

REVIEW ARTICLE

Transgenic rabbit models for cardiac disease research

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To study the pathophysiology of human cardiac diseases and to develop novel treatment strategies, complex interactions of cardiac cells on cellular, tissue and on level of the whole heart need to be considered. As *in vitro* cell-based models do not depict the complexity of the human heart, animal models are used to obtain insights that can be translated to human diseases. Mice are the most commonly used animals in cardiac research. However, differences in electrophysiological and mechanical cardiac function and a different composition of electrical and contractile proteins limit the transferability of the knowledge gained. Moreover, the small heart size and fast heart rate are major disadvantages. In contrast to rodents, electrophysiological, mechanical and structural cardiac characteristics of rabbits resemble the human heart more closely, making them particularly suitable as an animal model for cardiac disease research. In this review, various methodological approaches for the generation of transgenic rabbits for cardiac disease research, such as pronuclear microinjection, the sleeping beauty transposon system and novel genome-editing methods (ZFN and CRISPR/Cas9) will be discussed. In the second section, we will introduce the different currently available transgenic rabbit models for monogenic cardiac diseases (such as long QT syndrome, short-QT syndrome and hypertrophic cardiomyopathy) in detail, especially in regard to their utility to increase the understanding of pathophysiological disease mechanisms and novel treatment options.

KEYWORDS

hypertrophic cardiomyopathy, Long QT Syndrome, monogenic cardiac diseases, Short QT Syndrome, Transgenic rabbit models

Abbreviations: AR, arrhythmia; AV, atrioventricular; β -MHC, beta-myosin heavy chain; CAS9, CRISPR associated effector protein; CRISPR, clustered regularly interspaced short palindromic repeats; HERG/KCNH2, alpha subunit to I_{Kr} -conducting potassium channel; $I_{Ca,L}$, L-type calcium current; I_{Kr} , rapid delayed rectifier potassium current; I_{Ks} , slow delayed rectifier potassium current; I_{K1} , inward rectifier potassium current; KCNE1, beta subunit to I_{Ks} -conducting potassium channel; KCNQ1, alpha subunit to I_{Ks} -conducting potassium channel; LQTS, long QT syndrome; LQT2, long QT type 2; LQT5, long QT type 5; LQT2-5, combined long QT type 2 and type 5; LV, left ventricle; QT, QT interval in surface ECG; RR, interval between two consecutive R waves in ECG; RV, right ventricle; SCN5A, gene encoding the voltage-gated sodium channel alpha subunit 5; SQT1, short-QT type 1; STV_{QT}, short-term variability of QT interval; TALEN, transcription activator-like effector nuclease; ZFN, zinc finger nuclease; VT, ventricular tachycardia; VF, ventricular fibrillation; WT, wild type.

Tibor Hornyik and Marina Rieder contributed equally.

1 | ADVANTAGES AND DISADVANTAGES OF TRANSGENIC RABBIT MODELS FOR MONOGENIC CARDIAC DISEASES

1.1 | Advantages and disadvantages of animal models for monogenic cardiac diseases

Human subjects, their organs, tissues and cells would be ideal sources for studies on the pathophysiology of human cardiac diseases. In-depth mechanistic studies on alterations of cardiac function, however, can only be performed to a very limited extent in human patients for ethical reasons. The use of animal models has therefore been common practice in cardiac research for many decades (Hasenfuss, 1998; Russell & Proctor, 2006; Suzuki et al., 2010).

Although the initial focus has been on animal models of cardiac disease induced by drugs, surgical interventions or other environmental changes (Hasenfuss, 1998), more and more genetically modified animal models have been generated in recent years (Cesarovic et al., 2020b; Matsuhisa et al., 2020). In these models, genes of interest are knocked-out or knocked-in or are subjected to disease-specific point mutations. This is particularly important for research in the field of monogenic cardiac diseases such as inherited cardiomyopathies and channelopathies (highlighted in the following). Here, genetically altered animal models (e.g. knock-out, knock-in and particularly transgenic models expressing human pathogenic mutations) have been generated to mimic the human disease genotype and phenotype. This allowed to obtain insights into disease-specific cardiac function on cellular, tissue, organ and *in vivo* levels, that can be used for 'bench to

bedside' translation to improve diagnostic and therapeutic strategies in patients (Lang et al., 2016).

These animal models allow identification of pathophysiological mechanisms on multiple levels and to conduct longitudinal studies in subjects with a defined genetic background and without confounding comorbidities, allowing not only observations but also defined quantitative therapeutic interventions. This comes at the cost, however, of partially limited clinical transferability due to species differences in features of cardiac mechanical and electrical function (Baczko et al., 2016; Nerbonne et al., 2001; Salama & London, 2007) that are responsible for incomplete emulation of different aspects of human diseases.

1.2 | Advantages and disadvantages of animal models of different species for (monogenic) cardiac disease research

The first and currently still most commonly used animal model in cardiac disease research is the mouse. Mice offer great advantages thanks to their low husbandry costs, relatively easy handling and high reproduction rate already at young age, which allows a fast generation of large study cohorts (Camacho et al., 2016a). Besides this, 99% of human genes have direct murine orthologues and the generation of transgenic mouse strains is relatively straight forward as genetic manipulation can be easily performed in mice (Duranthon et al., 2012; Yutzey & Robbins, 2007). Also, the proof-of-principle studies for gene therapy of genetic disorders can be conducted easily in mice (Brunner et al., 2003; Kodirov et al., 2003) (Figure 1).

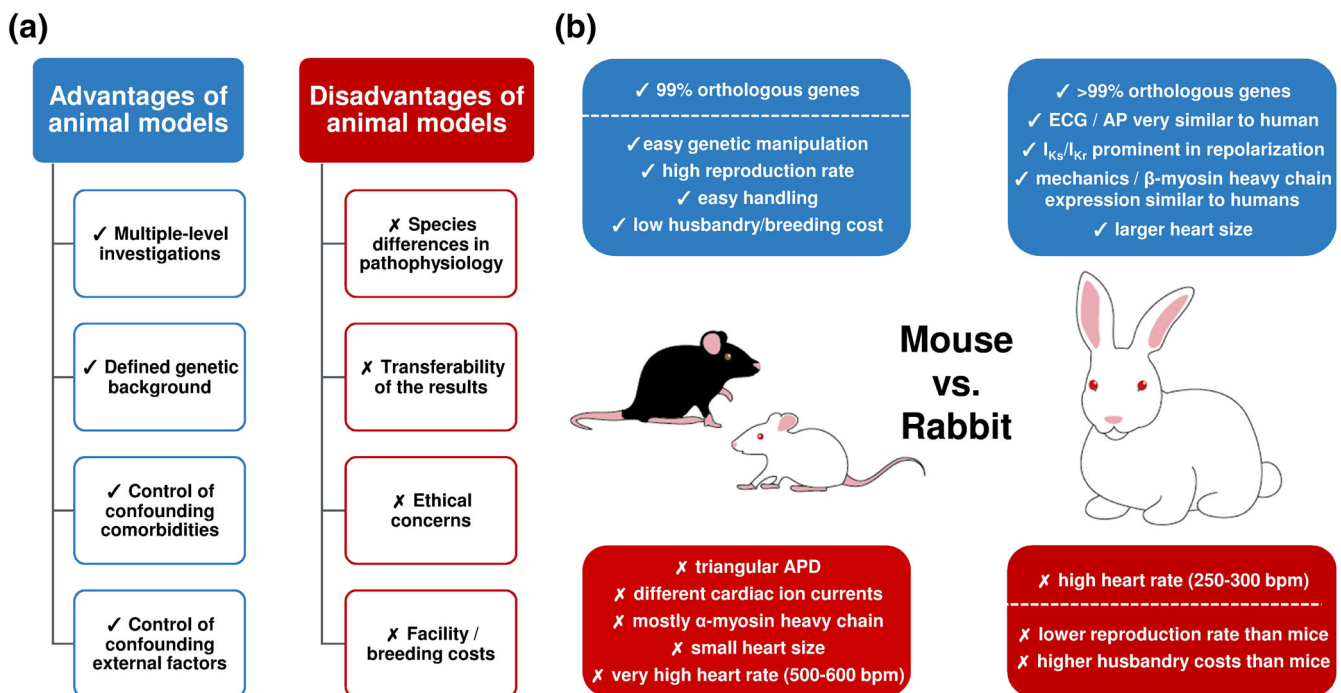


FIGURE 1 Advantages and disadvantages of animal models used for studying human cardiac diseases. Schematic figure summarising the main advantages and disadvantages of animal models in studying human cardiac diseases compared to humans (a). (b) The main benefits and drawbacks of mouse and rabbit models used in cardiac disease research from a translational point of view

A major limitation, however, are relevant physiological and/or anatomical differences in mice compared to humans, which limit their translational impact. The different dimensions of murine and human hearts not only make surgical cardiac interventions very challenging, but also prevent their use for testing of cardiac devices such as prosthetic valves or pacemakers (Laughner et al., 2013). Along with the small size of their heart, mice have remarkably high heart rates around 500–600 bpm, enabled by a different composition of ion channels (see below) and contractile proteins in cardiomyocytes compared to humans: Whereas humans express predominantly the β -myosin heavy chain, mice express mostly α -myosin heavy chain with rapid ATPase activity (Hasenfuss, 1998). In addition to the difference in the composition of contractile proteins, also contractile function differs between mice and human with different rotational spin in early systole (Jung et al., 2012).

