RESEARCH PAPER

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Transgenic LQT2, LQT5, and LQT2-5 rabbit models with decreased repolarisation reserve for prediction of drug-induced ventricular arrhythmias

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Background and Purpose: Reliable prediction of pro-arrhythmic side effects of novel drug candidates is still a major challenge. Although drug-induced pro-arrhythmia occurs primarily in patients with pre-existing repolarisation disturbances, healthy animals are employed for pro-arrhythmia testing. To improve current safety screening, transgenic long QT (LQTS) rabbit models with impaired repolarisation reserve were generated by overexpressing loss-of-function mutations of human *HERG* (*HERG-G628S*, loss of I_{Kr}; LQT2), *KCNE1* (*KCNE1-G52R*, decreased I_{Ks}; LQT5), or both transgenes (LQT2-5) in the heart.

Experimental Approach: Effects of K^+ channel blockers on cardiac repolarisation and arrhythmia susceptibility were assessed in healthy wild-type (WT) and LQTS rabbits using *in vivo* ECG and ex vivo monophasic action potential and ECG recordings in Langendorff-perfused hearts.

Key Results: LQTS models reflect patients with clinically "silent" (LQT5) or "manifest" (LQT2 and LQT2-5) impairment in cardiac repolarisation reserve: they were more sensitive in detecting I_{Kr}-blocking (LQT5) or I_{K1}/I_{Ks}-blocking (LQT2 and LQT2-5) properties of drugs compared to healthy WT animals. Impaired QT-shortening capacity at fast heart rates was observed due to disturbed I_{Ks} function in LQT5 and LQT2-5. Importantly, LQTS models exhibited higher incidence, longer duration, and more malignant types of ex vivo arrhythmias than WT.

Conclusion and Implications: LQTS models represent patients with reduced repolarisation reserve due to different pathomechanisms. As they demonstrate increased sensitivity to different specific ion channel blockers (I_{Kr} blockade in LQT5 and I_{K1} and I_{Ks} blockade in LQT2 and LQT2-5), their combined use could provide

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Abbreviations: APD, action potential duration; AR, arrhythmia; AV, atrioventricular; CL, cycle length; HERG, α-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, β-subunit to I_{Ks}-conducting potassium channel; KH, Krebs–Henseleit solution; 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; MAP, monophasic action potential; QT, QT interval in surface ECG; RR, interval between two consecutive R-waves in ECG; SCD, sudden cardiac death; STV_{QT}, short-term variability of QT interval; TdP, torsades de pointes; VEB, ventricular extra beat(s); VER, ventricular escape rhythm; VT, ventricular tachycardia; VF, ventricular fibrillation; WT, wild type.

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more reliable and more thorough prediction of (multichannel-based) pro-arrhythmic potential of novel drug candidates.

1 | INTRODUCTION

Pro-arrhythmia is a rare but potentially lethal side effect of various drugs and therefore a major safety concern for pharmaceutical industry during drug development. Most often, pro-arrhythmia is based on drug-induced prolongation of cardiac repolarisation. This "acquired long QT syndrome" (LQTS) predisposes to torsades de pointes (TdP) ventricular tachycardia (VT) that can lead to sudden cardiac death (SCD). TdP-induced SCD has been associated with a wide range of commonly used drugs (antipsychotics, antidepressants, antihistamines, and antibiotics; Fenichel et al., 2004; Haverkamp et al., 2000; Redfern et al., 2003), and many of them have been withdrawn from the market (Farkas & Nattel, 2010). Therefore, the need to minimise the pro-arrhythmic risk of novel drug candidates is overwhelming, although it remains largely unmet (Farkas & Nattel, 2010; Pugsley, Authier, & Curtis, 2008).

Roughly 20%–60% of novel chemical entities have the potential to modulate cardiac ion channels, thereby affecting cardiac repolarisation (Danker & Moller, 2014). The majority of these compounds inhibit the rapid delayed rectifier potassium current I_{Kr} (HERG); but interference with other ion currents such as the **slow delayed rectifier potassium current** I_{Ks} or the **inward rectifier potassium current** I_{K1} can also induce serious pro-arrhythmia—particularly in the setting of reduced repolarisation reserve as demonstrated for I_{Ks} blockers, such as **isoflurane, oxytocin**, and propionic acid and for the I_{K1} blocker, **midazolam**, in the context of LQTS (Bodi et al., 2016, 2019; Dunnink et al., 2010; Odening et al., 2008). Despite this risk, drug effects on I_{Ks} or I_{K1} are not yet routinely assessed in safety screening (Ponte, Keller, & Di Girolamo, 2010).

Drug-induced TdP are more frequently found in patients with cardiovascular and metabolic diseases that induce structural and/or electrophysiological remodelling of the heart, leading to reduction of the "repolarisation reserve," a term, defined as the ability of cardiomyocytes to maintain sufficient repolarisation despite repolarisation-prolonging (I_{Kr}, I_{Ks}, I_{K1}, and I_{to} blocking) effects by compensation via non-affected "reserve" outward K^+ currents (Roden, 1998; Varro & Baczko, 2011). In spite of this, preclinical safety tests are performed in healthy animals or their tissues and cells (Food et al., 2005a, 2005b). This approach has serious limitations due to false-positive and false-negative results that are very costly or highly dangerous. Therefore, novel animal models for safety testing should ideally mimic the pathophysiological conditions under which drugs usually display highest pro-arrhythmic risk. Animal models with remodelled myocardium (Nattel, Maguy, Le Bouter, & Yeh, 2007) and/or impaired repolarisation reserve (Varro & Baczko, 2011) due to reduced I_{Kr} or I_{Ks} appear to be more suitable (Lengyel, Varro, Tabori, Papp, & Baczko, 2007; London et al., 1998; Salama & London, 2007; Thomsen et al., 2004; Volders et al., 1999; Vos et al., 1998). Among

What is already known

- For more reliable screening of drug-induced pro-arrhythmia, models with impaired repolarisation reserve are needed.

What this study adds

- LQT5, LQT2, and LQT2-5 rabbits represent patients with impaired repolarisation reserve due to different pathomechanisms.
- LQTS models better detect K^+ channel blocker effects on pro-arrhythmic biomarkers and predict drug-induced arrhythmias.

What is the clinical significance

- LQTS rabbits provide better prediction of pro-arrhythmic potential of drug candidates than wild-type animals.

them, the rabbit has a prominent place in arrhythmia research for numerous reasons (Valentin, Hoffmann, De Clerck, Hammond, & Hondeghem, 2004). Thus, rabbit cardiac physiology resembles that of humans more closely than that of other small animals like rats or mice regarding (i) the shape of action potential (Varro, Lathrop, Hester, Nanasi, & Papp, 1993) and the underlying cardiac ion channels and currents (Nerbonne, 2000), (ii) the relative effective heart size relating cardiac mass to the frequency of VF (Panfilov, 2006), and (iii) their responses to pharmacological interventions (Harken et al., 1981).

Therefore, in this work, several transgenic LQTS rabbit models (LQT2: *HERG-G628S*, loss of I_{Kr}, Brunner et al., 2008; LQT5: *KCNE1-G52R*, decreased I_{Ks}, Major et al., 2016; and LQT2-5, containing both mutations) with impairment in cardiac repolarisation due to different mechanisms were used to model electrophysiological changes that occur in patients most susceptible for drug-induced arrhythmias. In these models, genotype differences in response to challenges on cardiac repolarisation, for example, different K^+ channel blockers and sympathomimetic drugs, were investigated in comparison to WT animals. Ultimately, their arrhythmia susceptibility was tested to estimate their potential use in drug-induced pro-arrhythmia risk prediction and/or assessment.

2 | METHODS

2.1 | Animals

All animal care and experimental procedures were performed in compliance with EU legislation (directive 2010/63/EU), the German (TierSchG and TierSchVersV), and Hungarian animal welfare laws, after approval by the local Institutional Animal Care and Use Committees in Germany (Regierungspraesidium Freiburg; approval number G14/111) and Hungary (Department of Animal Health and Food Control of the Csongrád County Government Office; approval number XIII/4227/2016). Animal housing and handling was in accordance with good animal practice as defined by the Federation of European Laboratory Animal Science Association, FELASA. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010) and with the recommendations made by the *British Journal of Pharmacology* (McGrath & Lilley, 2015).

New Zealand white WT female (purchased from Charles River Laboratory in Châtillon, France), transgenic LQT2 male and LQT5 male and female rabbits (*Oryctolagus cuniculus*), from our own colonies were used for breeding to generate littermate control and transgenic LQT5, LQT2, and LQT2-5 animals. Generation of the "founder" transgenic LQT2 and LQT5 rabbits was described earlier in detail (Brunner et al., 2008; Major et al., 2016). Rabbits were individually housed in stainless steel cages that fulfilled the legal requirements in terms of size and environmental enrichments. The animals were kept at standard temperature, humidity, and lighting. Food and drinking water were provided ad libitum.

2.2 | Description of model systems

Adult (aged 4–5 months, approximately 3–3.5 kg) transgenic long QT syndrome type 2 (LQT2; *HERG-G628S*; Brunner et al., 2008), type 5 (LQT5; *G52R-KCNE1*; Major et al., 2016), double transgenic long QT 2-5 (LQT2-5), and wild-type (WT) littermate control New Zealand white rabbits of both sexes were used for experiments. In LQT2 rabbits, the dominant-negative loss-of-function mutation in HERG—the α-subunit conducting I_{Kr} currents—leads to a complete loss of I_{Kr} as the co-assembly of one or more mutant HERG subunits even with up to three normal HERG subunits will cause a malfunctioning channel. In LQT5, the loss-of-function mutation in the β-subunit KCNE1, which may co-assemble with KCNQ1 or HERG, leads predominantly to an alteration in I_{Ks}.

