






# ESC working group on cardiac cellular electrophysiology position paper: relevance, opportunities, and limitations of experimental models for cardiac electrophysiology research

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## Abstract

Cardiac arrhythmias are a major cause of death and disability. A large number of experimental cell and animal models have been developed to study arrhythmogenic diseases. These models have provided important insights into the underlying arrhythmia mechanisms and translational options for their therapeutic management. This position paper from the ESC Working Group on Cardiac Cellular Electrophysiology provides an overview of (i) currently available *in vitro*, *ex vivo*, and *in vivo* electrophysiological research methodologies, (ii) the most commonly used experimental (cellular and animal) models for cardiac arrhythmias including relevant species differences, (iii) the use of human cardiac tissue, induced pluripotent stem cell (hiPSC)-derived and *in silico* models to study cardiac arrhythmias, and (iv) the availability, relevance, limitations, and opportunities of these cellular and animal models to recapitulate specific acquired and inherited arrhythmogenic diseases, including atrial fibrillation, heart failure, cardiomyopathy, myocarditis, sinus node, and conduction disorders and channelopathies. By promoting a better understanding of these models and their limitations, this position paper aims to improve the quality of basic research in cardiac electrophysiology, with the ultimate goal to facilitate the clinical translation and application of basic electrophysiological research findings on arrhythmia mechanisms and therapies.

## Keywords

Animal models • Experimental models • Arrhythmias • Atrial fibrillation • Mechanisms • Cardiac electrophysiology • Cellular electrophysiology • Ion channels • Position paper

## Introduction

Cardiac arrhythmias are a major cause of death and disability. Despite important advances in arrhythmia management, numerous knowledge gaps remain.<sup>1</sup> During the past few decades, a large number of experimental cell and animal models have been developed to study different arrhythmogenic diseases. These models have provided important insights into the underlying arrhythmia mechanisms and translational options for their therapeutic management. Each experimental model has specific advantages and limitations, making it more or less suitable to study specific arrhythmogenic diseases, but a comprehensive overview of the different experimental models, electrophysiological techniques, and their optimal use is currently lacking.

This position paper from the ESC Working Group on Cardiac Cellular Electrophysiology addresses this knowledge gap and provides an overview of currently available electrophysiological research methodologies, the most common experimental (cellular and animal) models for cardiac arrhythmias, and their relevance and suitability for certain research questions. Additionally, intrinsic model limitations, as well as opportunities to advance the current state-of-the-art, are discussed. By promoting a better understanding of these opportunities and their limitations, this position paper aims to improve the quality of basic research in cardiac electrophysiology, with the ultimate goal to facilitate the clinical translation and application of basic electrophysiological research findings on arrhythmia mechanisms and therapies, thereby contributing to the overall goal of the ESC to disseminate evidence-based scientific knowledge to cardiovascular professionals so they can provide better care to patients.

## Cellular and whole heart electrophysiology techniques

A variety of different techniques allow for a multi-scale investigation of electrophysiological features from *in vivo*, *ex vivo* whole heart, to the cellular level (Figure 1), providing insights into physiological and pathophysiological electrical activity and arrhythmogenesis on different levels.

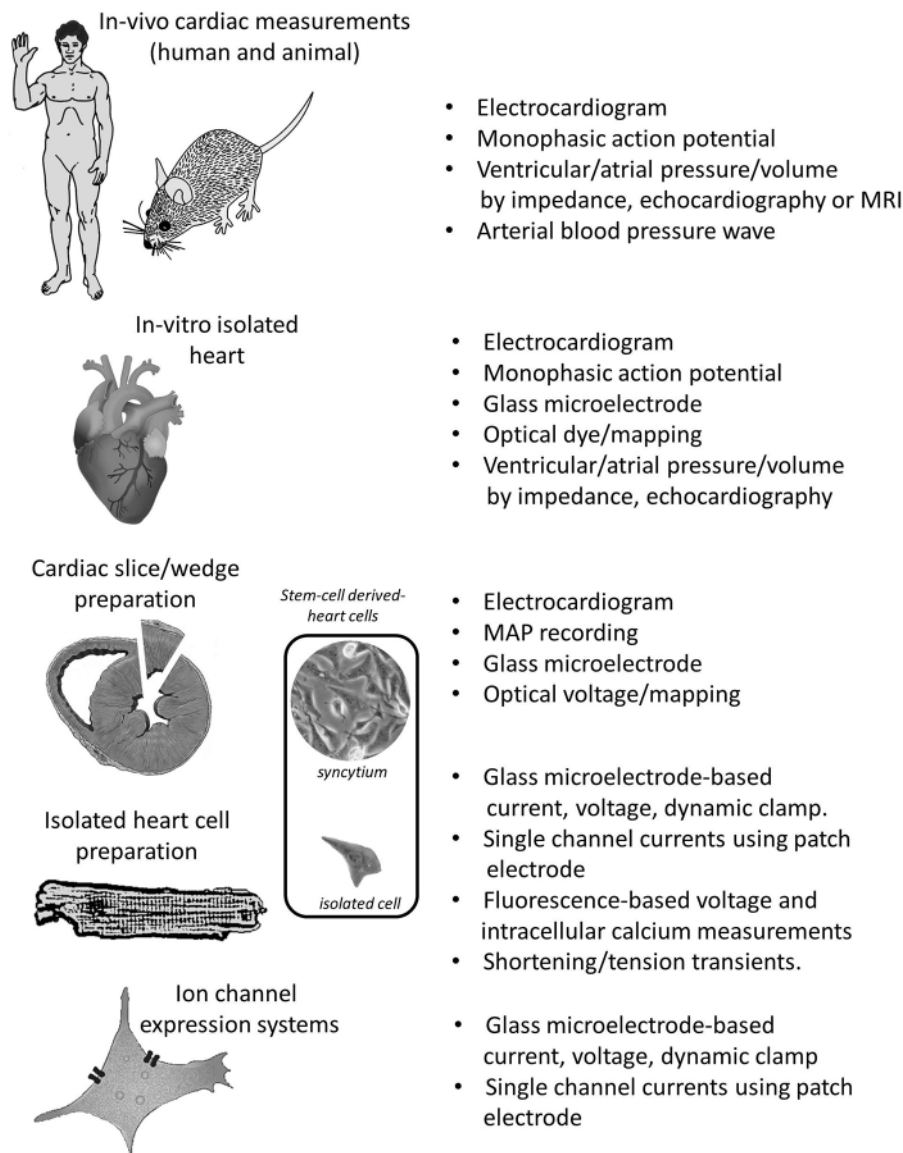
### Whole heart electrophysiology (*in vivo*)

Electrocardiography (ECG) has been successfully employed for monitoring and analysing cardiac electrical activity and arrhythmia in different species ranging from mice to horses.<sup>2</sup> In ECGs of anaesthetized animals complex effects of anaesthesia on the electro-mechanical function of the heart and on the autonomic nervous system must be taken into account. Advances in ECG telemetry have enabled reliable recordings in awake conscious animals.<sup>3</sup> Moreover, these ECG techniques allow investigation of both spontaneous and induced arrhythmia formation, e.g. after AV-block induction or during transvenous or transoesophageal arrhythmia induction.<sup>4,5</sup> Although ECG morphology is similar in different species, a number of interspecies differences exist that may hinder clinical translation (see 'Species differences in cardiac electrophysiology' section and Figure 2). Higher resolution non-invasive ECG imaging is possible in large animal models.<sup>6,7</sup> Monophasic action potentials (MAP) can be recorded *in vivo* invasively from endocardial and epicardial surfaces of the heart in anaesthetized animals using a contact electrode catheter

technique. Monophasic APs reproduce the repolarization time course of transmembrane APs, providing information on AP duration (APD) and configuration (including proarrhythmic early afterdepolarizations) but not on AP amplitude or upstroke velocity.<sup>8</sup> Advances in optical mapping in combination with a novel ratiometric voltage-sensor and a high-speed camera above the epicardial surface have more recently allowed high-resolution cardiac electrophysiology in large animals *in vivo*.<sup>9</sup>

### Whole-heart and cardiac tissue electrophysiology (*ex vivo*)

In 1895, Oscar Langendorff showed that an isolated mammalian heart can be kept alive for hours by blood perfusion through a cannula attached to the ascending aorta.<sup>10</sup> Nowadays, retrograde Langendorff perfusion<sup>11,12</sup> and its variants like the working heart setup<sup>13</sup> are considered invaluable analytic tools to assess many important aspects of cardiac physiology. In the Langendorff-perfused heart electrophysiological properties (such as MAP and conduction velocities), arrhythmia inducibility, contractility and metabolics can be quantified at the whole-heart level at baseline and at pro-arrhythmic conditions such as ischaemia–reperfusion injury, and cell-based regeneration. The Langendorff-perfused heart also allows to manipulate electrical activation and propagation at the whole heart level without systemic circulatory effects or haemodynamic instability that may be encountered *in vivo*. In addition to MAP measurements (see above), the conventional sharp microelectrode technique has been used in numerous studies to investigate cardiac action potential characteristics, applied for the first time by Coraboeuf and Weidman in isolated heart tissue.<sup>14</sup> This approach is however limited by the fact that only localized information is obtained. More recently, optical mapping has been emerged as a powerful tool to study electrical activity of the whole heart or intact parts of the cardiac conduction system: sinoatrial node (SAN),<sup>15</sup> atrioventricular node or the Purkinje-fibres network.<sup>16</sup> The principle of optical mapping is to irradiate the sample and detect the fluorescence emitted from fluorescent indicators (e.g. voltage and/or Ca<sup>2+</sup> sensors). It is based on wide-field illumination of the whole heart or cardiac tissue, while an objective lens is used to collect the fluorescence signal. A high-speed imaging system (at kHz frame rate) is used to capture and visualize small variations in fluorescence intensity associated with voltage or Ca<sup>2+</sup> transients. Multimodal (simultaneous) acquisition of voltage and Ca<sup>2+</sup> signals is also possible by using spectrally distinct fluorescence dyes.<sup>17</sup> Many electrophysiological properties can be assessed by mapping the heart on a single view, including detailed assessment of epicardial conduction velocities. In addition, multi-view panoramic mapping is particularly useful to study self-propagating rotors on the heart surface during arrhythmias. Excitation-contraction uncoupling agents, such as blebbistatin, have traditionally been used to avoid artefacts due to tissue movement. This is particularly useful in optical mapping of the pacemaker impulse in isolated atrio/sinus preparations. On the other hand, uncoupling molecules may affect arrhythmia inducibility due to mechano-electric feedback mechanisms<sup>18</sup> and recent work has investigated mechanical phase singularities during arrhythmia.<sup>19</sup> With novel optogenetics tools<sup>20</sup> it is possible to achieve full optical control of cardiac electrical activity or sinus node pacemaking.<sup>15</sup> To assess transmural cardiac electrophysiological heterogeneity and its



