar artery, and iliac artery, similar to the locations prone to atherosclerosis in humans (Jia et al. 2022; Zhao et al. 2022).

Techniques for evaluating plaque formation in humans are compatible with dogs. For example, ultrasonography detected plaque formation in half of 9–15 months old F2 and F3 homozygous mutants, 10% of 18–29 months old heterozygous mutants, but none in WT controls (Jia et al. 2022). RNA-seq analysis and immunostaining found proliferation of resident intimal and media-derived smooth muscle

cells in plaques (Shi et al. 2022). A thin fibrous, unstable cap covering a necrotic core was observed in the internal carotid of *ApoE* mutant dogs (Zhao et al. 2022). Rupture of the fibrous cap covering a necrotic core would lead to thrombosis events with immediate clinical manifestations. The C-reactive protein (CRP), a reporter for inflammation, increases risk prediction for atherosclerotic events in patients (Haverkate et al. 1997). We found a significantly increased level of CRP in *ApoE* KO dogs (Zhao et al. 2022). Thus, the pathological features in *ApoE* KO dogs closely resembled that in atherosclerotic CVD patients.

Zygote microinjection-based gene editing is so far the first choice in editing various genes (successful so far in editing *Myostatin, ApoE,* and *Glucokinase* genes) in dogs. With the development of more precise gene manipulation tools by Chen et al. (2021), it is now much easier and convenient to model diseases caused by point mutations, and even generate copy number variations in large animals such as dogs. The key point is to obtain a male mutant with the desired mutation at a high mutation rate. A single male mutant dog can be bred into a large colony in a relatively short period of time due to the rapid reproduction cycle (12 months to be sexually mature and approximately six progeny per pregnancy). Mosaicism and off-target mutations induced by genetic modification are problems of concern in F0 founder mutant dogs (Wang et al. 2021). Somatic cell nuclear transfer (SCNT) is a method to clone animals with desired mutation sequences to avoid such limitations. A *Dystrophin* mutant dog was generated by SCNT using male canine fetal fibroblasts in which the *Dystrophin* gene was edited by CRISPR/Cas9 (Oh et al. 2022). Zygotic injection of CRISPR/Cas9 combined with somatic cell cloning was used to propagate *ApoE* founder mutants (Feng et al. 2018). Cloned mutants showed less genetic background diversity and smaller individual variations in clinical symptoms than mutants obtained by colony breeding. Thus far, only a few research groups in South Korea and China have used gene-edited mutant dogs in their research. The next step is to further evaluate the *ApoE* dog model at different developmental stages and to use the model for developing therapies such as endovascular treatments for CVD.

Comparison of dogs with other animals for modeling CVD

Animal models of atherosclerosis serve as an essential tool to understand the pathophysiological mechanisms of CVD in humans. The popular species used for modeling atherosclerosis or CVD include mice, rats, hamsters, rabbits, quails, pigs, dogs, and non-human primates (Daugherty et al. 2017). The advantages and disadvantages of small animal models (mainly mice and rabbits) and large animal models (pigs and non-human primates) of atherosclerosis have been well summarized by Daugherty and colleges (Daugherty et al. 2017).

Comparison of dog models with small animal models

Genetically modified mice (mainly *ApoE* KO and *Ldlr* KO) with hyperlipidemia are the most widely used atherosclerosis models today (Plump et al. 1992; Ishibashi et al. 1993). The major problem for rodent models is the rarity of plaque rupture and thrombosis (Bentzon and Falk 2010; Libby et al. 2011). The most apparent atherosclerosis was observed in the aortic root in mutant mice (Plump et al. 1992; Zhang et al. 1992), which is different from the distribution of atherosclerosis in large and middle arteries such as the abdominal aorta, coronary, carotid, and cerebral arteries in human patients (Bentzon et al. 2014; Libby et al. 2011; Stary et al. 1995). Lifespan differs substantially among different animal species. Rodents normally live for less than 3 years, which is a short lifespan that may not be sufficient for the occurrence of plaque rupture and occlusion that usually takes decades to occur in humans (Gilmore and Greer 2015).