The rabbit is an ideal model system for the purpose of this study since (in contrast to other frequently utilised species such as mouse and rat) it demonstrates close similarities to human (patho)physiology in repolarising ion currents, action potential shape, and arrhythmia mechanisms (as detailed in Section 1).

2.3 | Drug treatments

For *in vivo* and ex vivo experiments, the following "selective" K^+ channel blockers were used: **dofetilide** to inhibit the rapid delayed rectifier potassium current (I_{Kr}), **HMR-1556** to inhibit the slow delayed rectifier potassium current (I_{Ks}), and **barium chloride** ($BaCl_2$) to inhibit the inward rectifier potassium current (I_{K1}; Figure S1C). PEG-400 (0.125 ml·kg^{-1}) was used as solvent to dissolve HMR-1556. The ex vivo and *in vivo* doses of dofetilide, HMR-1556, and $BaCl_2$ were determined based on published data dosages described in the literature (for references, see Figure S1C) with minimal or no effect in healthy, WT animals, but with expected repolarisation prolonging effects in the set of reduced repolarisation reserve in long QT models. Therefore, no dose-finding experiments were performed. For I_{Ks} activation, the sympathomimetic drug **isoprenaline** (Isuprel 0.2 mg·kg^{-1}, Hospira Inc., USA) was continuously perfused intravenously in a dose of 6–12 µg·h^{-1} (3–6 ml·h^{-1}) to increase the baseline heart rate by 20%–30%. **S-ketamine** (Pfizer, USA) and **xylazine** (Bayer, Germany) were used for anaesthesia (12.5 ml·kg^{-1}/3.5 ml·kg^{-1} i.m., followed by intravenous infusion) during ECG transmitter implantation, surface ECG recording, and prior to heart extraction, as this combination does not affect cardiac repolarisation (Odening et al., 2008).

2.4 | Genotype and phenotype verification

The presence of transgene(s) in the offspring was verified by PCR performed on genomic DNA obtained from blood taken at the age of 2–3 months as described earlier (Brunner et al., 2008; Major et al., 2016). The following primer pairs were used: LQT2: 5′- GAA CCA GCT TCT TCC GCT CAC TAC AGG TAC AG -3′ and 5′- GGG CAC ATC CAC CAG ACA TAG GAA GCA -3′; LQT5: 5′- ATG ATC CTG TGT AAC ACC ACA GAG-3′ and 5′- TTA GCC AGT GGT GGG GTT CA -3′.

The phenotypes were verified by conventional surface ECG in sedated rabbits at the age of 3–4 months. QT indexes were calculated (QTi; QTi (%) = (QT observed/QT expected) * 100) as published previously (Brunner et al., 2008). Rabbits from all genotypes (WT, LQT5, LQT2, and LQT2-5) with QTi of at least 95% or higher were used for further experiments (predetermined exclusion criteria; similarly as predefined in all previous studies with these transgenic LQTS rabbit models).

2.5 | ECG monitoring

For ECG monitoring of awake, unrestrained, free-moving animals, WT (*n* = 11), LQT5 (*n* = 11), LQT2 (*n* = 10), and LQT2-5 (*n* = 8) rabbits were subjected to ECG transmitter implantations (triple-lead ECG D70-EEE; Data Sciences International; Brunner et al., 2008; Odening et al., 2012). Subcutaneous ECG transmitter implantations were performed under general anaesthesia with ketamine and xylazine (induced with intramuscular administration of 12.5/3.75 ml·kg^{-1} ketamine/xylazine; maintained with intravenous administration of 2.5–5 ml·kg^{-1}·h^{-1} ketamine/xylazine) as described in detail in (Brunner et al., 2008; Odening et al., 2012). ECG recordings were started at least 2 weeks after device implantation

to ensure adequate recovery and good healing for artefact-free ECG signals.

Following 24-h baseline recordings, dofetilide (0.02 µg·kg^{-1}), BaCl$_2$ (0.3 mg·kg^{-1}), PEG-400 (0.125 ml·kg^{-1}), HMR-1556 (0.1 µg·kg^{-1} in 0.125 ml·kg^{-1} PEG400), and combinations of HMR-1556 and BaCl$_2$ were administered intramuscularly in 0.5 ml·kg^{-1} BW final injection volume, one drug per subsequent day in all monitored animals. ECGs were continuously recorded for 24-h following each injection to monitor the changes in conventional ECG parameters.

To calculate the QT/RR relationship for each individual rabbit, pairs of QT and RR intervals were averaged over 5 s every 30 min during the 24-h baseline recording period (48 pairs per animals). These QT-RR pairs were plotted, and a linear regression formula was obtained for each animal. Using this individual heart rate correction formula (QT (y) = a * RR (x) + b), individual QT expected (QT exp. (y) = a * RR (x) + b) and QT index (QTi (%) = 100 * (QT observed/QT expected)) were calculated for each animal (Brunner et al., 2008). For heart rate-corrected QT intervals (QTc) calculation, a modified version (QTc = QT observed – (a * (RR – 250), where "a" represents the slope of the individual QT/RR relationship) of the original Carlsson equation (QTc = QT observed – (0.175 * (RR – 300); Carlsson, Abrahamsson, Andersson, Duker, & Schiller-Linhardt, 1993) was used to better match the heart rate range of our telemetrically monitored rabbits.

As we observed no significant circadian alteration in the individual's QTc and QTi values, their 24-h averaged values during baseline were used as control to assess the effect of dofetilide and BaCl$_2$ on QTc/QTi. For HMR-1556 and HMR-1556 + BaCl$_2$, the averaged QTc and QTi values measured within 5 h after intramuscular injection of PEG 400 vehicle administration were used as "vehicle control" values. To obtain genotype-specific heart rate correction formulas, linear regression curves were fitted to all QT/RR pairs measured in all animals per genotype (Brunner et al., 2008).

Conventional 12-lead surface ECG was recorded to monitor changes in pro-arrhythmic biomarkers in sedated WT (n = 6), LQT5 (n = 9), LQT2 (n = 8), and LQT2–5 (n = 8). Sedation was performed with ketamine/xylazine (12.5/3.75 ml·kg^{-1} i.m.). ECG was recorded at baseline and after 20 min of intravenous administration of dofetilide (0.02 µg·kg^{-1}), BaCl$_2$, (0.3 mgkg^{-1}), HMR-1556 (0.1 µg·kg^{-1}), and combination of HMR-1556 and BaCl$_2$ (Figure S1C). T$_{peak}$–T$_{end}$ (T$_{p-e}$) and beat-to-beat variability of QT (short-term variability of the QT interval [STV$_{QT}$]) were calculated to assess changes in spatial and temporal heterogeneity of repolarisation. T$_{peak-end}$ was measured in V3 as duration (ms) from the peak to the end of the T wave. For STV$_{QT}$, 31 consecutive QT were measured, and STV$_{QT}$ was calculated using the following equation: STV$_{QT}$ = $\sum |D_{n+1} – D_n|(30 \times \sqrt{2})^{-1}$, where D is the duration of the QT intervals (Berger et al., 1997).

As the sympathetic nervous system plays a major role in triggering arrhythmias in (drug-induced and acquired) LQTS—particularly in the setting of absent or impaired I$_{Ks}$—we aimed at investigating if similar phenomenon could be observed in the LQT-5 and LQT5 models (with mildly reduced I$_{Ks}$). For these experiments, we used the sympathomimetic isoprenaline as it mimics the effect that an activation of the sympathetic nervous system will have in LQTS patients. Changes

in QTi (%) resulting from intravenous administration of I$_{Ks}$ activator isoprenaline followed by the I$_{Ks}$ blocker HMR-1556 were measured to assess I$_{Ks}$ function *in vivo*.

2.6 | Monophasic action potential measurement and ex vivo arrhythmia study

WT (n = 13), LQT5 (n = 15), LQT2 (n = 12), and LQT2-5 (n = 11) rabbits were anaesthetized with ketamine and xylazine (as described above). After additional injection of heparin (500 IE i.v.), animals were killed by intravenous administration of thiopental-sodium (40 mg·kg^{-1} i.v.). Immediately afterwards, beating hearts were excised, attached to a vertical Langendorff apparatus (Model IH5, Hugo Sachs Elektronik, Hugstetten, Germany), and paced at basic cycle length (CL) of 500 ms (Ziupa et al., 2014).

Monophasic action potentials (MAPs) were recorded by four epicardial contact MAP electrodes positioned onto different regions of the heart: MAP 1, apico-anterior; MAP 2, mid-anterolateral; MAP 3, base-inferolateral; and MAP 4, base-inferior positions (Figure 3). After an average of 20–30 min of equilibration, MAPs were recorded at 500 and 250 ms CLs of stimulation (2 and 4 Hz) at baseline and during the perfusion with dofetilide (1 nM), HMR-1556 (100 nM), BaCl$_2$ (10 µM), or with combination of BaCl$_2$ (10 µM) + HMR-1556 (100 nM) (Figure S1C).

