Transgenic Animal Models of Cardiac Channelopathies: Benefits and Limitations

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Abstract

Ideally, studies investigating pathophysiological mechanisms of human arrhythmia disorders should be performed in human subjects, their hearts, tissue, and cells. Human cardiac tissues, however, are not easily accessible to experimental electrophysiologists. Therefore, transgenic animal models (mouse, rabbit, and pig) mimicking (several aspects of) the human disease phenotype have been generated and utilized to gather mechanistic insight into cardiac channelopathies.

In this overview, we summarize advantages, limitations, and translational value of the different available genetic animal models (mouse, rabbit, and pig) for potassium channelopathies (long QT syndromes), sodium channelopathies (LQT3, Brugada syndrome, cardiac conduction disease, and overlap syndrome), and catecholaminergic polymorphic ventricular tachycardia (CPVT).

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15.1 Introduction

To increase our understanding of the pathophysiology of human diseases, ideally, human subjects, their organs, tissues, and cells should be studied. However, in-depth mechanistic studies on alterations of cardiac electrophysiology and arrhythmogenesis in channelopathies can only be performed to a very limited extent in human patients. Particularly, mechanisms of arrhythmogenesis can only be assessed on certain levels, e.g., in vivo and exceptionally—if available—on cellular levels in isolated or induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) (Hoekstra et al. 2012), while assessment of whole human hearts remains an exception (Opthof et al. 2017). Similarly, although the expression and biophysical characterization of mutated cardiac ion channels in heterologous cellular expression systems have increased our understanding of the electrophysiological basis of cardiac channelopathies (Nerbonne et al. 2001; Nerbonne and Kass 2005), these cells cannot reflect the endogenous cardiomyocyte environment and any potential electrical remodeling that may occur in it due to the disease-specific ion channel mutations.

The advantage of using animals over human patients (or cellular systems) for mechanistic studies is that animal models allow (1) to identify pathophysiological mechanisms on multiple levels and (2) to conduct longitudinal studies in subjects with a defined genetic background and without confounding comorbidities for the assessment of factors that may alter the arrhythmic disease phenotype, allowing not only observations but also defined quantitative pro- or anti-arrhythmic interventions. This comes at the cost, however, of partially limited clinical transferability due to some species differences in features of cardiac electrical function (Nerbonne et al. 2001; Salama and London 2007; Baczkó et al. 2016) that are responsible for incomplete recapitulation of different aspects of the human disease. Therefore, animal models that capture disease-specific essential aspects of human cardiac pathophysiology (e.g., repolarizing ion channel function for long QT syndromes, sodium channel function for Brugada syndrome and long QT type 3, and Ca^{2+} handling/ryanodine receptor properties for CPVT) are required for improving our understanding of the complex, multidimensional alteration of physiological cardiac function in channelopathies with the goal of "bench-to-bedside" translation (Odening and Kohl 2016).

Both drug-induced and genetically modified animal models of various species have been generated and utilized to investigate arrhythmic mechanisms in different channelopathies [reviewed in Nerbonne et al. (2001), Salama and London (2007), Nattel et al. (2008), Derangeon et al. (2012), Choy et al. (2016) and Lang et al. (2016b)]. One main shortcoming of drug-induced animal models, however, is the fact that most ion channel-blocking or ion channel-activating drugs are not channel-selective, thus causing a "mixed" disease phenotype. In addition, drugs have to be administered continuously to sustain the drug-induced "channelopathy," thus impeding detailed investigation of arrhythmic mechanisms and triggering free-moving, non-anesthetized animals. Therefore, transgenic or genetically modified animal models for channelopathies are generated aiming at (1) mimicking the human disease genotype and phenotype and (2) gathering insights into disease-

specific electrophysiological cardiac function and mechanisms of arrhythmogenesis on cellular, tissue, organ, and in vivo levels and (3) for "bench-to-bedside" translation to improve diagnostic and therapeutic strategies in patients with channelopathy.

Small animals such as mice and rabbits are among the most commonly used animal models in cardiac research, since they have relatively short generation times and their handling is rather cost-effective. Most importantly, they have the added advantage that they can be more easily subjected to genetic manipulation than larger animals. Therefore, despite apparent differences between human and mouse cardiac electrophysiology [reviewed in Nerbonne et al. (2001), Salama and London (2007) and Baczkó et al. (2016)], the first (and most) genetic channelopathy models were mouse models [reviewed in Nerbonne et al. (2001), Salama and London (2007), Derangeon et al. (2012) and Choy et al. (2016)]. Thanks to novel developments in animal transgenesis (Bősze et al. 2016), rabbits—representing a species that mimics human cardiac electrophysiology surprisingly well (Nerbonne 2000; Valentin et al. 2004; Hondeghem 2016)—have also entered the range of species in whom genetic manipulation can successfully replicate certain human cardiac diseases (Sanbe et al. 2005; Brunner et al. 2008; Major et al. 2016). Last but not least, a much larger species, the pig—which even more closely resembles humans—has recently been successfully modified genetically to represent a channelopathy model (Park et al. 2015).

In the following, we will summarize advantages, limitations, and translational value of the different currently available genetic (mouse, rabbit, and pig) animal models for cardiac channelopathies: potassium channelopathies (long QT syndromes; Tables 15.1, 15.2 and 15.3), sodium channelopathies (LQT3, Brugada syndrome, cardiac conduction disease, and overlap syndrome; Table 15.4), and catecholaminergic polymorphic ventricular tachycardia (CPVT; Table 15.5). The different diseases and their clinical manifestation and treatment options, however, are not reviewed again in detail; here the interested readers are encouraged to refer to the respective preceding chapters in this volume.

15.2 Available Transgenic Animal Models of Cardiac Channelopathies

15.2.1 Transgenic Animal Models for Long QT Syndrome Based on Potassium Channel Mutations

Long QT syndrome (LQTS) is an inherited channelopathy characterized by prolonged QT duration as manifestation of a prolonged cardiac repolarization (Priori et al. 2001a). The disease is predominantly caused by autosomal dominant mutations in genes encoding for repolarizing potassium channels (90%, *KCNQ1*: LQT1, *KCNH2*: LQT2) and depolarizing sodium channels (5%, *SCN5A*: LQT3) (Priori et al. 2001a). Patients are prone to develop polymorphic *torsade de pointes* ventricular tachycardia (VT) and sudden cardiac death (SCD).

Table 15.1 LQTS—mouse models with alterations of mouse potassium channels

Channel subunit	Modification	Current	APD prolongation	QT prolongation	Arrhythmia	Reference
Kv1.1	Kv1.1 DN	↓IK,slow	Yes	Yes	Yes, spontaneous and inducible	London et al. (1998a)
Kv1.4	$Kv1.4^{-/-}$	↓Ito,s	No	No	No	London et al. (1998b)
Kv1.5	Kv1.5 replaced with Kv1.1 (SWAP)	↓ IK, slow1 ↑ IK, slow2	No	No	No	London et al. (2001)
	Kv1.5 DN	↓ Ito,s ↓ IK, slow1	Yes	Yes	No	Li et al. (2004)
Kv2.1	Kv2.1 DN	↓ IK, slow2	Yes	Yes	Yes, spontaneous and inducible	Xu et al. (1999)
Kv4.2	Kv4.2 DN	↓ Ito,f	Yes	n.d.	n.d.	Wickenden et al. (1999)
	Kv4.2 DN	↓ Ito,f ↑ Ito,s	Yes	Yes	No	Barry et al. (1998)
	$Kv4.2^{-/-}$	↓ Ito,f ↑ Ito,s	No	No	n.d.	Guo et al. (2005)
KChIP2 (subunit to Kv4.2 and Kv4.3)	$KChIP2^{-/-}$	↓ Ito,f	Yes	No	Yes, inducible	Kuo et al. (2001)
Multiple channels (cross)						
Kv1.1 + Kv2.1	Kv1.1 DN × Kv2.1 DN	↓ Ito,s ↓ IK, slow1 ↓ IK, slow2	Yes	Yes	Yes, spontaneous and inducible	Kodirov et al. (2004)

Kv1.1 + Kv4.2	Kv1.1 DN × Kv4.2 DN	↓ Ito,f ↓ Ito,s ↓ IK, slow1	Yes	Yes	No	Brunner et al. (2001)
Kv1.4 + Kv4.2	$Kv1.4^{-/-}$ × Kv4.2 DN	↓Ito,f ↓Ito,s	Yes	Yes	Yes, spontaneous	Guo et al. (2000)

Abbreviations: −/−, homozygous knockout; *DN* dominant-negative, *n.d* not done

Table 15.2 LQTS—mouse models with alterations of "human" potassium channels or mouse-equivalent genes

Human disease and gene	Modification	Current	APD prolongation	QT prolongation	Arrhythmia	Reference
LQT1: Kcnq1/ KCNQ1	$Kcnq1^{-/-}$ (exon 1)	n.d.	n.d.	No	n.d.	Lee et al. (2000)
	$Kcnq1^{-/-}$ (exon 2)	n.d.	No	Yes	n.d.	Casimiro et al. (2001)
	KCNQ1 DN (TG)	↓ IKs	Yes	Yes[a]	No	Demolombe et al. (2001) and Lande et al. (2001)
LQT2: Kcnh1/ Merg/KCNH2/ HERG	$Merg^{+/-}$	n.d.	Yes	n.d.	Yes, induced VT	Salama et al. (2009)
	$Merg1b^{-/-}$	↓ IKr	n.d.	No	No	Lees-Miller et al. (2003)
	HERG1-G628S DN (TG)	↓ IKr	Yes/No[b]	No	No	Babij et al. (1998)
LQT5: Kcne1/ KCNE1	Kcne1/ $minK^{-/-}$	↓ IKs	n.d.	Yes[c]	n.d.	Drici et al. (1998)
	Kcne1/ $minK^{-/-}$	n.d.	No	n.d.	n.d.	Charpentier et al. (1998)
	Kcne1/ $minK^{-/-}$	n.d.	Yes + APD alternans/ dispersion	n.d.	Pacing- and isoprenaline-induced arrhythmia (but also in WT), anti-arrhythmic effect of nifedipine	Balasubramaniam et al. (2003) and Thomas et al. (2007)
	Kcne1/ $minK^{-/-}$	n.d.	No	n.d.	No	Salama et al. (2009)
	minK replaced with lacZ	↓ IKs	No	No	n.d.	Kupershmidt et al. (1999)