Rabbits were the first animal model for atherosclerosis research. Ignatowski in 1908 (Ignatowski 1908) demonstrated lesion formation in the aortic arch of rabbits when fed with an animal protein-enriched diet (mainly meat, milk, and egg yolk). Rabbits show a high level of CETP, which transfers cholesteryl esters from HDL to LDL, resulting in a high level of LDL cholesterol and a low level of HDL cholesterol similar to that in humans (Yin et al. 2012). However, rabbits lack hepatic lipase, an enzyme with both triglyceride lipase and phospholipid lipase activity. Consequently, a high-cholesterol diet causes massive cholesterol accumulation in chylomicron remnants and VLDL particles in rabbits (Chang and Borensztajn 1993). Dogs show advantages compared with small animal models due to the fact that the large size of arteries, lipid profile, and metabolism in dogs are more similar to humans. Thus, dog models show potential applications in various contexts including testing anti-atherosclerotic drugs and developing endovascular procedures.

Comparison of dog models with large animal models

Large animal models with atherosclerotic lesions more similar to humans are more suitable for studies on disease progression, screening for potential targets, and even developing endovascular procedures than small animal models (Emini Veseli et al. 2017). *ApoE* single KO pigs and *ApoE* and *LDL Receptor* (*Ldlr*) double KO pigs were developed using the CRISPR/Cas9 system; these pig models showed elevated serum LDL cholesterol and total cholesterol levels and signs of atherosclerosis (Fang et al. 2018; Huang

et al. 2017). The increased levels of total cholesterol and LDL cholesterol in *ApoE*/*Ldlr* double KO pigs are moderate (less than twofold increase) compared to the changes of total cholesterol (fivefold increase) and LDL cholesterol levels (30-fold increase) in *ApoE* KO dogs on a regular chow diet. Non-human primates fed HFHC diets are the closest model of human atherosclerosis on the basis of lipoprotein profiles and site of lesion formation (Daugherty et al. 2017; Manalo-Estrella et al. 1963). However, the limitations of non-human primate (NHP) models are ethical and cost issues and slow breeding. Dogs show advantages of a lower cost and fast breeding compared to non-human primates.

Advantages and limitations of dog models

The dog is, thus, an alternative model for studying CVD. Dogs can cooperate well with researchers after short-term training and are suitable for observation over a long period of time. Below, we summarize the advantages and limitations of using dogs for biomedical research, especially for research on atherosclerosis (Table 1). (1) The sites and pathology of the atherosclerotic lesions are very similar between humans and dog models. The regional propensity for atherosclerosis is possibly related to the origin and proliferation ability of smooth muscle cells in atherosclerosis prone arteries (Shi et al. 2022). The *ApoE* KO dogs and HFHC diet-fed dogs showed consistent and severe lesions in the abdominal aorta, and iliofemoral, coronary, and cerebral arteries (Mahley et al. 1976; Zhao et al. 2022; Fig. 1). Histologically, these lesions frequently invade the media of the vessel wall. The plaques of *ApoE* KO dogs and human patients contain a group of Tenascin-C-positive proliferative smooth muscle cells as evidenced by single-cell RNA-seq (Shi et al. 2022). The plaques in *ApoE* KO dogs also show calcification (Fig. 1) and an unstable necrotic core under a thin fibrous cap, which are signs of advanced atherosclerosis but rarely observed in other animal models. (2) *ApoE* KO dogs show stenosis and occlusion of arteries, resulting in human-like clinical complications of stroke and gangrene due to a severely damaged peripheral arteries before the age of 2 years (Zhao et al. 2022). Thrombosis occurs in HFHC diet-fed hypothyroid dogs in the iliofemoral artery, terminal aorta, and internal carotid artery (Mahley et al. 1976). In contrast, all other animal models seldom show human-like endpoint events of atherosclerotic diseases (Daugherty et al. 2017). (3) *ApoE* KO dogs spontaneously develop severe atherosclerotic lesions when kept on a normal diet (Zhao et al. 2022). (4) Because of the large size of arteries and heart, dogs are suitable for developing therapies of endovascular procedures that have become the main treatment options for atherosclerotic patients (Herrmann et al. 2019). (5) Atherosclerosis phenotype in dogs can be examined in vivo by non-invasive magnetic resonance imaging and ultrasonography commonly used in hospitals, which can facilitate the evaluation of disease progression and prognosis in mutant dogs (Zhao et al. 2022; Jia et al. 2022). (6) Dogs have contributed to the development of cholesterol-lowering drugs such as statins (Yin et al. 2012), which is a therapeutic milestone for CVD. In a study conducted in 1981, the treatment of dogs with mevinolin, a fungal HMG CoA reductase inhibitor, led to a marked reduction in plasma LDL cholesterol levels (Kovanen et al. 1981).