The ex vivo arrhythmia setting was developed based on a method described by Eckardt, Haverkamp, Borggrefe, and Breithardt (1998), in which bradycardia, low K$^+$ concentration, and a K$^+$ channel blocker were combined to prolong repolarisation and favour arrhythmias. Arrhythmia (AR) development was provoked in atrioventricular (AV)-ablated hearts (n = 7 WT, n = 8 LQT5, n = 6 LQT2, and n = 7 LQT2–5)—beating spontaneously in stable ventricular escape rhythm (VER) at a constant rate of 60–80 beats per minute—by perfusion with the following solutions: 5.4-mM K$^+$ Krebs–Henseleit (KH; baseline I, 10 min), 2.0-mM K$^+$ KH (5 min), 5.4-mM K$^+$ KH (baseline II, 10 min), 5.4-mM K$^+$ KH + 10-µM BaCl$_2$ (10 min), and 2.0-mM K$^+$ KH + 10-µM BaCl$_2$ (5 min). ECG was continuously recorded, and the duration (% of perfusion time) and incidence (as average number of AR events as well as % of total number of experiments) of arrhythmias were measured offline. Arrhythmias were categorised by "The Lambeth Conventions (II)" (Curtis et al., 2013) as ventricular extra beats (VEBs; ventricular "premature" extra beat(s)—ranging from a simple ventricular extrasystole to couplets or triplets in terms of complexity), bigeminy, VT, and ventricular fibrillation (VF).

2.7 | Data and statistical analysis

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018). All data that support the findings of this study are available from the corresponding author upon reasonable request. Data are expressed either as mean ± SEM or median with the lower (25th percentile) and upper (75th percentile) quartiles and the minimum and maximum values. Statistical and Power

analyses were performed by Prism 8.0 (Graphpad, San Diego, USA) and Prism StatMate. Graphs were created by Prism 8.0.

Power analyses were performed to determine sample sizes: the minimal number of animals per groups (WT, LQT5, LQT2, and LQT2-5) required for detecting a minimum difference of 15 (ms) in APD or QTc between genotypes (unpaired t test, expected SD = 8 ms) at a 90% power level was $N = 8$, and at an 80% power level $N = 6$. To detect a minimum change in QTc or APD of 10 ms in response to drug administration (paired t test, expected SD = 8 ms) at a 90% power level, a minimum of $N = 8$ animals per groups were required, and at an 80% power level $N = 6$. To detect significant difference in arrhythmia occurrence (chi-square test) between WT and LQTS (at arrhythmia rates of 5% in WT and 75% in LQTS), a minimum of $N = 5$ animals per groups were needed at 80% power level. On the basis of such considerations, we planned a sample size of six to eight animals for each genotype group. Slight differences among genotypes resulted mainly from variable availability of offspring with the different genotypes. Experiments were not designed to perform statistical analyses on potential sex differences.

Experiments were performed by several qualified and experienced operators. No randomisation was performed, as the same study protocol and the same procedures were performed in a standardised manner on each rabbit for every genotype group. Analyses could not remain completely blinded as the genotype differences (at least between LQT2/LQT2-5 and LQT5/WT) were immediately apparent when measuring QT or APD due to pronounced phenotype differences.

Measured data were reported as absolute values; QTi, QTc, STV_{QT}, duration, and incidence of ex vivo arrhythmias were calculated as described in detail above. Normal distribution of all data was checked prior to statistical analysis. To analyse normally distributed data, the following parametric tests were used: paired t test for comparisons before and treatment: one-way ANOVA for genotype-specific comparisons; repeated-measure ANOVA for intragroup comparisons (e.g., for regional comparisons between MAP 1-4 parameters).

Post hoc Tukey tests were conducted only if F was significant and there was no variance inhomogeneity. For not normally distributed data, nonparametric tests were used: Wilcoxon rank-sum test for comparisons before and after treatment; Kruskal–Wallis test for genotype-specific comparisons. Chi-square tests were used to compare arrhythmia incidences between different genotypes. A level of $P<.05$ was taken to show statistically significant differences between group means.

2.8 | Materials

The compounds used in these studies were supplied as follows: $BaCl_2$ and dofetilide by Sigma-Aldrich (Munich, Germany); heparin by Braun (Germany); HMR-1556 by Tocris Bioscience (Bristol, UK); isoprenaline by Hospira Inc., (USA); S-ketamine by Pfizer (USA); thiopental-sodium by Inresa (Germany); xylazine by Bayer (Germany).

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Mathie et al., 2019).

3 | RESULTS

3.1 | Baseline characteristics of different (LQTS) rabbit models with impaired repolarisation reserve

3.1.1 | ECG characteristics *in vivo*

For *in vivo* phenotypic characterisation of the different rabbit models with reduced repolarisation reserve, 24-h telemetric ECGs were recorded at baseline (drug-free) in awake, free-moving WT, LQT2, LQT5, and LQT2-5 rabbits. LQT2 and LQT2-5 rabbits showed prolonged QT intervals compared to WT or LQT5 animals (Figure 1a)— despite similar heart rates (Figure 1b and Table 1A). Similarly, heart rate-corrected QT interval was prolonged in LQT2 and LQT2-5 rabbit models, compared with that in WT or LQT5 animals — but did not differ between LQT5 and WT (Figure 1b and Table 1A).

Importantly, LQT2 and LQT2-5 rabbits also showed an increased QT/RR ratio steepness compared to WT or LQT5 (Figure 1c and Table 1A), indicating a particularly pronounced QT prolongation at lower heart rates. Of note, in LQT2-5 rabbits, flatter QT-RR regression curve with slightly higher QT values at high heart rates could be observed compared to LQT2, which may be the consequence of the impaired I_{Ks} function in LQT2-5, compared with LQT2.

Similarly to the free-moving animals, no genotype differences were seen in RR, PQ, or QRS in anaesthetised animals. Heart rate-corrected QTc and pro-arrhythmia markers SVT_{QT} and $T_{peak}-T_{end}$ (T_{p-e}), however, were significantly prolonged in LQT2 and LQT2-5 as compared with those in WT and LQT5 rabbits (Table 1B).

3.1.2 | Global and regional MAP characteristics ex vivo

MAP durations (APDs) recorded ex vivo in Langendorff-perfused hearts were also significantly longer in hearts from LQT2 and LQT2-5 animals than in those from WT or LQT5 animals (Figure 2a). LQT2 and LQT2-5 hearts showed prolonged APD_{75} compared to those from WT or LQT5 rabbits, particularly at longer stimulation CL, resulting in steeper APD/CL ratio in LQT2 and LQT2-5 hearts, than in those from WT or LQT5 animals (Figure 2b,c and Table 2). This phenomenon could be observed in all different left ventricle (LV) regions (Figure S2A).

Triangulation of the action potential (mean APD_{90-30}), an important marker of pro-arrhythmia (Hondeghem, Carlsson, & Duker, 2001; Hondeghem & Hoffmann, 2003), was also more prominent in LQT2 and LQT2-5 than in WT or LQT5 (Figure 2d and Table 2).

In addition to genotype differences in overall repolarisation characteristics (mean APD, mean APD_{90-30}), genotype differences in regional heterogeneities of APD and AP-triangulation were observed. In the ex vivo isolated hearts from all transgenic rabbits with reduced repolarisation reserve, shorter apical (MAP 1) than basal APD_{75} (MAP 3/4) was measured, but not in hearts from WT animals (Figure 3a). Furthermore, triangulation of APD (APD_{90-30}) was more pronounced in LV apex than in base only in LQT5 and LQT2-5 hearts (Figure 3b).

In summary, baseline AP parameters (APD_{75}, APD_{90-30}, and APD_{75}/CL ratio) were not significantly different in hearts from LQT5 rabbits, compared to those from WT animals but were similarly prolonged in LQT2 and LQT2-5 models. Assessment of regional AP parameters revealed further repolarisation disturbances, and in LQT5, LQT2, and LQT2-5 hearts, an apico-basal heterogeneity of repolarisation (APD) was detected. Regional heterogeneity in APD/CL ratio was measured in LQT2 but not in LQT2-5 hearts, whereas regional differences in AP triangulation were detected in LQT2-5 hearts but was absent in LQT2 hearts.

3.2 | Utility of different (LQTS) rabbit models with impaired repolarisation reserve to detect K^+ channel blocking effects

3.2.1 | K^+ blocker effects on QTc, STVQT, and $T_{peak}-T_{end}$ *in vivo*

To investigate the sensitivity of the different models with impaired repolarisation reserve to further drug-induced reduction of repolarising potassium currents, different "selective" K^+ channel blocking drugs were administered.