LQT7: Kcnj2/ KCNJ2	Kir2.1$^{-/-}$	↓ IK1	Yes	Yes[c]	Spontaneous AP in vitro, but no PVC/VT in vivo	Zaritsky et al. (2000)
	Kir2.1 DN (TG)	↓ IK1	Yes	Yes	No	McLerie and Lopatin (2003)

Abbreviation: −/− homozygous knockout, −/+ heterozygous knockout, *DN* dominant-negative; *TG* transgenic; *AP(D)* action potential (duration), *PVC* premature ventricular contraction, *n.d* not done, *WT* wild type

[a]No QT prolongation induced by dofetilide, E 4031, haloperidol, sultopride, astemizole, cisapride; QT shortening: lidocaine, nicardipine

[b]Yes in single cardiomyocytes, No in ventricular strips

[c]Only during bradycardia

Table 15.3 LQTS—rabbit models based on potassium channel mutations

Human disease and gene	Modification	Curr.	APD prolong.	QT prolong.	Arrhythmia	Clinical implication: alteration by drugs or hormones	Clinical implication: electromechanical insight	Reference
LQT1: KCNQ1	KCNQ1/ KvLQT1-Y315S DN (TG), pore region	↓ IKs (loss)	Yes	Yes	No	*Parameters analyzed*: Assessment of QT interval, EAD, conduction velocities, VERP and VERP dispersion, APD and APD dispersion/ triangulation/alternans *Drugs*: Isoflurane, thiopental, midazolam, propofol, ketamine, isoproterenol, dofetilide, nicorandil (+/− isoproterenol), NS-1643, E-4031, erythromycin, ryanodine, tetrodotoxin, ranolazine [as reviewed in Lang et al. (2016b)]	Electrical: – APD dispersion not increased – VT/VF inducible in tachymyopathy (continuous tachypacing) – Focal excitations arising from the RV initiates arrhythmia – EAD formation with continuous adrenergic stimulation	Brunner et al. (2008), Odening et al. (2008, 2013), Liu et al. (2012), Ziupa et al. (2014), Lau et al. (2015), Kim et al. (2015) and Lang et al. (2016b)
LQT2: KCNH2	KCNH2/ HERG-G628S DN (TG), pore region	↓ IKr (loss)	Yes, even more at slow	Yes, even more at slow heart rate	Yes: Spontaneous TdP resulting in SCD,	*Parameters analyzed*: Assessment of arrhythmia (TdP), QT interval, T wave alternans, EAD,	Electrical: – Increased APD dispersion, leading to unidirectional functional block,	Brunner et al. (2008), Odening et al. (2008, 2010, 2012, 2013), Ziv et al. (2009), Liu

			heart rate		inducible VT/VF	conduction velocities, VERP, VERP dispersion	reentry formation and VF – "discordant alternans" preceded VT/VF – EAD formation with sudden adrenergic surge	et al. (2012) and Lang et al. 2016a, b)
						Drugs: Isoflurane, thiopental, midazolam, propofol, ketamine, isoproterenol, dofetilide [as reviewed in Lang et al. (2016b)]	Mechanical: – Echo/MRI: Normal global function – TPM-MRI: Regional diastolic dysfunction at baseline; particularly prolonged contraction duration in symptomatic animals	
						Hormones: Pro-arrhythmic effect of estradiol (APD dispersion, EAD formation, lethal pVT), anti-arrhythmic effect of progesterone		
LQT5: KCNE1	KCNE1-G52R DN (TG)	↓ IKs	No	Slightly prolonged, increased STVQT	No	Dofetilide provoked TdP and further increase in STVQT	n.d.	Major et al. (2016)

Abbreviations: *DN* dominant-negative, *TG* transgenic, *STVQT* short-term variability of the QT interval, *TPM-MRI* tissue phase mapping MRI

Table 15.4 Animal models for sodium channelopathies (*Scn5a*—LQT3, Brugada syndrome, CCD, and overlap syndrome)

Human disease	Modification	Curr.	APD prolong.	QT prolong.	Conduction disease	Arrhythmia	Clinical implication: evaluation of drug effects	Reference
Features of LQT3 *(gain-of-function)*								
LQT3 (+CCD)	$Scn5a^{+/\Delta KPQ}$ (KI)	↑ INa ↑ INa, L	Yes, EAD in vitro after AV block	Yes	Lower heart rate, sinus pauses, AV block	Yes, spontaneous and induced	– Adrenergic agonists suppressed induced arrhythmias – No arrhythmias were provoked by physical stress, isoproterenol, or atropine – Carbachol induced bigemini and TdP – Propranolol and esmolol did not prevent arrhythmias (except in[a]) – Propranolol prevented carbachol-induced VT/VF[a] – Mexiletine and flecainide suppressed arrhythmias	Nuyens et al. (2001), Fabritz et al. (2003, 2010) and Calvillo et al. (2014)[a]
LQT3 (+CCD)	$Scn5a^{+/\Delta KPQ}$ (KI)	↑ INa ↑ INa, L	Yes, EAD in vitro	n.d.	n.d.	Yes, induced	– Propranolol did not suppress isoproterenol-induced arrhythmia – Mexiletine suppressed arrhythmia	Head et al. (2005)

LQT3 (+CCD)	SCN5A-N1325S (TG)	↑ INa, L	Yes, EAD in vitro	Yes	RR and PR shortened, QRS similar PR shortened	Yes, spontaneous	– Mexiletine shortened APD and suppressed arrhythmia	Tian et al. (2004)
LQT3 (+atrial fibrillation + structural changes)	SCN5A-F1759A (TG)	↑ INa, L	Yes	Yes	PR shortened	Yes, spontaneous PVC, pVT (and AF)	– Diminished use-dependent lidocaine block of INa – Persistent INa resistant to ranolazine – NCX inhibitor SEA-0400 reduced burden to PVC and atrial fibrillation	Wan et al. (2016)
Features of BrS and CCD (*loss-of-function*)								
CCD (+ BrS)	Scn5a$^{+/-}$ (KO)	↓ INa	No	No	P wave, PR, and QRS prolongation	Triggered VT (spontaneous in old)	n.d.	Papadatos et al. (2002)
CCD	Scn5a $_{\Delta SIV/\Delta SIV}$ (KI)	↓ INa[a]	No, but reduced AP upstroke	No	P wave, PR, and QRS prolongation	No	– Flecainid aggravated conduction disease	Shy et al. (2014)
Pig model[b] of BrS (+CCD)	Scn5a$^{E558X/+}$ (KI)	↓ INa	No	No	P wave, PR, and QRS prolonged, atrial-his and his-ventricular conduction delay	– No, in vivo (no SCD, no arrhythmia)– Yes, ex vivo (spontaneous and pacing-induced, VF at 39 °C, stable at 35 °C)	– Combination of propranolol and atropine did not provoke arrhythmia– Flecainide induced PR and QRS prolongation, but not BrS-type ECG changes	Park et al. (2015)

(continued)

Table 15.4 (continued)

Human disease	Modification	Curr.	APD prolong.	QT prolong.	Conduction disease	Arrhythmia	Clinical implication: evaluation of drug effects	Reference
Features of overlap syndromes (and biophysical overlap)								
Overlap: −LQT3—BrS—CCD	Scn5a $^{1798insD/+}$ (KI)	↓ INa ↑ INa, L	Yes	Yes	RR, PR, and QRS prolonged, RV conduction slowing	Sinus pauses in vivo, EADs in vitro	– Flecainide induced sinus bradycardia and/or sinus arrest	Remme et al. (2006)
Overlap: LQT3—CCD	SCN5A-D1275N(TG)	↓ INa ↑ INa, L	Yes	Yes	P wave, PR, and QRS prolongation	Yes, spontaneous mVT/pVT	n.d.	Watanabe et al. (2011)

Abbreviations: *KI* knock-in, *KO* knockout, *TG* transgenic, *CCD* cardiac conduction disease, *BrS* Brugada syndrome, *INa* peak sodium current, *INa,L* late sodium current, *PVC* premature ventricular contraction

[a]Decreased INa most likely due to defects in cell surface expression of sodium channel

[b]With the exception of this pig model, all other models are mouse models

Table 15.5 Mouse models for catecholaminergic polymorphic VT

Gene	Modification	Normal ECG at baseline	Arrhythmia (trigger)	Clinical implication: evaluation of drug effects	Reference
FKBP12.6	$FKBP12.6^{-/-}$ (KO)	Yes	Yes (exercise)	n.d.	Wehrens (2003)
RyR2	$RyR2^{R4496C/+}$ (KI)	Yes	Yes (catecholamines)	Mice pretreated with propranolol developed VT	Cerrone et al. (2005, 2007) and Fernández-Velasco (2009)
	$RyR2^{R176Q/+}$ (KI)	Yes	Yes (programmed stimulation, catecholamines, caffeine)	n.d.	Kannankeril (2006)
	$RyR2^{P2328S/+}$ $RyR2^{P2328S/P2328S}$ (KI)	Yes	Yes (programmed stimulation, catecholamines, caffeine)	Catecholamines and caffeine reduced myocardial conduction velocity	Goddard (2008) and Zhang (2013)
	$RyR2^{R2474S/+}$ (KI)	Yes	Yes (exercise, catecholamines)	Dantrolene prevented inducible VT	Kobayashi et al. (2010)
	$RyR2\text{-}^{S2246L/+}$ (KI)	Yes	Yes (exercise)	Dantrolene stopped the exercise-induced ventricular tachycardia	Suetomi et al. (2011)
	$RyR2^{+/Ex3\text{-}del}$ (KO)	No (bradycardia)	No	n.d.	Liu et al. (2014)
	$RyR2^{A4860G/+}$ (KI)	n.d.	Yes (catecholamines)	n.d.	Zhao (2015)
CASQ2	$CASQ2^{\Delta E9/\Delta E9}$ (KO)	Yes	Yes (spontaneous, exercise, catecholamines)	– Flecainide (but not lidocaine) suppressed VT (conflicting data in and) – Propafenone prevented exercise-induced CPVT (but not procainamide or lidocaine) – Propranolol and sotalol had little anti-arrhythmic effect during exercise	Knollmann (2006), Song et al. (2007), Watanabe et al. (2009), Katz et al. (2010), Hwang et al. (2011) and Kurtzwald-Josefson et al. (2014)