Furthermore, action potentials in murine cardiomyocytes are shorter than human action potentials and exhibit a different morphology with a rapid repolarization phase without a prominent plateau phase. These electrophysiological differences are due to pronounced species differences in repolarizing ion currents: In mice repolarization is 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 $I_{to,s}$; whereas the rapid and slow delayed rectifier potassium currents (I_{Kr} and I_{Ks}), the main repolarizing ion currents in human cardiomyocytes, are expressed but their functional relevance is reduced as the manipulation of *KCNE1*, *KCNQ1* and *KCNH2* genes has little effect on myocardial function and K^+ currents in the mouse heart (Nerbonne et al., 2001).

Besides small rodents, large animal models (such as pigs, dogs, sheep, goats and rarely non-human primates) are used in cardiac disease research. In such animals, the heart volume and weight as well as the overall body size more closely resemble human dimensions (Milani-Nejad & Janssen, 2014), thus allowing preclinical *in vivo* testing of cardiac devices or prostheses (Taramasso et al., 2015). Nevertheless, the experimental use of large mammals also has several disadvantages: Daily housing costs are up to 100 times higher for larger mammals than for mice. Ethical concerns and restrictive legislations may represent a major obstacle, especially concerning dogs or non-human primates (Cesarovic et al., 2020a). Further, and importantly, genetic manipulation of large mammalian animals is still technically challenging and resource-intensive, and hence rarely carried out (Whitelaw et al., 2016) but is particularly important for monogenic cardiac disease research.

In the following, we will therefore limit our species comparison to large animal used in monogenic cardiac disease research.

Canine hearts show close similarities to human hearts both on the organ and cellular level. Hearts have a similar weight and heart rate (Camacho et al., 2016b), and slow and fast delayed rectifier potassium currents play a similar major role in cardiac repolarization as in humans (Szabo et al., 2005; Szentadrassy et al., 2005). Dog models are thus particularly suitable for the preclinical definition of safety profiles for new drugs, especially regarding the arrhythmogenic

risk. Moreover, spontaneous canine models of monogenic diseases exist for Duchenne muscular dystrophy (Kornegay, 2017) with dilated cardiomyopathy and for arrhythmic right ventricular cardiomyopathy (Oxford et al., 2007). The latter shows increased risk for ventricular arrhythmias and histopathological lesion that closely resembled those of human patients affected by arrhythmic right ventricular cardiomyopathy.

Pig models also play an important role in cardiac research thanks to similarities in heart rate, cardiac size, action potential shape, and autonomic innervation (Stubhan et al., 2008). In addition, a genetic pig model of Brugada syndrome with mutant *SCN5A* has been generated, despite the difficulties in genetic manipulations in larger animals (Park et al., 2015). Although this model did not show a typical Brugada Syndrome ECG, even in the presence of high-dose sodium channel blocker **flecainide**, it mimicked the rhythm instability and conduction disturbances observed in human Brugada patients.

In the past decades, the laboratory rabbit (*Oryctolagus cuniculus*) has gained more and more significance in cardiac research as a useful alternative model to small rodents and larger animal models. Thanks to continued improvements in animal transgenesis (Bosze et al., 2016; Matsuhisa et al., 2020), rabbits have entered the range of species in whom genetic manipulation can successfully replicate human cardiac diseases.

Rabbits are bigger than mice, hence several experimental procedures such as the collection of blood or tissue samples, the cannulation of certain blood vessels, the production of larger amounts of a specific foreign protein and the implantation of external devices (e.g. pacemakers or ECG recorders) is technically much easier in rabbits than in mice (Fan et al., 1999; Hornyik et al., 2020; Laughner et al., 2013) (Figure 1). Importantly, electrophysiological, mechanical and structural cardiac characteristics, coronary architecture and cardiac responses to ischaemia and various drugs in rabbits more closely resemble humans than do rodents (Nerbonne, 2000; Odening et al., 2012; Odening & Kohl, 2016). Moreover, in both species, rabbits and humans, the β isoform of the myosin heavy chain is the predominant myosin heavy chain in the heart (Swynghedauw, 1986), making the rabbit an interesting model for human genetic cardiomyopathies (as described in detail in Section 3). Pronounced similarities between rabbits and humans also exist in cardiac electrophysiology with similar action potential shape and similar function and gating kinetics of various repolarizing potassium currents. In both species, the rapid and slow delayed rectifier K^+ currents (I_{Kr} and I_{Ks}) are the main repolarizing ion currents (Nerbonne, 2000; Varro et al., 1993). Therefore, rabbits are very useful models for the investigation of arrhythmogenic cardiac diseases (as described in detail in Sections 4 and 5).

Despite the many similarities, rabbit heart rates are still faster than those of humans, ranging from 200–300 beats per minute in awake and active animals and from 150–240 beats per minute under sedation. This relatively high heart rate leads to a reduced heart rate reserve, which limits the use of rabbits for the investigation of exercise-induced effects on the cardiovascular system (Milani-Nejad & Janssen, 2014). Partial solutions to this problem may be obtained by surgical ablation of the atrioventricular node (Hagiwara

et al., 2017; Tsuji et al., 2006), although this may predispose the rabbit heart to arrhythmias. Other disadvantages of rabbits, compared to mice, are their relative high housing/breeding costs, lower reproduction rate and lower efficacy of genetic manipulation, which is outweighed by their high clinical relevance as cardiac disease models as highlighted in the following.

2 | METHODOLOGICAL APPROACHES TO GENERATE TRANSGENIC RABBIT MODELS FOR CARDIAC DISEASE RESEARCH

In the field of cardiac disease research, thus far, rabbit models have been generated using classical transgenesis techniques with pronuclear microinjection, which allows not only to generate knock-out or knock-in models of certain genes but also to introduce disease-specific point mutations. However, the techniques to generate transgenic animal models have developed rapidly: Genome-editing technologies using nucleases have revolutionised the field of animal transgenesis also in non-murine species. Among these nucleases are the zinc finger nuclease (ZFN), the transcription activator-like effector nuclease (TALEN) and the newest, clustered regularly interspaced short palindromic repeats (CRISPR) with the CRISPR associated effector protein (CAS9). In 2011, the first knock-out rabbit model was generated using the ZFN technology (Flisikowska et al., 2011). Since then, the number of reports on knock-out rabbits used as human disease models has increased rapidly. The first genome-editing of rabbit using CRISPR/Cas9 system was reported in 2014 (Yang et al., 2014). Although these new techniques have already been used to knock-out genes in the field of lipid metabolism and vascular/atherosclerosis research (reviewed in (Matsuhisa et al., 2020)), they have not been used for the generation of transgenic rabbit models for monogenic cardiac diseases as these need more complex approaches to introduce targeted disease-specific point mutations. However, with further modification and improvement of this technique, the insertion of targeted point mutations will also be feasible rather than only knock-out or knock-in approaches of complete genes, thus allowing the future CRISPR/Cas9 based generation of transgenic rabbit models also for monogenic cardiac diseases.

2.1 | Pronuclear microinjection to generate transgenic rabbit models

The pronuclear microinjection method is based on the injection of a naked, linear DNA fragment (containing the gene of interest) into the pronucleus of a zygote (Hammer et al., 1985). From 1985, when the first transgenic rabbit was produced by pronuclear microinjection (Hammer et al., 1985), until the 2010s, pronuclear microinjection remained the only available and applied method for rabbit transgenesis. Even though pronuclear microinjection resulted in numerous important rabbit models of human diseases, the efficiency of transgenesis was generally low (Bosze et al., 2016; Duranthon

et al., 2012). In general, the efficiency of transgenesis was reported to be lower than 5% of total born rabbits and in the range of 0.5% of total injected and transplanted embryos (Matsuhisa et al., 2020). Despite several attempts to improve the efficiency of microinjection-mediated transgenic animal production (Houdebine, 2002), one of the disadvantages of the pronuclear injection-based transgenesis could not be solved, namely random integration, this means that the pronuclear microinjection technology is unable to control the copy number and integration site of the transgene in the genome. Therefore, there is a high chance of a position effect, which might lead to undesired ectopic expression or transgene silencing (Ivics et al., 2014; Matsuhisa et al., 2020).

Novel genome-editing and gene-editing technologies that are summarised in the following represent promising solutions to overcome limitations of the traditional pronuclear microinjection technique (Matsuhisa et al., 2020).