In awake, free-moving animals, slight I_{Kr} blockade by low-dose administration of dofetilide prolonged QTc only in LQT5 but not in healthy WT, nor in LQT2 and LQT2-5 rabbits that both lack I_{Kr} (Figure 4a). The I_{K1} blocker $BaCl_2$ prolonged QTc in all groups (Figure 4a) and this effect was particularly pronounced in LQT2
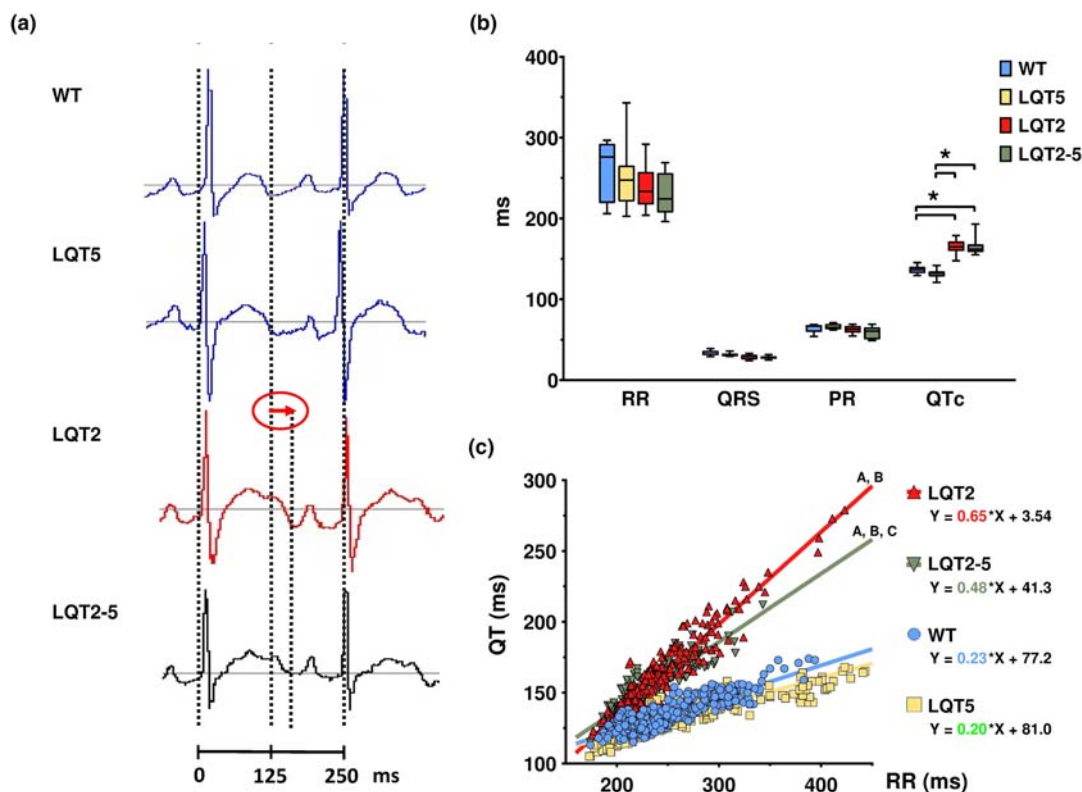

FIGURE 1 Baseline ECG characteristics in vivo. (a) Telemetric ECG: representative telemetric ECG recordings (lead II) in WT, LQT5, LQT2, and LQT2-5 rabbits at comparable RR intervals. (b) ECG parameters: RR, QRS, PR, QT, and QTc intervals of awake, free-moving rabbits during 24-h baseline (drug-free) telemetric ECG recordings in WT (*n* = 11), LQT5 (*n* = 11), LQT2 (*n* = 10), and LQT2-5 (*n* = 8) animals. *P < .05, significant differences between genotypes. Data are shown as box and whisker plots, with median, the lower (25th percentile) and upper (75th percentile) quartiles and the minimum and maximum values. (c) QT/RR relationship: QT/RR relationships of WT (*n* = 11), LQT5 (*n* = 11), LQT2 (*n* = 10), and LQT2-5 (*n* = 8) rabbits. Colour-coded lines indicate linear regression curves best-fit to all data points per genotype. Linear regression formulas with QT/RR ratio steepness (as colour coded numbers) are also shown. [A]P < .05 significantly different from WT; [B]P < .05 significantly different from LQT5; [C]P < .05 significantly different from WT

TABLE 1 ECG parameters at baseline

(A) Mean RR, QRS, PR, QT, and QTc intervals and QT/RR steepness of awake, free-moving rabbits during 24-h baseline (drug-free) telemetric ECG recordings						
Genotype (no. of animals)	RR	QRS	PR	QT	QTc	QT/RR steepness
WT (11)	265.5 ± 10.9	33.2 ± 1.2	64.4 ± 1.9	136.5 ± 2.9	136.8 ± 1.6	0.23 ± 0.007
LQT5 (11)	248.9 ± 11.9	31.6 ± 0.9	66.1 ± 1.3	129.1 ± 2.9	131.6 ± 1.7	0.20 ± 0.005
LQT2 (10)	239.4 ± 9.3	29.0 ± 1.1	62.6 ± 1.7	153.9[a,b] ± 6.1	165.4[a,b] ± 2.9	0.65[a,b] ± 0.013
LQT2-5 (8)	230.2 ± 9.3	28.0 ± 0.9	59.0 ± 3.1	151.7[a,b] ± 4.7	165.7[a,b] ± 4.2	0.48[a,b] ± 0.017

(B) Mean RR, QT, and QTc intervals, STV_{QT}, and $T_{peak}-T_{end}$ of anaesthetized rabbits at baseline ECG recordings					
Genotype (no. of measurements; no. of animals)	RR	QT	QTc	STV_{QT}	T_{p-e}
WT (18; 6)	347.2 ± 10.4	159.3 ± 3.5	137.0 ± 2.4	1.9 ± 0.1	29.8 ± 0.8
LQT5 (27; 9)	359.9 ± 6.0	167.7 ± 1.6	145.7 ± 1.2	1.9 ± 0.1	30.6 ± 0.6
LQT2 (20; 8)	357.8 ± 7.7	233.1[a,b] ± 8.2	163.0[a,b] ± 5.2	2.8[a,b] ± 0.1	40.9[a,b] ± 1.4
LQT2-5 (20; 8)	354.0 ± 8.0	218.1[a,b] ± 5.6	168.2[a,b] ± 3.2	2.5[a,b] ± 0.1	39.2[a,b] ± 1.2

Note. Number of animals are indicated in the table. Data are shown as mean ± SEM.

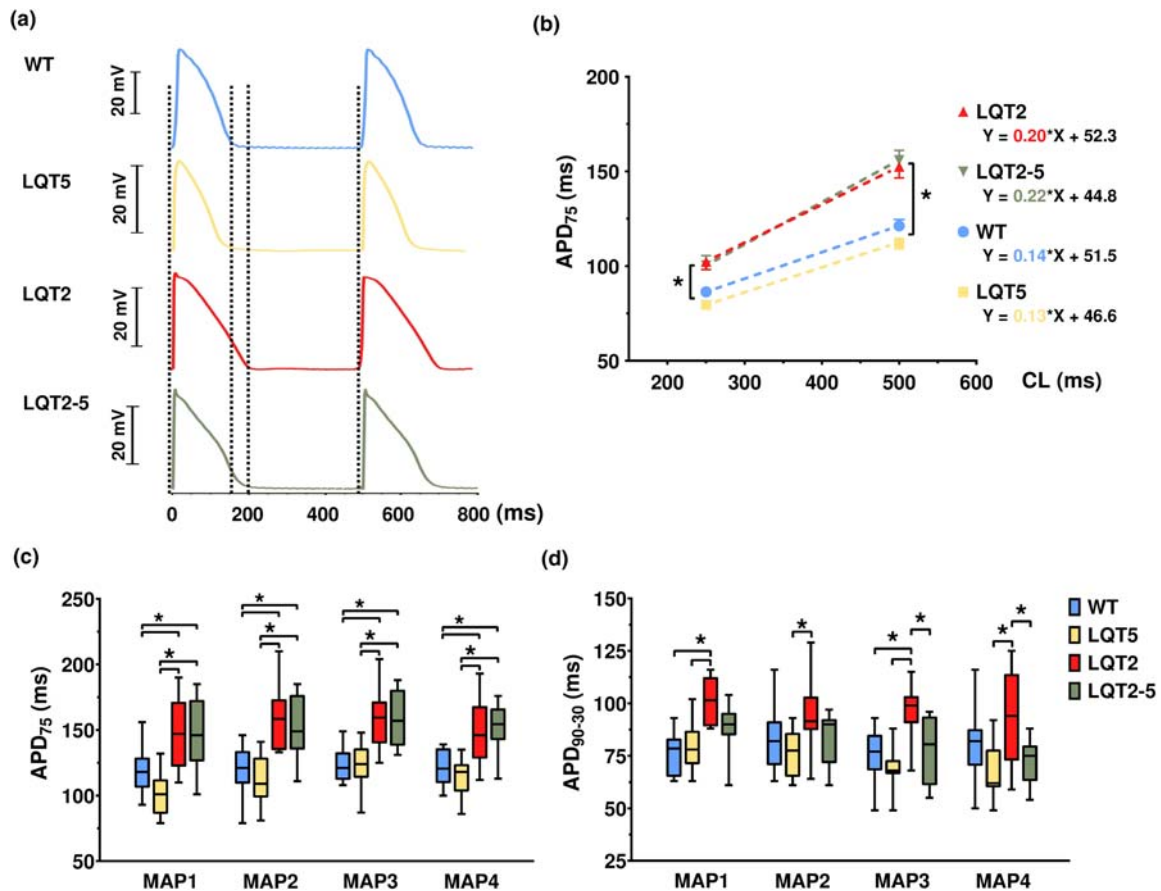[a]$P < 0.05$ versus WT.

[b]$P < 0.05$ versus LQT5.