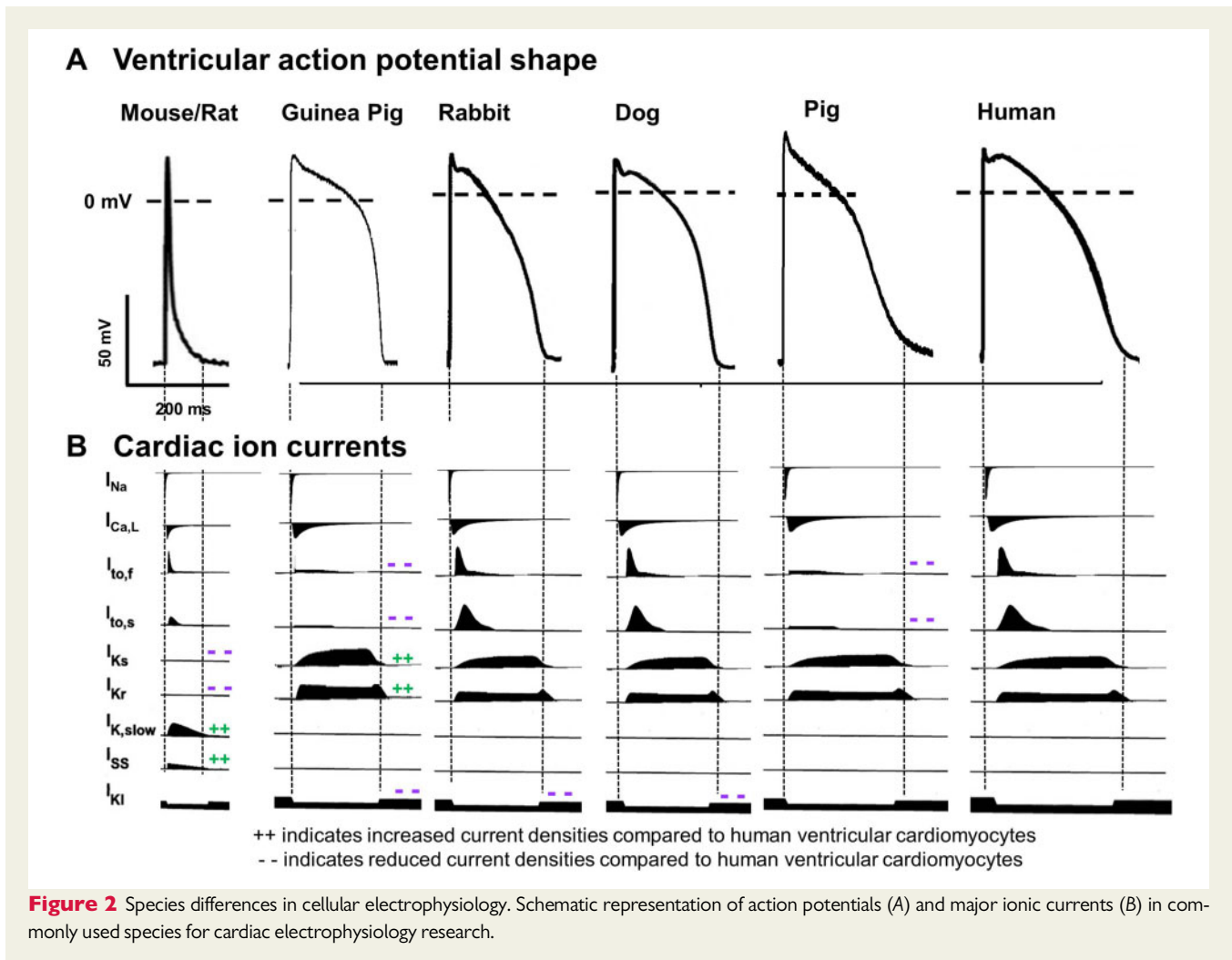
**Figure 1** Hierarchy of preparations and techniques in cardiac electrophysiology. Preparations range from organism (mouse-to-human), isolated heart, multicellular preparations (e.g. slice/wedge or papillary/trabeculae preparations), isolated cardiac cells (e.g. atrial, ventricular, nodal or Purkinje cells) to ion-channel expression systems (e.g. HEK). The associated techniques for measuring cardiac function are shown on the right. Comparable preparations (syncytium and single cell) for stem-cell-derived cardiomyocytes are indicated in a separate panel.

consequences for arrhythmogenesis, canine isolated coronary-perfused ventricular wedge preparation and floating microelectrodes have been employed.<sup>21</sup>

## Cellular electrophysiological techniques (in vitro)

In isolated cardiomyocytes, impaling with a sharp micro-electrode can accurately measure membrane voltage while minimally disturbing the intracellular environment, but the resistance associated with the small size of the electrode tip poses technical challenges for the injection of current and accurate voltage-clamp recordings of some

currents. The patch-clamp methodology consecutively developed by Neher and Sakmann uses larger glass electrodes and allows single-channel recordings from the patch underneath the electrode (cell-attached patch), as well as whole-cell recordings after opening the membrane within the patch. In general, two different patch-clamp modes are used: current clamp in which currents are applied and resulting changes in membrane potentials are assessed (e.g. to measure APs), and voltage clamp which applies predefined membrane voltage protocols together with pipette/bath solutions and blockers to measure specific ion currents. With the conventional whole-cell approach, the opening of the membrane allows the pipette solution to dialyse the intracellular environment, thus providing experimental



**Figure 2** Species differences in cellular electrophysiology. Schematic representation of action potentials (A) and major ionic currents (B) in commonly used species for cardiac electrophysiology research.

control over, e.g. intracellular ion concentrations. Alternatively, the whole-cell configuration can be established using pharmacological agents in the patch pipette that form pores in the cell membrane. This perforated patch-clamp methodology aims to maintain a more physiological intracellular milieu. Recent developments have also led to the dynamic-clamp technique, in which the current injected into a cell via a low-resistance electrode is a mathematical function of the instantaneous  $E_m$  of the cell.<sup>22</sup> This can be used to mimic the coupling of other cells (myocytes/non-myocytes) to an isolated heart cell<sup>23</sup> or modulation of the biophysical properties of one or more currents.<sup>24</sup> There are several limitations to this technique including the fact that dynamic clamp can simulate the electrical but not the ionic aspects of ion-channel activity, e.g.  $Ca^{2+}$  current without the  $Ca^{2+}$  ions. In addition to the more traditional patch-clamp approach, fluorescence-based techniques are increasingly used to measure membrane potential, as well as cellular  $Ca^{2+}$  transients and pro-arrhythmic  $Ca^{2+}$ -release events.<sup>15,25,26</sup> While such approaches allow for more high-throughput, non-invasive analyses, they have certain limitations: in particular, voltage-sensitive dyes do not provide information on actual membrane voltage (only relative values), which may

potentially impact on AP results. Overall, single-cell cardiac electrophysiology continues to provide electrophysiologists with key information and technical challenges now and for decades to come. For a detailed overview of cardiac ion channels and APs, the interested reader is referred to the comprehensive recent review by Varro *et al.*<sup>27</sup>

## Species differences in cardiac electrophysiology

Depolarizing cardiac ion channels/currents are highly conserved among species, with  $I_{Na}$  currents conducted by voltage-gated  $SCN5A/Nav1.5$  channels as main depolarizing ion currents in atrial and ventricular cardiomyocytes and an important role for  $I_{Ca,L}$  currents ( $Cav1.2$ ) as depolarizing ion current in the conduction system. Despite these similarities in depolarizing ion currents, pronounced species differences exist in heart rate, ranging from 600 b.p.m. resting heart rate in small animals such as mice, 300 b.p.m. in rats, and 150–200 b.p.m. in rabbits, to similar heart rates as in humans in bigger

animals such as pigs and dogs (~60–80 b.p.m.). Accordingly, AP duration is also different between species, largely due to important species-specific differences in repolarizing ion currents/channels (Figure 2).<sup>28</sup>

### Mouse/rat

In rodents, the AP shape is markedly different than in human and other bigger species: the plateau phase of the action potential is missing, resulting in a more triangular AP shape with a very short APD. In contrast to humans, repolarization in mice and rats is mainly driven by the rapidly activating, slowly inactivating delayed rectifier potassium currents  $I_{K,slow1}$  and  $I_{K,slow2}$  and the fast and slow components of the transient outward potassium current  $I_{to,f}$  and  $I_{to,s}$ ,<sup>28</sup> while the rapid and slow delayed rectifier  $K^+$  currents ( $I_{Kr}$  and  $I_{Ks}$ )—the main repolarizing ion currents in human cardiomyocytes—are functionally irrelevant. In addition, other repolarizing ion currents such as the atrial  $I_{Kur}$  are differentially expressed in rodents.<sup>29</sup>

### Rabbit

Pronounced similarities exist in AP shape, as well as function and gating kinetics of various cardiac potassium channels between rabbits and humans. In both species, the rapid and slow delayed rectifier  $K^+$  currents ( $I_{Kr}$  and  $I_{Ks}$ ) conducted by KCNQ1/KCNE1 and KCNH2 are the main repolarizing ion currents.<sup>28–31</sup> In other potassium currents, however, some inter-species differences exist: In humans,  $I_{to}$  is formed of two distinct subtypes named as  $I_{to,fast}$  and  $I_{to,slow}$ —with fast and slow recovery from inactivation, determined by Kv3.4 and Kv1.4, respectively.<sup>32</sup> In contrast, in rabbits  $I_{to,slow}$  is the primary transient Kv current in the left ventricle (LV),<sup>33</sup> while in the right ventricle (RV)  $I_{to,fast}$  and its role in LQT1-related arrhythmogenesis has recently been confirmed.<sup>34</sup>

