

## Review Article

# Small mammalian animal models of heart disease

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Received April 18, 2016; Accepted May 12, 2016; Epub September 15, 2016; Published September 30, 2016

**Abstract:** There is an urgent clinical need to develop new therapeutic approaches for treating cardiovascular disease, but the biology of cardiovascular regeneration is complex. Model systems are required to advance our understanding of the pathogenesis, progression, and mechanisms underlying cardiovascular disease as well as to test therapeutic approaches to regenerate tissue and restore cardiac function following injury. An ideal model system should be inexpensive, easily manipulated, reproducible, physiologically representative of human disease, and ethically sound. The choice of animal model needs to be considered carefully since it affects experimental outcomes and whether findings of the study can be reasonably translated to humans. This review presents a guideline for the commonly used small animal models (mice, rats, rabbits, and cats) used in cardiac research as an effort to standardize the most relevant procedures and obtain translatable and reproducible results.

**Keywords:** Mouse, rat, rabbit, cat, myocardial infarction, heart failure, diabetic cardiomyopathy

## Introduction

Cardiovascular disease is considered the major cause of morbidity and mortality worldwide. Over the past several years, enormous achievements have been made in the management of cardiovascular disease, which has depended on the use of experimental animal models [1]. However, there are still no permanent cures for most cardiovascular diseases. For example at present, the only cure for heart failure (HF) is a heart transplant; however, its therapeutic potential is limited by very small numbers of donor hearts available relative to the need and is complicated by long-term allograft vasculopathy. Tissue-engineered biomaterials and autologous or allogeneic cell transplant therapy may have the potential to restore function and obviate the necessity for transplant [1, 2]). In recent years, gene therapy and cell transplant technologies have advanced remarkably. If proven efficient and safe, these therapies might revolutionize the treatment of myocardial failure [3].

A major challenge of preclinical testing is to establish clinically relevant models for myocar-

dial failure and infarction [3]. Donated human hearts, either non-transplantable (ranging from healthy to diseased) or end-stage failing hearts, typically obtained at the time of transplantation, are great tools for addressing this issue. These tissues are, however, available in very limited quantities and exhibit great variability due to differences in factors such as genetics, medication, diet, social habits, and disease. Therefore, it is necessary to have a suitable animal model where cardiac physiology and disease can be studied efficiently and reliably with translational applicability to humans [4].

Depending on the cardiovascular process being studied, the choice of animal model needs to be considered carefully since it affects experimental outcomes and whether findings of the study can be reasonably translated to humans. As a simple rule, the closer the heart or body weight of the animal model to human heart or body weight, the more similar are the hearts [4]. Both small and large animal models of heart disease have advantages and disadvantages. The main problems with using small animals are how to establish functional benefit of the treatment and the translation of results. In

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large-animal models, the ethical requirement for limiting the number of animals influences study protocols, especially concerning non-human primates or dogs [3].

Determining the best experimental model of a human condition requires a number of decisions and compromises - especially in relation to obtaining the optimal balance between the quantity and quality of the data produced and the relevance of the data to the condition under investigation. In assessing the utility of an investigative model, it is necessary to identify the research objectives. The nature of the question under study will greatly influence selection of the most appropriate investigative models and endpoints of cardiac function and malfunction [5]. It is also important to standardize the procedures used so as to obtain relevant and reproducible results that can be compared with other findings.

Rodent models are often used in cardiovascular research since they are easier to handle and house, have a short gestation time, genetically manipulate to generate transgenic strains, and have low maintenance costs; therefore, they are more suitable for "high-throughput" studies than large animal models [6]. These characteristics make small rodent models the most used model for cardiac physiology and disease, genetics, pharmacology, and long-term survival studies [4]. However, rodents are phylogenetically very distant from humans and some pathophysiological features of disease and their response to pharmacological treatments may not be reliable predictors [4, 6].

### Mouse

During the past 15 years, the mouse has become the model organism of choice to study human heart disease. 99% of human genes have direct murine orthologs and mice are suitable for selection of genetically modified individuals within a relatively short time because of their high breeding rate. They also have a short life span, allowing investigators to follow the natural history of the disease at an accelerated pace [6]. Use of genetically modified mouse models allows for rapid establishment of proof-of-principle, which can later be extended into larger animal models and, eventually, into humans [4].

Despite their widespread use, mice represent a heart model that is farthest from human contractile function, mainly due to their small size and short lifespan. Thus, translational aspects and the value of genetic mouse models must be interpreted with caution. Although these models may recapitulate some of the characteristics of the human cardiac phenotype of a disease, they typically do not recapitulate all aspects of human cardiovascular disease [4].

### *Ligation-induced myocardial infarction (MI)*

MI animal model is one of the most commonly used one to mimic human heart attack. Mortality after MI is relatively high and surviving patients are often severely compromised due to insufficient heart-pump function. At the cellular level, damage to the heart's contractile constituents, the cardiomyocytes, is irreversible, and treatments merely serve to reduce symptoms. Only a minority of patients receives heart transplants [7].