(continued)

Table 15.5 (continued)

Gene	Modification	Normal ECG at baseline	Arrhythmia (trigger)	Clinical implication: evaluation of drug effects	Reference
				but not after epinephrine – Verapamil significantly lowered VT prevalence – Phentolamine (alpha-antagonist) or labetalol (alpha/beta-antagonist) abolished exercise- and epinephrine-induced arrhythmia – Mg2+ significantly lowered the incidence of catecholamine-induced sustained VT	
	CASQ2 $^{D307H/D307H}$ (KO)	Yes	Yes (spontaneous, exercise, catecholamines)	– Flecainide, procainamide, and lidocaine were ineffective in controlling arrhythmia – Propranolol and sotalol had little anti-arrhythmic effect during exercise but not after epinephrine – Verapamil completely abolished arrhythmia in D307H mice – Mg2+ significantly lowered the incidence of catecholamine-induced sustained VT	Song et al. (2007), Katz et al. (2010) and Kurtzwald-Josefson et al. (2014)

Abbreviations: *KO* knockout, *KI* knock-in, n.d. not done

15.2.1.1 Genetically Modified Mouse Models for Long QT Syndromes with Potassium Channel Mutations

Since genetic manipulation has for a long time nearly exclusively been feasible in mice and not in other larger mammals, the first transgenic (and knockout) animal models for LQTS based on potassium channel mutations were mouse models (London et al. 1998a, b). However, it has to be noted that pronounced species differences in the expression and functional relevance of ion channel proteins and currents conveying cardiac repolarizing, action potential shape and duration, and consecutive surface ECG morphology have been identified between mice and humans [reviewed in Nerbonne et al. (2001), Salama and London (2007) and Baczkó et al. (2016)]. Briefly (and simplified), in mouse cardiomyocytes, the major repolarizing currents are the fast and slow components of the transient outward K^+ current $I_{to,f}$ and $I_{to,s}$ and the rapidly activating, slowly inactivating delayed rectifier currents $I_{K,slow1}$ and $I_{K,slow2}$ (Nerbonne 2000; Nerbonne et al. 2001). In human cardiomyocytes, in contrast, repolarization is driven by the transient outward K^+ current I_{to}, the slow delayed rectifier K^+ current I_{Ks}, the rapid delayed rectifier K^+ current I_{Kr}, and the inward rectifier K^+ current I_{K1}—with I_{Kr} and I_{Ks} being by far the most important repolarizing currents (Nerbonne 2000; Nerbonne et al. 2001).

Since the 1990s a variety of different models (1) targeting mouse repolarizing potassium currents or (2) introducing mutated human repolarizing potassium channels have been generated and investigated in detail (Nerbonne et al. 2001; Salama and London 2007) (see sections "Genetically Modified Mouse Models for Long QT Syndrome with Alterations of Mouse Potassium Channels" and "Genetically Modified Mouse Models for Long QT Syndrome with Alterations of "Human" Potassium Channels"). In these models, the so-called "dominant-negative" (DN) transgenic strategy, e.g., the fact that the co-assembly of mutated and normal channel subunits completely disrupts ion channel function, was utilized to decrease the expression of functionally normal repolarizing potassium channel proteins.

Genetically Modified Mouse Models for Long QT Syndrome with Alterations of Mouse Potassium Channels

(Partial) Imitation of Long QT Phenotype: APD, QT, and Arrhythmia

Mouse models with altered expression of mouse potassium channel α-subunits have been crucial for our understanding of the functional relevance of molecularly distinct subunits of repolarizing potassium channels (Nerbonne et al. 2001). Moreover, these models either overexpressing dominant-negative loss-of-function mutations in mouse repolarizing potassium channel genes or harboring targeted deletions of these genes were able to partially mimic the human LQTS disease phenotype. Dominant-negative transgenic mice expressing (1) an N-terminal fragment of Kv1.1 (lack of the 4-aminopyridine (4-AP)-sensitive current $I_{K,slow1}$), (2) a Kv1.5 pore mutation (similar lack of the 4-AP-sensitive current $I_{K,slow1}$), (3) a Kv2.1 mutation (lack of $I_{K,slow2}$), or (4) a truncated Kv4.2 as well as a Kv4.2 pore mutation (lack of $I_{to,f}$) all demonstrated prolongation of the action potential duration (APD) and/or the QT interval (Table 15.1; London et al. 1998a; Barry et al. 1998; Xu et al.

1999; Wickenden et al. 1999; Li et al. 2004). Similarly, the targeted disruption of KChIP2—an auxiliary subunit of the Kv4.x family necessary for regular trafficking of channel proteins to the cell membrane—that results in a lack of I_{to} led to a prolongation of APD and QT (Kuo et al. 2001). Other mice with targeted deletions of mouse channel subunits (Kv1.4$^{-/-}$ lack of $I_{to,s}$; Kv4.2$^{-/-}$ lack of $I_{to,f}$), in contrast, had no APD or QT prolongation at all (London et al. 1998b; Guo et al. 2005).

However, only some of these models exhibited short spontaneous and/or inducible ventricular arrhythmia (London et al. 1998a; Xu et al. 1999; Kuo et al. 2001; Kodirov et al. 2004), particularly when dominant-negative mutations in several ion channel genes were combined (Guo et al. 2000; Kodirov et al. 2004)—while others seemed to be protected from arrhythmia despite their prolonged cardiac repolarization (Li et al. 2004; Barry et al. 1998; Brunner et al. 2001). Of note, interestingly, in Kv1.1DN mice, the major arrhythmia was monomorphic and not polymorphic *torsade de pointes* tachycardia that typically develops in human LQTS (London et al. 1998a), indicating potentially different mechanisms of arrhythmogenesis in murine and human hearts. SCD due to VT/VF, however, was not observed in any of these LQTS mouse models.

Electrical Remodeling

A compensatory upregulation of repolarizing currents not affected by the mutation was observed in many of these mouse models and may be (partially) responsible for a lack of APD/QT prolongation or lack of arrhythmia: Kv4.2 DN transgenic mice lacked $I_{to,f}$ but demonstrated a compensatory upregulation of Kv1.4/$I_{to,s}$ leading to APD and QT prolongation but without arrhythmia (Barry et al. 1998). Similarly, mice with a targeted deletion of Kv4.2 completely lacked $I_{to,f}$ and showed compensatory upregulation of $I_{to,s}$ and downregulation of the accessory subunit KChIP2 and consequently lacked APD or QT prolongation (Guo et al. 2005). Gene-targeted mice in which Kv1.5 was replaced by Kv1.1 (SWAP) lacked $I_{K,slow1}$ and demonstrated upregulation of Kv2.1/$I_{K,slow2}$ resulting in a lack of APD prolongation (London et al. 2001). Whether a likewise electrical remodeling with compensatory upregulation of other repolarizing ion currents also occurs in human cardiomyocytes is unclear and remains to be elucidated.

Mechanisms of Long QT-Related Arrhythmia

To gather insights into mechanisms responsible for long QT-related arrhythmia, not only the abovementioned "monogenic" LQTS mouse models were generated, but experiments intercrossing two specific models were also performed to investigate the impact of the combined lack of several repolarizing ion currents on arrhythmogenesis. Cross-breeding Kv4.2 DN mice with Kv1.4$^{-/-}$ mice yielded mice that completely lacked I_{to} ($I_{to,s}$ and $I_{to,f}$) and had very pronounced APD/QT prolongation, with increased early afterdepolarization (EAD) formation and spontaneous ventricular arrhythmia (Guo et al. 2000). Similarly, crossing Kv1.1 DN and Kv2.1 DN mice resulted in mice lacking both $I_{K,slow1}$ and $I_{K,slow2}$ with pronounced APD/QT prolongation and spontaneous and inducible arrhythmia (Kodirov et al. 2004). Cross-breeding Kv1.1 DN with Kv4.2 DN mice (lack of $I_{K,slow1}$ and both components

of I_{to}), in contrast, let to mice with pronounced prolongation of cardiac repolarization but lack of arrhythmia (Brunner et al. 2001). These models revealed the importance of a heterogeneously prolonged cardiac repolarization for LQTS-related arrhythmia formation and an anti-arrhythmic effect of a regionally more homogenous AP prolongation (Baker et al. 2000; Brunner et al. 2001; Kodirov et al. 2004; London et al. 2007).

However, due to the above indicated differences in cardiac electrophysiology, the clinical relevance of mechanistic findings gathered in these potassium channel-targeting mouse models to human LQT1 and LQT2 remains elusive. Moreover, in transgenic or gene-targeted mouse models of LQTS in general, electrical remodeling (compensatory upregulation of other ion currents) and structural remodeling (fibrosis) are common, which may itself affect arrhythmogenesis, thus limiting their transferability to human pathophysiology (Koren 2004; Salama and London 2007).

Genetically Modified Mouse Models for Long QT Syndrome with Alterations of "Human" Potassium Channels

Another approach to investigate LQTS in mouse models is to modify human potassium channel genes or their mouse equivalents. Several groups have generated mouse models with dominant-negative loss-of-function mutations of human voltage-gated potassium channels KvLQT1/*KCNQ1* (LQT1), HERG/*KCNH2* (LQT2), minK/*KCNE1* (LQT5), or Kir2.1/*KCNJ2* (LQT7) or knockouts of the mouse-equivalent genes (Table 15.2), aiming at a better imitation of the human LQTS phenotype [reviewed in Nerbonne et al. (2001) and Salama and London (2007)]. As different repolarizing voltage-gated potassium currents determine cardiac repolarization in murine and human cardiomyocytes (Nerbonne 2000), however, these mouse models representing LQT types 1, 2, 5, or 7 have failed to completely mimic the human disease phenotype. Only some of the models demonstrated APD/QT prolongation, and all failed to show any spontaneous ventricular arrhythmia (Table 15.2).