FIGURE 2 Baseline action potential parameters ex vivo. (a) Monophasic action potentials: representative MAP recordings in isolated hearts from WT, LQT5, LQT2, and LQT2-5 rabbits, recorded at 500-ms cycle length (CL) of stimulation. (b) APD/CL relationship: CL dependence of averaged APD_{75} (representing the averaged APD_{75} values measured simultaneously by four epicardial electrodes of n = 15 WT, n = 17 LQT5, n = 14 LQT2, n = 11 LQT2-5). Data are shown as mean ± SEM. (c) Regional action potential duration: action potential durations were defined as APD_{75}. WT, n = 15; LQT5, n = 17; LQT2, n = 13; and LQT2-5, n = 11. (d) Regional AP triangulation: AP triangulation was defined as APD_{90}-APD_{30}. WT, n = 12; LQT5, n = 15; LQT2, n = 13; and LQT2-5, n = 9. *$P < .05$, significant differences between genotypes. Data are shown as box and whisker plots, with median, the lower (25th percentile) and upper (75th percentile) quartiles, the minimum and maximum values. Abbreviations: MAP 1, apico-anterior; MAP 2, mid-anterolateral; MAP 3, base-inferolateral; and MAP 4, base-inferior positions

TABLE 2 APD_{75}, APD_{90-30}, and APD_{75}/CL ratio values at baseline

| Genotype (no. of animals) | Cycle lengths of stimulation | | | | APD$_{75}$/CL steepness |
| | 250 ms | | 500 ms | | |
	APD$_{75}$	APD$_{90-30}$	APD$_{75}$	APD$_{90-30}$	
WT (13)	86.4 ± 3.0	62.8 ± 1.9	121.2 ± 3.7	78.4 ± 2.7	0.14 ± 0.018
LQT5 (15)	79.5 ± 2.7	58.5 ± 1.0	112.5 ± 3.7	73.0 ± 2.0	0.13 ± 0.017
LQT2 (12)	102.3^{a,b} ± 4.6	70.4^b ± 3.5	152.6^{a,b} ± 6.3	94.9^{a,b} ± 4.1	0.20^{a,b} ± 0.029
LQT2-5 (11)	98.6^{a,b} ± 5.2	66.0^{a,b} ± 1.4	152.5^{a,b} ± 5.2	82.1 ± 2.7	0.22^{a,b} ± 0.029

Note: Number of animals are indicated in the table. Data are shown as mean ± SEM.
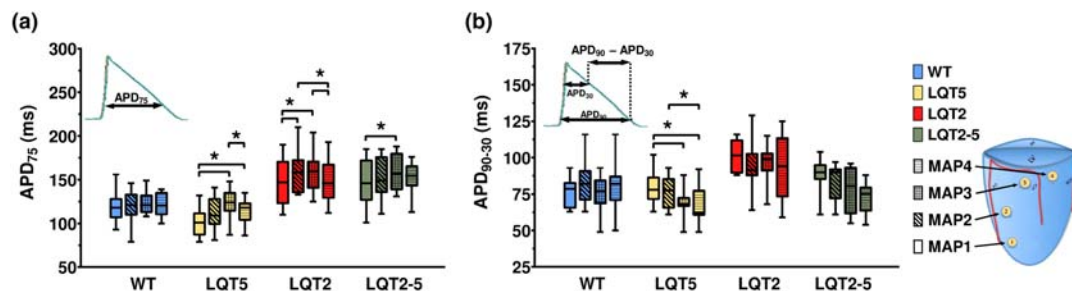^{a}$P < 0.05$ versus WT.
^{b}$P < 0.05$ versus LQT5.

FIGURE 3 Regional heterogeneities of baseline action potential parameters ex vivo. Regional differences in (a) action potentials (APD_{75}) and (b) AP triangulation. WT, $n = 13$–15; LQT5, $n = 15$–17; LQT2, $n = 13$–14; and LQT2-5, $n = 9$–12. $^*P < .05$, significant differences between regions. Data are shown as box and whisker plots, with median, the lower (25th percentile) and upper (75th percentile) quartiles, the minimum and maximum values. Abbreviations: MAP 1, apico-anterior; MAP 2, mid-anterolateral; MAP 3, base-inferolateral; and MAP 4, base-inferior positions

rabbits, compared with data from WT, LQT5 or LQT2-5 animals. I_{Ks} blockade alone (HMR-1556) did not have any significant effect on QTc in any genotype. Combined blockade of I_{K1} (BaCl$_2$) and I_{Ks} (HMR-1556), in contrast, prolonged QTc in all groups (Figure 4a).

In anaesthetised animals, similar changes in QTc were observed (Figure 4b). I_{K1} blocker BaCl$_2$ prolonged QTc significantly in all genotypes, but this effect was more prominent in LQT2 and LQT2-5 than in WT or LQT5. HMR-1556 and HMR-1556 + BaCl$_2$ effects were similar to those observed in free-moving animals. Dofetilide prolonged QTc in LQT5 but, surprisingly, it also prolonged QTc in LQT2-5 rabbits. Pro-arrhythmia markers STV$_{QT}$ and T_{peak}–T_{end} were more pronouncedly affected by K$^+$ channel blockers in LQTS rabbits with impaired repolarisation reserve. Thus, dofetilide and HMR-1556 increased STV$_{QT}$ and prolonged T_{peak}–T_{end} only in LQT5 and LQT2-5 rabbits (Figure 4c,d). BaCl$_2$-induced and combined HMR-1556 and BaCl$_2$-induced increases in STV$_{QT}$ and T_{peak}–T_{end} were more pronounced in all LQTS animals than in WT (Figure 4c,d).

It is important to note that series of VEBs and nonsustained VTs were observed in one LQT2 rabbit and one LQT2-5 rabbit—which both had exceptionally severe phenotype (QTi > 110%) even at baseline—during BaCl$_2$ exposure, demonstrating increased susceptibility *in vivo* to arrhythmia induced by K$^+$ channel blockers (Figure 4e).

3.2.2 | I_{Ks} function in the different models with reduced repolarisation reserve

The sympathomimetic isoprenaline was administered to activate I_{Ks} and to investigate differences in cardiac repolarisation, which may occur upon sympathetic activation in the different LQTS rabbit models. Normally, QT shortening is observed as a consequence of physiological QT adaptation, a process in which the interplay between simultaneously activated repolarising I_{Ks} (QT shortening) and depolarizing $I_{Ca,L}$ (QT prolongation) plays a major role (Liu et al., 2012). Due to the presence of the mutant *KCNE1* (*KCNE1-G52R*) encoding an abnormal β-subunit of the I_{Ks}-conducting channel complex in LQT5 and LQT2-5, the malfunctioning I_{Ks} could not counterbalance the QT-prolonging effect of activated $I_{Ca,L}$, thus resulting in significantly more pronounced QT prolongation in LQT5 and LQT2-5 than in WT and LQT2 with normally functioning I_{Ks} (Figure 5a). On the other hand, the I_{Ks} blocker HMR-1556-induced QT prolongation was much more prominent in WT or LQT2 rabbits, in which the normally functioning I_{Ks} was properly "pre"-activated by isoprenaline, than it was in LQT5 or LQT2-5 rabbits, in which I_{Ks} could not be "pre"-activated (Figure 5b).

These results suggest impaired I_{Ks} function in LQT5 and LQT2-5 animals, a fact that should be taken into consideration when testing
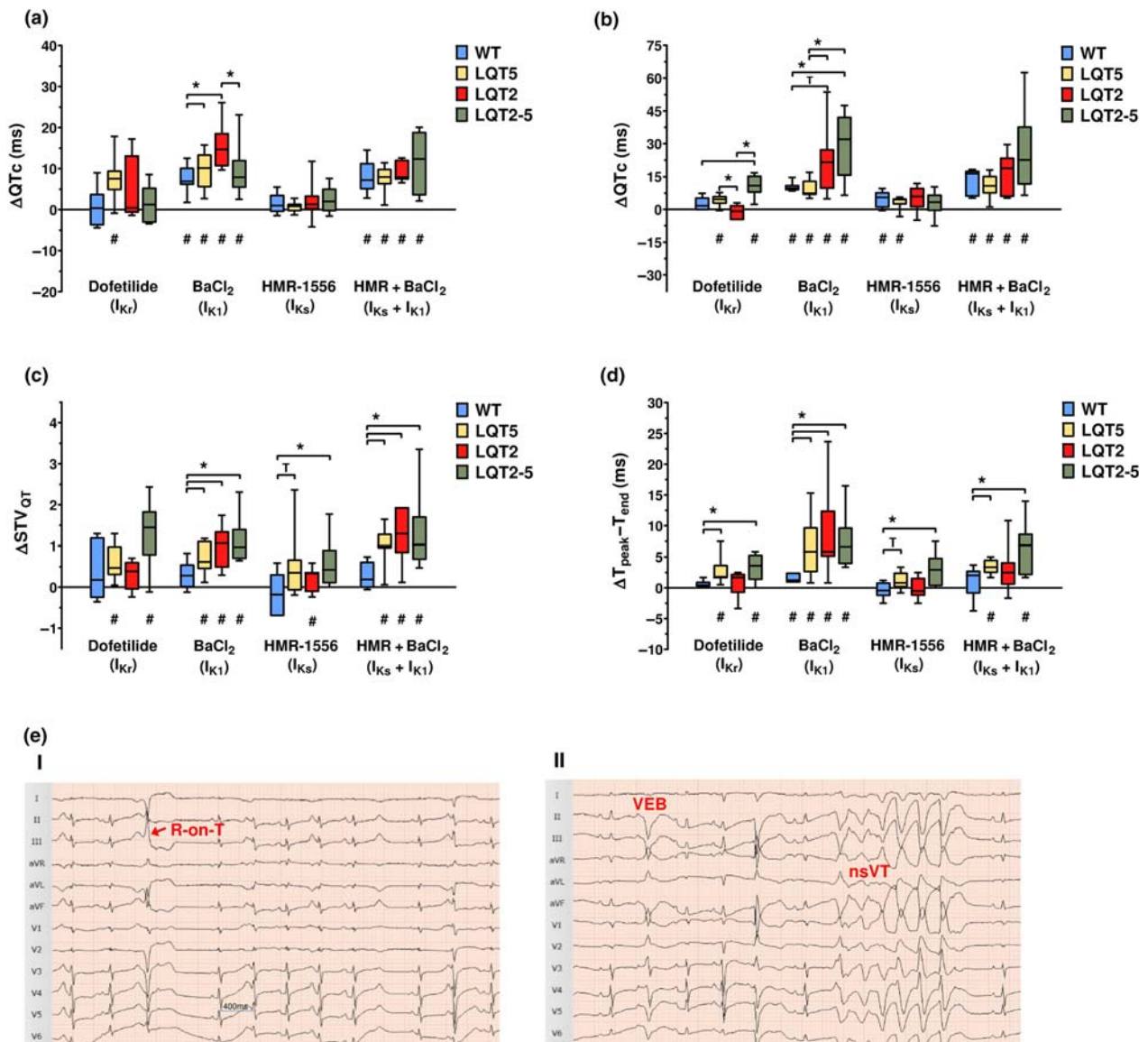
FIGURE 4 I_K blocker-induced changes in ECG parameters and arrhythmia formation in vivo. (a) Changes in QTc in awake, free-moving animals. N = 6–8 in each genotype. Changes in QTc (b), STV_{QT} (c), and $T_{peak}-T_{end}$ (d) in anaesthetised animals. Box and whisker graphs show maximal changes in the indicated parameters after intramuscular injection of I_{Kr}, I_{K1}, I_{Ks}, and $I_{Ks} + I_{K1}$ blockers dofetilide, $BaCl_2$, HMR-1556, and HMR + $BaCl_2$, respectively in WT, LQT2, LQT5, and LQT2-5 rabbits. $^*P < .05$, significant differences between genotypes; $^\#P < .05$, significantly different from baseline, n = 6–9 in each genotype. Data are shown as median, the lower (25th percentile) and upper (75th percentile) quartiles, the minimum and maximum values. (e) Examples of ECG recordings demonstrate I_{K1} blocker $BaCl_2$ induced ventricular extra beat (VEB) with R-on-T phenomenon in LQT2-5 (I) and VEB and non-sustained ventricular tachycardia (nsVT) in LQT2 (II) rabbits in vivo