The neonatal mouse MI model represents a useful tool for evaluating mammalian cardiac regeneration and cellular/molecular mechanisms that govern cardiomyocyte proliferative capacity only in the early stage of neonatal mice [8]. To generate MI, neonates are briefly sedated using an isoflurane induction chamber followed by a short cooling period (a few minutes) in ice water. The animals are then taped to a cooling bag in the right lateral position to ensure continued hypothermic anesthesia during the surgical procedure. The skin is cut a few millimeters below the left foreleg, the thorax opened in the 4th intercostal space, and the left anterior descending (LAD) artery is ligated. MI is indicated by a light pallor of the myocardium below the ligature after suturing. The musculoskeletal thorax and the skin are closed, and mice are put onto a 37°C heated pad to recover from the anesthesia [8-10].

It is commonly found that the complete regeneration of cardiomyocytes can be reached when the LAD artery is ligated in newborn mice. Myocardial necrosis is usually observed at Day 3 after ligation as ~75% of the myocardium below the ligature becomes nonviable. There is also a marked decline in left ventricular (LV) systolic function at Day 4 after injury. Cardiac systolic function is able to restore to sham operating levels at Day 21 after MI as the myo-

cardium is repaired to greater than 95% viable [8]. However, the complete regeneration is age-dependent. For 1 day old mice, they are able to clear fibrotic tissue and replenish all cardiomyocytes while for 7 days old mice, their capacity for the capacity for complete morphological and functional cardiac regeneration was lost and retained significant scar tissue at day 21 after MI [9]. Very few proliferating cardiomyocytes were identified in 7 day old mice after MI and the remodeling response of 14 day old mice was characterized by extensive fibrosis, wall thinning, and ventricular dilation after MI [8].

The adult mouse infarct model better represents the cardiac response of human patients to an ischemic injury than neonatal mouse model. To induce heart infarction, mice are anesthetized by inhaling isoflurane or injecting anesthetics (e.g., Ketamine/Xylazine). The mice are then intubated using a catheter and ventilated with a rodent ventilator. The surgical procedure is similar to the one described above for neonatal mice. A left-sided thoracotomy is performed and the LAD artery is permanently ligated, approximately ~4 mm distal to the origin of the artery under the left atrium. Infarction is verified by LV blanching distal to the suture. The chest and skin are then closed, and mice are allowed to recover in a heated chamber [8, 11, 12]. Inflammation and scar tissue formation replacing the necrotic myocardium after MI are essential to preventing cardiac wall rupture. It is possible that ischemic injury, with subsequent cell necrosis, apoptosis, and damage to the extracellular matrix, dictates the course of inflammation and inhibits complete regeneration [13].

An inherent limitation of the LAD ligation model both in neonates and adults makes standardizing injury size difficult. Given that a proximal LAD occlusion can result in infarction of as much as 40% of the LV, it is highly plausible that larger ischemic injuries, similar to larger resection injury, are incompatible with complete regeneration [14].

### *Cryogenic injury*

Cryogenic injury is another mouse model for studying heart regeneration and cellular remodeling. Unlike MI, the cryoinjury model induces confluent necrosis but does not spare

cells within the center of the lesion. It can also have interdigitating viable and necrotic tissue [15]. Interestingly, cardiomyocytes are capable to proliferate sufficiently to effectuate myocardial regeneration after extensive cryoinjury of the LV myocardium in adult mice. High levels of macrophages precede and coincide with the highest levels of cardiomyocyte proliferation, suggesting a functional role of these cells in myocardial regeneration [16].

Cryogenic injury can be induced in several places throughout the heart, depending on the investigators' area of interest. An open thoracotomy approach is used to induce cryoinjury on the LV, because this allows direct visualization of the freezing process and immediate validation of the resulting freeze-thaw injury. A liquid nitrogen-cooled aluminum probe is applied directly onto the anterior LV for ~10 seconds. The frozen myocardium thaws completely 10-15 seconds following probe removal, and the injured area exhibits a deep red coloration [12]. Three 1-min exposures cause an extensive lesion on the midventricular portion of the anteriolateral LV wall [11].

Another approach is through an abdominal incision to induce cryoinjury on the right ventricle (RV). Mice are anesthetized and the abdomen is opened through a transverse laparotomy. The translucent diaphragm is exposed by lifting the chest with a divaricator. The surface of the heart is now visible through the diaphragm and the desired area for the injury can be selected. A blunt tip stainless steel Von Graefe hook is directly placed on the diaphragm after cooling for ~2 min in liquid nitrogen to make an apical or midventricular RV injury. Two sequential 10-s exposures (with an intermittent 30-s interval) cause an extensive, transmural lesion on the lateroapical portion of the RV wall with an extension to the apical LV wall. A single 30-s exposure causes an extensive, transmural lesion on the midventricular RV wall with no extension to the septum [11]. This procedure has also been described in rats [17].