The common limitations for using dogs in biomedical research are high cost, ethical issues, and time consumption in breeding compared to rodents. Specifically, the following limitations have been noted: (1) There is particular public concern about the use of dogs for invasive analysis. Compared to the other model systems, dogs are the most popular pet loved by their human owners and receive exceptional care. Ethical and cultural reasons have affected the use of dogs in biomedical research (Taylor and Alvarez 2019). (2) Another limitation of using dog models is the lack of behavior analytical tools, antibodies, and virus vectors for delivering genes into dog cells. Despite the high degree of protein sequence homology between dogs and humans, our experience shows that only a limited number of antibodies work in dogs (data not shown). (3) Dogs express minimal CETP, hence, most of plasma cholesterol is in the form of HDL particles, while humans have a high LDL cholesterol and low HDL cholesterol profile (Yin et al. 2012). A high

Table 1 Advantages and limitations of dog models for CVD

Advantage	Limitation
Conserved atherosclerotic lesion sites and pathology with patients	Plasma lipid profiles different from human
Large size arteries for endovascular procedures	Ethical concerns
Non-invasive magnetic resonance imaging and ultrasonography applicable	Not efficient for mechanism studies and large scale drug screening
Multiple offspring per pregnancy, short gestation and quick sexual maturation compared with NHP	High cost in genetic modification and cloning
Sensitive to cholesterol-lowering drugs such as statins	