### Zebrafish

Zebrafish also share pronounced similarities with human cardiac electrophysiology in terms of ventricular AP shape, AP/QT duration, and repolarizing ion currents, with an important role for  $I_{Kr}$  as main repolarizing current.<sup>35</sup> While zebrafish have provided ground-breaking insights into (early) principles of heart development, the adult cardiac structure differs significantly between mammals and fish, with fish having only one atrium and one ventricle. Moreover, the body temperature is much lower in zebrafish, which affects biophysical ion channel properties, potentially decreasing its translational relevance regarding cardiac conduction and arrhythmia mechanisms.<sup>36</sup>

### Pig

Porcine ventricular APs resemble those of humans in many aspects: configuration with dominant plateau phase, duration, rate dependence and transmural heterogeneity with populations of M-like cells.<sup>37</sup> Major contributing ionic currents (fast  $Na^+$  current,  $I_{Na}$ ; L-type  $Ca^{2+}$  current,  $I_{CaL}$ ; and  $I_{Kr}$ ,  $I_{Ks}$  and inward-rectifier  $K^+$  current,  $I_{K1}$ ) similar to those of human have been reported,<sup>38,39</sup> but atrial and ventricular ion currents and calcium handling are less well-characterized than for other species. One major difference is the lack of voltage-dependent, 4-aminopyridine-sensitive  $I_{to}$  in porcine myocytes.<sup>40</sup> A similar ventricular AP pattern was also shown in minipigs.<sup>41,42</sup>

### Dog

From all experimental species, canine ventricular action potential probably shows the highest level of similarity to human.<sup>43–46</sup> Various aspects of cardiac electrophysiology and arrhythmogenesis have been addressed in canine preparations with potential translational relevance.<sup>47–49</sup> However, although contributions of major ionic currents in canine cardiac cells seem very similar to human, also significant quantitative differences exist—for example in the sensitivity to  $I_{Kr}$ -block—which have to be considered when translating to human conditions.<sup>30,50</sup>

## Animal models used in cardiac electrophysiology research

Historically, large animals have been preferentially used for cardiac electrophysiological studies, given their relative similarity to human hearts and their heart size allowing for the use of multiple electrodes and (transmural) needles. Dogs are arguably the most often used large animal model, followed by pigs, rabbit, guinea pigs, sheep, cats, and goats. Dogs are easily accustomed to experimental conditions (e.g. to chronic and conscious instrumentation). Pigs have a heart anatomy closely resembling human, and are therefore increasingly used to refine novel (catheter-based) arrhythmia mapping, ablation, and device-based pacing techniques. Porcine preparations are generally more susceptible to (ventricular) arrhythmias and SCD than human hearts,<sup>51</sup> whereas sheep and goats are more resilient (stable). In a proof-of-concept study in chronically instrumented conscious goats, transition from paroxysmal atrial fibrillation (AF) to sustained AF due to chronic atrial pacing was documented ('AF begets AF'<sup>52</sup>) allowing to elucidate underlying electrophysiological, contractile and structural remodelling in detail.<sup>53,54</sup> Mechanisms of ventricular 'torsades-de-pointes' arrhythmias, both spontaneous and drug-induced, have been addressed in hypertrophied hearts following chronic atrioventricular block in dogs<sup>55,56</sup> and in genetically modified rabbit models of inherited arrhythmia syndromes.<sup>57</sup> In a multi-scaled effort, contributions of remodelled ionic currents,<sup>58</sup> calcium handling,<sup>59</sup> spatial and temporal electrophysiological heterogeneity,<sup>60,61</sup> and autonomic modulation<sup>62</sup> to triggering and maintenance of ventricular arrhythmia and sudden cardiac death have been established.

Due to increasing costs and ethical restrictions for large-animal research, and promoted by the miniaturization of *in vivo* equipment, rats and mice are increasingly used. Mice carry certain limitations due to intrinsic differences compared to humans, including ion current characteristics (see 'Species differences in cardiac electrophysiology' section), heart rate, and (basic) sympathetic tone. To their advantage, mice are easy to breed, relatively cheap to house, and can be genetically modified, e.g. by overexpression of genes of interest using a (cardiac-specific) promoter, by deletion through knock-out strategies or CRISPR-Cas technology, by tamoxifen-induced conditional targeting, or AAV-based gene transfer to specific cardiac regions of interest.<sup>63</sup> Mice are typically bred by inbreeding, resulting in identical genetic backgrounds. However, mice can also be outbred to enable identification of potential genetic modifiers.<sup>64</sup> Similar approaches include the use of outbred mice, recombinant inbred rodents, or randomly

mutagenized mice to identify novel genes modulating cardiac traits including electrical function.<sup>65,66</sup>

Wild-type or genetically modified mice are easily subjected to well-established interventions simulating clinical triggers of arrhythmogenic cardiac remodelling, e.g. LV hypertrophy (by aortic banding, nephrectomy/volume overload or chronic administration of isoproterenol or angiotensin-II) or ischaemia/infarction (coronary artery ligation), ultimately resulting in heart failure (HF). These models have been very useful in elucidating pro-arrhythmic mechanisms through detailed *in vivo*, *ex vivo*, whole heart, and cellular electrophysiological studies (as described in detail in 'Cellular and whole heart electrophysiology techniques' section), and molecular investigations, often performed in the same hearts or in distinct cardiac regions, including conduction system, atria, LV vs. RV, transmural, etc. Ageing studies are more feasible given the short murine life-span of around 18–24 months. Interestingly, despite their small heart size, mice are able to develop sustained complex arrhythmias such as AF and ventricular fibrillation (VF).<sup>67,68</sup>

Thanks to novel developments in animal transgenesis, rabbits—that more closely resemble humans in terms of cardiac electrophysiology<sup>28</sup>—have also entered the range of species in whom genetic manipulation can successfully replicate certain (genetic) human cardiac diseases such as hypertrophic cardiomyopathy (HCM) or channelopathies.<sup>57,69–71</sup> In addition, pigs, which also closely resemble humans, have recently been successfully modified genetically to mimic Brugada syndrome (BrS).<sup>72</sup>

In summary, despite their intrinsic limitations indicated above, animal models allow to examine, modulate and dissect different components of arrhythmogenicity, i.e. increased triggered activity, alterations in the myocardial substrate (conduction, repolarization) and neurohumoral/systemic modulation. Of note, ECG and cardiac phenotype of the chosen animal model should mimic clinical features to facilitate translation of experimental results into clinical concepts.

## Cellular models used in electrophysiology research

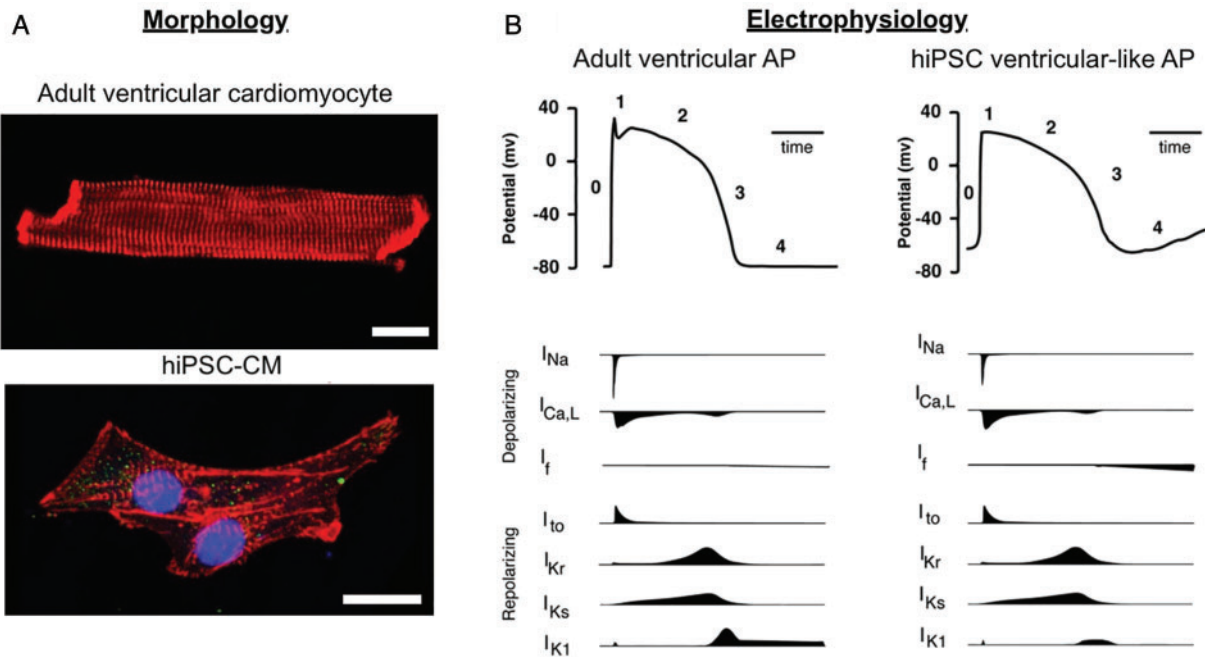
Various cellular models can be used for electrophysiological studies. To study characteristics and pharmacology of specific ion channels, these can be transiently expressed in heterologous expression systems such as Chinese Hamster Ovary (CHO) cells, Human Embryonic Kidney (HEK293) cells, and *Xenopus* oocytes. These cells are inexpensive, can be kept in culture for a long period of time, and are relatively easy to transfect and patch. By co-transfecting cDNA of the ion channel in question with accessory subunits, interacting proteins, and/or other genes of interest, their modulatory effect can be investigated. Furthermore, expression systems allow for functional investigation of the consequences and putative pathogenicity of mutations identified in patients with inherited cardiac arrhythmias. However, these heterologous cell systems do not fully recapitulate the cardiomyocyte environment and may have distinct differences in intracellular pathways and ion channel trafficking systems and/or lack certain interacting proteins. This can be (partly) overcome by using for instance HL-1 cells, which originate from the AT-1 mouse atrial cardiomyocyte tumour lineage, and partly retain cardiac morphological and functional properties of atrial cardiomyocytes. HL-1 cells can

be cultured, transfected and transduced, and studied by (electro)-physiological analyses. Similarly, neonatal cells from rat, mouse or rabbit cardiomyocytes can be kept in culture for days and are relatively easy to transfect or transduce, allowing overexpression or knock-down of genes followed by electrophysiological assessment. However, the immature nature of these cells results in certain differences in, e.g. cardiac ion channel isoform expression, t-tubule structure, and post-translational modification compared to adult cardiomyocytes.