### *Doxorubicin (DOX)-induced HF*

Nonischemic cardiomyopathy accounts for approximately one-third of HF cases. Unlike ischemic forms, which are amenable to palliative procedures like revascularization and remodeling operations, nonischemic cardiomyopa-

thies, once they have reached an end stage of drug refractoriness, can only be treated radically by heart transplantation. DOX-treated mice receive a single dose of DOX at 15-20 mg/kg i.p., which causes clinical symptoms of HF [18]. Mice are studied five days later because at this time point more than 5 half-lives of elimination of DOX from both plasma and cardiac tissue in mice has taken place and, at the time of functional and immunohistochemical assessment, DOX is no longer present in the blood or cardiac tissues. LV fractional shortening and cardiac output are significantly reduced (35 and 23%, respectively) relative to control animals. This murine model of severe LV dysfunction by DOX cardiotoxicity mimics the human pathology. Changes are consistent with clinical observations, suggesting that this murine model is appropriate for mechanistic evaluations [19].

### *Transgenic lines*

One of the most advantageous aspects of utilizing mice is the ability to make genetic models. Although such models can be produced in larger species, mouse models can be developed in a shorter time period due to their short gestation age at a substantially lower cost [4].

*Dilated cardiomyopathy (DCM):* Two strains of mice have been established to model the development, progression, and regression of DCM in humans. Muscle LIM protein (MLP) null mice are engineered by a deletion of MLP, an actin-associated cytoskeletal protein and Ca-sequestrin (CQS) mice have a cardiac-restricted overexpression of the calcium binding protein CQS. Both produce a HF phenotype that replicates various aspects of human DCM. The MLP<sup>-/-</sup> mice appear to closely replicate human DCM, with progressive reduction in cardiac function beginning at 4-6 months, whereas CSQ mice develop rapid-onset DCM resulting in mortality by 9-14 weeks [20]. Echocardiography shows normal contractility in 4-week-old MLP null mice and a progressive decrease in contractility with increasing age. This suggests that contractile dysfunction is not the primary defect in this mouse model of DCM. Rather, the lack of MLP causes hypertrophy, resulting in HF with an increase in connecting tissue and increased myocardial stiffness [21].

*Hypertrophic cardiomyopathy (HCM):* Mouse models in which cMyBP-C is homozygously ablated (cMyBP-C<sup>-/-</sup>) have been used to study the role of cMyBP-C in normal cardiac contractility and the development of HCM. Alterations in contractile kinetics serve as the primary pathophysiologic trigger for the development of hypertrophy in this model [22]. Another transgenic mouse model of HCM that overexpresses myotrophin in the heart, the Tg mouse, was also developed. This model showed hypertrophy as early as 4 weeks of age that progressively led to HF with severe compromised function. All the symptoms in this model mimic human HF. Tg mice demonstrated prevalent DNA damage in hearts during transition from long standing hypertrophy to HF and the induction of the cell regenerative machinery, especially in cardiac myocytes. Human HCM heart samples show similar changes in several genes for apoptosis and cell regeneration. Data in the Tg mouse model convincingly suggest that cell death and regeneration take place simultaneously during the transition of hypertrophy to HF [23].

*Autoimmune cardiomyopathy (AICM):* To develop an animal model for AICM, DQ8 transgenic non obese diabetic (NOD) mice were crossed with a NOD Major Histocompatibility Complex (MHC) class II  $\beta$ -chain knockout (KO) line. Animals from the original DQ8 transgenic NOD line developed spontaneous autoimmune diabetes at the same rate as the regular NOD/LtJ strain. However, when the DQ8-NOD animals were crossed with the NOD MHC class II KO line, the resulting DQ8<sup>+/+</sup>, IAB $\beta$ <sup>-/-</sup> NOD animals no longer developed diabetes or insulinitis; instead, they developed progressive DCM (hearts three to four times than normal size) and HF leading to premature death in both males and females. As the animals aged, first-degree atrioventricular (AV) block became widespread in the population, and by 18 weeks, the majority of animals had progressed to second degree or complete AV block. Histological examination of end-stage hearts showed pancarditis, with grossly dilated atria and only a few live cardiomyocytes remaining in mostly fibrotic atrial walls, which in places were paper thin [24].

*Duchenne muscle dystrophy (DMD):* DMD is an X-linked neuromuscular disorder caused by a mutation in the dystrophin gene [25]. Although limb muscle weakness and loss of ambulation

are usually the initial clinical signs of the disease, patients with DMD die from respiratory failure or HF. To improve lifespan and quality of life, progressive loss of contractile function in the heart also needs to be prevented or halted [26].

The genotypic murine model for DMD is the dystrophin-deficient Mdx mouse. The disease phenotype is milder in Mdx mice in contrast to human counterparts [25]. They have a normal life span and do not show significant muscle weakness or cardiac alterations nor any of the skeletal or cardiac muscle lesions that develop at late time points in DMD patients, such as fibrosis and muscular atrophy [27]. This is partially due to the compensatory effects of utrophin, a dystrophin homolog that can offset the loss of dystrophin in mice [26]. Another transgenic mouse was developed to better model the severe cardiac dysfunction in humans with DMD. In this double knock-out mouse, where both dystrophin and utrophin are absent, cardiac contractile function is severely affected at 8 weeks-of-age, displaying the classic pathophysiological hallmarks of end-stage human cardiac failure [28].