2.2 | Sleeping Beauty transposon system to generate transgenic rabbit models

Even though different transposon-based systems were used to create genetically modified mammals, 'Sleeping Beauty' was the first successfully applied system in rabbit (Ivics et al., 2014). DNA transposons are mobile genetic elements, which can integrate into the host genome by a simple 'cut and paste' mechanism (Bosze et al., 2016). One of the main advantages of transposon-based transgenesis compared to pronuclear microinjection is its improved efficiency:- a rate of 15.1% transgenic animals out of total animals born alive and a rate of 1.48% transgenic animals of total injected and transplanted embryos was reported. The advantage is that the sleeping beauty transposon system provides similar gene expression levels of the (mutated) gene of interest in the transgenic rabbit as in humans as a single copy of the mutant human gene is integrated in the rabbit genome creating a hemizygous mutation (Bosze et al., 2016). The problem of insertional bias, however, could not be overcome with this technique (Bosze et al., 2016).

2.3 | Genome-editing methods (ZFN, TALEN and CRISPR/Cas9) to generate transgenic rabbit models

Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks specifically at the target DNA sequences. These programmable proteins are composed of a target specific domain fused to a nuclease domain of the type II endonuclease *FokI* (Bibikova et al., 2001). The first ZFN rabbit was created via the disruption of the immunoglobulin M (IgM) locus resulting in a functional knock-out phenotype (Flisikowska et al., 2011). In addition, targeted sequence replacement (knock-in) was performed in the target site in the presence of a linear DNA donor (Bosze et al., 2016). Certain factors

restrict the practical application of ZFNs in genome editing, such as the expensive zinc finger library, the laborious design and assembly of ZFN arrays, the cumbersome ZF-DNA identification and the site selection as design of ZFNs targeting guanine-poor sequences is complicated (Chadwick & Musunuru, 2017).

Transcription activator-like effector nucleases (TALENs) are programmable nucleases that share similar features with ZFNs, but with easier design and production (Song et al., 2013). The structure of TALENs are similar to ZFNs, each comprising a DNA-binding domain fused to a FokI nuclease domain (Miller et al., 2011). The DNA binding of TALENs, however, relies on so called TAL repeats containing a highly conserved 33–35 amino acids long repeat with variable amino acids at the 12th and 13th position. The method is limited by the need to screen and fine tune potential TALEN pairs. Only few rabbit models have been generated using this technique. Among them are rabbit models used for arteriosclerosis research such as a new rabbit with knock-in of human apoAII (Koike et al., 2021).

The accuracy of genome editing in rabbits has dramatically increased in the past 6 years which can be attributed to CRISPR/Cas9 technology. CRISPR-Cas9 system was first identified as an adaptive immune system in bacteria (Cong et al., 2013). The CRISPR-Cas9 system consists of the Cas9 nuclease and the synthetic guide RNA, which is about 100 nucleotides in length and can target and introduce mutations at specific genomic sites (Chadwick & Musunuru, 2017; Cong et al., 2013). CRISPR-based technologies take advantage of a DNA endonuclease which generates site-specific double-stranded DNA breaks. Repair of double-stranded DNA breaks can occur via non-homologous end joining, which may cause frameshift mutations and may result in functional KO of the selected gene. Homology directed repair is another option for the repair of double-stranded DNA breaks, by which a mutation or a DNA insert can be integrated at a specific locus (Chadwick & Musunuru, 2017; Song et al., 2013).

The CRISPR/Cas9 technology has already been used to create knock-out models for a variety of different transgenic rabbit models for diseases associated with lipid metabolism and atherosclerosis (reviewed in (Fan et al., 2015; Matsuhisa et al., 2020). Despite its huge potential, this technique still awaits being employed for the generation of transgenic rabbit models for (monogenic) cardiac diseases in which more complex approaches to introduce targeted disease-specific point mutations are warranted.

In the following sections, the different currently available transgenic rabbit models for monogenic cardiac diseases will be introduced in detail and their utility to increase the understanding of pathophysiological disease mechanisms and novel treatment options will be highlighted.

3 | TRANSGENIC CARDIOMYOPATHY RABBIT MODELS TO STUDY HUMAN GENETIC CARDIOMYOPATHIES

Familial hypertrophic cardiomyopathy (HCM) is a genetic disease characterised by left ventricular (LV) and/or right ventricular

(RV) hypertrophy with predominant involvement of the inter-ventricular septum, in the absence of other causes of hypertrophy such as hypertension or valvular heart disease (Wigle et al., 1995). Myocyte disarray, interstitial fibrosis and ventricular dysfunction are the predominant pathophysiological characteristics of the disease.

The clinical spectrum ranges from asymptomatic cases to severe heart failure and/or sudden cardiac death due to ventricular tachycardia/ventricular fibrillation (Wigle et al., 1995). The estimated prevalence in the population of young adults is approximately 0.2% (Maron et al., 1995). Hypertrophic cardiomyopathy is of major clinical importance as it is the most common cause of sudden cardiac death in young adults, especially in previously apparently healthy and athletic individuals (Maron, 1997; Maron et al., 1982, 1986). To date, there are no curative therapeutic approaches available. Current treatment, such as the use of non-dihydropyridine calcium antagonists or β -adrenoceptor antagonists (Beta blockers) combined with standard heart failure medication focuses on symptomatic therapy to prevent heart failure and arrhythmias. In patients with a high degree of outflow obstruction, transcatheter ablation of septal hypertrophy or surgical myectomy can be performed. Depending on the estimated risk for arrhythmias, implantable cardioverter defibrillators are implanted to prevent sudden cardiac death (Makavos et al., 2019).

Mutations in various genes encoding sarcomeric proteins have been identified as causes of hypertrophic cardiomyopathy (Marian & Roberts, 1998). The most common gene linked to hypertrophic cardiomyopathy is the cardiac β -myosin heavy chain gene, in which different missense mutations can be found in approximately 50% percent of families with hypertrophic cardiomyopathy (Watkins et al., 1992). Mutations in alpha-tropomyosin or cardiac troponin are even more rare and only account for 3–15% of all hypertrophic cardiomyopathy cases (Watkins et al., 1995). Hypertrophic cardiomyopathy is inherited in an autosomal-dominant manner, the penetrance, however, is highly variable and phenotypes may vary even within families with the same genetic defect (Marian & Roberts, 1995). Thus, despite the availability of arrhythmogenic risk score calculators, the assessment of the individual patient's risk and the consecutive need for treatment to prevent sudden cardiac death is challenging—particularly as the impact of different mutations on the arrhythmic risk or atypical forms of hypertrophy such as apical hypertrophic cardiomyopathy are not yet reflected in these risk calculator (Authors/Task Force Members et al., 2014).

Transgenic animal models for hypertrophic cardiomyopathy have been created to gain further insights into the patho-mechanisms of the disease, to increase the understanding of the individual's arrhythmic risk and identify novel targets for therapy.

The first genetic cardiomyopathy animal models were transgenic mice expressing mutations in various sarcomeric proteins. These showed a broad spectrum of disease phenotypes, however, they did not develop LV hypertrophy, the key element of hypertrophic cardiomyopathy in humans (Muthuchamy et al., 1999; Oberst et al., 1998; Peng, 2012; Vikstrom et al., 1996). This is due to the previously mentioned difference in the composition of cardiac sarcomeric proteins

between humans and mice: Whereas β -MHC is predominant in human ventricles, α -MHC is the predominant sarcomeric protein in mice (Swynghedauw, 1986). As the various MHC subtypes show significant differences in actin-activated myosin-ATPase activity and cross-bridge kinetics, the composition of cardiac sarcomeric proteins affects the clinical spectrum of the disease (Peng, 2012; Sugiura et al., 1998).

As mentioned above, the myosin composition in rabbits was found to be similar as in humans with β -MHC as the major isoform (Kavinsky et al., 1984). Furthermore, rabbit β -MHC is approximately 98% homologous to the human protein (Jaenicke et al., 1990; Peng, 2012). This makes the rabbit a particularly suitable species for studying hypertrophic cardiomyopathy.

In 1999, the first transgenic rabbit model for hypertrophic cardiomyopathy was generated (Marian et al., 1999) (Table 1). These rabbits show a targeted cardiac-specific expression of the mutant β -MHC-Q⁴⁰³, which is frequently associated with hypertrophic cardiomyopathy in humans (Watkins et al., 1995). The phenotype of these transgenic rabbits is similar to the phenotype of human patients with cardiac hypertrophy, interstitial fibrosis, histological evidence of myocyte and myofibrillar disarray, and premature arrhythmic death (Marian et al., 1999).

Other transgenic hypertrophic cardiomyopathy rabbit models have been generated subsequently, over-expressing other mutant genes that have been identified to cause hypertrophic cardiomyopathy in human subjects. Different mutations in the ventricular isoform of the essential light chain (*ELC1v*) have been identified to cause hypertrophic cardiomyopathy (Lee et al., 2001; Poetter et al., 1996). This is due to the fact that essential light chain binds to the neck region of MHC and changes in essential light chain alter the myosin and sarcomeric function (James et al., 2002). A transgenic rabbit model expressing a human essential light chain mutation (M149V *ELC1v*), however, did not develop a hypertrophic cardiomyopathy phenotype for unknown reasons (Table 1).