For electrophysiological assessments under more physiological conditions, adult cardiomyocytes isolated from animal models or human heart samples are generally considered most appropriate for investigating ion channel (dys)function and AP characteristics by patch-clamp analysis, as well as fluorescence-based quantification of intracellular calcium homeostasis. Freshly isolated cardiomyocytes of the working myocardium, the SAN or of the atrioventricular conduction system retain most of their anatomical and functional features, including (stable) resting membrane potentials, contractile properties, and subcellular distribution of ion channels, although dissociation-induced changes have been described. They furthermore allow for isolation and investigation of cardiomyocytes from various regions of the heart, including right and left atria,<sup>73</sup> LV vs. RV, (sub)epicardium vs. (sub)endocardium vs. (mid)myocardium, and Purkinje fibres from large mammals<sup>74</sup> or mice expressing EGFP-labelled connexin-40.<sup>75</sup> Nevertheless, the disruption of cardiomyocytes from adjacent cells and the extracellular matrix likely does have functional consequences: for instance, Na<sup>+</sup>-current density is higher at the intercalated disc region of coupled cells than in isolated cells.<sup>76</sup> Primary adult cardiomyocytes isolated from some animal models<sup>77</sup> and human samples<sup>78</sup> can also be kept in culture during a few days for gene modification studies. However, in both neonatal and adult cardiomyocytes, the culturing process may itself induce structural and functional remodelling. On the other hand, studies in human cardiomyocytes critically depend on availability of patient tissues and there is often a limited availability of appropriate controls. Electrophysiological properties of the intact SAN region can be characterized using the sharp intracellular electrode technique on SAN tissue strips,<sup>79,80</sup> using surface electrograms in combination with optical mapping of membrane potential in isolated SAN to record impulse conduction<sup>81,82</sup> or confocal live imaging of intracellular Ca<sup>2+</sup> release.<sup>83</sup>

## Human-induced pluripotent stem-cell-derived cardiomyocytes in electrophysiology research

The use of human-induced pluripotent stem-cell-derived cardiomyocytes (hiPSC-CMs) has recently gained prominence to screen novel drugs for potential pro-arrhythmic effects as a consequence of drug-induced block of  $I_{Kr}$ .<sup>84</sup> In the comprehensive *in vitro* pro-arrhythmia assessment (CiPA) initiative, as well as to generate experimental models for basic research into human genetic diseases that predispose to lethal cardiac arrhythmias. In addition to conventional patch-clamp and microelectrodes approaches, a variety of invasive and non-invasive methods exist for the electrophysiological analysis



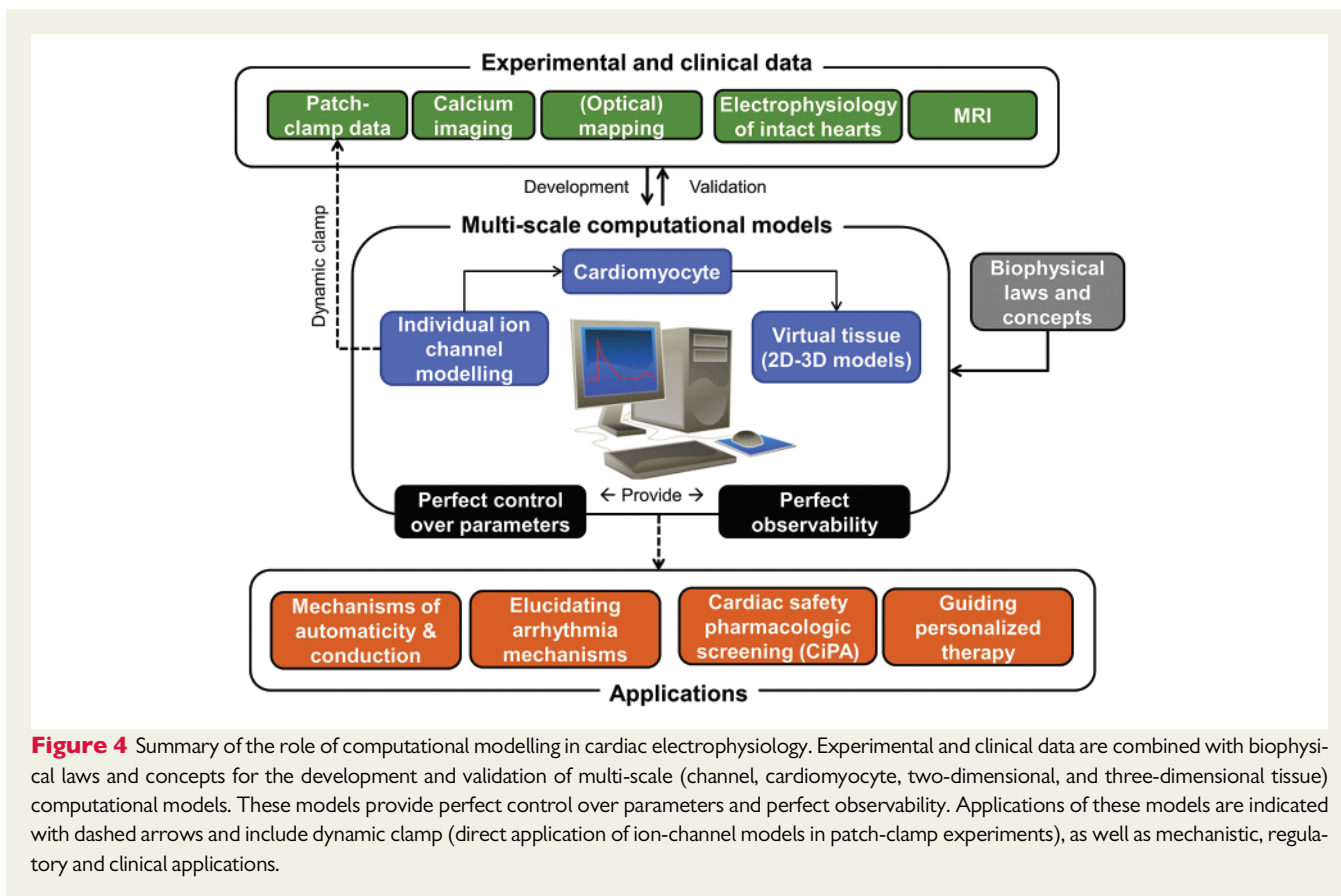
**Figure 3** Comparison of morphological and electrophysiological features of adult ventricular cardiomyocytes and human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes. (A) Microscopic image of an adult cardiomyocyte from mouse stained with anti- $\alpha$ -actinin (red) and a hiPSC-derived cardiomyocyte stained with anti- $\alpha$ -actinin (red), DAPI (blue), and Nav1.5 (green). Scale bars are 20  $\mu$ m. (B) Schematic of ventricular action potentials (APs) during rapid upstroke (Phase 0), early repolarization (1), plateau (2), late repolarization (3), and diastole (4). The ionic currents involved are shown below the AP traces.

of hiPSC-CMs, which have both advantages and limitations depending on the research question to be investigated. While a promising tool for (compound) screening purposes, automated patch clamp requires high density, homogeneous single-cell suspensions which can be challenging when working with hiPSC-CMs. Multi-electrode arrays (MEAs) allow long-term measurements from clusters and monolayers of hiPSC-CMs, with field-potential duration used as measure of QT and AP duration, and activation maps for conduction velocity measurements. Similarly, fluorescence-based measurements using voltage- and calcium-sensitive dyes allow for more high-throughput, non-invasive analyses.

A frequent criticism of iPSC-CMs is their embryonic electrophysiological phenotype (Figure 3). Human-induced pluripotent stem-cell-derived cardiomyocytes lack T-tubules [where the  $Ca^{2+}$  released by ryanodine receptors (RyR2) locally affects sarcolemmal ion channels and transporters], hampering analyses on the mechanisms linking intracellular  $Ca^{2+}$  handling abnormalities and triggered activity.<sup>26</sup> This problem can be partly solved by obtaining more mature hiPSC-CMs with hormonal treatment<sup>85</sup> or using nanostructured/biomimetic substrates.<sup>86,87</sup> APs in hiPSC-CMs are generated by a sequential activation of  $I_{Na}$ ,  $I_{Ca,L}$ , and repolarizing  $K^+$  currents similar to adult ventricular cardiomyocytes, and accordingly have demonstrated similar mutation-induced electrophysiological consequences as adult mutant mouse cardiomyocytes,<sup>88</sup> as well as clear similarities between *in vitro* effects of pharmacological interventions in hiPSC-CMs and their reported clinical efficacy.<sup>89,90</sup> However, hiPSC-CMs display an

unstable diastolic potential caused by insufficient expression of  $I_{K1}$  and significant expression of the funny current ( $I_f$ ).<sup>25</sup> To overcome this,  $I_{K1}$  density can be artificially enhanced by cells transduction with Kir2.1<sup>91</sup> or injection of an *in silico*  $I_{K1}$ , inducing a more physiological and stable resting membrane potential, in addition to a ventricular-like, more 'mature' action potential morphology.<sup>92,93,94</sup>  $I_{Ks}$  density and APD-prolongation in response to  $I_{Ks}$  blockade are generally small and highly variable among hiPSC-CMs. Although  $I_{Ks}$  with proper kinetics can be recorded in hiPSC-CMs,<sup>94,95</sup> in some reports the current pharmacologically identified as  $I_{Ks}$  has kinetics sharply diverging from that observed in mature myocytes.  $I_{Kr}$  can be more consistently recorded in hiPSC-CMs, whose APD has been proposed as a reporter of drugs' arrhythmogenic risk.<sup>96</sup> It also remains unclear whether stem-cell-derived cardiomyocytes possess one general phenotype or whether the process results in a mixture of nodal, atrial and ventricular cells.<sup>97</sup> Certainly, it appears that the differentiation conditions can be manipulated to generate cells with a predominately atrial or ventricular phenotype.<sup>98,99</sup>

Human-induced pluripotent stem-cell-derived cardiomyocytes can be cultured as a monoculture or mixed with other cell types, typically cardiac fibroblasts. While isolated hiPSC-CMs have a variable electrophysiological phenotype, monolayers of electrically coupled cells generally have a more stable spontaneous rate and electrophysiology. Three-dimensional tissue preparations, referred to as engineered heart tissue, can be created from hiPSC-CMs held within a matrix (e.g. fibrinogen) to create microspheres, trabeculae or sheets