Several studies using dystrophin-deficient mice bred with other strains were conducted to clarify mechanisms leading to cardiomyopathy in DMD. Different therapies have been used to treat the symptoms of cardiomyopathy in DMD, but these agents cannot replace lost or damaged cardiomyocytes resulting from lack of fully functional dystrophin. Generation of new cardiomyocytes in dystrophin-deficient cardiac muscle from an endogenous population of cardiac stem cells suggests a potential mechanism that may be exploited to delay or prevent DCM in DMD [29]. Macrophages are necessary for repair of damage in the heart, as they stimulate new cardiomyocytes [29]. Also, IL-10 might be an important immune-modulator in dystrophic muscle. Cardiac inflammation induced by IL-10 ablation induced cardiac dysfunction and decreased LV function with LV and RV dilatation [30].

**Atrial fibrillation (AF):** Cardiac-specific LKB1 KO mice are used to study AF and associated pathologies. 95% of KO mice developed AF by 12 weeks. They also demonstrated variable heart rhythm disorders including first- and second-degree AV block, bundle branch block, pre-

mature atrial and ventricular contractions, and atrial flutter. AF resulted in increased fibrosis, apoptosis, and disrupted ultrastructure [31]. KO mouse heart developed dilated atrial cardiomyopathy, which was vulnerable to electrical and structural remodeling. Atrial dilation, stretch, fibrosis, loss of muscle mass, cellular and matrix remodeling, and disruption of gap junctions are documented in this model. AF caused LV systolic dysfunction with depressed LV ejection fraction and clinical HF. It accurately represents human AF with characteristic structural and electrical remodeling in atria [31].

### Rat

#### *Ligation-induced myocardial infarction (MI)*

The procedure to induce rat MI is very similar to the one described above for mice. Briefly, after rats are anaesthetized, intubated, and connected to a ventilator, a left thoracotomy is performed in the fourth intercostal space, the heart exposed, and the LAD artery ligated 2-3 mm from its origin between the pulmonary artery conus and the LA [32-34].

#### *Overload-induced cardiac hypertrophy*

One of the most common and successful surgical models to create pressure overload HF is constriction of the ascending aorta. This model is clinically relevant because of its slow but steady progression from compensated cardiac hypertrophy to the decompensated phase and, finally, to the HF stage. Ascending aortic constriction can be performed in two ways, either by using sutures or by application of metallic clips, which is less surgically complicated. In this procedure, one has to locate the supra-valvular ascending aorta and insert the clip around the aorta to obtain the desired level of constriction [35, 36].

#### *Diabetic cardiomyopathy (DbCM)*

Little attention has been devoted to defining the initial response of myocardial tissue to a short period of hyperglycemia in terms of proliferative properties of myocytes and alterations of cardiac stem cell storage before the appearance of the cardiomyopathic phenotype. A detailed knowledge of changes occurring at the very beginning of diabetes, when cardiac elec-

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tromechanical performance is still normal, may suggest appropriate therapeutic approaches aimed at preventing the development of mechanical dysfunction and arrhythmogenesis, which characterizes more advanced stages of DCM [37].

To create an animal model for diabetes, a single intra-peritoneal injection of streptozotocin (STZ, 60 mg/kg) is applied to rats. This is one of the most commonly used experimental models of diabetes. Rats exhibit hyperglycemia and hyperlipidemia coupled with hyperinsulinemia [38]. Ventricular dysfunction and marked structural damage appear 12 weeks after STZ treatment. The first detrimental effect of metabolic changes is a marked loss of ventricular mass, in the absence of cardiomyocyte hypertrophy or accumulation of extracellular matrix [37]. However in STZ-injected rats, it is difficult to definitively exclude the possibility that the pathogenesis is related, at least in part, to a permanent toxic action of STZ [39].

### *Transgenic lines*

*MI in hypertensive rats:* Coronary occlusion in spontaneously hypertensive rats (SHR) presents many similarities to MI in humans and represents an adequate model for the investigation of the role and mechanisms of stem cell therapy [40]. Hypertension in SHR causes higher LV systolic pressure, blood pressure, and RV and LV weight compared with their normotensive counterparts. After MI, hypertension accelerates LV dilatation and haemodynamic alterations, indicating that this animal model presents many similarities to MI in humans [32, 40].