As mutations in cardiac troponin can also cause hypertrophic cardiomyopathy, more recently, a transgenic rabbit model expressing a human cardiac troponin I mutation (cTnI^{146Gly}) has been generated (Table 1). This rabbit model showed aberrant connexin organisation, morphological deficits and an altered pattern of the repolarization phase. However, it led to only subtle defects without severely affecting cardiac function (Sanbe et al., 2005).

Taken together, the β -MHC-Q⁴⁰³ rabbit model is currently the only available hypertrophic cardiomyopathy model mimicking the human phenotype.

The effect of a variety of diagnostic and therapeutic options in hypertrophic cardiomyopathy has been studied in this β -MHC-Q⁴⁰³ hypertrophic cardiomyopathy rabbit model (Table 1). One diagnostic method studied in the β -MHC-Q⁴⁰³ rabbit model was Doppler imaging. It was revealed to be a sensitive tool to evaluate myocardial contraction and relaxation abnormalities in hypertrophic cardiomyopathy (Nagueh et al., 2000). Then, a preclinical trial confirmed these findings in humans (Nagueh et al., 2001). Besides this diagnostic approach developed in the animal model, a variety of

pharmacological treatment options have been evaluated in the β -MHC-Q⁴⁰³ transgenic rabbit: It was reported that treatment with N-acetylcysteine reversed the clinical hypertrophic cardiomyopathy phenotype in the β -MHC-Q⁴⁰³ rabbit model. Transgenic rabbits treated with N-acetylcysteine showed less cardiac and myocyte hypertrophy and interstitial fibrosis and presented lower incidence of arrhythmias than a placebo-treated group (Lombardi et al., 2009). A consecutive study in humans showed small effect sizes on indices of cardiac hypertrophy or fibrosis, however the small sample size of the study prevented further conclusions for daily clinical practice (Marian et al., 2018).

Simvastatin induced a reduction of hypertrophy and fibrosis by reducing ERK1/2 activity in the β -MHC-Q⁴⁰³ transgenic hypertrophic cardiomyopathy rabbit model. In consequence, this led to improved cardiac function (Patel et al., 2001). Similarly, atorvastatin prevented development of cardiac hypertrophy at organ, cellular and molecular levels in β -MHC-Q⁴⁰³ rabbits by reducing active Ras and p44/42 MAPK (Senthil et al., 2005). Despite these promising results in the rabbit model, however, atorvastatin was shown not to be effective in humans (Hersi et al., 2016), indicating that some of these signalling pathways may be regulated differently among species.

4 | TRANSGENIC LONG QT SYNDROME RABBIT MODELS TO STUDY HUMAN LONG QT SYNDROME

Long QT syndrome (LQTS), both the inherited (congenital) and the acquired (drug-induced) form, is characterised by prolonged heart rate corrected QT duration (QTc) in surface ECG (QTc > 470–480 ms) as manifestation of a prolonged cardiac repolarization (Priori et al., 2001) (Figure 2a) and is associated with high risk for ventricular arrhythmias. Although to date 17 genes have been identified as causal for congenital LQTS, the disease is predominantly caused by loss-of-function mutations in genes encoding for repolarizing potassium channels (90%, *KCNQ1*: LQT1, *KCNH2*: LQT2) and gain-of-function mutations in depolarizing sodium channels (5%, *SCN5A*: LQT3) (Priori et al., 2001). Patients are prone to developing polymorphic torsade-de-pointes ventricular tachycardia (VT) and sudden cardiac death. Currently available therapeutic options (β -adrenoceptor antagonists and cardioverter defibrillator implantation) either target the pro-arrhythmic trigger or terminate the arrhythmia once it occurs; but fail to protect from the development of arrhythmias in up to one third of patients (Moss et al., 2000). Therefore, better understanding of the pathophysiology of the disease is needed to develop more efficient mechanism-based novel therapeutic strategies.

In the following, we highlight mechanistic findings on the arrhythmic substrate, pro-arrhythmic triggers, pro-arrhythmic and anti-arrhythmic agents, and electro-mechanical dysfunction obtained in transgenic long QT syndrome (LQTS) rabbit models (of different LQTS subtypes) and their potential translational application in the clinical management of LQTS patients in more detail.

TABLE 1 Transgenic rabbit models of monogenic cardiac diseases

Monogenic cardiac disease	Rabbit model	Phenotype	Key (translational) findings	Reference
HCM	Expression of mutant β -MHC-Q ⁴⁰³	Mimics human phenotype with - cardiac hypertrophy - interstitial fibrosis - histological evidence of myocyte and myofibrillar disarray Premature arrhythmic death	- Evaluation of the sensitivity of Doppler imaging to determine abnormalities in myocardial contraction and relaxation - Evaluation of therapeutic potential of N-acetylcysteine and statins in HCM	Lombardi et al. (2009); Marian et al. (1999); Nagueh et al. (2000); Patel et al. (2001); Senthil et al. (2005); Watkins et al. (1995)
HCM	Expression of human ELC1v mutation (M149V ELC1v)	No HCM phenotype	- Not suitable as HCM model	James et al. (2002)
HCM	Expression of human cardiac troponin I mutation (cTnI ^{T46G/V})	Lack of clear phenotype, no cardiac hypertrophy - aberrant connexin organisation - morphological deficits - altered pattern of repolarization	- Only subtle defects without severely affected cardiac function	Sanbe et al. (2005)
LQT1	Expression of mutant human KCNQ1/KvLQT1-Y315S (dominant negative mutation in K ⁺ channel α - subunit [KvLQT1] \rightarrow loss of I _{Ks})	Mimics human phenotype with: - prolonged QT/APD - but: No increased risk in arrhythmia or SCD	- Electrical function: • APD dispersion not increased • VT/VF inducible in tachyopathy (continuous tachypacing) • Focal excitations arising from RV initiates arrhythmia • EAD formation with continuous adrenergic stimulation - mechanical function: normal - proarrhythmic/anti-arrhythmic potential of anaesthetics, I _{KACH} -opener (Nicorandil) and I _{Kr} -opener (NS1643) assessed - potential use in pro-arrhythmia testing (increased sensitivity to I _{Kr} blockers)	Bentzen et al. (2011); Biermann et al. (2011); Brunner et al. (2008); Kim et al. (2015); Lang et al. (2016); Lau et al. (2015); Liu et al. (2012); Odening et al. (2008); Ziupa et al. (2014, 2019)
LQT2	Expression of mutant human KCNH2/HERG-G628S (dominant negative mutation in K ⁺ channel α - subunit [HERG] \rightarrow loss of I _{Kr})	Mimics human phenotype with: - prolonged QT/APD and steeper QT/RR relationship - increased risk of arrhythmia and SCD	- Electrical function: • Increased APD dispersion, leading to unidirectional functional block, re-entry formation and VF • 'Discordant alternans' preceded VT/VF surge • EAD formation with sudden adrenergic surge - Mechanical function: • Impaired regional diastolic function, prolonged contraction duration • Increased mechanical dispersion • Correlation between more serious (regional) diastolic dysfunction and higher arrhythmic risk	Brunner et al. (2008); Lang et al. (2016); Liu et al. (2012); Odening et al. (2008, 2010, 2012, 2013); Ziv et al. (2009)

(Continues)

TABLE 1 (Continued)

Monogenic cardiac disease	Rabbit model	Phenotype	Key (translational) findings	Reference
LQTS	Expression of mutant human KCNE1-G52R (dominant negative mutation in K ⁺ channel β -subunit [MinK] \rightarrow decreased I_{Ks})	Incomplete LQTS phenotype: - Normal QT/APD—increased short-term QT variability - Increased drug-induced arrhythmia	<ul style="list-style-type: none"> - Pro-arrhythmic/anti-arrhythmic potential of anaesthetics and sex hormones assessed - Potential use in pro-arrhythmia testing (increased sensitivity to I_{Ks} and I_{K1} blockers) - Mimics 'concealed' human LQTS - Potential use in pro-arrhythmia testing: Increased sensitivity to I_{Kr} blocker agents (Dofetilide provoked TdP and increased STV_{QT}) 	Hornyik et al. (2020); Major et al. (2016)
LQT2-5	Expression of mutant human KCNH2/HERG-G628S and KCNE1-G52R (dominant negative mutations in K ⁺ channel α - (HERG) and β - (MinK) subunits \rightarrow loss of I_{Kr} , decreased I_{Ks})	Mimics human phenotype with: - Prolonged QT/APD and steeper QT/RR relationship	<ul style="list-style-type: none"> - Increased spatial dispersion of APD - Potential use in pro-arrhythmia testing (increased sensitivity to I_{Ks} and I_{K1} blockers and to sympathomimetics) 	Hornyik et al. (2020)
SQT1	Expression of the mutant human KCNH2/HERG-N588K (gain-of-function mutation in K ⁺ channel α - subunit (HERG) \rightarrow increased I_{Kr})	Mimics human phenotype with: - shortened QT/APD and flutter QT/RR relationship - increased risk of AF, VT/VF and SCD	<ul style="list-style-type: none"> - Mimics the full spectrum of human SQTS phenotype - Electrical function: <ul style="list-style-type: none"> • Increased spatial APD dispersion - Mechanical function: <ul style="list-style-type: none"> • Altered regional diastolic function Increased mechanical dispersion 	Odening et al. (2019)