pro-arrhythmic potential of drugs at different sympathetic activity levels of the animal models.

3.2.3 | Effects of K⁺ channel blockers on MAP characteristics ex vivo

To investigate global and regional sensitivity of the different hearts with impaired repolarisation reserve to further drug-induced reduction of K⁺ currents, hearts were Langendorff-perfused with different "selective" potassium channel blockers.

Following perfusion with a very low concentration of the I_{Kr} blocker dofetilide (1 nM), a slight prolongation of mean APD_{75} was observed in all groups (Figure 6a). However, as this prolongation was very slight, below a "threshold" of 10 ms, it is likely to be due to the experimental set-up and not of clinical relevance. The I_{Ks} blocker HMR-1556 (100 nM) induced a more pronounced APD_{75} prolongation in LQT2 and LQT2-5, than in WT or LQT5 hearts (Figure 6a). Similarly, the I_{K1} blocker $BaCl_2$ (10 μM) or combined I_{K1}/I_{Ks} blockade by $BaCl_2$ (10 μM) + HMR-1556 (100 nM) prolonged APD_{75} significantly more in LQT2 and LQT2-5 than in WT or in LQT5 hearts (Figure 6a). This prolongation of APD was particularly pronounced at slower rates,
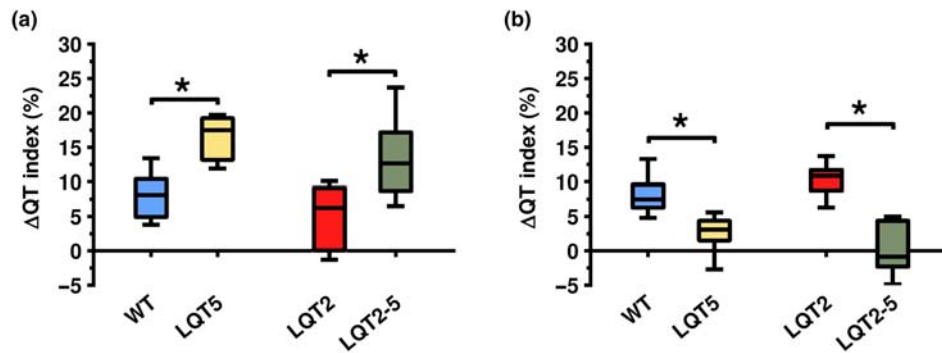
FIGURE 5 I_{Ks} function in vivo. Change of heart rate-corrected QT (QT index (QTi) = 100 * (QT_{observed}/QT_{expected})) after intravenous administration of isoprenaline (a) and HMR-1556 + isoprenaline (b) in WT, LQT5, LQT2, and LQT2-5 animals. *$P < .05$, significant differences between genotypes; $n = 6$–9 in each group. Data are shown as box and whisker plots, with median, the lower (25th percentile) and upper (75th percentile) quartiles, and the minimum and maximum values

leading to an increased APD/CL ratio steepness upon I_{K1}, I_{Ks}, or combined I_{K1}/I_{Ks} blockade in LQT2 and LQT2-5 hearts (Figure 6b).

Importantly, mean AP triangulation (APD_{90-30}) was more pronounced following I_{K1} or combined I_{K1}/I_{Ks} blockade in LQT2 and LQT2-5 hearts than in those from WT rabbits (Figure 6c).

3.2.4 | Regional differences in response to combined I_{K1}/I_{Ks} blocker effects on MAP characteristics ex vivo

APD changes induced by combined I_{K1}/I_{Ks} inhibition were regionally different and varied in the different LQTS models. While prolongation of APD was more pronounced in hearts from LQT2 and LQT2-5 than in those from WT animals, in most MAP positions, this difference was not found in base-inferolateral (MAP 3) region at 2 Hz in LQT2-5 hearts (Figure 7a), leading even to a less pronounced steepness of APD/CL ratio in LQT2-5, than in LQT2 hearts, in this region (Figure 7b). I_{K1}/I_{Ks} blocker-induced increased AP triangulation was also more pronounced in LQT2 and LQT2-5 than in WT hearts, in nearly all regions, except the apico-anterior area (MAP 1; Figure 7c). The LQT5 hearts, in contrast, were, in general, equally sensitive to I_{K1}/I_{Ks} inhibition as WT hearts. The extent of APD prolongation in LQT5, however, was significantly

higher in base-inferolateral (MAP 3) position as in any other regions (Figure S3). Quite uniquely, such regional heterogeneity in the extent of APD prolongation was only present in hearts from LQT5 rabbits.

3.2.5 | Effects of low [K⁺]ₒ and K⁺ channel blockers on arrhythmia development ex vivo

To test our hypothesis of an increased sensitivity of models with reduced repolarisation reserve to drug-induced pro-arrhythmia, AV-ablated hearts were perfused with low [K⁺]ₒ KH solution and/or I_{K1} blocker BaCl₂. At baseline (5.4 mM [K⁺]ₒ KH I), the AV-ablated hearts were beating on their own stable VER (heart rates in average 69.1 ± 3.5 in all groups). No major arrhythmia events were observed.

Five-minute perfusion with 2.0-mM [K⁺]ₒ KH resulted in longer duration of bigeminy and VTs in transgenic animals with reduced repolarisation reserve than in healthy WT (% of perfusion time; bigeminy: LQT2 38.8 ± 11.7, LQT2-5 37.9 ± 7.0 compared with WT 11.1 ± 6.8; VT: LQT2 25.0 ± 11.1, LQT2-5 30.2 ± 10.5 compared with WT 1.7 ± 1.1; Figure 8a). The effects were reversible, and the original VER was regained in all group after 10-min perfusion with normal KH solution (5.4 mM [K⁺]ₒ KH II as second baseline).
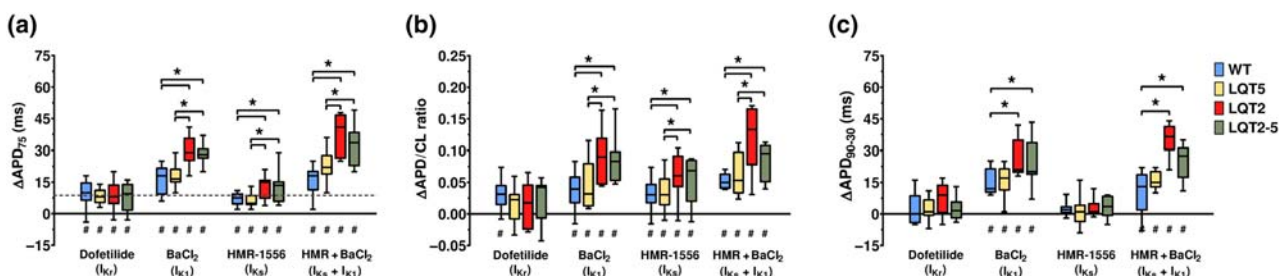

FIGURE 6 I_K blocker-induced changes in action potential parameters ex vivo. Bar graphs of mean (average MAP 1-4) changes in (a) action potential duration (ΔAPD_{75}), (b) APD/CL ratio (ΔAPD/CL ratio), and (c) triangulation of action potential (ΔAPD_{90-30}). *$P < .05$, significant differences between genotypes; #$P < .05$ significantly different from baseline; $n = 6$–7 in each group. Data are shown as box and whisker plots, with median, the lower (25th percentile) and upper (75th percentile) quartiles, and the minimum and maximum values