**Figure 4** Summary of the role of computational modelling in cardiac electrophysiology. Experimental and clinical data are combined with biophysical laws and concepts for the development and validation of multi-scale (channel, cardiomyocyte, two-dimensional, and three-dimensional tissue) computational models. These models provide perfect control over parameters and perfect observability. Applications of these models are indicated with dashed arrows and include dynamic clamp (direct application of ion-channel models in patch-clamp experiments), as well as mechanistic, regulatory and clinical applications.

of tissue.<sup>100</sup> These 3D preparations are commonly stable in beating rate and electrophysiology over many weeks.<sup>101</sup>

## Integration of experimental electrophysiology data into computational models

Since the first computational models of the cardiac AP were developed in the 1960s, *in silico* modelling of cardiac electrophysiology has advanced significantly.<sup>102</sup> Ion channel and AP models are available for most cell types and many species.<sup>103,104</sup> Tissue and organ-level models have been developed and can be used to simulate atrial and ventricular arrhythmias in the presence of different structural and functional substrates.<sup>105</sup> Computer models provide perfect control over individual parameters, overcoming limited selectivity of most pharmacological tools or compensatory effects in genetic animal models (Figure 4). Computer models also offer perfect observability, enabling detailed analyses of multiple components (e.g. ion currents, membrane potential, intracellular concentrations) at the same moment in time, which is not possible during experiments. Accordingly, computer models have been used extensively to study the mechanisms of heart automaticity<sup>106,107</sup> and arrhythmia.<sup>103,104</sup> In addition, computer models have been used to improve our knowledge of the contribution of individual ionic currents in combination with dynamic clamp recording systems.<sup>22,108</sup> Recently, the first applications of *in*

*silico* models with important clinical implications have emerged. Initial proof-of-concept studies with prospective, simulation-guided ablation of atrial and ventricular arrhythmias have been conducted.<sup>109–111</sup> Cardiac cellular electrophysiology models play a central role in predicting the proarrhythmic risk of new drugs as part of the CiPA initiative.<sup>112,113</sup>

The role of computational models in clinical arrhythmia management and drug development is expected to increase, raising important questions regarding model complexity, validation, and uncertainty quantification.<sup>113,114</sup> Traditionally, the distinction between model calibration (parameter estimation) and validation using independent data has been largely ignored for cardiomyocyte models, due to the paucity of experimental data. Moreover, only a single ‘representative’ model was generated and analysed. However, it has become increasingly common to study populations of models that reflect cell-to-cell variability.<sup>115</sup> In addition, several studies have employed true validation data sets in the development of new cardiomyocyte models,<sup>116</sup> as well as in their application (e.g. predicting the proarrhythmic risk of drugs that were not used to calibrate the model in the CiPA initiative<sup>112</sup>). As our understanding of cardiac cellular electrophysiology expands, models are becoming increasingly complex, e.g. integrating molecular ion-channel dynamics, localized changes in calcium handling and post-translational regulation through signalling cascades,<sup>117,118</sup> making extensive validation and establishing the context of use<sup>114</sup> increasingly challenging. At the same time, this level of complexity can currently not be simulated at the organ level, so that new approaches are needed to span the different spatial and



temporal scales involved in cardiac electrophysiology. For example, recent approaches have made it possible to investigate the interaction between re-entry-induced  $\text{Ca}^{2+}$  loading, subsequent triggered activity and re-induction of re-entry in tissue-level models with spontaneous  $\text{Ca}^{2+}$ -release events based on detailed subcellular models.<sup>119</sup> Above all, close collaboration between experimental cardiac electrophysiologists, computational modellers, and clinicians is needed for the development of well-validated models and their clinical applications.

## Suitability of individual models for specific research questions

### Simplification vs. integration

Although pro-arrhythmic mechanisms may occur on the single cell-level, arrhythmogenesis *in vivo* is strongly affected by factors at the tissue/organ level and by neurohumoral activity. The challenging task is integration from molecular function (artificial membranes → heterologous expression systems) through the subsequent levels of complexity (myocyte → tissue → organ). A crucial question in this process is whether the detected molecular abnormality is 'biologically relevant'. This depends on its magnitude, but also on system responsiveness. The latter may vary, for example, according to the AP phase on which the abnormality impacts,<sup>120</sup> the presence of buffering mechanisms,<sup>121</sup> connectivity to neighbouring cells,<sup>122</sup> etc. *In silico* modelling may provide a powerful tool to this end, but there is room for improvement also at the experimental level. One aspect that may deserve attention is system behaviour under realistic ('dynamic') conditions, not considered in classical biophysical evaluation. At cardiomyocyte level, 'action-potential clamp' and 'dynamic-clamp' are simple and powerful tools; they can provide stringent optimization of numerical models<sup>123</sup> and experimentally test AP response to channel dysfunction/modulation.<sup>120</sup> At the tissue level, integrated information on dynamic response can be obtained from 'electrical restitution' of propagation and repolarization, which have been directly linked to arrhythmogenesis.<sup>124</sup>

### Disease-modelling of common (acquired) arrhythmogenic disorders

Beside the biological relevance of an abnormal finding in the whole body context, the relevance of the animal model is critical for clinical translation. Key features of the clinical phenotype that are relevant for diagnosis, therapy, and/or prognosis must be reproduced by the animal model (Table 1). Fortunately, this seems to be the case in many instances, surprisingly even when species differences in electrical activity are substantial (e.g. for murine models).<sup>125,126</sup>

#### Atrial fibrillation

As the most prevalent clinically relevant arrhythmia with significant impact on morbidity and mortality, numerous studies have attempted to model AF.<sup>127,128</sup> However, all models used to study AF have limitations in terms of predictability, reliability or transferability. Due to the multifactorial nature of AF, to date even the most sophisticated model is unable to fully recapitulate the diversity of aetiologies and

pathological mechanisms of human AF. Thus, it is important to keep in mind the question being addressed and the limitations of each model.

Rodents are widely used models due to their easy handling, low costs, and easy genetic manipulation, despite the aforementioned electrophysiological differences to humans limiting transferability. Nonetheless, a large number of transgenic mouse models with either spontaneous AF or increased vulnerability to burst-pacing induced AF have been developed.<sup>129</sup> In addition, rodents have been used to study AF promotion due to a large number of risk factors, including endurance exercise and sleep-disordered breathing.<sup>130</sup> Rodent models are suitable to perform *in vivo* and *ex vivo* experiments in isolated hearts and to study new therapeutic interventions early in the development pipeline.<sup>129</sup>

Large animal models are commonly used in preclinical AF studies.<sup>127,128</sup> AF is easily induced and relatively stable in goats, making this model suitable to study the progressive nature of AF.<sup>52</sup> Goat, dog, pig, and rabbit models have also been used to study atrial tachycardia-related electrical and structural remodelling using rapid atrial pacing.<sup>127,131</sup> The atrial burst-pacing model can be used for simulating paroxysmal AF but is also commonly used to study AF inducibility in the presence of disease-related background remodelling. For example, atrio-ventricular (AV) block in goats leads to progressive atrial dilatation and prolonged AF.<sup>127</sup> Dogs and sheep are typically used for vagal or ischaemic AF promotion and in sterile pericarditis as a model of post-operative AF.<sup>127,132</sup> Furthermore, the dog ventricular tachypacing model of congestive HF has provided important insight in the relation between AF and HF.<sup>127,128</sup> Although this model does not present big changes in refractoriness, it has pronounced structural remodelling and impaired conduction. Models investigating AF promotion after myocardial infarction are available in several species and have revealed complex changes in atrial electrophysiology, which depend on the presence of atrial ischaemia and timing of the experiments (acute vs. chronic setting).<sup>133–135</sup> Recently, horses have emerged as an interesting model for AF because, like humans, they spontaneously develop AF.<sup>128</sup>

Many cell lines are available for AF research, including fibroblasts, stem cells, HEK-293T, and HL-1 cells.<sup>136</sup> Human iPSC-derived cardiomyocytes can model familial AF, but atrial-like cells are required and their similarity to adult human atrial cardiomyocytes is limited. Importantly, none of these cellular models can capture the decades of atrial remodelling present in most AF patients. Isolated human atrial cardiomyocytes may therefore represent the most clinically relevant cellular AF model for pharmacological testing or adenovirus-based gene-therapy studies. Nevertheless, tissue is generally only available from patients undergoing open heart surgery and is restricted to parts of the atria (e.g. appendages). In addition, these models lack organ-dependent environmental factors.<sup>136</sup>

*In silico* models are powerful tools for testing hypotheses, predicting effects of new therapeutic targets or capturing dynamic systems at different scales (cells-tissue-organ-person),<sup>104</sup> and are increasingly used in AF research, e.g. to study antiarrhythmic drug<sup>137,138</sup> and ablation therapy.<sup>109,111</sup> Combined, these experimental and computational models can increase our understanding of AF mechanisms and facilitate the development of novel therapeutic approaches.

