Spatial correlation of action potential duration and diastolic dysfunction in transgenic and drug-induced LQT2 rabbits

Katja E. Odening, MD,[*] Bernd A. Jung, PhD,[†] Corinna N. Lang, MD,[*] Rocio Cabrera Lozoya, MSc,[‡] David Ziupa, MD,[*] Marius Menza, MSc,[†] Jatin Relan, PhD,[‡] Gerlind Franke, PhD,[*] Stefanie Perez Feliz,[*] Gideon Koren, MD,[§] Manfred Zehender, MD,[*] Christoph Bode, MD,[*] Michael Brunner, MD,[*] Maxime Sermesant, PhD,[‡] Daniela Föll, MD[*]

From the [*]Department of Cardiology and Angiology I, Heart Center Freiburg University, Freiburg, Germany, [†]Department of Radiology, Medical Physics, University Hospital Freiburg, Freiburg, Germany, [‡]INRIA, Asclepios Research Project, Cardiac Modelling, Sophia Antipolis, France, and [§]Division of Cardiology, Cardiovascular Research Center, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, Rhode Island.

BACKGROUND Enhanced dispersion of action potential duration (APD) is a major contributor to long QT syndrome (LQTS)-related arrhythmias.

OBJECTIVE To investigate spatial correlations of regional heterogeneities in cardiac repolarization and mechanical function in LQTS.

METHODS Female transgenic LQTS type 2 (LQT2; n = 11) and wild-type littermate control (LMC) rabbits (n = 9 without E4031 and n = 10 with E4031) were subjected to phase contrast magnetic resonance imaging to assess regional myocardial velocities. In the same rabbits' hearts, monophasic APDs were assessed in corresponding segments.

RESULTS In LQT2 and E4031-treated rabbits, APD was longer in all left ventricular segments ($P < .01$) and APD dispersion was greater than that in LMC rabbits ($P < .01$). In diastole, peak radial velocities (Vr) were reduced in LQT2 and E4031-treated compared to LMC rabbits in LV base and mid (LQT2: -3.36 ± 0.4 cm/s, $P < .01$; E4031-treated: -3.24 ± 0.6 cm/s, $P < .0001$; LMC: -4.42 ± 0.5 cm/s), indicating an impaired diastolic function. Regionally heterogeneous diastolic Vr correlated with APD (LQT2: correlation coefficient [CC] 0.38, $P = .01$; E4031-treated: CC 0.42, $P < .05$). Time-to-diastolic peak Vr were prolonged in LQT2 rabbits (LQT2: 196.8 ± 2.9 ms, $P < .001$; E4031-treated: 199.5 ± 2.2 ms, $P < .0001$, LMC 183.1 ± 1.5), indicating a prolonged contraction duration. Moreover, in transgenic LQT2 rabbits, diastolic time-to-diastolic peak Vr correlated with APD (CC 0.47, $P = .001$). In systole, peak Vr were reduced in LQT2 and E4031-treated rabbits ($P < .01$) but longitudinal velocities or ejection fraction did not differ. Finally, random forest machine learning algorithms enabled a differentiation between LQT2, E4031-treated, and LMC rabbits solely based on "mechanical" magnetic resonance imaging data.

CONCLUSIONS The prolongation of APD led to impaired diastolic and systolic function in transgenic and drug-induced LQT2 rabbits. APD correlated with regional diastolic dysfunction, indicating that LQTS is not purely an electrical but an electromechanical disorder.

KEYWORDS Long QT syndrome; Rabbits; Repolarization; Diastolic function; Cardiac electrophysiology; Cardiac MRI

ABBREVIATIONS **APD** = action potential duration; **APD_{75}** = action potential duration at 75% of repolarization; **$[Ca^{2+}]_i$** = cytoplasmic Ca^{2+} concentration; **CC** = correlation coefficient; **ECG** = electrocardiogram; **EPS** = electrophysiological study; **$I_{Ca,L}$** = L-type Ca2+ current; **I_{Kr}** = rapid delayed rectifier potassium current; **IV** = intravenously; **LMC** = wild-type littermate control; **LQT2** = long QT syndrome type 2; **LQTS** = long QT syndrome; **LV** = left ventricle/ventricular; **MAP** = monophasic action potential; **MRI** = magnetic resonance imaging; **pVT** = polymorphic ventricular tachycardia; **QTc** = heart-rate corrected QT; **SD** = standard deviation; **TTP** = time-to-peak velocities; **Vr** = radial velocities; **Vr_dia** = diastolic peak radial velocities; **Vz** = long-axis velocities; **Vz_dia** = diastolic peak long-axis velocities

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Introduction

The inherited long QT syndrome (LQTS) is an arrhythmogenic disease characterized by impaired cardiac repolarization that clinically manifests with QT prolongation, polymorphic ventricular tachycardia (pVT), syncopes, and sudden cardiac death.[1] A spatially heterogeneous prolongation of repolarization leading to an enhanced dispersion of action potential duration (APD) is considered a major contributor to LQT-related arrhythmias.[2] As a result of their prolonged repolarization, patients with LQTS have globally prolonged cardiac contraction duration with transmural differences.[3,4] However, the spatial relationship between electrical and mechanical (dys) function remains to be elucidated.