FIGURE 7 Differences in combined I_{Ks} + I_{K1} blocker-induced regional changes in action potential parameters ex vivo. Bar graphs of regional changes (in all different MAP leads) in (a) action potential duration (ΔAPD_{75}), (b) APD/CL ratio ($\Delta APD/CL$ ratio), and (c) triangulation of action potential (ΔAPD_{90-30}). $^*P < .05$, significant differences between genotypes; n = 6–9 in each group. Data are shown as box and whisker plots, with median, the lower (25th percentile) and upper (75th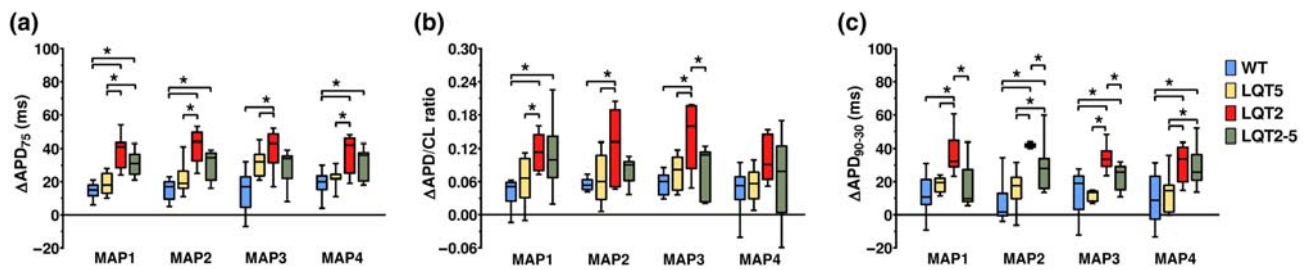 percentile) quartiles, and the minimum and maximum values. Abbreviations: MAP 1, apico-anterior; MAP 2, mid-anterolateral; MAP 3, base-inferolateral; and MAP 4, base-inferior positions

I_{K1} blocker $BaCl_2$ induced longer duration and higher incidence of arrhythmias in LQT2 and LQT2-5 rabbits than in WT (Figure 8a,b). Combined $BaCl_2$ and 2-mM $[K^+]_o$ perfusion resulted in even more pronounced (longer duration and higher incidence of) arrhythmia formation in transgenic animals than in WT (total duration of all AR events as % of perfusion time: LQT5 53.7 ± 11.3, LQT2 86.3 ± 5.3, LQT2-5 83.0 ± 5.1, compared with WT 16.2 ± 5.9; average incidence [No.] of all AR events; LQT2 52.0 ± 16.1, LQT2-5 46.9 ± 13.2 compared with WT 3.6 ± 1.6; Figure 8a,b).

Overall, a more malignant type of arrhythmia development (VT and VF) was seen in LQT2 and LQT2-5 than in LQT5 or WT (occurrence of AR: $BaCl_2$: LQT2 [VT: 100%] and LQT2-5 [VT: 86%, VF: 57%] compared with WT [VT: 0%, VF: 0%]; $BaCl_2$ + 2-mM $[K^+]_o$: LQT2 [VT: 100%, VF: 60%] and LQT2-5 [VT: 83%, VF: 57%] compared with WT [VT: 0%, VF: 0%]; Figure S4).

4 | DISCUSSION

4.1 | Animal models with reduced repolarisation reserve

Several *in vivo* models with reduced repolarisation reserve have been developed—such as the chronic AV-block model in dogs (Vos et al., 1998) with reduced I_{Ks} currents due to electrical remodelling and increased susceptibility to TdP arrhythmias (Thomsen et al., 2004; Volders et al., 1999) and anaesthetised rabbit and conscious dog models with impaired repolarisation reserve following pharmacological inhibition of I_{Ks} (by HMR-1556; Lengyel et al., 2007); but none of them are routinely used in pro-arrhythmia research. Another approach to reduce repolarisation reserve is the generation of transgenic LQTS animals with genetic alteration of cardiac ion channels. The first LQTS rabbit models were generated by overexpression of human mutant *KCNQ1*/ *KvLQT1* (*KvLQT1-Y315S*, loss of I_{Ks}, LQT1) or *KCNH2*/HERG (*HERG-G628S*, loss of I_{Kr}, LQT2) in the heart (Brunner et al., 2008). These LQT1 and LQT2 models mimic human LQTS with severely prolonged QT, spontaneous TdP, and in rare cases SCD (in LQT2).

It is important to note, however, that in a relatively stress-free housing environment, nearly *no spontaneous* TdP/SCD occurred—especially within the first year of their life—rendering them a suitable tool for the assessment of drug-induced pro-arrhythmia.

Later, the LQT5 transgenic rabbit model was generated by over-expression of human mutant *KCNE1* (*KCNE1-G52R*, impaired I_{Ks}; Major et al., 2016). This model reflects "silent" LQTS, in which the slight reduction of repolarisation reserve does not lead to clinically manifested QT prolongation but increases vulnerability to repolarisation-prolonging drugs (Major et al., 2016).

In this work, we investigated the potential benefits of using different transgenic LQTS rabbit models with reduced repolarisation reserve in pro-arrhythmia research. We demonstrated that transgenic LQT5, LQT2, and the newly generated double-transgenic LQT2-5 models more reliably detected I_K-blocking properties of drugs, for example, blockade of I_{Kr}, I_{K1}, and I_{Ks}. Importantly, transgenic LQTS rabbit models also demonstrated increased drug-induced arrhythmia susceptibility, compared with healthy animals. Therefore, LQTS rabbits could be used to assess the pro-arrhythmic potential of drug candidates in a more complex and more reliable manner.

4.2 | Baseline characteristics of LQTS rabbit models with impaired repolarisation reserve

Several electrophysiological features are important for arrhythmia formation in (acquired and genetic) LQTS patients. Among them, (i) increased temporal instability and regional heterogeneity of repolarisation form the electrical "substrate" that facilitates re-entry formation, and (ii) increased sympathetic nervous system activity serves as "trigger" for early afterdepolarizations and mediates additional acute effects on I_{Ks} and $I_{Ca,L}$ currents (Antzelevitch, 2007; Brunner et al., 2008; Ziv et al., 2009).

LQT2 and LQT2-5 rabbits demonstrated a pronounced LQTS phenotype and pro-arrhythmic substrate even at baseline: prolonged QTc/APD, steeper QT/RR slope, increased temporal instability (STV_{QT}), transmural ($T_{peak-end}$), and apico-basal heterogeneity of repolarisation and more AP triangulation—similar to those
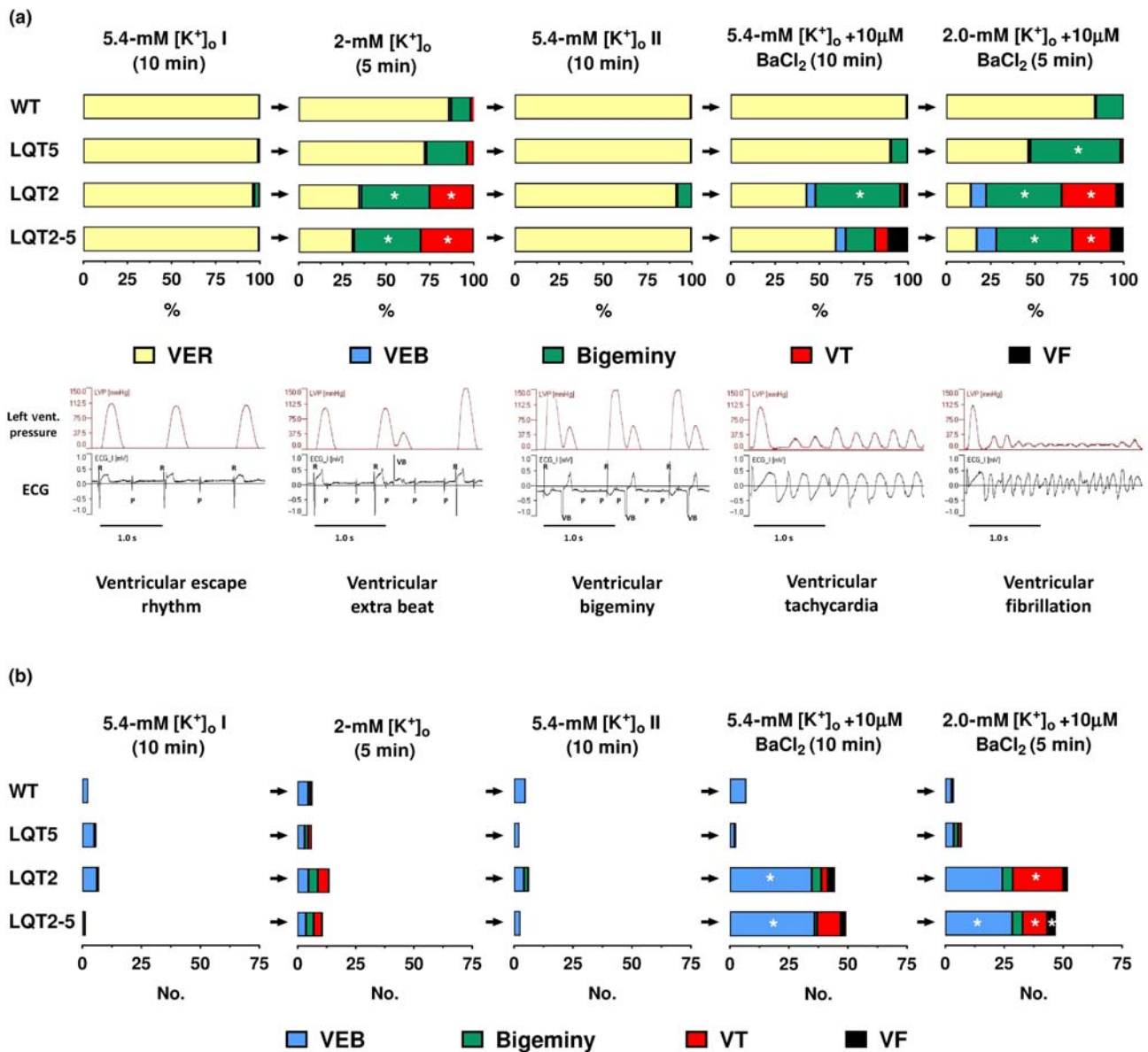
FIGURE 8   Low potassium- and drug-induced ex vivo arrhythmia development. Graphs indicating the (a) duration (% of perfusion time) and (b) incidence (average number of events) of arrhythmias provoked by perfusing the AV-ablated non-stimulated hearts with low $[K^+]_o$ (2 mM) KH, I_{K1} blocker $BaCl_2$ (10 μM), or with their combination in WT ($n = 7$), LQT5 ($n = 8$), LQT2 ($n = 6$), and LQT2-5 ($n = 7$) animals. Insets show representative left ventricular pressure curves and ECG recordings of ventricular escape rhythm (VER), ventricular extra beats (VEB), bigeminy, ventricular tachycardia (VT), and ventricular fibrillation (VF). $^*P < .05$, significant differences between genotypes

observed in human LQTS patients. These characteristics all favour increased re-entry-based arrhythmias, especially during bradycardia. The overall severity of the phenotype was similar in LQT2-5 and LQT2 at baseline. Only I_{Ks} function was more impaired in LQT2-5 rabbits with decreased QT-shortening capacity at higher heart rates.