**Table 1** Disease-specific experimental models for cardiac electrophysiology research

Species	Experimental model	Important facts	References
Common (acquired) arrhythmogenic disorders			
Atrial fibrillation			
Mouse	Genetic models with mutations in ion channels or fibrosis-related genes	<ul style="list-style-type: none"> <li>Spontaneous AF or increased susceptibility for AF-inducibility</li> </ul>	127–130
Goat, dog, pig, rabbit	Atrial tachy-pacing-induced AF	<ul style="list-style-type: none"> <li>Electrical and structural remodelling</li> </ul>	52,127,128
Rat, rabbit, dog, sheep	Myocardial-infarction/ischaemia-induced AF	<ul style="list-style-type: none"> <li>Disease-specific AF mechanisms</li> <li>Electrical and structural remodelling</li> </ul>	133–135
Dog, sheep	Vagal-induced AF	<ul style="list-style-type: none"> <li>Disease-specific AF mechanisms</li> <li>Role of autonomous nervous system</li> </ul>	127
Dog, sheep	Sterile pericarditis	<ul style="list-style-type: none"> <li>Model of post-operative AF</li> </ul>	132
Dog	Ventricular tachypacing-induced heart failure	<ul style="list-style-type: none"> <li>Heart failure-induced AF</li> <li>Interactions between heart failure and AF</li> <li>Structural remodelling</li> </ul>	127,128
Horse	Spontaneous AF (no alterations necessary)	<ul style="list-style-type: none"> <li>Spontaneous AF</li> </ul>	128
Cell lines	Fibroblasts, HEK-cells, HL-1 cells	<ul style="list-style-type: none"> <li>Cellular mechanisms</li> <li>No representation of remodelling</li> </ul>	136
Human	hiPSC-CM	<ul style="list-style-type: none"> <li>Familial AF mechanisms</li> </ul>	136
Human	Isolated human atrial CM	<ul style="list-style-type: none"> <li>Best cellular model for human AF</li> <li>Pharmacological testing</li> <li>Limited availability</li> </ul>	136
<i>In silico</i>	Cellular, tissue, and organ models	<ul style="list-style-type: none"> <li>Investigation of novel drug- or ablation therapies</li> </ul>	104,109,111,137,138
Heart failure with reduced ejection fraction			
Rat	Spontaneous hypertensive rats or salt-sensitive rats	<ul style="list-style-type: none"> <li>Hypertension-related LV-hypertrophy and heart failure</li> </ul>	141
Mouse, rat, rabbit, pig, sheep	Coronary-artery ligation	<ul style="list-style-type: none"> <li>Ischaemia-related heart failure</li> </ul>	141,142,144,146
Mouse, rat	Aortic constriction	<ul style="list-style-type: none"> <li>Increased-afterload induced heart failure</li> </ul>	141
Rabbit, dog, pig	Ventricular tachypacing	<ul style="list-style-type: none"> <li>Tachyarrhythmia-related heart failure</li> </ul>	143,146
Mouse	Chemical anticancer therapy	<ul style="list-style-type: none"> <li>Anticancer-therapy related/toxic heart failure</li> </ul>	141
Human	LV samples, explanted hearts	<ul style="list-style-type: none"> <li>Limited availability of appropriate healthy heart tissue as control</li> </ul>	147,148
Heart failure with preserved ejection fraction			
Mouse, rat	Disease models for hypertensive heart disease + metabolic syndrome, chronic kidney failure	<ul style="list-style-type: none"> <li>Disease-specific HFpEF models</li> </ul>	151,154,155
Rat	Chronic volume-overload (high-salt diet) model for HFpEF	<ul style="list-style-type: none"> <li>Disease-specific HFpEF models</li> <li>Identification of arrhythmogenic mechanisms</li> <li>Age-related atrial remodelling</li> </ul>	152,153,156
Myocarditis			
Mouse	Coxsackie B-virus	<ul style="list-style-type: none"> <li>Acute infectious myocarditis</li> </ul>	161

Continued

**Table 1** Continued

Species	Experimental model	Important facts	References
Mouse	Immunization with cardiac myosin	<ul style="list-style-type: none"> <li>• Automimmune myocarditis</li> </ul>	162,163
Dog	Canine parvovirus or non-viral pathogens (Chagas)	<ul style="list-style-type: none"> <li>• Acute infectious myocarditis</li> </ul>	164
Sinus and AV node diseases			
Mouse	Genetic mouse models with mutations in ion channels (and ankyrin B) involved in SAN pacemaking	<ul style="list-style-type: none"> <li>• Primary familial SND</li> <li>• Mechanistic insights</li> </ul>	167–173
Mouse, rat, rabbit, dog	Heart failure, tachypacing-induced AF, diabetes-models	<ul style="list-style-type: none"> <li>• Secondary SND associated with heart failure, AF, age</li> </ul>	177–183
Inherited arrhythmogenic disorders			
Ion channel diseases with altered Na <sup>+</sup> -channel function			
HEK/CHO cells	Transfected HEK/CHO-cells expressing various <i>SCN5A</i> mutations	<ul style="list-style-type: none"> <li>• Biophysical properties of human <i>SCN5A</i> mutations</li> </ul>	187–190
Dog	Arterially perfused RV wedges with Na <sup>+</sup> /Ca <sup>2+</sup> blockade or activation of <i>I<sub>Na, late</sub></i>	<ul style="list-style-type: none"> <li>• Mechanistic insights into Brugada syndrome or LQT3</li> <li>• Pharmacological interventions</li> </ul>	184,186,187
Mouse	Genetic mouse models with various <i>scn5a</i> mutations	<ul style="list-style-type: none"> <li>• Models for LQT3 or LQT3/BrS overlap syndrome</li> <li>• Complete recapitulation of human phenotype</li> <li>• Effect of genetic background and ageing</li> <li>• Pharmacological studies</li> </ul>	168,176,189,191–197
Pig	Genetic pig model with <i>SCN5A</i> (E558X/+) mutation	<ul style="list-style-type: none"> <li>• Model for Brugada syndrome</li> <li>• Recapitulation of conduction disturbances, no arrhythmias</li> </ul>	72
Human	<i>SCN5A</i> mutant hiPSC-CM lines	<ul style="list-style-type: none"> <li>• Patient-specific exploration of disease mechanisms and therapies</li> <li>• Limitations of hiPSC-CM</li> </ul>	88–90,198
<i>In silico</i>	Incorporation of various <i>SCN5A</i> mutations in computational models	<ul style="list-style-type: none"> <li>• Prediction of biophysical properties of human <i>SCN5A</i> mutations (with limitations)</li> </ul>	199,200
Ion channel diseases with altered K <sup>+</sup> -channel function			
HEK/CHO cells	Transfected HEK/CHO-cells expressing various K <sup>+</sup> channel mutations	<ul style="list-style-type: none"> <li>• Biophysical properties of human <i>KCNQ1</i>, <i>KCNH2</i>, <i>KCNE1</i>, <i>KCNE2</i>, <i>KCJN2</i> mutations</li> </ul>	
Mouse	Transgenic mouse models expressing human K <sup>+</sup> channel mutations ( <i>KCNQ1</i> , <i>KCNH2</i> )	<ul style="list-style-type: none"> <li>• Incomplete recapitulation of human LQTS phenotype: prolongation of APD but lack of VT/VF/SCD</li> </ul>	204–206
Mouse	Mouse models with altered murine K <sup>+</sup> channels ( <i>Kv1.4</i> , <i>Kv4.2</i> )	<ul style="list-style-type: none"> <li>• LQTS phenotype with prolongation of APD</li> <li>• Arrhythmias only in models with increased APD dispersion</li> <li>• Different K<sup>+</sup> channels than in human LQTS</li> </ul>	207
Zebrafish	Zebrafish models with loss-of-function in <i>KCNH2</i>	<ul style="list-style-type: none"> <li>• LQT2 phenotype with APD prolongation and AV 2:1 block</li> <li>• Lack of VT/VF</li> </ul>	208,209

Continued

**Table 1** Continued

Species	Experimental model	Important facts	References
Zebrafish	Zebrafish models with gain-of-function in <i>KCNH2</i>	<ul style="list-style-type: none"> <li>• SQT1 phenotype with APD shortening</li> <li>• Lack of VT/VF</li> </ul>	210
Rabbit	Transgenic rabbit models expressing loss-of-function mutations in K <sup>+</sup> channels ( <i>KCNQ1</i> -Y315S; <i>KCNH2</i> -G628S; <i>KCNE1</i> -G52R)	<ul style="list-style-type: none"> <li>• LQTS phenotype with long APD/QT</li> <li>• In LQT5 only slightly prolonged QT</li> <li>• VT/VF and SCD only in LQT2</li> <li>• Similar hormonal influences as in human patients</li> <li>• Electro-mechanical dysfunction</li> <li>• Pharmacological studies</li> </ul>	57,70,211–213
Rabbit	Transgenic rabbit models expressing gain-of-function mutations in K <sup>+</sup> channels ( <i>KCNH2</i> -N588K)	<ul style="list-style-type: none"> <li>• SQT1 phenotype with APD/QT shortening, inducible AF/VT</li> <li>• Pharmacological studies</li> </ul>	71
Human	LQT1/LQT2/SQT1/SQT2 hiPSC-CM lines with mutant <i>KCNQ1</i> / <i>KCNH2</i>	<ul style="list-style-type: none"> <li>• Patient-specific exploration of disease mechanisms and therapies</li> <li>• Limitations of hiPSC-CM</li> </ul>	93–95,214–216
<i>In silico</i>	Incorporation of various K <sup>+</sup> channel mutations in computational models	<ul style="list-style-type: none"> <li>• Prediction of biophysical properties of human K<sup>+</sup> channel mutations</li> </ul>	
Catecholaminergic polymorphic ventricular tachycardia			
HEK/CHO cells	Transfected HEK/CHO-cells expressing various <i>RyR2</i> or <i>Casq1</i> mutations	<ul style="list-style-type: none"> <li>• Biophysical properties of human <i>RyR2</i> or <i>Casq1</i> mutations</li> <li>• Mechanistic link to arrhythmias cannot be assessed</li> </ul>	
Mouse	Heterozygous knock-in mouse models with various <i>RyR2</i> or <i>Casq1</i> mutations	<ul style="list-style-type: none"> <li>• Recapitulation of CPVT phenotype with VT/VF induced by sympathetic activation</li> <li>• Identification of novel therapies</li> </ul>	218–221
Human	hiPSC-CM lines with mutant <i>RyR2</i> or <i>Casq1</i>	<ul style="list-style-type: none"> <li>• Patient-specific exploration of disease mechanisms and therapies</li> <li>• Limitations of hiPSC-CM</li> </ul>	26
<i>In silico</i>	Incorporation of various <i>RyR2</i> or <i>Casq1</i> mutations in computational models	<ul style="list-style-type: none"> <li>• Prediction of biophysical properties of human <i>RyR2</i> mutations (with limitations)</li> </ul>	
Genetic cardiomyopathies			
Mouse	Mouse models with sarcomeric mutations	<ul style="list-style-type: none"> <li>• Recapitulation of HCM features</li> <li>• Cardiac hypertrophy only in some mouse models</li> <li>• Pharmacological studies</li> </ul>	222,223,225–228
Mouse	Mouse models with desmosomal mutations	<ul style="list-style-type: none"> <li>• Recapitulation of some ARVC features</li> <li>• But: no fibro-fatty replacement</li> <li>• Identification of arrhythmogenic mechanisms</li> </ul>	231–233
Rabbit	Rabbit models with sarcomeric mutations ( $\beta$ -MHC-Q <sup>403</sup> , <i>ELC1v</i> , or <i>cTnl</i> )	<ul style="list-style-type: none"> <li>• Recapitulation of HCM phenotype</li> <li>• Clear cardiac hypertrophy only in <math>\beta</math>-MHC-Q<sup>403</sup> model</li> <li>• Pharmacological studies</li> </ul>	229

AF, atrial fibrillation; APD, action potential duration; AV, atrio-ventricular; CHO, Chinese Hamster Ovary; CM, cardiomyocyte; HCM, hypertrophic cardiomyopathy; hiPSC-CM, human-induced pluripotent stem-cell-derived cardiomyocyte; HFpEF, heart failure with preserved ejection fraction; LV, left ventricle; RV, right ventricle; SAN, sinoatrial node; SND, sinoatrial node dysfunction; VT/VF, ventricular tachycardia/ventricular fibrillation.