*Type II diabetic rats:* A rat model of Type II diabetes is the Goto-Kakizaki (GK) rat. GK rats have a stable, inheritable form of Type II diabetes characterized by mild hyperglycemia and hyperinsulinemia, with no obesity, hypertension, or marked hyperlipidemia. Under normoxic conditions, cardiac function in the GK rat is indistinguishable from that of control rats. A contractile defect in both systolic and diastolic LV function can be elicited in the GK heart during even brief moderate hypoxia. Regardless the level of insulin, a high level of extracellular glucose is sufficient for development of contractile abnormalities in diabetic cardiomyocytes, which may explain the absence of these abnormalities in the GK rat model where the

level of hyperglycaemia is mild. Contractile dysfunction in the GK heart has a primarily metabolic basis [39].

Another rat model is the JCR: LA-cp rat. It is a normotensive, hyperinsulinaemic model of Type II diabetes, which differs from the GK rat in being obese and markedly hyperlipidaemic. In addition, these rats develop early atherosclerotic lesions of major blood vessels and occlusive coronary thrombi at later stages of the disease [39].

*Duchenne muscular dystrophy (DMD):* A new model of dystrophin-deficient rats was made by microinjecting a mixture of TALE nuclease mRNA for DMD in rat zygotes, allowing for the generation of two DMD/Mdx rat lines. The lesions in heart and skeletal muscle in this model closely mimics those observed in DMD patients. As observed in DMD patients but not in Mdx mice, fibrosis is severe in all skeletal muscle examined as well as in cardiac muscle of DMD/Mdx rats. Cardiac muscles are affected with necrosis and fibrosis and show signs of progressive DCM. Echocardiography showed significant concentric remodeling and alteration of diastolic function. These results indicate that DMD/Mdx rats represent a new, invaluable small animal model for pre-clinical research on DMD [27].

### **Rabbit**

#### *Spontaneous watanabe heritable hyperlipidemic myocardial infarction (WHHL-MI)*

Rabbit myocardium shares more similarities with human myocardium than small rodent myocardium; therefore, rabbit genetic models, albeit expensive, can be used as a stepping-stone to determine whether a particular study can be extended to humans and larger animal models. Although, other large species such as canine and sheep more closely resemble the human heart, the cost of acquiring and housing rabbits is still significantly lower, making them an attractive alternative to larger animal models for cardiac research. Differences between rabbit and human myocardium remain, possibly resulting in a particular study or therapeutic intervention having differential effects in rabbits and humans. For example, rabbits might not serve as the best animal model for studying the effects of exercise on the cardiovascular

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**Table 1.** Advantages and disadvantages of small animal models

Advantages	Disadvantages
<ul style="list-style-type: none"><li>• Lower maintenance costs</li><li>• Easier to handle and house</li><li>• Shorter gestation time and lifespan</li><li>• Suitable for proof-of-concept and “high-throughput” studies</li><li>• ischemia-reperfusion induced arrhythmias are infrequent and easy to reverse when they occur</li><li>• Suitable for genetic selection and production of transgenic strains</li></ul>	<ul style="list-style-type: none"><li>• Phylogenetically distant from humans</li><li>• Pathophysiology of disease may not be translatable to humans</li><li>• Different response to pharmaceuticals</li><li>• Not suitable for chronic studies</li></ul>

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**Table 2.** Relevant small animal models for cardiac disease (only one reference is listed for each model. See the Main text for more references)

Animals	Induced models	Spontaneous models
Mouse	Cryoinjury [11]	AF [31]
	DOX induced HF [18]	AICM [24]
	MI [8]	DCM [21] DMD [28] HCM [23]
Rat	Cardiac hypertrophy [35]	Hypertensive rat [40]
	Cryoinjury [17]	DbCM [39]
	DbCM [38] MI [33]	DMD [27]
Rabbits		WHHL-MI rabbits [41]
Cats		HCM [42]

Abbreviations: AF: Atrial fibrillation, AICM: Autoimmune cardiomyopathy, DbCM: Diabetic cardiomyopathy, DCM: Dilated cardiomyopathy, DMD: Duchenne muscular dystrophy, DOX: Doxorubicin, HF: Heart failure, HCM: Hypertrophic cardiomyopathy, MI: Myocardial infarction, WHHL-MI: Watanabe heritable hyperlipidemic myocardial infarction.

system mainly because its heart rate reserve is much less than that in human and large animal models, such as canines, which would serve as better animal models in such cases [4].

A rabbit spontaneous MI model could be obtained from the selective breeding descendants of coronary atherosclerosis-prone WHHL-MI rabbits. Because the coronary lumen area stenosis was enhanced in these rabbits, the incidence of spontaneous WHHL-MI was increased in proportion to the serial nearly-occluded coronary lesions. Coronary plaques with complicated lesions are the major determinant for MI in these rabbits. However, observations indicate that the mechanisms for MI in WHHL rabbits are different from those in humans [41].

### Cats

#### *Hypertrophic cardiomyopathy (HCM)*

Spontaneous cardiac diseases similar or identical to those in humans are extremely common in companion animals and are vastly underutilized as models of human cardiac disease. HCM is currently the most common heart disease in cats, and its incidence appears to be increasing. As in humans, feline HCM is characterized by hypertrophy of the (LV, impaired diastolic filling, secondary left atrial enlargement is usually evident, and variable right heart enlargement or hypertrophy may develop over time. Arrhythmias are uncommon unless severe left atrial enlargement is present. In cats with HCM, congestive HF and arterial thromboembolism are common clinical manifestations [42].