Note: The mutant (human) trans-genes used for generation of the various models as well as a short description of their main pathophysiological characteristics and key translational findings are indicated. Abbreviations: AF, atrial fibrillation; APD, action potential duration; EAD, early afterdepolarization; ELC1v, essential light chain; HCM, hypertrophic cardiomyopathy; SCD, sudden cardiac death; TdP, Torsades-de-Pointes; VT, ventricular tachycardia

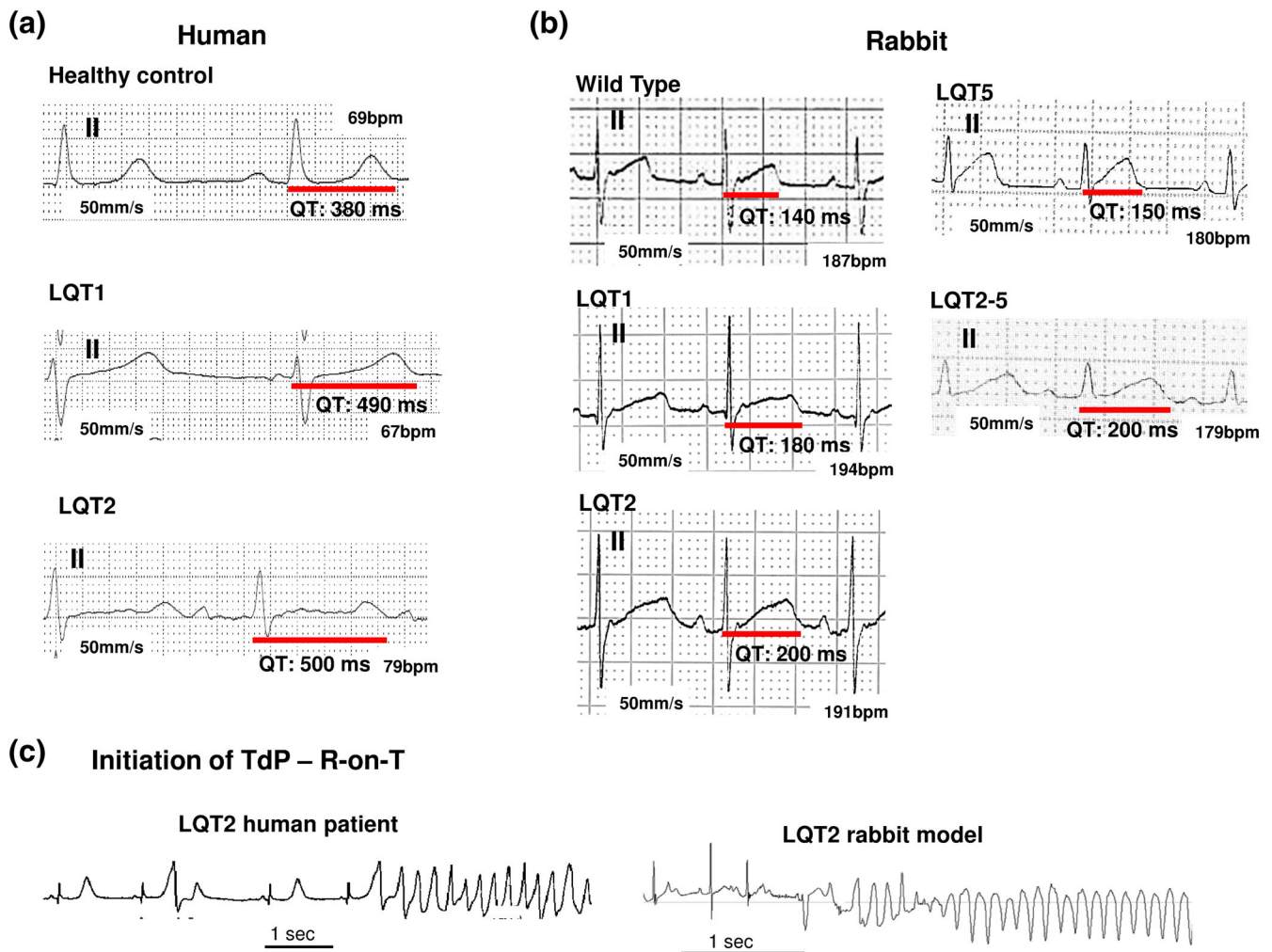


FIGURE 2 ECG characteristics of human LQTS patients and transgenic LQTS rabbit models. Representative ECG recordings of LQT1 and LQT2 patients show prolonged QT intervals compared to healthy control (a). Similar to human LQTS patients, transgenic LQT1, LQT2, LQT5 and LQT2-5 rabbit models also demonstrate prolonged QT intervals—at various extents in the different models—compared to wild-type control animals (b). (c) The presence of similar arrhythmia (TdP) initiation mechanism—R-on-T phenomenon—in human LQT2 patient and transgenic LQT2 rabbit model

4.1 | Electrophysiological characteristics of transgenic LQT1, LQT2, LQT5 and LQT2-5 rabbit models - Imitation of human long QT phenotype

All available transgenic LQTS rabbit models have been engineered by cardio-selective over-expression of dominant negative mutated human genes encoding for voltage-gated K^+ channels *KCNQ1/KvLQT1* (*KvLQT1-Y315S*, LQT1), *KCNH2/HERG* (*HERG-G628S*, LQT2) or *KCNE1/minK* (*KCNE1-G52R*) driven by beta-myosin heavy chain promoters (Brunner et al., 2008; Major et al., 2016) (Table 1).

In LQT1 or LQT2 rabbit cardiomyocytes I_{Ks} (LQT1) or I_{Kr} (LQT2), respectively, were completely eliminated by the loss-of-function mutation, resulting in prolongation of action potential duration (APD) on cellular and whole heart levels and prolongation of ventricular refractoriness and QT duration *in vivo* (Brunner et al., 2008; Odening et al., 2010) (Figure 2b). The phenotype of both LQT1 and LQT2 was even further aggravated as a result of decrease in the remaining

reciprocal potassium currents, I_{Kr} in LQT1 and I_{Ks} in LQT2, due to interactions of the mutant human *KvLQT1-Y315S* with the native rabbit *HERG* and of the mutant *HERG-G628S* with rabbit *KvLQT1* proteins, respectively (Ren et al., 2010). These protein interactions may thus play an important role in the arrhythmogenesis of the models. The prolongation of repolarisation was, just as in human LQTS patients, more pronounced at slow heart rates leading to increased steepness of the QT-RR ratio compared to wild-type healthy animals, especially in LQT2 (Brunner et al., 2008). In LQT2 rabbit hearts, an increased spatial dispersion of action potential duration was observed (Brunner et al., 2008; Odening et al., 2013), leading to increased VT/VF inducibility and even spontaneous polymorphic VT and sudden cardiac death (Brunner et al., 2008; Odening et al., 2012), thus representing the first transgenic animal models mimicking the complete electrical phenotype of LQT2 (Table 1). Transgenic LQT1 rabbits, in contrast, presented a more homogeneously prolonged action potential duration without

dispersion of repolarization and developed no spontaneous VT or sudden cardiac death (Brunner et al., 2008) (Table 1).

In transgenic LQT5 rabbits (Major et al., 2016), the mutation in *KCNF1* caused alterations of the biophysical properties of I_{Ks} with accelerated deactivation kinetics. These rabbits demonstrated only a very slightly prolonged QT (Figure 2b) and an increase in short-term beat-to-beat variability of the QT (Major et al., 2016), a newly proposed pro-arrhythmia prediction biomarker (Varkevisser et al., 2012), hence, exhibited no spontaneous arrhythmias at baseline. Due to their reduced repolarization reserve, however, the phenotype could be augmented by I_{Kr} -blocking drug **dofetilide**, which further increased short-term variability of QT and promoted drug-induced VT (Major et al., 2016). Based on above, LQT5 could represent an ideal model to mimic 'concealed' human LQTS with nearly normal baseline phenotype but with an increased susceptibility to drugs with I_{Kr} -blocking properties (Table 1).

Recently, double-transgenic LQT2-5 rabbits have been generated by cross-breeding LQT2 males and LQT5 females. Phenotypically, this LQT2-5 model closely resembles LQT2 due to the lack of I_{Kr} , but it also exhibits pronounced reduction in the function of I_{Ks} that is activated by sympathetic stimuli (Hornyik et al., 2020) (Figure 2b). Therefore, LQT2-5 demonstrates the following added value over LQT2:- (i) it may provide insights into the role of sympathetic nervous system in LQTS-related arrhythmogenesis, (ii) could mimic diseases, such as heart failure and diabetes, with high arrhythmogenic risk due to impaired I_{Ks} in the context of clinically manifest LQTS and (iii) the pro-arrhythmic/anti-arrhythmic effects of pharmacological reduction or increase of I_{Ks} could be investigated (Hornyik et al., 2020) (Table 1).