### Arrhythmias in heart failure with reduced ejection fraction

Heart failure often develops secondary to other pathologies such as myocardial infarction, hypertension, diabetes, anticancer therapy, kidney failure, infection, or genetic cardiomyopathies. About half of the patients diagnosed with HF die within 5 years, around 50% due to pump failure and 50% by sudden cardiac death due to fatal arrhythmias.<sup>139</sup>

Several animal experimental models mimicking the various aetiologies have been developed to explore the mechanisms involved both in cardiac failure and arrhythmogenesis. Cardiac remodelling in HF is often characterized by ventricular hypertrophy of the remaining viable tissue. It has to be kept in mind, however, that only a minority of patients with LV hypertrophy develop HF.<sup>140</sup> Most HF animal models employ rodents and concern heart failure with reduced ejection fraction (HFrEF).<sup>141</sup> To reproduce hypertension-related HF, genetically selected rats, e.g. spontaneous hypertensive rats or salt-sensitive rats (Dahl), are used. However, in most animal models, enhanced afterload is obtained by surgery via aortic constriction. Other surgical techniques include coronary artery ligation, which can be permanent to induce a myocardial infarction, or temporary to induce ischaemia reperfusion and mimic reperfusion in the hospital in patients suffering an infarction. Chemical treatment with anticancer therapies, including monocrotalin to induce RV HF and pancreatic toxic compounds to induce type-1 diabetes are also commonly used, together with dietary interventions and genetic models to induce glucose intolerance and type 2 diabetes. Rodent models of HF are reviewed in Gomes *et al.*<sup>141</sup>

Besides small rodents, other experimental animal models of HF are used in an attempt to get closer to human heart function, including rabbits with combined pressure and volume overload or coronary artery ligation,<sup>142</sup> cats with pressure overload, ventricular tachypacing in dogs<sup>143</sup> and pigs, pigs with myocardial infarction,<sup>144</sup> and sheep.<sup>145</sup> More details, as well as advantages and disadvantages of these bigger animal experimental models have recently been reviewed.<sup>146</sup>

Finally, human LV samples or complete explanted hearts are used by some groups to investigate HF-related electrophysiological remodelling,<sup>144,147,148</sup> but these studies are often limited by the limited availability of appropriate healthy heart tissue as control.

### Arrhythmias in Heart Failure with Preserved Ejection Fraction

Incidence of arrhythmias (in particular AF) and sudden cardiac death is also increased in Arrhythmias in heart failure with preserved ejection fraction (HFpEF) patients,<sup>149</sup> although the true prevalence of arrhythmogenic sudden death requires further study.<sup>150</sup> Developing appropriate animal models for HFpEF has been complicated by the heterogeneity of clinical phenotypes. However, in the past decade models of hypertensive heart disease, metabolic syndrome, chronic kidney failure, and ageing have been developed that mimic relevant clinical features of HFpEF.<sup>151</sup> Yet, to date only few studies, mainly in rodents, have investigated pathomechanisms of arrhythmias in these models. In rats with HFpEF due to chronic volume overload (high salt diet), spontaneous ventricular tachycardia (VTs) were documented

and related to delayed repolarization.<sup>152,153</sup> Increased intracellular  $\text{Ca}^{2+}$  load and altered activity of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger have been implicated in cellular arrhythmogenesis in a cardiorenal HFpEF model.<sup>154,155</sup> In rats predisposed to HFpEF, age-related atrial remodelling (fibrosis, enlargement, conduction abnormalities) facilitates AF.<sup>156</sup> Clearly, the complex interaction between cardiac remodelling and arrhythmogenesis in HFpEF warrants further research in clinically relevant animal models.

### Myocarditis

Arrhythmias are a common manifestation of myocarditis (incidence up to 45%<sup>157</sup>). Most common arrhythmias are VT, VF, and AV block.<sup>158</sup> Arrhythmogenic mechanisms are mainly triggered activity (due to inflammatory modulation of ion-channel properties in acute myocarditis) and re-entry (scar-related in chronic myocarditis).<sup>159</sup> Transition to dilated cardiomyopathy occurs in up to 50% of cases.<sup>160</sup> In mice, infection with a Coxsackie Virus B strain (Nancy strain) is the most commonly used model of acute infectious myocarditis, but a variety of other rodent models exist.<sup>161</sup> Non-infectious autoimmune myocarditis is often induced by immunization of cardiac myosin.<sup>162,163</sup> Of note, time course and extent of remodelling in myocarditis in mice are strongly strain dependent.<sup>161,163</sup> Dogs are the most common large animal model for investigating arrhythmias in myocarditis, facilitating ambulatory monitoring.<sup>164</sup> Canine parvovirus is the best studied natural pathogen associated with myocarditis, whereas experimental canine models have focused on non-viral pathogens (e.g. Chagas disease). Future studies may profit from more standardized experimental protocols as a basis for pooled data analyses.<sup>161</sup>

### Sinus and atrioventricular node disease

Sinoatrial node dysfunction (SND) and AV block together account for over half of the permanent electronic pacemaker implantations worldwide. Sinoatrial node dysfunction can be distinguished into primary familial or secondary forms, which are associated with cardiovascular or systemic disease,<sup>165</sup> and age-related SND.<sup>166</sup> During the last two decades, models of primary and secondary SND forms have been developed. Genetically modified mouse models of inherited SND due to mutations in ion channels involved in SAN pacemaking have been created by global or conditional gene knockout,<sup>167–170</sup> or by heart specific and time controlled expression of mutant ion channel proteins.<sup>171</sup> These include  $I_f$  (HCN4),<sup>171,172</sup> L-type Cav1.3,<sup>167</sup> Nav1.5 channels,<sup>168</sup> and RyR2.<sup>83</sup> All these models except RyR2 also present with variable degrees of AV block, thus providing mechanistic insights about the origin of primary heart block. In addition, mouse models of primary SND due to lack of the scaffolding protein Ankyrin-B have been developed.<sup>173</sup> These mouse models have provided insights about the mechanisms of primary familial SND forms, such as SND associated with ventricular non-compaction,<sup>174</sup> the sinus node dysfunction and deafness syndrome,<sup>169</sup> autoimmune congenital heart block,<sup>175</sup> and SND associated with Lev-Lenègre syndrome.<sup>176</sup>

Numerous models of secondary SND exist. Models of SND secondary to HF have been developed using dogs,<sup>177</sup> rabbits,<sup>178</sup> and mice.<sup>179</sup> A canine AF model induced by rapid atrial pacing also presents with SND<sup>180</sup> and has provided insights into the relationship

between AF and SND. Animal models of diabetes also often present with SND,<sup>181</sup> enabling studies into the mechanistic link between these pathologies. Recently, models of bradycardia<sup>182</sup> and AF<sup>130</sup> secondary to long-term intensive exercise have been developed. Finally, age-related SND has been studied using old mice and rats.<sup>183</sup>

## Disease-modelling of inherited arrhythmogenic disorders

### Ion channel diseases with altered Na<sup>+</sup>-channel function

Mutations in *SCN5A* encoding Na<sub>v</sub>1.5 lead to disease entities associated with reduced peak *I*<sub>Na</sub> (cardiac conduction/Lev-Lenègre disease; BrS), increased late *I*<sub>Na</sub> (LQTS type 3, LQT3), or a combination of these (overlap syndrome).

Pharmacological interventions that interfere with the inactivation of Na<sup>+</sup> channel (sea anemone toxin II, veratridine, anthopleurin) have been exploited for understanding the contribution of late *I*<sub>Na</sub> to AP repolarization and arrhythmogenesis on cellular, multicellular and organ levels. Such experiments in canine arterially perfused LV wedges<sup>184</sup> and rabbit Langendorff-perfused hearts<sup>185</sup> have revealed mechanistic insights into LQT3 arrhythmogenesis. Pharmacological modulation of canine arterially perfused RV wedges with a combination of Na<sup>+</sup> and Ca<sup>2+</sup>-channel blockers and K<sup>+</sup>-channel openers has also been employed to model BrS.<sup>186,187</sup>

Expressing *SCN5A* mutant channels in expression systems (e.g. HEK293/CHO) allows detailed assessment of the biophysical consequences,<sup>187,188</sup> but may not fully recapitulate the cardiomyocyte environment.<sup>189,190</sup> *Scn5a* transgenic mice may overcome such shortcomings, with Na<sup>+</sup>-channel contribution to APs being relatively comparable between mouse and human. Indeed, cardiomyocytes from *Scn5a*-1798insD mice showed biophysical properties in line with a clinical overlap syndrome, whereas previous studies in expression systems did not.<sup>189</sup> *Scn5a* mouse models also allow assessment of the functional impact in distinct regions of the myocardium and conduction system.<sup>168,189,191,192</sup> Furthermore, their use has provided insight into pro-arrhythmic intracellular Na<sup>+</sup> and Ca<sup>2+</sup> dysregulation and (age-dependent) electrical and structural remodelling.<sup>176,192,193</sup> Indeed, Na<sup>+</sup>-channel gain of function (late *I*<sub>Na</sub> enhancement as in LQT3) is arrhythmogenic mainly because it perturbs intracellular Na<sup>+</sup> homeostasis, leading to Ca<sup>2+</sup>-store instability and energy imbalance.<sup>194</sup> Finally, they allow exploration of the modulatory role of the autonomic nervous system, co-morbidities and genetic modifiers, and enable chronic pharmacological studies.<sup>195–197</sup> More recently, a pig model carrying a BrS-associated *SCN5A* mutation was successfully generated, displaying conduction slowing and increased susceptibility to ventricular arrhythmias.<sup>72</sup> While such large animal models may have some benefits over mice in terms of clinical transferability, their generation is time-consuming and costly, thus limiting their availability for research.