NHP: non-human primates

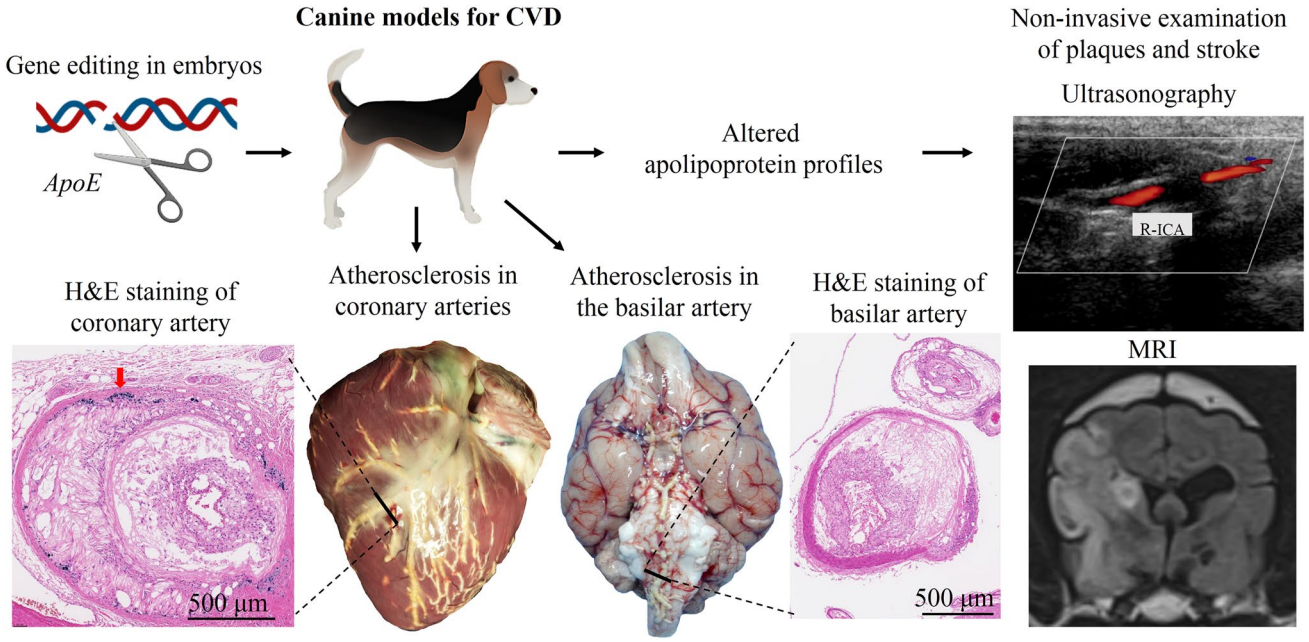

Fig. 1 Dog models for cardiovascular diseases. *ApoE* KO dogs show pathological changes of atherosclerotic plaques and occlusion in the coronary and basilar arteries. Red arrows indicate calcification (blue puncta) in the paraconal interventricular branch. Scale bars denote 500 μm. R-ICA indicates right-sided internal carotid artery

HDL cholesterol level is considered athero-protective to reduce the risk of coronary heart disease (Tsutsumi et al. 2001). (4) The efficiency of genetic modification is still low and the process to obtain bi-allelic mutant offspring is time-consuming. Despite these limitations, dogs show apparent advantages such as multiple offspring per pregnancy, short gestational period (60 – 65 days), and quick sexual maturation (10 – 12 months) compared to non-human primates. Specifically, it takes less than one year to breed one generation of dogs, compared to at least 5 years required to breed one generation of monkeys.

Conclusion

For cardiovascular research aimed at clinical translation, studies typically start with rodent models, but findings from rodent studies need to be evaluated in a large animal model that more closely resembles the human heart. The dog model is a valuable option to perform complementary and validation studies for preclinical evaluations, but not suitable for high throughput screening. The advantages and limitations of using dog models need to be considered in advance of the experiment. *ApoE* KO dogs develop advanced atherosclerosis and associated clinical manifestations that can be accelerated by supplementing HFHC diet. The large size of dog heart allows almost all in vivo techniques for human hearts to be utilized in dogs, while rodent models are not favored to develop endovascular treatments because of size limitations and substantial differences in brain and cerebrovascular anatomy (Hermann et al. 2019). Delivering CRISPR gene-editing tools to muscles by adeno-associated viruses was successful in treating Duchenne muscular dystrophy in dogs (Amoasii et al. 2018). Niemann–Pick C1-like protein 1 (NPC1L1) plays a critical role in the absorption of intestinal cholesterol, thus, affecting blood cholesterol levels and CVD risk (Libby et al. 2011). The proprotein convertase subtilisin/kexin type 9 (PCSK9) escorts LDLR for lysosomal degradation to regulate plasma cholesterol homeostasis (Libby et al. 2011). Thus, targeting NPC1L1 and PCSK9 to lower LDL concentrations in the blood might be beneficial for ameliorating atherosclerosis in dog models.

An ideal animal model for human diseases should mimic the complex phenotypes of human diseases to the largest extent (face validity) and the underlying causality (construct validity). Many animal models involve the artificial induction of a disease in a previously healthy tissue, such as surgical occlusion of the coronary artery, which lacks the complex changing progression of human CVD. The *ApoE* mutant dog models show unique pathological changes in arteries because of severe atherosclerotic lesions (Fig. 1). Further examination and application of genetically modified dogs including *ApoE* mutants will accelerate the development of therapeutics for CVD in humans and dogs.

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Data availability All data are available by contacting the corresponding author Y.Q. Zhang (yqzhang@genetics.ac,cn).

Declarations

Conflict of interest The authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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