4.2 | Transgenic LQTS rabbits to study LQTS-related arrhythmogenic mechanisms

Clinical registry data suggest genotype-specific arrhythmogenic triggers in LQTS patients. A constantly elevated adrenergic tone during physical exercise (particularly during swimming) has been identified to promote arrhythmia in LQT1, whereas a sudden sympathetic surge in episodes of rest through emotional stress and (auditory) startle (e.g. a ringing alarm clock) may trigger arrhythmia in LQT2 (Morita et al., 2008). In addition, changes in serum ion concentrations (Pezhouman et al., 2015) or in hormone levels may increase arrhythmogenicity. Adult women with LQT1 and LQT2 are at a higher arrhythmogenic risk than men and women with LQT2 have a particularly high arrhythmogenic risk during the postpartum period (Sauer et al., 2007). In addition to the presence of these pro-arrhythmic triggers that initiate triggered activity, an arrhythmogenic substrate, for example an enhanced spatial and temporal dispersion of repolarization, has been proposed as a prerequisite for arrhythmia formation in human LQTS (Antzelevitch, 2007; Liu et al., 2012).

In *transgenic LQT2 rabbit models*, ventricular arrhythmia and sudden cardiac death often occur in stressful situations such as mating or after anaesthesia (Brunner et al., 2008; Odening et al., 2012) and were

particularly pronounced in situations of altered sex hormone levels during the postpartum phase and with chronic **estradiol** treatment (Brunner et al., 2008; Odening et al., 2012), suggesting the existence of similar arrhythmia-triggering mechanisms as in human LQTS patients (Figure 2c). These LQTS rabbit models are thus particularly suitable for use in investigating arrhythmogenic substrate, pro-arrhythmic triggers and potential anti-arrhythmic treatment options in detail.

4.2.1 | Arrhythmia substrate: role of spatial and temporal dispersion of repolarization

Human data: The hallmark of LQTS is a prolonged QT interval. However, this prolongation of repolarization is not homogeneous in the heart. In LQTS patients, increased dispersion of QT and prolonged $T_{peak-end}$ intervals has been recorded, denoting regional and transmural heterogeneities in the prolongation of action potential duration (Lubinski et al., 1998; Priori et al., 1994). Similarly, new non-invasive imaging techniques (electrocardiographic imaging, ECGi) revealed regional dispersion of repolarization also between LV and RV in LQTS patients (Vijayakumar et al., 2014). In addition, temporal dispersion of repolarization has been evidenced by increased short-term variability of the QT in LQTS patients (Hinterseer et al., 2009) and by T wave alternans, which often precede ventricular tachyarrhythmia in LQTS patients (Wilson & Rosenbaum, 2007; Zareba et al., 1994).

Studies in transgenic LQT1 and LQT2 rabbits highlight the major role of an enhanced dispersion of repolarization in LQTS-related arrhythmogenesis: In LQT2 rabbit hearts, a pronounced dispersion of repolarization was identified in LV and RV (Brunner et al., 2008; Odening et al., 2010, 2013), which caused unidirectional functional block and re-entry formation (Brunner et al., 2008) (Figure 3). Dispersion of repolarization can also occur in a dynamic spatio-temporal fashion with pronounced beat-to-beat alternations and 'out-of-phase' heterogeneities between adjacent regions, termed 'discordant alternans'. In transgenic LQT2 rabbit hearts, these discordant alternans preceded VT / VF formation (Ziv et al., 2009). It is important to note, that the interaction of mutant HERG with wild-type α -subunits of rabbit KvLQT1 that led to decreased I_{Ks} as well contributed to an even more pronounced reduction of repolarisation reserve and potentially aggravated arrhythmogenic phenotype in LQT2. In contrast, in LQT1 hearts lacking regional or temporal dispersion of repolarization (Brunner et al., 2008; Odening et al., 2010, 2013; Ziupa et al., 2014), no VT/VF could be induced, suggesting that a regionally more homogeneous action potential duration prolongation may exert a protective anti-arrhythmic effect (Figure 3). When LQT1 hearts were further stressed, however, by continuous tachypacing to evoke tachycardia-induced cardiomyopathy, action potential duration dispersion increased, spatially discordant alternans developed and VT/VF was easily inducible (Lau et al., 2015). Importantly, discordant action potential duration alternans was preceded by particularly pronounced Ca^{2+} alternans; and both Ca^{2+} and voltage alternans could be

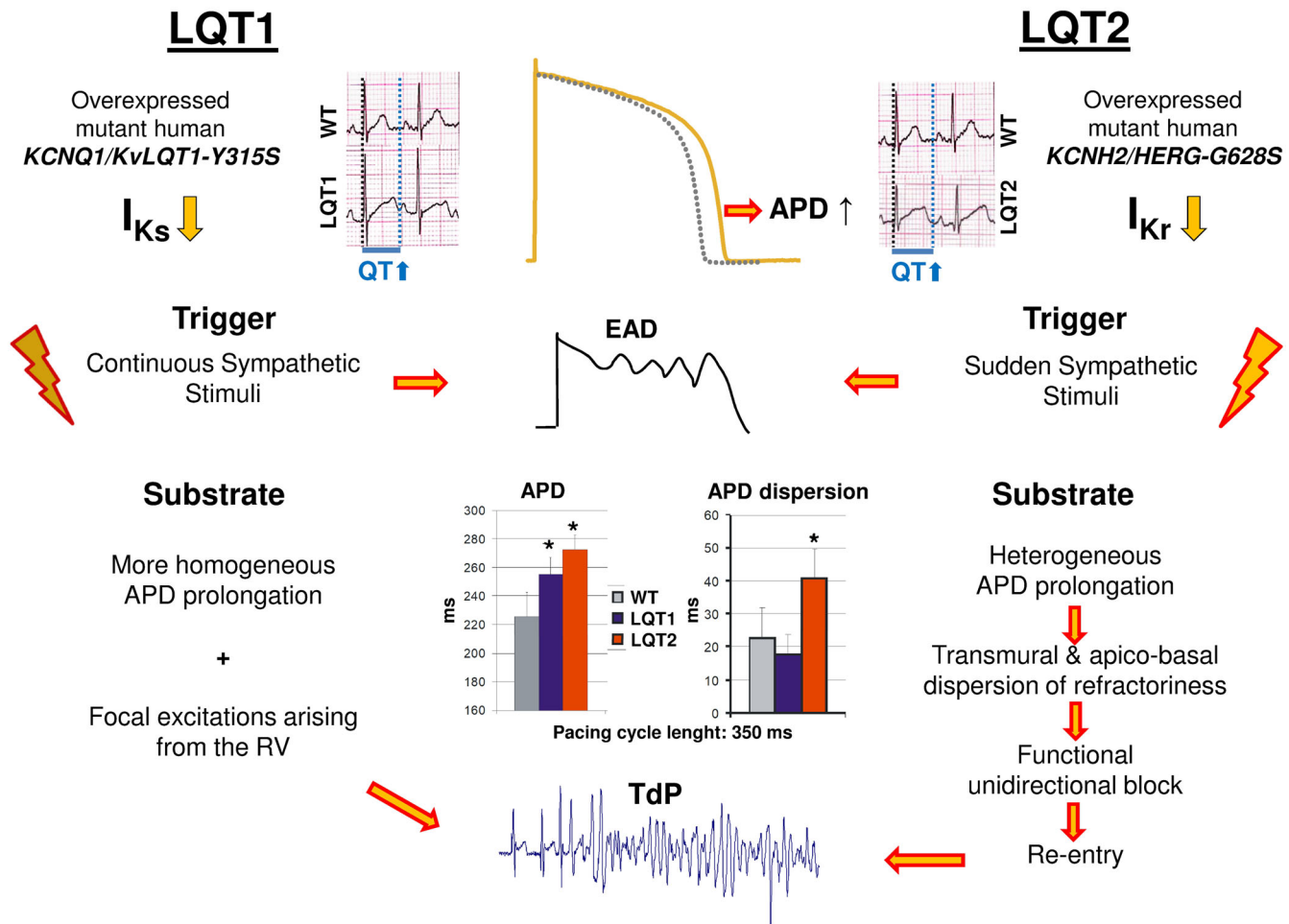


FIGURE 3 Differential arrhythmia mechanisms in transgenic LQT1 and LQT2 rabbits. Schematic illustration of the different genetic backgrounds leading to action potential duration (APD)/QT prolongation (due to loss of I_{Ks} or I_{Kr}) and differential arrhythmia initiating (trigger) and maintaining (substrate) mechanisms in LQT1 and LQT2 models

abolished by **ryanodine**, underlining the importance of Ca^{2+} -handling for discordant alternans and arrhythmia formation in LQTS (Lau et al., 2015).

Thus, these data suggest that an ‘arrhythmogenic substrate’ with an enhanced spatial and/or temporal dispersion of repolarization is of major importance in LQTS-related arrhythmogenesis.