In recent years, a number of *SCN5A* mutant hiPSC-CM lines have been generated, which recapitulate nicely the LQT3, BrS, and/or overlap syndrome phenotypes.<sup>88,89</sup> These hiPSC-CMs have so far been predominantly used for pharmacological studies<sup>90,198</sup> but may also be useful for prediction of mutation pathogenicity and patient-specific arrhythmia risk.<sup>89</sup> Sophisticated computational modelling could theoretically also predict pathogenicity,<sup>199</sup> or at least *I*<sub>Na</sub> abnormalities,<sup>200</sup> directly from genetic variants. Unfortunately, the accuracy

of such predictions is still inadequate for *SCN5A*; thus, variant's biophysical descriptors must still be obtained experimentally. Whereas interpreting loss of Na<sup>+</sup>-channel function in terms of conduction disturbance (Lenegre's syndrome) is straightforward, modelling BrS is far more challenging. The BrS phenotype can be reproduced as a 'repolarization'<sup>201</sup> or 'propagation'<sup>202</sup> disorder, further underscoring the increasingly recognized complexity of the disorder.

### Ion channel diseases with altered K<sup>+</sup>-channel function

Mutations in genes encoding for cardiac repolarizing K<sup>+</sup> currents lead to disease entities associated with altered cardiac repolarization (LQTS and SQTs).

Mice were the first species utilized to generate genetic models of K<sup>+</sup> channelopathies. They, however, do not represent an optimal model organism for modelling cardiac K<sup>+</sup> channel-related diseases (LQTS, SQTs) due to the aforementioned differences in cardiac repolarization patterns and responsible K<sup>+</sup> currents between mice and humans ('Species differences in cellular electrophysiology' section and Figure 2).<sup>203,204</sup> Consequently, mouse models with genetic manipulation of human channel subunits usually do not show a proarrhythmic phenotype,<sup>204,205</sup> unless significant remodelling of other channels relevant for murine cardiac repolarization occurs.<sup>206</sup> Genetic manipulations of K<sup>+</sup> channels contributing to murine cardiac repolarization (Kv 4.2, Kv.1.4), in contrast, may result in arrhythmias when associated with increased dispersion of repolarization.<sup>207</sup>

Another animal model frequently used in LQTS and SQTs-related research is the zebrafish. Spontaneous LQTS and SQTs zebrafish mutants exist and can be found by screening-approaches. The zebrafish *breakdance*, which carries the trafficking-deficient *KCNH2*-I59S mutation, recapitulates severe forms of human LQT2 with 2:1 atrio-ventricular block due to prolonged ventricular APD.<sup>208,209</sup> The zebrafish *reggae* expresses the missense mutation *KCNH2*-L499P, producing similar *I*<sub>Kr</sub> dysfunction and shortened QT as observed in human SQT1.<sup>210</sup> But these models do have limitations due to their differences in cardiac morphology and structure as highlighted in 'Species differences in cellular electrophysiology' section.

Several transgenic rabbit models for K<sup>+</sup> channel-related LQTS have been generated by over-expression of human loss-of-function mutated K<sup>+</sup> channels: LQT1 (*KCNQ1*/*KvLQT1*-Y315S), LQT2 (*KCNH2*/*HERG*-G628S),<sup>57</sup> and LQT5 (*KCNE1*/*minK*-G52R).<sup>70</sup> Similarly, a short-QT syndrome (SQTs) rabbit model has been engineered based on over-expression of gain-of-function mutated *KCNH2*/*HERG*-N588K (SQT1).<sup>71</sup> In LQT1 and LQT2 rabbits, *I*<sub>Ks</sub> (LQT1) or *I*<sub>Kr</sub> (LQT2) were completely eliminated due to a dominant-negative effect, resulting in APD/QT prolongation in both, and the development of spontaneous VT, sudden cardiac death, and sex differences in arrhythmogenic risk with pro-arrhythmic effects of oestradiol in LQT2.<sup>57,211</sup> In transgenic LQT5 rabbits, in contrast, *I*<sub>Ks</sub> was altered with accelerated deactivation kinetics<sup>70</sup> but not reduced, leading to a partial phenotype with only slightly prolonged QT-intervals and no spontaneous arrhythmias. In SQT1 rabbits, steady-state *I*<sub>Kr</sub> was increased due to impaired channel inactivation,<sup>71</sup> leading to shortened atrial and ventricular APD and QT, and increased VT/VF and AF inducibility; thus mimicking the human disease phenotype on atrial and ventricular levels. These transgenic rabbit models have

been used to investigate mechanisms of arrhythmogenesis and pro- and anti-arrhythmic effects of various drugs, hormones, and metabolites.<sup>212,213</sup>

Human-induced pluripotent stem-cell-derived cardiomyocytes from LQT1 and LQT2 patients have been shown to recapitulate clinical phenotypes,<sup>214</sup> disclose 'modifier genes',<sup>93,94</sup> and allowed to devise and test therapeutic approaches.<sup>95,215,216</sup> Nonetheless, because of hiPS-CMs immaturity and variability, some caveats should be considered, as discussed in 'Human-induced pluripotent stem-cell-derived cardiomyocytes in electrophysiology research' section.

Apart from these genetic models for K<sup>+</sup> channelopathies, several species such as dogs and guinea pigs are often employed as 'drug-induced' long-QT models, particularly for safety pharmacology research. Due to space limitations, we cannot comprehensively cover all these models in this position paper.<sup>217</sup>

### Ion channel diseases with altered susceptibility to sympathetic stimulation (CPVT)

CPVT is caused by mutations in *RyR2* or other genes that code for proteins within the RyR2 macromolecular complex.

Different experimental models have been used to determine the mechanisms involved in catecholaminergic polymorphic ventricular tachycardia (CPVT) by different mutations. Plasmids for *RyR2* carrying mutations identified in CPVT patients have been transfected into heterologous expression systems. Given that the *RyR2* is an intracellular channel, its biophysical properties have also been analysed by incorporating single channels into lipid bilayers of cell lines. However, as these cells lack all the components that make up the *RyR2* complex in cardiomyocytes, the link to arrhythmia generation is difficult to assess.

Heterozygous knock-in mouse models have been shown to be a valuable tool for exploring the underlying cause of VT and replicate many features associated with CPVT, including disease progression, and drug response.<sup>218–221</sup> However, there are significant differences in Ca<sup>2+</sup> handling between mice and larger mammals, with a much smaller contribution of the electrogenic Na<sup>+</sup>/Ca<sup>2+</sup> exchanger in mice. Rabbits have more similar ion-channel and Ca<sup>2+</sup>-handling patterns to humans, but no heterozygous knock-in CPVT rabbit model has been reported so far. Human-induced pluripotent stem-cell-derived cardiomyocyte from CPVT patients can generate cells with typical nodal/pacemaker, atrial, and ventricular electrical properties, expressing channels and transporters involved in Ca<sup>2+</sup> handling (see 'Human-induced pluripotent stem-cell-derived cardiomyocyte' section). Although they have an immature phenotype compared to adult cardiomyocytes, they retain channels and transporters from human cardiomyocytes, making them a valuable tool to study mechanisms underlying CPVT.<sup>26</sup>

### Genetic arrhythmogenic cardiomyopathies

Mutations in genes encoding sarcomeric proteins such as cardiac  $\beta$ -myosin heavy-chain,  $\alpha$ -tropomyosin, cardiac troponin and others can cause familial HCM characterized by myocyte disarray, interstitial fibrosis, ventricular dysfunction, and increased risk of VT and SCD.

The first genetic cardiomyopathy animal models were transgenic mice expressing mutations in various sarcomeric proteins. These showed a broad spectrum of disease phenotypes, however, several

did not develop LV hypertrophy, the key element of HCM in humans,<sup>222,223</sup> likely due to pronounced differences in the composition of cardiac sarcomeric proteins between humans and mice with  $\beta$ -MHC as predominant form in human ventricles and  $\alpha$ -MHC in mice.<sup>224</sup> Nevertheless, recent studies in HCM mouse models have implicated intracellular Ca<sup>2+</sup> dysregulation and enhanced late Na<sup>+</sup> current in the generation of pro-arrhythmic early and delayed afterdepolarizations.<sup>225</sup> Moreover, these preclinical models have been instrumental in developing novel therapeutic strategies<sup>226</sup> including the cardiac myosin inhibitor mavacamten.<sup>227,228</sup> In 1999, the first transgenic rabbit model for HCM was generated by targeted cardiac-specific expression of the mutant  $\beta$ -MHC-Q<sup>403</sup>. These HCM rabbits showed cardiac hypertrophy, interstitial fibrosis, myocyte disarray, and premature arrhythmic death,<sup>229</sup> but thus far no electrophysiological studies have been performed in these models to better understand arrhythmogenic mechanisms in HCM.

Arrhythmogenic cardiomyopathy (ACM), caused by mutations in predominantly desmosomal genes is an inherited, familial disorder characterized by progressive replacement of cardiomyocytes by fibrofatty tissue, ultimately resulting in ventricular dilation, cardiac dysfunction, life-threatening arrhythmias and sudden cardiac death.<sup>230</sup> Disturbed desmosomal organization in the setting of ACM leads to myocardial fibrosis formation, fibro-fatty replacement and cardiac dilation, setting the stage for arrhythmias. While most mouse models of ACM do not fully recapitulate the human disease phenotype (such as for instance fibro-fatty replacement),<sup>231</sup> they have been vital for identifying Ca<sup>2+</sup> dysregulation as a contributing factor and for establishing reduced Na<sup>+</sup> current as a pro-arrhythmic feature during early disease stages prior to the development of overt cardiomyopathy.<sup>232,233</sup>

## Outlook

Current *in silico*, *in vitro*, and *in vivo* models offer a wide variety of electrophysiological research techniques. Strategies that combine different methodological approaches are expected to offer the most comprehensive assessment of cardiac electrophysiology, at the same time reducing animal experiments as much as possible. Personalized disease understanding and individualized, mechanism-based therapy planning is one major goal of next-generation electrophysiological research. While conventional cellular electrophysiological techniques will remain essential tools for detailed analyses, future scientific efforts in cardiac electrophysiology will certainly require additional novel methods and technologies that allow for (i) the identification of mechanisms underlying physiology and disease at system scale (e.g. '-omics' approaches combined with computational simulations, with particular focus on epigenetic effects); (ii) *in vitro* validation employing advanced cellular models; (iii) pre-clinical translation of interventions derived from (i) and (ii) in animals that model the disease of interest as closely as possible; and (iv) optimization of (i)–(iii) using artificial intelligence and machine learning where appropriate. The present and future armamentarium of techniques and models will allow basic, translational, and clinical electrophysiological researchers to employ optimized approaches tailored to individual, 'personalized' scientific needs.

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