(Partial) Imitation of Long QT Phenotype—APD, QT, and Arrhythmia: KCNQ1—LQT1

Consequences of a reduction or elimination of I_{Ks} have been investigated in *KCNQ1* knockout and dominant-negative mouse models. Similarly as human patients with Jervell and Lange-Nielsen syndrome—an autosomal recessive form of LQT1—homozygote mice with targeted disruption of *Kcnq1* (*Kcnq1*$^{-/-}$) had bilateral sensorineural deafness (Lee et al. 2000; Casimiro et al. 2001). The cardiac phenotype, however, was less clear. While some *Kcnq1*$^{-/-}$ mice [deletion of exon 1 (Lee et al. 2000)] had normal QT, other *Kcnq1*$^{-/-}$ mice [deletion of exon 2 (Casimiro et al. 2001)] demonstrated QT prolongation but no corresponding changes in endocardial or epicardial monophasic action potentials (Table 15.2), indicating a less predominant role for KCNQ1/I_{Ks} in ventricular repolarization in mice than in human. In dominant-negative *KCNQ1* DN mice (Demolombe et al. 2001), in contrast, prolonged QT and APD were observed and were associated with sinus node dysfunction and alterations of AV nodal conduction, suggesting that KCNQ1/I_{Ks} may play a role in sinus node automaticity and impulse propagation through the AV node in murine hearts. *KCNQ1* DN mice were further

utilized to investigate differential effects of I_{Kr} and I_{to} blocking drugs (Lande et al. 2001), demonstrating a QT prolonging effect of I_{to} blockers (but—contrasting with effects observed in humans—not of I_{Kr} blockers) and slowing of sinus automaticity with I_{Kr} blockade, thus further dissecting the functional role of various repolarizing ion currents in different parts of the heart.

(Partial) Imitation of Long QT Phenotype—APD, QT, and Arrhythmia: KCNH2—LQT2

Similarly as suggested by pharmacological experiments, the selective knockout of mouse Merg1b potassium channel (*Kcnh2/Merg*$^{-/-}$) (Lees-Miller et al. 2003) or the elimination of I_{Kr} due to a cardiac-specific overexpression of the dominant-negative pore mutation HERG-G628S (Babij et al. 1998) [which confers a pronounced phenotype in human patients (Sanguinetti et al. 1996)] both did not prolong QT intervals but caused sinus bradycardia (Table 15.2). These findings indicate a very limited role of I_{Kr} in murine ventricular repolarization but some importance in sinus node electrophysiology. Similarly as in models targeting mouse potassium channels, the lack of a cardiac phenotype may also be partly due to compensatory upregulation of other ion currents such as I_{Ks} (Lees-Miller et al. 2003). In contrast to these observations, a more recent study (Salama et al. 2009) demonstrated that a partial reduction of Merg1a protein/I_{Kr} current in heterozygous *Merg1*$^{+/-}$ mice may cause APD prolongation and increased base-to-apex dispersion of repolarization thus predisposing the heart to arrhythmia as demonstrated by increased VT inducibility.

(Partial) Imitation of Long QT Phenotype—APD, QT, and Arrhythmia: KCNE1—LQT5

Consequences of *Kcne1/minK*$^{-/-}$ knockout on murine cardiac electrophysiology are conflicting: Several groups reported that *minK*$^{-/-}$ mouse models have reduced I_{Ks} current densities but lack changes in QT (Kupershmidt et al. 1999) or in (right and left ventricular) APD (Charpentier et al. 1998; Salama et al. 2009)—while others have described QT abnormalities in response to heart rate changes with longer QT at slow heart rates and a paradoxical shorter QT at fast heart rates (Drici et al. 1998) (Table 15.2). In line with these observations, a recent study demonstrated prolonged epicardial APD, resulting in increased transmural dispersion of repolarization (Thomas et al. 2007). Earlier studies by the same group had additionally identified slowing and increased dispersion of conduction velocities (Balasubramaniam et al. 2003). However, whether these are due to potential fibrotic remodeling or stem directly from the knockout remains unclear. In *Kcne1/minK*$^{-/-}$ mice demonstrating prolongation and increased dispersion of APD and conduction velocities, an increased incidence of EADs and of inducible and spontaneous VT (particularly upon complete cessation of ventricular pacing and hence during bradycardia) was observed in Langendorff-perfused hearts ex vivo (Balasubramaniam et al. 2003; Thomas et al. 2007). Of note, these ventricular tachycardias, however, were monomorphic [similarly as observed in Kv1.1DN mice (London et al. 1998a)] and not polymorphic thus not corresponding to *torsade de pointes* tachycardia typically observed in human LQTS patients. Interestingly, in these models the Ca^{2+} channel blocker nifedipine exerted a pronounced anti-arrhythmic effect (Balasubramaniam et al. 2003; Thomas et al. 2007). In another study, in which optical mapping

experiments were performed in *Kcne1/minK*$^{-/-}$ mouse hearts that lacked APD prolongation, in contrast, no arrhythmia was observed neither spontaneously nor after ventricular stimulation (Salama et al. 2009).

(Partial) Imitation of Long QT Phenotype—APD, QT, and Arrhythmia: KCNJ2—LQT7
The effects of a reduction or elimination of Kir2.1/I_{K1} have been investigated using gene-targeted knockout and dominant-negative transgenesis (Table 15.2): Ventricular cardiomyocytes from *Kcnj2/Kir2.1*$^{-/-}$ knockout mice lacked I_{K1} and demonstrated a pronounced APD prolongation and increased rates of spontaneous APs. No spontaneous ventricular arrhythmia, however, was observed in *Kcnj2/Kir2.1*$^{-/-}$ mice in vivo (Zaritsky et al. 2000). Similarly, cardio-selective overexpression of dominant-negative Kir2.1 channel subunits (Kir2.1 DN mice) led to a nearly absent I_{K1} and prolonged APD and QT (McLerie and Lopatin 2003). In addition, a prolongation of PR intervals and QRS duration was observed, indicating a role of I_{K1} in controlling repolarization and impulse propagation. Despite the prolonged cardiac repolarization in this model, however, no ectopic electrical activity and no VT were observed (McLerie and Lopatin 2003), further stressing the electrophysiological differences between mice and human in terms of arrhythmogenesis.

Mechanisms of Long QT-Related Arrhythmia
Despite their lack of complete imitation of the human disease phenotype, insights into mechanisms underlying long QT-related arrhythmia were also gathered with these mouse models: the importance of an increased dispersion of repolarization for arrhythmia formation (Thomas et al. 2007; Salama et al. 2009) was similarly highlighted as with mouse models with alterations in mouse potassium channels.

15.2.1.2 Genetically Modified Rabbit Models for Long QT Syndromes Based on Potassium Channel Mutations

Although the above described LQTS mouse models were able to partly mimic the human LQTS phenotype, these models failed to show spontaneous sustained ventricular arrhythmia or SCD thus limiting their value for the investigation of arrhythmic mechanisms or anti-arrhythmic therapeutic approaches. This calls for a species that more closely resembles humans such as the rabbit, which demonstrates pronounced similarities to humans in terms of ion currents determining cardiac repolarization, intracellular ion concentrations, and responses to electrophysiologically relevant pharmacological interventions (Nerbonne 2000; Valentin et al. 2004; Hondeghem 2016). In rabbit cardiomyocytes, cardiac repolarization is mainly driven by HERG/I_{Kr} and (to a lesser degree) KvLQT1/I_{Ks}—similar as in human cardiomyocytes (Salata et al. 1996; Nerbonne et al. 2001; Baczkó et al. 2016). In addition, regional contractile and diastolic behavior of rabbit hearts resembles that of human (Jung et al. 2012), and similar cardiac mechano-electrical coupling mechanisms have been described (Quinn and Kohl 2016).

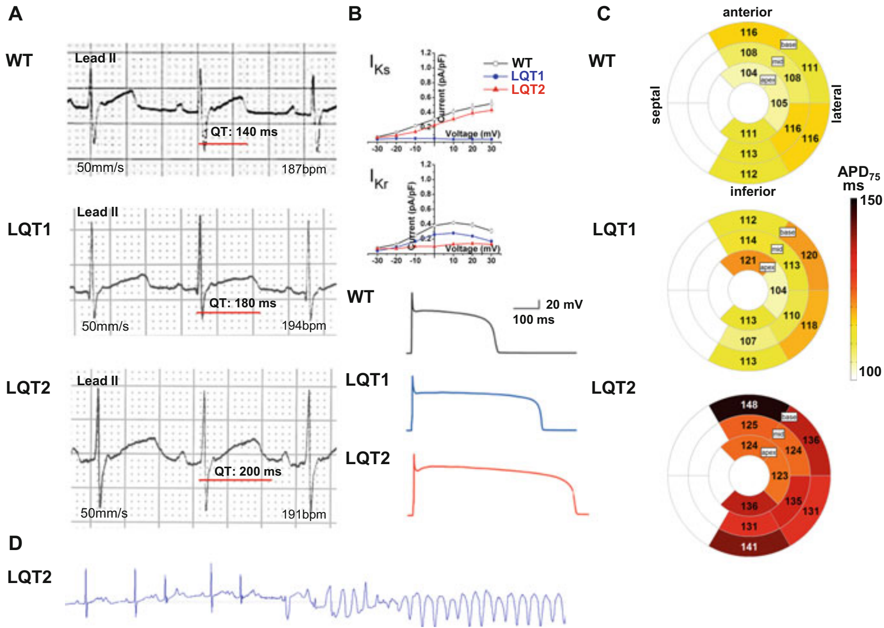

Fig. 15.1 Electrical phenotype of transgenic LQT1 and LQT2 rabbits. (**a**) Representative ECGs from sedated wild-type (WT), transgenic LQT1 and LQT2 rabbits depicting prolonged QT intervals in LQT1 and LQT2 rabbits. (**b**) Current-voltage diagrams of I_{Kr} (top) and I_{Ks} (mid) current densities in cardiomyocytes from WT, LQT1, and LQT2 rabbits demonstrating loss of I_{Kr} in LQT2 and loss of I_{Ks} in LQT1. Representative cellular action potentials (bottom) from WT, transgenic LQT1 and LQT2 rabbits with prolonged action potential durations in LQT1 and LQT2. (**c**) Bull's-eye plots from average monophasic APD at 75% of repolarization (APD_{75} in ms) in different segments of the left ventricular base, mid, and apex derived from Langendorff-perfused hearts of wild-type ($n = 10$), LQT1 ($n = 12$), and LQT2 ($n = 9$) rabbits. (**d**) Exemplary ECG episode of ventricular fibrillation in a free-moving LQT2 rabbit (telemetric ECG monitoring). Modified and adapted from Brunner et al. (2008), Odening et al. (2012, 2013), Ziupa et al. (2014) and Lang et al. (2016a, b)