A specific breed, the Maine Coon, has a genetic mutation that makes the breed prone to suffer from HCM. The prevalence of HCM in cats homozygous for this mutation is much higher than in cats heterozygous for the mutation. However, it is highly likely that other causes (genetic or not) are also responsible for the disease in the Maine Coon [43]. Individuals of this breed can be used as relevant animal models to study the development and pathophysiology of HCM.

### Conclusions

Small animal models are commonly used in cardiovascular research because of their many advantages over large animal models (see **Table 1**). They have a short life span, allowing the investigators to follow the natural history of the disease at an accelerated pace. Also, the development of genetically modified models allows for rapid establishment of proof-of-principle that can later be extended into larger animal models. However, rodents have a few disadvantages as well. They are phylogenetically very distant from humans and some pathophysiological features of disease and their response to pharmacological treatments may not be reliable predictors for humans. Thus, translational aspects and value of genetic small animal models must be interpreted with caution. Although they may display some of the characteristics of human cardiac disease, they typically do not recapitulate all aspects of it.

Determining the best experimental model of a human condition requires a number of deci-



sions and compromises - especially in relation to obtaining the optimal balance between the quantity and quality of the data produced vs the relevance of the data to the condition under investigation. In assessing the utility of an investigative model, it is necessary to identify the research objectives. The question under study will greatly influence the choice of the most appropriate model and determination of cardiac function and malfunction. It is also important to standardize the procedures used in order to obtain relevant and reproducible results that can be compared with other findings. **Table 2** summarizes the most relevant small animal models for cardiac disease and whether the disease is artificially induced or spontaneous. This provides a basis in which to decide what model best suits a specific investigation.

### Acknowledgements

We thank Dr. Janet Webster of the Fralin Life Science Institute at Virginia Tech for providing a review of the manuscript. This work was supported by the Institute for Critical Technology and Applied Science (ICTAS) at Virginia Tech (#JFC2014\_JIAHE958451025). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Disclosure of conflict of interest

None.

### Abbreviations

AF, atrial fibrillation; AICM, autoimmune cardiomyopathy; AV, atrioventricular; CSQ, calsequestrin; DbCM, diabetic cardiomyopathy; DCM, dilated cardiomyopathy; DMD, Duchenne muscular dystrophy; DOX, doxorubicin; GK, Goto-Kakizaki; HCM, hypertrophic cardiomyopathy; HF, heart failure; KO, knockout; LAD, left anterior descending; LV, left ventricle; MHC, major histocompatibility complex; MI, myocardial infarction; MLP, muscle LIM protein; NOD, non obese diabetic; RV, rights ventricle; SHR, spontaneously hypertensive rat; STZ, streptozotocin; WHHL-MI, Spontaneous Watanabe heritable hyperlipidemic myocardial infarction.

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### References

- [1] Ou L, Li W, Liu Y, Zhang Y, Jie S, Kong D, Steinhoff G and Ma N. Animal models of cardiac disease and stem cell therapy. *Open Cardiovasc Med J* 2010; 4: 231-239.
- [2] Cui J, Li J, Mathison M, Tondato F, Mulkey SP, Micko C, Chronos NA and Robinson KA. A clinically relevant large-animal model for evaluation of tissue-engineered cardiac surgical patch materials. *Cardiovasc Revasc Med* 2005; 6: 113-120.
- [3] Ikonen TS, Patila T, Virtanen K, Lommi J, Lappalainen K, Kankuri E, Krogerus L and Harjula A. Ligation of ameroid-stenosed coronary artery leads to reproducible myocardial infarction—a pilot study in a porcine model. *J Surg Res* 2007; 142: 195-201.
- [4] Milani-Nejad N and Janssen PM. Small and large animal models in cardiac contraction research: advantages and disadvantages. *Pharmacol Ther* 2014; 141: 235-249.
- [5] Hearse DJ and Sutherland FJ. Experimental models for the study of cardiovascular function and disease. *Pharmacol Res* 2000; 41: 597-603.
- [6] Recchia FA and Lionetti V. Animal models of dilated cardiomyopathy for translational research. *Vet Res Commun* 2007; 31 Suppl 1: 35-41.
- [7] Andersen DC, Ganesalingam S, Jensen CH and Sheikh SP. Do neonatal mouse hearts regenerate following heart apex resection? *Stem Cell Reports* 2014; 2: 406-413.
- [8] Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, Mammen PP, Rothermel BA, Olson EN and Sadek HA. Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *Proc Natl Acad Sci U S A* 2013; 110: 187-192.
- [9] Haubner BJ, Adamowicz-Brice M, Khadayate S, Tiefenthaler V, Metzler B, Aitman T and Penninger JM. Complete cardiac regeneration in a mouse model of myocardial infarction. *Aging (Albany NY)* 2012; 4: 966-977.
- [10] Dayan V, Yannarelli G, Filomeno P and Keating A. Human mesenchymal stromal cells improve scar thickness without enhancing cardiac function in a chronic ischaemic heart failure model. *Interact Cardiovasc Thorac Surg* 2012; 14: 516-520.
- [11] Grisel P, Meinhardt A, Lehr HA, Kappenberger L, Barrandon Y and Vassalli G. The MRL mouse