LQT5 rabbits showed no overall QT prolongation but increased apico-basal heterogeneity of APD and AP triangulation compared to WT— results similar to those from the model's first characterisation (Major et al., 2016), in which accelerated I_{Ks} and I_{Kr} deactivation kinetics and only slightly increased baseline STV_{QT} were described.

Therefore, LQT5 rabbits could be a model to mimic "silent" LQTS condition with nearly normal baseline phenotype.

4.3 | LQTS rabbit models with impaired repolarisation reserve detect K^+ channel blocking effects on pro-arrhythmia markers

LQTS patients with reduced repolarisation reserve are more sensitive to drugs that (further) disturb cardiac repolarisation than normal individuals, and are more prone to develop drug-induced ventricular

arrhythmias (Schwartz & Woosley, 2016). Similarly, we observed an increased sensitivity to QT/APD-prolonging side effects of known K^+ channel blockers in different LQTS rabbits mimicking human LQTS characteristics.

LQT1 rabbits—with complete lack of I_{Ks}—were proposed as useful tools for better detection of I_{Kr}-blocking properties of drug candidates (Odening et al., 2010). Similarly, LQT5 animals with mild impairment in I_{Ks} were also more sensitive to the I_{Kr} blocker dofetilide than healthy WT rabbits, demonstrating increased QTc, $T_{peak-end}$, and STV_{QT}. These results are in line with earlier findings (Major et al., 2016).

It is known that inhibition of I_{K1} can result in prolonged APD, increased resting membrane potential and early and delayed afterdepolarisations (Maruyama et al., 2011; Varro & Baczko, 2011; Zhang et al., 2005), Despite of this, current safety tests still do not concentrate on detecting I_{K1}-blocking properties of drug candidates. According to our findings with the I_{K1} blocker BaCl₂, LQT2 and LQT2-5 rabbits could be ideal models to detect such I_{K1}-blocking side effects of drugs. This is in line with our previous work, in which increased QT prolongation and VEBs were observed only in LQT2, but not in WT animals, following midazolam administration—a well-known sedative-anxiolytic drug with I_{K1}-blocking properties (Odening et al., 2008).

In our *in vivo* experiments, application of the sympathomimetic (I_{Ks} and $I_{Ca,L}$ activating) isoprenaline resulted in less I_{Ks} activation and, therefore, in more pronounced QTi prolongation in LQT5 and LQT2-5 than in WT or LQT2, suggesting that LQT5 and LQT2-5 animals could be especially sensitive to **adrenoceptor** agonist-induced arrhythmias. Having both decreased I_{Ks} and lack of I_{Kr}, the new LQT2-5 model may provide additional insights into arrhythmias caused by sympathetic stimulation in the setting of impaired repolarisation reserve. In this regard, LQT2-5 may serve as an important model (i) for diseases with high arrhythmic risk due to (remodelling-based) impaired I_{Ks} function—such as heart failure and diabetes—and (ii) to investigate the effect of pharmacological reduction or increase of I_{Ks} in clinically manifest LQTS. Of note, in contrast to the isoprenaline-induced (slight) lengthening of heart rate-corrected QT index in LQT2 rabbits in our present study, a shortening of this parameter was observed in some of our earlier work (Brunner et al., 2008; Odening et al., 2010), due to differences in anaesthetic regimens and resulting differences in the effects on the autonomic nervous system and cardiac ion currents.

4.4 | LQTS rabbit models with impaired repolarisation reserve detect drug-induced arrhythmias

Pro-arrhythmic effects of various extrinsic (anaesthetics; Odening et al., 2008) and intrinsic (sex hormones; Odening et al., 2012) factors have been demonstrated in transgenic LQTS rabbits. The direct assessment of arrhythmias in response to provocation factors—such as bradycardia, hypokalaemia, or K^+ channel blockers that are crucial for drug-induced pro-arrhythmia in the clinical setting—however, has not been completely performed to date. In this project, we have closed this gap.

We chose the I_{K1} blocker BaCl₂ in our experimental setting, (i) as I_{K1} plays an important role in repolarisation reserve (Varro & Baczko, 2011) and (ii) as only this drug caused prolongation of repolarisation in all genotypes, therefore making it possible to compare sensitivities.

Application of BaCl₂ increased the incidence and duration of complex VEBs and more malignant arrhythmias such as VT and VF in LQT2 and LQT2-5, while in LQT5, only bigeminy occurred; and no serious arrhythmias were observed in WT. In LQTS hearts, the pre-existing temporal and regional heterogeneity in repolarisation prolongation, which was even further aggravated by the provocation factors, increased the sensitivity for re-entry formation.

These observations are in good agreement with earlier findings (Frommeyer, Brucher, et al., 2016; Frommeyer, von der Ahe, et al., 2016), demonstrating no arrhythmias in AV-ablated Langendorff-perfused WT hearts either at baseline or during hypokalaemia and serious VT only when repolarisation-prolonging drugs were added. Similarly, increased arrhythmia susceptibility was demonstrated in various LQTS models: spontaneous VT and SCD in LQT2 rabbits (Brunner et al., 2008), increased TdP development in pharmacologically (dofetilide + HMR-1556) induced acquired LQTS rabbit and dog models (Lengyel et al., 2007), and increased dofetilide-induced TdP in LQT5 rabbits (Major et al., 2016).

It is important to note that during ex vivo experiments, LQTS hearts were much more sensitive to arrhythmia development than *in vivo* since pro-arrhythmic factors were present: (1) the hearts were beating with a stable—very slow—VER after AV ablation, and (2) low potassium concentrations were used for the perfusion.

5 | CONCLUSIONS

LQT2 and LQT2-5 rabbits with lack of I_{Kr} (and decrease in I_{Ks} in case of LQT2-5) demonstrate increased sensitivity to I_{K1}- and I_{Ks}-blocking drugs and drug-induced ventricular arrhythmias. Phenotypically, the new LQT2-5 model closely resembles LQT2. However, it also shows characteristic differences due to its impaired I_{Ks} function. LQT5 rabbits with reduced I_{Ks} demonstrate increased sensitivity to I_{Kr}-blocking drugs.

This increased sensitivity of different LQTS models to specific ion channel blockers could be utilised for more reliable prediction of the (multichannel-based) pro-arrhythmic potential of novel drug candidates. Here, these models could play a role in safety testing, using ex vivo whole perfused hearts and *in vivo*, following the detailed in vitro determination of multichannel blocking properties in cells; by which the ideal combination of LQTS types for further assessment of drug effects on QT/APD and ex vivo pro-arrhythmia can be determined.

5.1 | Limitations

Previous studies demonstrated that females—both in patients (Lehmann, Hardy, Archibald, Quart, & MacNeil, 1996) and in LQTS rabbits (Odening et al., 2008)—may be more susceptible to

drug-induced QT prolongation/pro-arrhythmia. In this study, sex proportions within groups were equal in most experiments. The significant male dominance in telemetric ECG and ex vivo Langendorff experiments, however, might have caused less pronounced changes in pro-arrhythmia markers as could have been detected if sex ratio had been the same or had shown female dominance.

In general, our in vivo and ex vivo results were in good agreement. However, some differences were observed in HMR-1556 effects that may due to (i) the effect of the anaesthetics on the sympathetic/parasympathetic tone in vivo and (ii) differences in used drug concentrations.

In these proof-of-principle experiments, LQTS rabbit models detected pro-arrhythmia with increased sensitivities compared to animals with normal repolarisation—which from a clinical point of view is particularly important to prevent drug-induced SCD. We are aware, however, that further detailed assessment of their sensitivity and specificity would be mandatory prior to its use for pro-arrhythmia screening.

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AUTHOR CONTRIBUTIONS

T.H. performed and analysed experiments and wrote the manuscript; A.C. performed experiments and wrote the manuscript; G.F. performed experiments; S.P.-F. performed genotyping and phenotyping of the transgenic animals; P.M. generated the LQT5 rabbits and edited the manuscript; L.H. edited the manuscript; G.K. generated the LQT2 rabbits and edited the manuscript; Z.B. generated the LQT5 rabbits and edited the manuscript; A.V. edited the manuscript; M.Z. edited the manuscript; M.B. wrote the manuscript; C.B. edited the manuscript; I.B. designed the study and wrote the manuscript; K.E.O. conceived/designed the study and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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