Transgenic Rabbit Models for LQT1, LQT2, and LQT5—Imitation of Long QT Phenotype

All available transgenic LQTS rabbit models have been engineered by cardio-selective overexpression 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 β-myosin heavy chain promoters (Brunner et al. 2008; Major et al. 2016) [reviewed in detail in Lang et al. (2016b)] (Table 15.3). In LQT1 or LQT2, rabbit cardiomyocytes I_{Ks} (LQT1) or I_{Kr} (LQT2), respectively, were completely eliminated resulting in prolongation of APD on the cellular and whole heart levels and prolongation of ventricular refractoriness and QT duration in vivo (Brunner et al. 2008; Odening et al. 2010) (Table 15.3, Fig. 15.1a–c). In LQT2 rabbit hearts, an increased spatial dispersion of APD was observed (Brunner et al. 2008; Odening et al. 2013), and VT/VF were easily inducible with left ventricular (LV) epicardial

stimulation (Brunner et al. 2008). Importantly, LQT2 rabbits even developed spontaneous polymorphic VT and SCD (Brunner et al. 2008; Odening et al. 2012) (Fig. 15.1d), thus representing the first transgenic animal models mimicking the complete electrical phenotype of LQT2. Transgenic LQT1 rabbits with a more homogeneously prolonged APD without dispersion of repolarization, in contrast, developed no spontaneous VT or SCD (Brunner et al. 2008). In transgenic LQT5 rabbits (Major et al. 2016), biophysical properties of I_{Ks} were altered with accelerated deactivation kinetics. These rabbits exhibited only a very slightly prolonged QT but an increased short-term beat-to-beat variability of the QT (Major et al. 2016). Due to their reduced repolarization reserve, the phenotype was augmented by I_{Kr}-blocking drug dofetilide, which further increased short-term variability of QT and promoted drug-induced VT (Major et al. 2016) (Table 15.3).

In the following, we highlight mechanistic findings on the arrhythmic substrate, pro-arrhythmic triggering factors, anti-arrhythmic agents, and electromechanical dysfunction in transgenic LQTS rabbit models and their potential translational application in the clinical management of LQTS patients in more detail.

Arrhythmic Substrate: Role of Spatial and Temporal Dispersion of Repolarization

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 left and right ventricles (Brunner et al. 2008; Odening et al. 2010, 2013), and optical mapping visualizing VF initiation demonstrated that this enhanced regional dispersion of repolarization may cause unidirectional functional block and reentry formation (Brunner et al. 2008). Dispersion of repolarization can also occur in a dynamic spatiotemporal fashion with pronounced beat-to-beat alternations and “out-of-phase” heterogeneities between adjacent regions, the so-called discordant alternans. In transgenic LQT2 rabbit hearts, this discordant alternans developed at physiological heart rates and preceded VT/VF formation (Ziv et al. 2009). 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 APD prolongation may exert a protective effect. When LQT1 hearts were further stressed, however, by continuous tachypacing to induce cardiac tachymyopathy, APD dispersion increased, spatially discordant alternans developed, and VT/VF was easily inducible (Lau et al. 2015).

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, while a sudden sympathetic surge in episodes of rest through emotional stress and (auditory) startle may trigger arrhythmia in LQT2 (Schwartz et al. 2001; Morita et al. 2008). In line with these observations, genotype-specific differences in the mechanisms of EAD formation and arrhythmia initiation were demonstrated in

LQT1 and LQT2 rabbits. In LQT2 cardiomyocytes, EADs developed during sudden sympathetic surge, while continuous perfusion with isoproterenol prevented EAD formation. In LQT1 cardiomyocytes, in contrast, continuous adrenergic stimulation facilitated the occurrence of EADs (Liu et al. 2012). Different time courses in sympathetic activation of cardiac ion currents may explain why different sympathetic modes are associated with arrhythmia formation in different genotypes of LQTS: Upon sympathetic stimulation, activation of $I_{Ca,L}$ that may elicit EADs is faster than the activation of I_{Ks} that shortens APD in LQT2 and acts as anti-arrhythmic mechanism upon continuous adrenergic stimulation in LQT2. In addition, different modes of arrhythmia initiation and maintenance in different LQTS genotypes were identified. While in LQT2 reentry formation played an important role (Brunner et al. 2008), in LQT1 hearts, a novel mechanistic concept of LQTS-related arrhythmogenesis was 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).

Role of Electrical Remodeling

In transgenic LQTS rabbit models, a distinct interaction between I_{Kr} and I_{Ks} was identified. In contrast to transgenic mouse models, in which electrical remodeling with a partially compensatory upregulation of reciprocal repolarizing ion currents has been observed (Koren 2004), in transgenic LQT1 and LQT2 rabbits, the impaired repolarization reserve was further limited and the phenotype aggravated by downregulation of the reciprocal repolarizing currents (Brunner et al. 2008; Ren et al. 2010) due to direct KCNQ1 and HERG protein interactions (Organ-Darling et al. 2013). Likewise, in transgenic LQT5 rabbits, the faster deactivation of I_{Ks} was paralleled by a faster deactivation of I_{Kr} (Major et al. 2016). Whether electrical remodeling mechanisms occurring in human LQTS cardiomyocytes more closely resemble the changes in transgenic rabbit or mouse models still warrants detailed investigation.

Investigation of Pro- and Anti-arrhythmic Effects of Drugs and Hormones in LQTS

As transgenic LQTS rabbit models demonstrate a reduced repolarization reserve, they may serve as particularly sensitive tools for in vivo and ex vivo drug testing to identify potential pro-arrhythmic ion channel-blocking drugs and may be similarly used to investigate genotype-specific efficacy of ion channel-activating drugs and their potential application as genotype-specific therapies.

LQT1 rabbits lacking I_{Ks} and LQT5 rabbits with impaired I_{Ks} were demonstrated to be particularly sensitive in identifying I_{Kr}-blocking properties of drugs showing prolonged APD/QT, increased spatial dispersion of APD, increased short-term variability of QT, and increased arrhythmia formation (Odening et al. 2008, 2010; Ziupa et al. 2014; Major et al. 2016). Similarly, transgenic LQT2 rabbits demonstrated a particularly high sensitivity to I_{Ks}- or I_{K1}-blocking anesthetic agents (Odening et al. 2008).

Pronounced sex differences in arrhythmic risk have been identified 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- 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 and Koren 2014). As in transgenic LQT2 rabbits ventricular arrhythmia and SCD 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 utilized to explore sex hormone effects on arrhythmic triggers and substrate (Odening et al. 2012): Estradiol exerted a pro-arrhythmic effect with an increased incidence of lethal pVT by changing the pattern of APD dispersion and increasing EAD formation upon sympathetic stimuli, while progesterone had an anti-arrhythmic, protective effect that was based on a shortening of cardiac refractoriness, a reduced formation of EAD, and stabilizing Ca^{2+} effects (decreased $I_{Ca,L}$ density, increased SERCA expression) (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.

Insights into Electro-mechanical Dysfunction in LQTS

Because electrical and mechanical cardiac functions are closely coupled, it stands to reason that LQTS also causes mechanical dysfunction. Standard echocardiography techniques have demonstrated globally normal mechanical function in structurally normal hearts in LQTS. With the help of novel echocardiography or MRI techniques that allow to investigate regional tissue velocities and strain, however, more and more evidence is accumulating that diastolic relaxation may be impaired and contraction duration prolonged (Nador et al. 1991; Haugaa et al. 2010; Leren et al. 2015). Using *phase-contrast*-based MRI, a regionally heterogeneously reduced diastolic function (reduced peak diastolic relaxation velocities and prolonged time-to-diastolic peak as a marker for contraction duration) was demonstrated in transgenic LQT2 rabbits (Odening et al. 2013) (Fig. 15.2a–e) thus supplementing the clinical data with important insights into regional mechanical heterogeneity. In addition, a spatial correlation between the extent of electrical dysfunction (prolongation of APD) and the impaired diastolic function (Odening et al. 2013) and a correlation between mechanical dysfunction and arrhythmic risk were revealed (Lang et al. 2016a): LQT2 rabbits exhibiting arrhythmia featured a more prolonged time-to-diastolic peak and a more pronounced mechanical dispersion than those without arrhythmia (Fig. 15.2e) with time-to-diastolic peak proving to be a better predictor than QT/APD (Lang et al. 2016a). This strongly suggests a potential future role for the assessment of mechanical dysfunction for risk stratification. Transgenic LQT rabbit models may offer vast possibilities for further research investigating