4.2.2 | Arrhythmia triggers: role and mechanisms of early afterdepolarization

Clinical registry data suggest genotype-specific arrhythmic triggers in LQTS patients: The constantly elevated adrenergic tone during physical exercise (particularly during swimming) has been determined to promote arrhythmia in LQT1, whereas a sudden sympathetic surge in episodes of rest through emotional stress and (auditory) startle may trigger arrhythmia in LQT2 (Morita et al., 2008; Schwartz et al., 2001).

In line with these observations, genotype-specific differences in the mechanisms of early afterdepolarization formation and arrhythmia

initiation were demonstrated in *LQT1* and *LQT2* rabbits. In LQT2 cardiomyocytes, early afterdepolarizations developed during sudden sympathetic surge, whereas continuous perfusion with **isoproterenol** prevented early afterdepolarization formation. In LQT1 cardiomyocytes, in contrast, continuous adrenergic stimulation facilitated the occurrence of early afterdepolarizations (Liu et al., 2012) (Figure 3). Different time courses in the sympathetic modulation of cardiac ion currents may explain why different sympathetic modes are associated with arrhythmia formation in different genotypes of LQTS. Upon sudden sympathetic surge, the activation of inward $I_{Ca,L}$ (Liu et al., 2012), that may elicit early afterdepolarizations, is faster than the activation of outward I_{Ks} , therefore the more slowly activated I_{Ks} cannot counterbalance the effect of increased $I_{Ca,L}$, which favours arrhythmia formation in LQT2. On the other hand, continuous adrenergic stimulation, during sportive activity, will result in activation of both inward $I_{Ca,L}$ and outward I_{Ks} , hence leading to no arrhythmia in LQT2. Whereas, the lack of I_{Ks} in LQT1 in the same setting (continuous sympathetic tone) results in unopposed activation of $I_{Ca,L}$, and thereby, to arrhythmia formation (Liu et al., 2012). In addition, different modes of arrhythmia initiation and maintenance were identified in

different LQTS genotypes. Although in LQT2, re-entry formation played an important role (Brunner et al., 2008), in LQT1 hearts, a novel mechanistic concept of LQTS-related arrhythmogenesis has been identified. Arrhythmia was initiated by focal excitations arising particularly from the RV and was maintained by multiple shifting excitation foci and bi-excitability (Kim et al., 2015) (Figure 3). In transgenic LQT5 rabbits with accelerated I_{Ks} deactivation, an I_{to} -mediated alternans formation (that is based on remodelling-based I_{to} enhancement) has been revealed as another mechanism of arrhythmogenesis in LQTS (Kim et al., 2020).

4.2.3 | Bradycardia and AV conduction alterations

Clinical observations indicate that bradycardia and pause-related short-long-short sequences precede ventricular tachyarrhythmia, predominantly in LQT2 patients (Noda et al., 2004; Tan et al., 2006). AV conduction block due to markedly prolonged ventricular refractoriness, termed ‘pseudo-AV block’ by Rosenbaum and Acunzo (Rosenbaum & Acunzo, 1991), as well as true infranodal AV block, have been described in LQTS (Ben Caref et al., 2008). The occurrence of AV conduction block is infrequent but associated with elevated arrhythmogenic risk (Gorgels et al., 1998; Pruvot et al., 1999; Rosenbaum & Acunzo, 1991; Sakaguchi et al., 2008).

In LQT2 rabbits, slow heart rates, short-long-short sequences, bigeminy and R-on-T phenomena were recorded prior to VT (Brunner et al., 2008), similarly as in human LQTS patients. In addition, several episodes of AV conduction block were detected prior to VT/VF in LQT2, but not in LQT1 rabbits (Brunner et al., 2008; Odening et al., 2008). *In vivo* electrophysiology studies revealed that LQT2 rabbits developed infra-His blocks and decremental His conduction during **isoflurane** anaesthesia, whereas LQT1 rabbits exhibited altered slowed His conduction and intra-His block when exposed to I_{Kr} blockade with dofetilide (Odening et al., 2010), suggesting genotype-specific differences also in the His–Purkinje system.

4.3 | Transgenic LQTS rabbits to investigate pro-arrhythmic and anti-arrhythmic effects of drugs and hormones

The term ‘repolarization reserve’ indicates the physiological ability of cardiomyocytes to maintain a sufficiently normal repolarization even when exposed to drugs blocking one repolarizing K^+ current by compensation via non-affected ‘reserve’ outward K^+ currents (Roden, 1998; Varro & Baczko, 2011). As transgenic LQTS rabbit models demonstrate a reduced repolarization reserve (due to a genetic reduction of repolarizing ion currents), they may serve as particularly sensitive tools for *in vivo* and *ex vivo* drug testing to identify potential pro-arrhythmic ion channel blocking drugs. Similarly, they may be used to investigate genotype-specific efficacy of ion channel-activating drugs and their potential application as genotype-specific therapies in LQTS.

4.3.1 | Investigation of pro-arrhythmic effects of drugs

LQT1 rabbits lacking I_{Ks} and LQT5 rabbits with impaired I_{Ks} currents both proved particularly sensitive in identifying I_{Kr} -blocking properties of drugs; demonstrating pronounced I_{Kr} blocker-induced action potential duration/QT prolongation, increased spatial dispersion of action potential duration, increased short-term-variability of QT and increased arrhythmia formation (Major et al., 2016; Odening et al., 2008, 2010; Ziupa et al., 2014) (Table 1). Similarly, transgenic LQT2 rabbits lacking I_{Kr} demonstrated a particularly high sensitivity to I_{Ks} - or I_{K1} -blocking anaesthetic agents or other drugs (Hornyik et al., 2020; Odening et al., 2010) (Table 1). Recently, a new study demonstrated that various transgenic LQTS rabbit models (LQT2, LQT5 and LQT2-5) did not only show more pronounced changes in pro-arrhythmia markers in response to different K^+ channel blockers but also exhibited higher drug-induced incidence, longer duration and more malignant type of *ex vivo* arrhythmias than wild-type rabbit hearts (Hornyik et al., 2020) (Table 1). This was particularly pronounced in LQT2 and LQT2-5 rabbit models (Hornyik et al., 2020). Therefore, the combined use of different LQTS models were proposed as potential tools for more reliable and more thorough prediction of (multi-channel-based) pro-arrhythmic potential of novel drug candidates.

4.3.2 | Investigation of anti-arrhythmic effects of drugs

Once heterogeneously prolonged repolarization was identified as a prerequisite for LQTS-related arrhythmogenesis, its pharmacological reduction was investigated as a possible (genotype-specific) therapeutic strategy in LQTS rabbit models. The effects of cardiac ion channel activators **nicorandil** (an antianginal drug that opens ATP-sensitive K^+ channels) and **NS1643** (a HERG/ I_{Kr} activator) were assessed in transgenic LQT1 rabbits (Bentzen et al., 2011; Biermann et al., 2011) (Table 1). Although both activators shortened cardiac repolarization, NS1643 displayed pro-arrhythmic effects in LQT1, underlining the difficulty of rescuing the LQTS phenotype with ion channel activators. They may have an ‘over-compensatory’ effect on cardiac repolarization and may actually increase dispersion of repolarization, just as the disease-causing mutation itself. I_{NaLate} was also identified as a target for novel anti-arrhythmic agents in LQT1 and LQT2 rabbits: The blockade of Na^+ currents through **tetrodotoxin** (blocks I_{NaLate} and I_{Na}) and **ranolazine** (blocks I_{NaLate}) prevented early afterdepolarization formation and transformed polymorphic VTs into monomorphic VTs in a LQT1 tachypacing-induced cardiomyopathy model (Lau et al., 2015). Similarly, the more specific I_{NaLate} inhibitor **GS-458967** was recently shown to suppress polymorphic VT formation in LQT2 rabbit hearts by accelerating Na^+ / Ca^{2+} exchanger (I_{NCX})-mediated Ca^{2+} efflux, shortening Ca^{2+} transient duration and reducing Ca^{2+} -mediated early afterdepolarization formation (Hwang et al., 2020).

4.3.3 | Investigation of pro-arrhythmic and anti-arrhythmic effects of hormones

Clinical registry data have provided evidence for pronounced sex differences in arrhythmic risk in LQTS patients with an increased risk for cardiac arrhythmic events in women after puberty and a particularly high risk during the postpartum (particularly in LQT2 patients) (Sauer et al., 2007), strongly suggesting that changing sex hormone levels may affect LQTS-related arrhythmogenesis. Consequently, identifying pro-arrhythmic and anti-arrhythmic effects of sex hormones has become an emerging field of interest in LQTS research, with the goal of revealing underlying molecular mechanisms and identifying novel potential therapeutic targets (Odening & Koren, 2014).