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- repairs both cryogenic and ischemic myocardial infarcts with scar. *Cardiovasc Pathol* 2008; 17: 14-22.
- [12] Robey TE and Murry CE. Absence of regeneration in the MRL/MpJ mouse heart following infarction or cryoinjury. *Cardiovasc Pathol* 2008; 17: 6-13.
- [13] Konfino T, Landa N, Ben-Mordechai T and Leor J. The type of injury dictates the mode of repair in neonatal and adult heart. *J Am Heart Assoc* 2015; 4: e001320.
- [14] Sen S and Sadek HA. Neonatal heart regeneration: mounting support and need for technical standards. *J Am Heart Assoc* 2015; 4: e001727.
- [15] Murry CE, Kay MA, Bartosek T, Hauschka SD and Schwartz SM. Muscle differentiation during repair of myocardial necrosis in rats via gene transfer with MyoD. *J Clin Invest* 1996; 98: 2209-2217.
- [16] van Amerongen MJ, Harmsen MC, Petersen AH, Popa ER and van Luyn MJ. Cryoinjury: a model of myocardial regeneration. *Cardiovasc Pathol* 2008; 17: 23-31.
- [17] Ren C, Wang F, Li G, Jiao Q, Bai J, Yu D, Hao W, Wang R and Cao JM. Nerve sprouting suppresses myocardial I(to) and I(K1) channels and increases severity to ventricular fibrillation in rat. *Auton Neurosci* 2008; 144: 22-29.
- [18] Agbulut O, Menot ML, Li Z, Marotte F, Paulin D, Hagege AA, Chomienne C, Samuel JL and Menasche P. Temporal patterns of bone marrow cell differentiation following transplantation in doxorubicin-induced cardiomyopathy. *Cardiovasc Res* 2003; 58: 451-459.
- [19] Weinstein DM, Mihm MJ and Bauer JA. Cardiac peroxynitrite formation and left ventricular dysfunction following doxorubicin treatment in mice. *J Pharmacol Exp Ther* 2000; 294: 396-401.
- [20] Bullard TA, Protack TL, Aguilar F, Bagwe S, Massey HT and Blaxall BC. Identification of Nogo as a novel indicator of heart failure. *Physiol Genomics* 2008; 32: 182-189.
- [21] Unsold B, Schotola H, Jacobshagen C, Seidler T, Sossalla S, Emons J, Klede S, Knoll R, Guan K, El-Armouche A, Linke WA, Kogler H and Hasenfuss G. Age-dependent changes in contractile function and passive elastic properties of myocardium from mice lacking muscle LIM protein (MLP). *Eur J Heart Fail* 2012; 14: 430-437.
- [22] de Lange WJ, Grimes AC, Hegge LF and Ralph JC. Ablation of cardiac myosin-binding protein-C accelerates contractile kinetics in engineered cardiac tissue. *J Gen Physiol* 2013; 141: 73-84.
- [23] Sarkar S, Chawla-Sarkar M, Young D, Nishiyama K, Rayborn ME, Hollyfield JG and Sen S. Myocardial cell death and regeneration during progression of cardiac hypertrophy to heart failure. *J Biol Chem* 2004; 279: 52630-52642.
- [24] Elliott JF, Liu J, Yuan ZN, Bautista-Lopez N, Wallbank SL, Suzuki K, Rayner D, Nation P, Robertson MA, Liu G and Kavanagh KM. Autoimmune cardiomyopathy and heart block develop spontaneously in HLA-DQ8 transgenic IAbeta knockout NOD mice. *Proc Natl Acad Sci U S A* 2003; 100: 13447-13452.
- [25] Fayssoil A, Renault G, Guerchet N, Marchiol-Fournigault C, Fougereousse F and Richard I. Cardiac characterization of mdx mice using high-resolution doppler echocardiography. *J Ultrasound Med* 2013; 32: 757-761.
- [26] Delfin DA, Xu Y, Peterson JM, Guttridge DC, Rafael-Fortney JA and Janssen PM. Improvement of cardiac contractile function by peptide-based inhibition of NF-kappaB in the utrophin/dystrophin-deficient murine model of muscular dystrophy. *J Transl Med* 2011; 9: 68.
- [27] Larcher T, Lafoux A, Tesson L, Remy S, Thepenier V, Francois V, Le Guiner C, Goubin H, Dutilleul M, Guigand L, Toumaniantz G, De Cian A, Boix C, Renaud JB, Cherel Y, Giovannangeli C, Concordet JP, Anegon I and Huchet C. Characterization of dystrophin deficient rats: a new model for Duchenne muscular dystrophy. *PLoS One* 2014; 9: e110371.
- [28] Janssen PM, Hiranandani N, Mays TA and Rafael-Fortney JA. Utrophin deficiency worsens cardiac contractile dysfunction present in dystrophin-deficient mdx mice. *Am J Physiol Heart Circ Physiol* 2005; 289: H2373-2378.
- [29] Berry SE, Andruszkiewicz P, Chun JL and Hong J. Nestin expression in end-stage disease in dystrophin-deficient heart: implications for regeneration from endogenous cardiac stem cells. *Stem Cells Transl Med* 2013; 2: 848-861.
- [30] Nitahara-Kasahara Y, Hayashita-Kinoh H, Chiyo T, Nishiyama A, Okada H, Takeda S and Okada T. Dystrophic mdx mice develop severe cardiac and respiratory dysfunction following genetic ablation of the anti-inflammatory cytokine IL-10. *Hum Mol Genet* 2014; 23: 3990-4000.
- [31] Ozcan C, Battaglia E, Young R and Suzuki G. LKB1 knockout mouse develops spontaneous atrial fibrillation and provides mechanistic insights into human disease process. *J Am Heart Assoc* 2015; 4: e001733.
- [32] Nishikimi T, Yamagishi H, Takeuchi K and Takeda T. An angiotensin II receptor antagonist attenuates left ventricular dilatation after myocardial infarction in the hypertensive rat. *Cardiovasc Res* 1995; 29: 856-861.
- [33] Di Lascio G, Harmelin G, Targetti M, Nanni C, Bianchi G, Gasbarri T, Gelsomino S, Bani D,