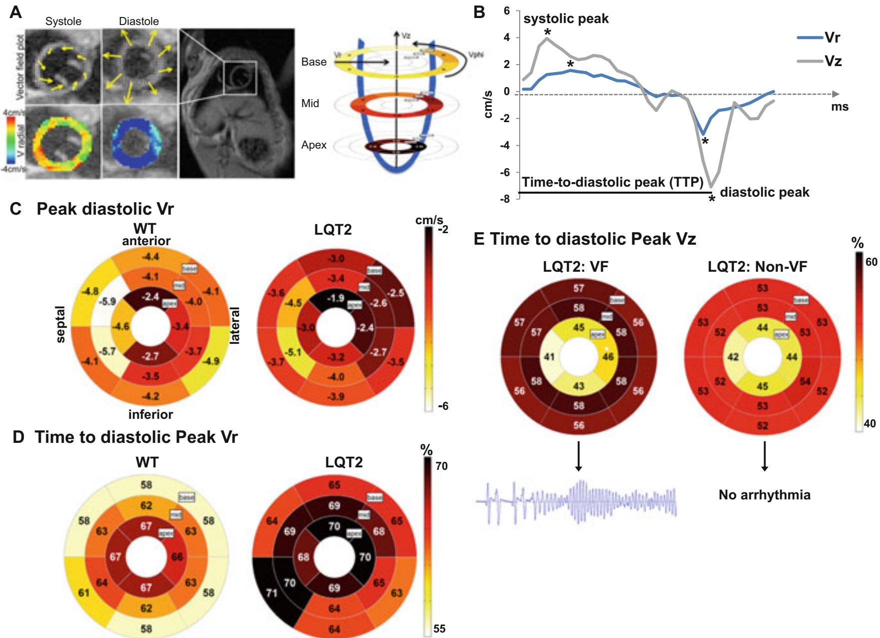

Fig. 15.2 Mechanical phenotype of transgenic LQT1 and LQT2 rabbits. (**a**) Color-coded radial velocities and summation vectors in the left ventricle in early systole and diastole are illustrated as recorded during tissue phase mapping (TPM) MRI. (**b**) Representative velocity graphs (velocities over time) in radial (Vr) and longitudinal (Vz) directions are displayed. Also indicated are systolic and diastolic peak radial velocities (asterisk) in the left ventricle and time to diastolic peak velocity (TTP) calculated from TPM. (**c**) Bull's-eye plots displaying averaged diastolic peak radial velocities (cm/s) in LV base, mid, and apical segments from TPM in wild-type (WT) ($n = 10$) and LQT2 rabbits ($n = 9$). LQT2 rabbits demonstrate a diastolic dysfunction with heterogeneously reduced peak radial velocities compared to wild type. (**d**) Bull's-eye plots displaying averaged heart rate—corrected time to diastolic radial peak velocities (TTP, %) from TPM. Regional TTP—a marker for contraction duration—prolonged in LQT2 ($n = 9$) compared to WT females ($n = 10$). (**e**) Bull's-eye plots displaying averaged heart rate—corrected time to diastolic longitudinal peak velocities (%). TTP are prolonged in LQT2 rabbits with ventricular fibrillation during Langendorff perfusion (VF, $n = 10$) compared to those without arrhythmia (Non-VF, $n = 33$). Modified and adapted from Odening et al. (2013) and Lang (2016a, b)

mechanisms underlying the observed mechanical dysfunction and its causative link to arrhythmogenesis.

15.2.2 Transgenic Animal Models for Sodium Channelopathies: Long QT Syndrome Type 3, Brugada Syndrome, Cardiac Conduction Disease, and Overlap Syndrome

The *SCN5A* gene encodes the alpha subunit of the cardiac voltage-gated sodium channel. While mutations in potassium channel genes alter cardiac repolarization

(see Sect. 15.2.1), *SCN5A* sodium channel mutations may influence cardiac depolarization and repolarization, thus causing different channelopathies such as LQT3 syndrome (Schwartz et al. 2001), Brugada syndrome (BrS) (Priori et al. 2002a), cardiac conduction diseases (CCD), and overlap syndromes (Remme et al. 2008).

Mutations causing LQT3 are considered to disrupt fast inactivation of the sodium current, allowing for a persistent (late) sodium current ($I_{Na,L}$) during the plateau phase of the action potential (*gain-of-function* mutations), whereas mutations causing Brugada syndrome and cardiac conduction disease are considered to reduce the total amount of available sodium current (*loss-of-function* mutations). Additionally, an overlap phenotype may result from biophysical overlap with opposing alterations of the peak sodium current (I_{Na}) and the persistent sodium current component ($I_{Na,L}$) (Remme et al. 2006, 2008; Watanabe et al. 2011).

Notably, a single mutation in *SCN5A* may even present with different phenotypes (LQT3, BrS, or CCD) in different family members of one family (Remme et al. 2008). Hence, in addition to the concept that defined mutations of *SCN5A* result in a *gain-of-function* (LQT3) or *loss-of-function* (BrS or CCD) of the voltage-gated sodium channel, other modifiers—such as sex, age, alternative splicing, single nucleotide polymorphisms, or coinheritance of other genetic variations—may contribute to disease penetrance and phenotype (Remme et al. 2008; Derangeon et al. 2012).

Since the cardiac voltage-gated sodium channel constitutes the main depolarizing ion channel in various species including human and mice (Nerbonne and Kass 2005), genetically modified mouse models have been largely used to investigate the electrical consequences of different *SCN5A* mutations. Similarly as in human subjects, mouse models of sodium channelopathies may demonstrate prominent QT prolongation (LQT3 pattern), features of BrS, cardiac conduction disease, or even an overlap syndrome [reviewed in detail in Salama and London (2007), Charpentier et al. (2008), Remme et al. (2008) and Derangeon et al. (2012); Table 15.4]. Therefore, in the following, the different mouse (and pig) models are presented and discussed based on their "main" clinical and electrical features.

15.2.2.1 Genetically Modified Mouse Models with Features of LQT3 (QT Prolongation)

Imitation of Long QT Phenotype—APD, QT, and Arrhythmia

Genetically altered or transgenic LQT3 mouse models are either heterozygous for the knock-in +/Δ KPQ deletion in *Scn5a* (Nuyens et al. 2001; Head et al. 2005) or overexpress the human *SCN5A* point mutations N1325S or F1759A (Tian et al. 2004; Wan et al. 2016). The +/Δ KPQ mouse model demonstrated increased sodium current (I_{Na} and $I_{Na,L}$) and prolonged APD and QT intervals and also mimicked the arrhythmic phenotype with increased EAD formation and spontaneous and inducible polymorphic VT (pVT) (Nuyens et al. 2001; Head et al. 2005). Additionally, the +/Δ KPQ mutation was associated with AV block in both human subjects and mice (Zareba et al. 2001; Fabritz et al. 2010).

Similar to these knockout models, both transgenic mouse models (*SCN5A*-N1325S and *SCN5A*-F1759A) demonstrated prolonged APD and QT interval and

imitated LQT3 with the occurrence of spontaneous premature ventricular contractions (PVC) and pVT (Tian et al. 2004; Wan et al. 2016). *SCN5A*-N1325S mice even died from premature cardiac death, and *SCN5A*-F1759A mice developed atrial fibrillation (the primary focus of the study by Wan et al.) (Tian et al. 2004; Wan et al. 2016). In cardiomyocytes of both transgenic models, only the persistent sodium current ($I_{Na,L}$) was increased, and peak sodium current (I_{Na}) was not altered (Wan et al. 2016).

LQT3 mouse models demonstrate prolonged APD and QT intervals; they develop spontaneous and/or inducible ventricular arrhythmia and thus mimic the human LQT3 phenotype considerably well. Additionally, the *SCN5A*-F1759A mice impressively demonstrated $I_{Na,L}$-induced damage to the heart, since beside electrical alterations, these mice developed pronounced macro- and microscopic alterations (atrial and ventricular enlargement, myofibril disarray, fibrosis, and mitochondrial injury) (Wan et al. 2016).

Mechanisms of Long QT-Related Arrhythmia

In human LQT3 patients, arrhythmia typically occurs during sleep and rest (Schwartz et al. 2001). Similarly, cholinergic stimulation with carbachol (imitating parasympathetic tone as during sleep and rest) induced arrhythmia in +/Δ KPQ mice (Fabritz et al. 2003). Increase in APD dispersion and EAD formation during bradycardia were detected as factors responsible for ventricular ectopy and pVT in these mice. Accordingly, ventricular pacing suppressed EADs and prevented pVT by reducing APD dispersion (Fabritz et al. 2003). However, +/Δ KPQ and *SCN5A*-N1325S mouse models also displayed APD prolongation with (sudden) acceleration of heart rate that was associated with EADs and triggered arrhythmia (Nuyens et al. 2001; Tian et al. 2004), indicating an additional pro-arrhythmic mechanism in these mouse models compared to human subjects.

Investigation of Anti-arrhythmic Therapeutic Drug Effects

Aiming at optimizing therapeutic strategies for LQT3 patients, beta-blockers and sodium channel blockers were extensively assessed in LQT3 mouse models (Table 15.4).

Conflicting data has been presented regarding the efficacy of beta-blockers in LQT3. Earlier studies in LQT3 mouse models found that propranolol and esmolol did not exert anti-arrhythmic effects (Head et al. 2005; Fabritz et al. 2010), while adrenergic agonists suppressed arrhythmia (Nuyens et al. 2001; Fabritz et al. 2010). However, a more recent study using (the same) +/Δ KPQ mice demonstrated that pretreatment with propranolol protected +/Δ KPQ mice from carbachol-induced VT/VF (Calvillo et al. 2014). Additionally, a recent study in LQT3 patients found a reduced risk for arrhythmia with beta-blocker therapy in female LQT3 patients, while the efficacy in males could not be determined conclusively due to the low number of arrhythmic events (Wilde et al. 2016). To date, beta-blockers are recommended as a cornerstone for the treatment of all human LQTS patients—including LQT3 (Priori et al. 2015).

Since an increased late $I_{Na,L}$ has been demonstrated to be causally linked to LQT3, the pharmacological blockade of this current should exert a mechanism-directed, genotype-specific anti-arrhythmic effect. Indeed, it was demonstrated in *Scn5a* +/Δ KPQ and *SCN5A*-N1325S mouse models that sodium blockers mexiletine and flecainide suppressed arrhythmia in LQT3 (Tian et al. 2004; Head et al. 2005; Fabritz et al. 2010). Similarly, recent clinical data demonstrate that LQT3 patients might benefit from sodium channel blockers (Moss et al. 2005, 2008; Priori et al. 2015). The ESC guidelines for the prevention of SCD (2015) thus mention sodium channel blockers (mexiletine, flecainide, or ranolazine) as a considerable add-on therapy in patients with LQT3 and a QTc greater than 500 ms with a class IIb recommendation (Priori et al. 2015). Since available agents do not only block late sodium current but to some extent also peak sodium current, this therapeutic approach should only be applied with caution in patients presenting with LQT3 and overlap syndrome (Moreno and Clancy 2012). However, more recently GS967 has been demonstrated to be a more selective inhibitor of $I_{Na,L}$ in *Scn5a*$^{1798insD/+}$ mice (Portero et al. 2017) and wild-type rabbit (Belardinelli et al. 2013).