As in *transgenic LQT2 rabbits* ventricular arrhythmia and sudden cardiac death also often occurred postpartum-related (Brunner et al., 2008; Odening et al., 2012), suggesting the existence of similar arrhythmia-triggering mechanisms as in human LQTS patients. These models were further utilised to explore sex hormone effects on arrhythmic triggers and the arrhythmic substrate (Odening et al., 2012) (Table 1): Estradiol exerted a pro-arrhythmic effect by changing action potential duration dispersion and increasing early afterdepolarization formation upon sympathetic stimuli, whereas **progesterone** had an anti-arrhythmic, protective effect due to a shortening of cardiac refractoriness, reduced formation of early afterdepolarization and stabilising Ca^{2+} effects (Odening et al., 2012). Further studies revealed that progesterone increased **SERCA** by slowing its degradation, thereby shortening the decay and duration of Ca^{2+} transients (Moshal et al., 2014). These studies suggest that progesterone-based therapies may be considered as novel anti-arrhythmic approaches in female LQTS patients. Recently, potentially harmful, pro-arrhythmic effects of the postpartum hormones **oxytocin** and **prolactin**, which both acutely block I_{Ks} , have been revealed in LQT2 rabbit models (Bodi et al., 2019) that might contribute to the clinical observation of an increased postpartum arrhythmic risk—particularly in LQT2 (Sauer et al., 2007).

4.4 | Transgenic LQTS rabbits provide insights into electro-mechanical dysfunction in LQTS

In recent years, clinical evidence has accumulated that in the ‘electrical’ disease LQTS mechanical function is also sub-clinically altered—due to electro-mechanical interactions. With the help of novel echocardiography or MRI techniques that allow to investigate regional tissue velocities and strain, an impaired diastolic relaxation, prolonged contraction duration and negative electro-mechanical window has been identified in LQTS patients (Brado et al., 2017; Haugaa et al., 2010; Nador et al., 1991; ter Bekke et al., 2015). As contraction duration was even more prolonged in symptomatic than in asymptomatic patients and differed depending on the specific LQTS genotype (more pronounced in LQT2 than in LQT1) (Haugaa et al., 2009, 2010; Leren et al., 2015),

these mechanical parameters may be helpful as new variables for risk stratification in LQTS.

In transgenic LQTS rabbits, phase-contrast-based cardiac MRI was employed to further investigate regional mechanical cardiac function. These studies demonstrated a regionally heterogeneously reduced diastolic function and prolonged contraction duration, leading to an increased mechanical dispersion in transgenic LQT2 rabbits (Odening et al., 2013) (Table 1). In addition, a spatial correlation between the extent of electrical dysfunction (prolongation of action potential duration) and impaired diastolic function (Odening et al., 2013; Ziupa et al., 2019), and a correlation between the extent of mechanical dysfunction/heterogeneity and arrhythmic risk was revealed (Lang et al., 2016). Moreover, studies in LQT2 rabbits could demonstrate sex differences and sex hormone effects not only on electrical function but also on mechanical function with longer contraction duration in female and estradiol-treated animals. Importantly, similarly as in human LQTS patients, genotype differences were observed with less pronounced mechanical/diastolic relaxation impairment in LQT1 (Ziupa et al., 2019) compared to LQT2 rabbit models. LQTS rabbit models may thus help to reveal mechanisms underlying the observed mechanical dysfunction and its potential causative link to arrhythmogenesis.

5 | TRANSGENIC SHORT-QT SYNDROME RABBIT MODELS TO STUDY HUMAN SHORT-QT SYNDROME

Short-QT syndrome (SQTS) is a rare, but potentially lethal inherited channelopathy characterised by accelerated cardiac repolarization and shortened QT interval duration in ECG ($\text{QTc} < 320\text{--}340\text{ ms}$) (Giustetto et al., 2006). The phenotype of SQTS patients is highly variable—ranging from asymptomatic patients to those presenting with arrhythmia-associated symptoms such as dizziness, syncope or sudden cardiac death (Mazzanti et al., 2014). 40% of patients present with sudden cardiac death due to VT/VF as their initial symptom (Mazzanti et al., 2014). In addition to ventricular arrhythmia, patients are prone to develop early-onset, familial atrial fibrillation (AF) (Giustetto et al., 2006). Causative mutations have been identified in eight different genes: SQTS types 1–3 are associated with gain-of-function mutations in K^+ channel genes, whereas SQTS types 4–6 are caused by loss-of-function mutations in Ca^{2+} channel genes and the extremely rare SQTS types 7 and 8 are caused by mutations in Na^+ or $\text{Cl}^-/\text{HCO}_3^-$ channel genes (Campuzano et al., 2018).

Clinical data indicate that arrhythmias often occur at rest, that the QT interval remains relatively short at slow heart rates (shallow QT-RR ratio) and that arrhythmias can be evoked during invasive electrophysiological studies by programmed ventricular stimulation (Borggrefe et al., 2005). Therapeutic options are currently limited to cardioverter defibrillator implantation or hydroquinidine therapy (Mazzanti et al., 2017). In general, our knowledge on arrhythmia mechanisms, triggers and consequences for risk stratification and therapy is limited, likely due to the small number of SQTS cases

characterised to date (<200). This calls for adequate animal models to further explore disease mechanisms.

Recently, a *transgenic rabbit model for SQTs type 1*, carrying the disease-specific human mutation *KCNH2-N588K* has been generated and characterised (Odening et al., 2019) (Table 1). Using the same approach as for the transgenic LQTS models, this SQT1 rabbit model has been engineered by cardio-selective over-expression of a dominant gain-of-function mutated human *KCNH2* gene under the control of the beta-myosin heavy chain promoter (Odening et al., 2019). The SQT1 rabbit model mimics the human disease phenotype with shortened QT interval, shallower QT/RR slope, shortened atrial and ventricular refractoriness and shortened atrial and ventricular action potential duration, as well as increased VT/VF and AF inducibility. This phenotype results from an increase in steady-state I_{Kr} current due to impaired channel inactivation (Odening et al., 2019). Cellular and *in vivo* electrophysiological experiments revealed that the multi-channel blocker **hydroquinidine** normalised QT and action potential duration in SQT1 rabbits by reducing the pathologically enhanced I_{Kr} . Moreover, also in this ‘electrical’ disease, mechanical alterations such as improved/fastened diastolic relaxation and mechanical dispersion have been revealed (Odening et al., 2019). The model will help to further characterise arrhythmia mechanisms and test potential novel anti-arrhythmic treatment options.

6 | OUTLOOK—POTENTIAL NOVEL TRANSGENIC RABBIT MODELS FOR CARDIAC DISEASE RESEARCH

In the field of cardiac disease research, thus far, rabbit models have been generated using classical transgenesis techniques with pronuclear microinjection. These classical transgenesis models, however, have limitations in transgenic efficiency, the size of the genes that can be targeted and the fact that these models overexpress the human mutant gene and two copies of the endogenous rabbit gene and thus do not mimic the genetic heterozygous pattern observed in patients with autosomal-dominant diseases, as indicated in detail in Section 2. An example that clearly illustrates these limitations in relation to the gene size is the attempt to generate a rabbit model for catecholaminergic polymorphic ventricular tachycardia (CPVT) that is based on mutations in the ryanodine receptor gene (**RYR2-R4497C**): the attempt failed as only a truncated part of the initial very big construct was integrated and expressed as mRNA (Wakula et al., 2011).

In the field of atherosclerosis research, genetically modified rabbit disease models have already been generated using CRISPR/Cas9 to knock-out genes of interest (reviewed in (Matsuhisa et al., 2020). With further modification and improvement of this technique, the insertion of targeted point mutations will also be feasible, thus allowing the generation of transgenic rabbit models for monogenic cardiac diseases that not only mimic the human diseases phenotypically but also mimic the underlying genetic heterozygote structure. Important to note, however, the genetically relatively homologue

laboratory transgenic rabbit model lines cannot fully ‘phenocopy’ the broad diversity of genetic backgrounds of humans with monogenic cardiac diseases and therefore cannot recapitulate, for example single nucleotide polymorphisms of human patients which may influence the risk of arrhythmia. Novel models for channelopathies like Brugada syndrome or catecholaminergic polymorphic ventricular tachycardia with point mutations in larger genes, such as the *SCN5A* or the **RyR2** gene, might thus be feasible. In general, the generation of additional autosomal-dominant channelopathies or cardiomyopathies rabbit models will be obtained much faster than with the techniques that have been used to date.

Additionally, transgenic rabbit models are not only developed to mimic human cardiac diseases. Attempts are made to specifically target specific cardiac cell types to mechanistically investigate cell–cell (cardiomyocyte–cardiofibroblast) interactions in a variety of cardiac diseases in which fibrosis plays a pathophysiological role.

In summary, transgenic rabbits have proven to be an indispensable tool to study complex *in vivo* effects of diseases not properly emulated by other animals or models, and hold promise to further our knowledge substantially in the future.

6.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY <http://www.guidetopharmacology.org> and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Kelly, et al., 2019; Alexander, Mathie, et al., 2019).

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AUTHOR CONTRIBUTIONS

T. Hornyik, M. Rieder, A. Castiglione, P. Major and K. Odening wrote and edited the manuscript; I. Baczko, M. Brunner and G. Koren edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

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