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- Orlandini SZ and Bonacchi M. Cellular retrograde cardiomyoplasty and relaxin therapy for postischemic myocardial repair in a rat model. *Tex Heart Inst J* 2012; 39: 488-499.
- [34] Liu JF, Wang BW, Hung HF, Chang H and Shyu KG. Human mesenchymal stem cells improve myocardial performance in a splenectomized rat model of chronic myocardial infarction. *J Formos Med Assoc* 2008; 107: 165-174.
- [35] Gs AK, Raj B, Santhosh KS, Sanjay G and Kartha CC. Ascending aortic constriction in rats for creation of pressure overload cardiac hypertrophy model. *J Vis Exp* 2014; e50983.
- [36] Berni R, Cacciani F, Zaniboni M, Savi M, Bocchi L, Lapucci S, Razzetti R, Pastore F, Musso E and Stilli D. Effects of the alpha2-adrenergic/DA2-dopaminergic agonist CHF-1024 in preventing ventricular arrhythmogenesis and myocyte electrical remodeling, in a rat model of pressure-overload cardiac hypertrophy. *J Cardiovasc Pharmacol* 2006; 47: 295-302.
- [37] Stilli D, Lagrasta C, Berni R, Bocchi L, Savi M, Delucchi F, Graiani G, Monica M, Maestri R, Baruffi S, Rossi S, Macchi E, Musso E and Quaini F. Preservation of ventricular performance at early stages of diabetic cardiomyopathy involves changes in myocyte size, number and intercellular coupling. *Basic Res Cardiol* 2007; 102: 488-499.
- [38] Okamoto T, Kanemoto N, Ohbuchi Y, Okano M, Fukui H and Sudo T. Characterization of STZ-Induced Type 2 Diabetes in Zucker Fatty Rats. *Exp Anim* 2008; 57: 335-345.
- [39] El-Omar MM, Yang ZK, Phillips AO and Shah AM. Cardiac dysfunction in the Goto-Kakizaki rat. A model of type II diabetes mellitus. *Basic Res Cardiol* 2004; 99: 133-141.
- [40] Braga LM, Rosa K, Rodrigues B, Malfitano C, Camassola M, Chagastelles P, Lacchini S, Fiorino P, De Angelis K, Schaan BD, Irigoyen MC and Nardi NB. Systemic delivery of adult stem cells improves cardiac function in spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* 2008; 35: 113-119.
- [41] Shiomi M, Ito T, Yamada S, Kawashima S and Fan J. Development of an animal model for spontaneous myocardial infarction (WHHLM rabbit). *Arterioscler Thromb Vasc Biol* 2003; 23: 1239-1244.
- [42] Freeman LM and Rush JE. Nutrition and cardiomyopathy: lessons from spontaneous animal models. *Curr Heart Fail Rep* 2007; 4: 84-90.
- [43] Mary J, Chetboul V, Sampedrano CC, Abitbol M, Gouni V, Trehou-Sechi E, Tissier R, Queney G, Pouchelon JL and Thomas A. Prevalence of the MYBPC3-A31P mutation in a large European feline population and association with hypertrophic cardiomyopathy in the Maine Coon breed. *J Vet Cardiol* 2010; 12: 155-161.