15.2.2.2 Genetically Modified Mouse and Pig Models with Features of Brugada Syndrome, Cardiac Conduction Disease, and Overlap Phenotype

Imitation of Brugada Syndrome and Cardiac Conduction Disease Phenotype
Brugada syndrome (BrS) is characterized by typical right precordial coved-type ST segment elevations and an increased risk for VT and SCD (Priori et al. 2002a). Besides the characteristic ECG changes, patients with BrS frequently have conduction abnormalities, including prolonged PR and QRS intervals, particularly in case of *SCN5A* mutations (Antzelevitch et al. 2005). Importantly, arrhythmia can be triggered by increased body temperature (Antzelevitch and Brugada 2002).

In the heterozygous *Scn5a*$^{+/-}$ knockout mouse model and the *Scn5a*$^{\Delta SIV/\Delta SIV}$ knock-in mouse models, I_{Na} was decreased and significant cardiac conduction disease with PR and QRS prolongation as well as AV conduction block was present. QT intervals, however, were normal in both models (Papadatos et al. 2002; Shy et al. 2014) (Table 15.4). In *Scn5a*$^{+/-}$ mice ventricular refractoriness was prolonged, and VT was inducible with programmed ventricular stimulation and even occurred spontaneously upon aging (Papadatos et al. 2002), thus also imitating some “Brugada-like” features. *Scn5a*$^{\Delta SIV/\Delta SIV}$ mice, in contrast, did not develop arrhythmia, and decreased I_{Na} was attributed to defective cell surface expression of sodium channels (Shy et al. 2014).

Recently a transgenic pig model with features of BrS was generated using a human *SCN5A*$^{E558X/+}$ mutation resulting in decreased peak I_{Na} (Park et al. 2015) (Table 15.4). Typical BrS-type ST elevations were not present at baseline and could not be induced by sodium channel blocker flecainide, and no SCD was observed during the first 2 years of life in *SCN5A*$^{E558X/+}$ pigs. In Langendorff-perfused *SCN5A*$^{E558X/+}$ pig hearts, however, VT/VF was inducible by ventricular pacing and by short-coupled ventricular premature beats. Of note, the arrhythmia

often initiated in the RV free wall (Park et al. 2015)—similarly as observed in human patients in whom the right ventricular outflow tract (RVOT) seems to be the source of PVCs and arrhythmia (Rudic et al. 2016). The development of arrhythmia during fever, a classical feature of BrS, was also imitated in *SCN5A*$^{E558X/+}$ pigs' hearts ex vivo: Spontaneous and inducible VF occurred at 39 °C, while hearts were stable and non-inducible at 35 °C (Park et al. 2015). Other non-specific features of BrS, e.g., conduction abnormalities such as a prolonged atrial-His and His-ventricular conduction intervals, were also present (Park et al. 2015). This model thus demonstrated some characteristic features of BrS, such as the sensitivity to increased temperature and increased VF inducibility (ex vivo), yet imitated the human phenotype incompletely. Due to pronounced similarities of the pig's cardiac physiology and anatomy to human with similar heart rate, heart size, ion channels/currents, action potential shape, and autonomic innervation (Park et al. 2015), the *SCN5A*$^{E558X/+}$ pig model may reveal more mechanistic insights into BrS and potential (pharmacological and interventional) therapeutic strategies in the future.

Imitation of Overlap Syndromes

In contrast to the above described sodium channel mutations leading to distinct features of LQT3 (predominant QT prolongation) or BrS (e.g., temperature sensitivity, VF inducibility), other mutations in *Scn5a* resulted in an "overlap" phenotype—clinically as well as biophysically (Table 15.4): In heterozygous *Scn5a*$^{1798insD/+}$ knock-in and transgenic *SCN5A*-D1275N mouse models, peak I_{Na} current (I_{Na}) was decreased, and significant cardiac conduction disease with PR and QRS prolongation as well as AV conduction block was present (Remme et al. 2006; Watanabe et al. 2011). In addition, preferential conduction slowing in the right ventricle was observed—similarly as in BrS (Remme et al. 2006). Persistent I_{Na} current ($I_{Na,L}$), however, was increased in both mouse models resulting in prolonged APD and QT intervals as in LQT3 (Remme et al. 2006; Watanabe et al. 2011)—similar as observed in the human overlap phenotype. These mouse models were thus able to demonstrate that one single *SCN5A* mutation may indeed be sufficient to cause an overlap syndrome of cardiac sodium channelopathy by differentially altering peak and late sodium current components (Remme et al. 2006; Watanabe et al. 2011). Moreover, further insights into BrS-associated arrhythmogenesis could be gathered with *Scn5a*$^{1798insD/+}$ models, in which the reduced peak I_{Na} current unmasked the maintenance of embryonic slow conduction in the adult RVOT that may account for the preferentially slowed conduction and arrhythmia initiation in RVOT observed in BrS (Boukens et al. 2013).

15.2.3 Genetically Modified Mouse Models for Catecholaminergic Polymorphic VT

The clinical characteristics of catecholaminergic polymorphic VT (CPVT) are physical or psychological stress-induced bidirectional (bVT) or polymorphic VT (pVT) and SCD in patients with normal baseline ECG and a structurally normal heart (Priori et al. 2002b). CPVT is caused by mutations in genes encoding the cardiac

ryanodine receptor (*RyR2*) or calsequestrin 2 (*CASQ2*), leading to defective ("leaky") RyR2 channels with enhanced Ca^{2+} releases from the sarcoplasmic reticulum (SR) during adrenergic stimulation, delayed afterdepolarizations, and triggered activity (Priori et al. 2001b; Lahat et al. 2001).

Several mouse models of CPVT have been generated by genetic modification of *RyR2*, *CASQ2*, and the RyR2 stabilizer *FKBP12.6* (Wehrens 2003; Cerrone et al. 2005; Song et al. 2007; Katz et al. 2010) (Table 15.5). All these mouse models (except $RyR2^{+/Ex3\text{-}del}$ (Liu et al. 2014)) demonstrated bidirectional/polymorphic VT and ventricular fibrillation (VF) during physical activity and after adrenergic stimulation or caffeine challenge and thus mimicked the human CPVT phenotype. Consecutively, these models have led to a better understanding of the pathophysiology of CPVT, and several insights into pro- and anti-arrhythmic mechanisms have been successfully implemented into improved anti-arrhythmic therapies in the clinics (Watanabe et al. 2009; van der Werf et al. 2011).

15.2.3.1 Imitation of CPVT Phenotype and Insights into Arrhythmic Mechanisms

The first model of CPVT was generated in 2003 by knockout of *FKBP12.6*, which normally stabilizes RyR2. $FKBP12.6^{-/-}$ mice died from exercise-induced ventricular arrhythmia (Wehrens 2003). Although reduced binding of FKBP12.6 to RyR2 is acknowledged as part of the pathophysiology of CPVT, thus far to the best of our knowledge, no mutations in *FKBP12.6* have been identified as causative for CPVT in human patients (Song et al. 2007; Katz et al. 2010; Priori et al. 2013; Sumitomo 2016).

In 2005, the first *RyR2* knock-in mouse model was generated containing a heterozygous $RyR2^{R4496C}$ mutation, the murine equivalent of the human $RyR2^{R4497C}$ mutation (Cerrone et al. 2005). $RyR2^{R4496C/+}$ mice imitated the human CPVT phenotype and developed typical bVT and pVT during exercise stress testing and administration of catecholamine or caffeine. Optical mapping demonstrated concentric epicardial breakthrough patterns with focal origin in the His-Purkinje network as origin of the VT (Cerrone et al. 2007). Moreover, in these mice a mechanistic link between the gene defect and arrhythmia was established demonstrating abnormal Ca^{2+} release during diastole that was further increased by beta-adrenergic stimulation (Fernandez-Velasco 2009). Several other CPVT mouse models have been generated successfully based on other *RyR2* mutations ($RyR2^{R176Q/+}$ (Kannankeril 2006), $RyR2^{P2328S/+}$ and $RyR2^{P2328S/P2328S}$ (Goddard 2008; Zhang 2013), $RyR2^{R2474S/+}$ (Kobayashi et al. 2010), $RyR2^{S2246L10/+}$ (Suetomi et al. 2011), and $RyR2^{A4860G/+}$ (Zhao 2015).

Furthermore, two CPVT mouse models have been created involving mutations in *CASQ2* gene, which encodes a calcium-binding reservoir protein within the SR (Lahat et al. 2001). Both mouse models, $CASQ2^{\Delta E9/\Delta E9}$ and $CASQ2^{D307H/D307H}$, imitate the human CPVT phenotype with exercise- and catecholamine-induced bidirectional and polymorphic VT (Knollmann 2006; Song et al. 2007; Katz et al. 2010; Kurtzwald-Josefson et al. 2014) (Table 15.5). *RyR2* and *CASQ2* mouse models all mimicked the human CPVT phenotype and were consecutively used for pharmacological investigations of anti-arrhythmic treatment options (see below).

15.2.3.2 Investigation of Anti-arrhythmic Therapeutic Drug Effects

Beta-blockers are recommended in human CPVT patients as first-line therapy (Priori et al. 2015). However, clinical studies have shown that despite being treated with beta-blockers, 47% of CPVT patients still develop arrhythmia (Priori et al. 2002b). Accordingly, propranolol prevented epinephrine- and exercise-induced VT only unreliably in *RyR2*$^{R4496C/+}$, *CASQ2*$^{\Delta E9/\Delta E9}$, and *CASQ2*$^{D307H/D307H}$ mice (Cerrone et al. 2005, 2007; Katz et al. 2010). Due to only partial anti-arrhythmic efficacy of beta-blockers, potential anti-arrhythmic effects of various other agents were studied in detail in CPVT mouse models.

Recent data gathered in *CASQ2*$^{\Delta E9/\Delta E9}$ mice suggest that catecholamines may trigger arrhythmia not only via beta-receptor stimulation but also via alpha-receptor stimulation: The alpha-agonist phenylephrine provoked VT in *CASQ2*$^{\Delta E9/\Delta E9}$ mice, while the alpha-antagonist phentolamine or the alpha-/beta-antagonist labetalol abolished exercise- and epinephrine-induced arrhythmia, indicating that concomitant block of beta- and alpha-adrenergic receptors could become a therapeutic option for patients suffering from beta-blocker refractory arrhythmia (Kurtzwald-Josefson et al. 2014).

Among class I anti-arrhythmic drugs, flecainide and propafenone effectively suppressed exercise- and catecholamine-induced ventricular arrhythmia in CPVT mice (Watanabe et al. 2009; Hwang et al. 2011), whereas lidocaine and procainamide had no anti-arrhythmic effects (Watanabe et al. 2009; Katz et al. 2010; Hwang et al. 2011). Interestingly, direct inhibition of RyR2 by flecainide with suppression of spontaneous Ca^{2+} release from SR in addition to the suppression of triggered beats by sodium channel block was identified as its underlying anti-arrhythmic mechanisms (Watanabe et al. 2009). Based on these experimental and first clinical data (Watanabe et al. 2009; van der Werf et al. 2011) (as described in detail in the Chap. 10), the ESC guidelines for the prevention of SCD (2015) recommend the use of flecainide as add-on therapy in CPVT patients that remain symptomatic with syncope or VT while on beta-blocker (Priori et al. 2013). Of note, however, in another study flecainide surprisingly did not exert a relevant anti-arrhythmic effect in *CASQ2*$^{\Delta E9/\Delta E9}$ and *CASQ2*$^{D307H/D307H}$ mice (Katz et al. 2010).

Calcium blocker verapamil significantly lowered VT prevalence in *CASQ2*$^{\Delta E9/\Delta E9}$ and *CASQ2*$^{D307H/D307H}$ mice and similarly effectively abolished stress-induced ventricular arrhythmia in roughly half of CPVT patients still symptomatic despite propranolol therapy (Katz et al. 2010). Besides classical anti-arrhythmic agents, magnesium and dantrolene—both inhibiting RyR2 – decreased the incidence of catecholamine-induced VT in *CASQ2*$^{\Delta E9/\Delta E9}$, *CASQ2*$^{D307H/D307H}$ (Song et al. 2007), *RyR2*$^{S2246L10/+}$ (Suetomi et al. 2011), and *RyR2*$^{R2474S/+}$ mice (Kobayashi et al. 2010).

15.3 Advantages and Limitations of Current Transgenic Animal Models for Channelopathies

Small animal models have the advantage of allowing conducting longitudinal studies in subjects with a defined genetic background and without confounding comorbidities for the assessment of factors that may alter the arrhythmic disease phenotype. This particularly applies to mouse models since they are genetically identical, resulting in a less heterogeneous phenotype (Davisson 1999). Moreover, different genetic backgrounds of different mouse strains can be used to study the modulatory effect of genetic modifiers on disease severity (Remme et al. 2009). As mice have relatively short generation times, their handling is rather cost-effective, and they can be more easily subjected to genetic manipulation than larger animals. Thanks to novel developments in animal transgenesis (Bősze et al. 2016), rabbits that have the added advantage of more closely mimicking human cardiac electrophysiology (Nerbonne 2000) have also entered the range of species in whom genetic manipulation can be more easily performed.

However, as described in detail in the introduction and the disease-specific subchapters, species differences exist in cardiac electrophysiological properties, channel composition, and Ca^{2+} handling (Nakata and Hearse 1990; Williams et al. 2000; Nerbonne et al. 2001; Salama and London 2007; Baczkó et al. 2016). Therefore, most animal models of channelopathies can only mimic certain aspects of the disease phenotype (see details in Sects. 15.2.1–15.2.3).

Apart from these species differences in cardiac ion channels and Ca^{2+} handling properties, some other aspects may limit the use and transferability of findings to human disease management:

1. A variety of different mutations in different genes can cause the abovementioned channelopathies (or overlap syndromes), which can have very different impacts on biophysical ion channel properties (e.g., reduction vs. complete loss of current, changes in voltage dependency, changes in gating properties) and on arrhythmic risk. When designing transgenic animal models for a given disease, one must choose a single disease-causing mutation—ideally one that produces a pronounced phenotype. Mutation-specific aspects on electrical dysfunction and arrhythmogenesis, however, cannot be assessed with the limited amount of different disease subtypes currently available or even one single animal model for each channelopathy subtype. Here, "high-throughput" approaches combined with computational modeling may add important information.
2. Moreover, species differences may exist also in electrical remodeling mechanisms occurring secondary to ion channel mutations. In mouse models of LQTS, compensatory upregulation of non-affected repolarizing ion currents—partially restoring the reduced repolarization reserve—has been described (Koren 2004; Salama and London 2007), while in rabbit models likewise, remodeling of repolarizing ion currents was observed that may aggravate the disease phenotype (Brunner et al. 2008; Major et al. 2016). Whether electrical remodeling mechanisms

occurring in human LQTS cardiomyocytes more closely resemble the changes in transgenic rabbit or mouse models still remains to be elucidated.

3. In addition, the current techniques used to generate genetic animal models, e.g., the complete knockout of cardiac ion channels or the dominant-negative approach with cardio-selective overexpression of (mouse or human) disease-causing genes rather than replacement of an endogenous gene with the human disease-causing gene thus replicating the heterozygous situation found in human patients, may cause additional differences in electrical remodeling. In the future, this limitation may be overcome with novel genetic techniques such as CRISPR/Cas9, which allows integration of the mutant human gene into the animal DNA [as reviewed in Bősze et al. (2016)], to generate future transgenic animal models for channelopathies that more closely resemble human patients genetically.

15.4 Clinical Implications/Translational Aspects

To improve medical anti-arrhythmic therapy in patients with inherited channelopathies—particularly in those who are refractory to standard therapy—novel (mechanistic) therapeutic concepts have to be evaluated preclinically. Since in vitro testing in cellular systems cannot assess all multidimensional factors of arrhythmogenesis, animal models of channelopathies are indispensable tools for preclinical identification of anti-arrhythmic mechanisms and drug testing to improve patient safety. Indeed, in all currently available genetic animal models for channelopathies, mechanisms underlying anti-arrhythmic effects of currently used standard therapies have been revealed, and several novel anti-arrhythmic treatment options have been identified; some of these even have already entered the guidelines:

1. The identification of anti-arrhythmic effects of progesterone in LQT2 models (Odening et al. 2012) has raised the awareness of the potential benefit of contraceptive drugs (minipills) in female LQTS patients (Moss 2012; Odening et al. 2016).
2. Studies identifying the anti-arrhythmic properties of late $I_{Na,L}$ blockers in LQT3 models (Fabritz et al. 2003, 2010; Tian et al. 2004; Head et al. 2005) have led to the development of more selective late $I_{Na,L}$ inhibitors [such as GS967 (Belardinelli et al. 2013) and GS6615 (Rajamani et al. 2016)], which have already entered phase 1 and 2 clinical trials (NCT01849003).
3. The identification of anti-arrhythmic effects of beta-blockers even in LQT3 models and the underlying mode of action (Calvillo et al. 2014) has led to a change in the clinical practice in the treatment of LQT3 patients.
4. The identification of anti-arrhythmic effects of flecainide in CPVT models (Hwang et al. 2011) has resulted in a class IIa recommendation to use flecainide in CPVT patients still symptomatic while on beta-blocker therapy (Priori et al. 2015).

15.5 Outlook

Despite their above discussed limitations, transgenic animal models for human channelopathies have already been instrumental for identifying arrhythmic mechanisms on the whole heart, cellular, and molecular levels (see details in disease-specific Sects. 15.2.1–15.2.3) and will certainly further improve our mechanistic insights into arrhythmogenesis in channelopathies. This knowledge will be crucial to develop novel, mechanism-directed, genotype-specific therapeutic strategies in the future. However, due to limitations intrinsic to the currently available transgenic animal models (see Sect. 15.3), several additional experimental and clinical steps have to be taken to be able to transfer these insights from bench to bedside.

1. Additional techniques such as the integration of experimental in vivo, whole-heart, cellular, and ion channel data into computational models are warranted—particularly to assess potential mutation-specific aspects since only limited animal models with different mutations are currently available for the different channelopathies.
2. Novel techniques such as CRISPR/Cas9 must be employed to generate future transgenic animal models for channelopathies that more closely resemble human patients genetically [as reviewed in Bősze et al. (2016)]. Here, the generation of additional animal models for the above presented channelopathies with different mutations (that will allow experimentally assessing and comparing mutation-specific disease patho-mechanisms and treatment options) as well as novel transgenic animal models for other channelopathies are clearly warranted. In this regard, transgenic rabbit models for short QT syndrome and LQT3 are already in the pipeline to be published.
3. As genetic techniques develop further (and get less time-consuming and less expensive), other species that more closely resemble (all) the different aspects of human cardiac pathophysiology may be used for future generation of transgenic animal models for channelopathies that may facilitate bench-to-bedside translation.
4. Most importantly, several of the molecular findings gathered in transgenic channelopathy animal models must still be verified in human diseased tissue and cells (such as iPS-CM from patients) prior to their clinical application. Although these iPS-CM also have some limitations in regard to ion current composition compared to mature ventricular cardiomyocytes, here, quite some work has already been done to generate and characterize disease- and patient-specific iPS-CM for LQTS, BrS, and CPVT [as reviewed in Hoekstra et al. (2012)], which will certainly supplement the insights gathered on the in vivo and whole heart levels using animal models.

Compliance with Ethical Standards

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Conflict of Interest Katja E. Odening and David Ziupa declare that they have no conflict of interest.

Ethical Approval All animal studies summarized and reviewed in this article were conducted based on international, national, and/or institutional guidelines for the care and use of animals.

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