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Address reprint requests and correspondence: Dr Katja E. Odening, Department of Cardiology and Angiology I, Heart Center Freiburg University, Hugstetter Str 55, 79106 Freiburg, Germany. E-mail address: katja.odening@uniklinik-freiburg.de.

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Phase contrast magnetic resonance imaging (MRI) has been used successfully to determine regional differences in myocardial contraction and relaxation velocities in human patients.[5] Healthy women, who physiologically have longer repolarization than men, have altered peak velocities and different regional patterns of relaxation compared to men,[6] suggesting that it may be possible to determine changes and spatial differences in mechanical function in LQTS by using this technique.

Rabbits exhibit pronounced similarities in the expression and function of voltage-gated K^+ channels[7] and Ca^{2+} cycling proteins[8] as humans, suggesting that rabbits can fairly appropriately mimic the human phenotype of cardiac diseases with electrical and/or mechanical impairment. Indeed, several transgenic rabbit models of human diseases such as LQTS and hypertrophic cardiomyopathy have demonstrated pronounced similarities with the human phenotype.[9,10] Transgenic LQTS type 2 (LQT2) rabbits mimic human LQTS with QT prolongation, pVT, sudden cardiac death, and an increased APD dispersion as major arrhythmogenic mechanism.[9] Moreover, despite some species differences in myocardial motion between humans and small animals, we could recently show similarities between humans and rabbits in regional contractile behavior with a similar rotational motion,[11] indicating that LQT2 rabbits may be a useful tool to assess mechanical function in LQTS.

By using in vivo phase contrast MRI, ex vivo monophasic action potential (MAP) measurements, and machine learning approaches, we aimed at elucidating the spatial relationship between regional electrical and mechanical cardiac function and their spatial dispersion in transgenic and drug-induced LQT2 rabbits.

Methods

A more detailed method description can be found in the Online Supplement.

Rabbits

Adult female transgenic LQT2 (HERG-G628S, 4.1 ± 0.6 months, 2.4 ± 0.3 kg) and wild-type littermate control (LMC) rabbits (without drugs, 6.3 ± 1.0 months, 3.8 ± 0.6 kg; with E4031, 4.0 ± 0.2 months, 2.9 ± 0.3 kg) were subjected to MRI followed by MAP measurements.

Phase contrast MRI

To assess myocardial velocities, transgenic LQT2 (n = 11) and LMC rabbits (n = 9 without drugs, n = 10 with E4031, bolus 10 μg/kg and infusion 1 μg/(kg · min)[12]) were subjected to phase contrast MRI at 1.5 T (Avanto, Siemens, Germany).[11] Animals were anesthetized with (*S*)-ketamine/xylazine (12.5/3.75 mg/kg intramuscularly, followed by 1–2.5 mL/kg/h intravenously [IV]), which does not affect cardiac repolarization,[13] and positioned in a 12-channel head coil.

Experiments were performed with a black blood prepared gradient echo sequence with prospective electrocardiographic (ECG) gating and high temporal (7.6 ms) and spatial resolution (1.0 × 1.2 × 4 mm).[11] For data postprocessing, customized MATLAB software was used. For regional analysis, the left ventricle (LV) was partitioned into 16 segments in the base, mid, and apex (the American Heart Association model[14]). Mean radial (Vr) and long-axis (Vz) systolic and diastolic peak and time-to-peak (TTP) were determined. The standard deviation (SD) of TTP from 16 segments was calculated as a measure for mechanical dispersion. Heart rate-corrected diastolic TTP were computed as the percentage of RR.

MAP measurements in Langendorff-perfused rabbit hearts

To correlate electrical and mechanical function, MAPs were acquired in the same LQT2 (n = 10) and LMC rabbits (n = 9 without drugs; n = 10 with E4031; 0.1 μM[15]). Rabbits were anesthetized with (*S*)-ketamine/xylazine (12.5/3.75 mg/kg) intramuscularly and received 1000 IU heparin IV. After euthanasia with sodium thiopental (40 mg/kg) IV, hearts were excised rapidly and mounted on the Langendorff-perfusion setup (IH5, Hugo Sachs Electronic-Harvard Apparatus, Hugstetten, Germany). Hearts were retrogradely perfused with modified Krebs-Henseleit solution.[16] ECG, MAP, coronary flow, and pressures were continuously recorded with Isoheart software (Hugo Sachs Electronic, Version 1.1.1.218(32)).

After mechanical atrioventricular node ablation, hearts were stimulated with 2, 3, and 4 Hz to obtain APD at heart rates comparable to MRI (130–189 beats/min). Four MAP electrodes were repetitively positioned on all different LV segments except for the septal segments (see the Online Supplemental Figure 2). APDs at 75% of repolarization (APD_{75}) and standard deviations (SD) of APD_{75} within all LV segments were calculated as measures for electrical dispersion.

Machine learning for image-based classification

Multivariate data analyses were performed by using random forest machine learning algorithms to explore the possibility to differentiate between LQT2, E4031-treated, and LMC rabbits based on MRI data. Image-based features included peak velocities, TTP, regional velocity statistics, and correlations with normal velocity curves (over 700 features per rabbit). Feature space dimension reduction was performed by using the χ^2 test. Only top-relevant features were considered. Cross-validation was used to obtain an estimate of classifier accuracy.[17]

Since heart-rate corrected QT (QTc) duration[18] and QT dispersion[19] are known arrhythmogenic risk factors in LQTS and since we observed correlations between APD and diastolic dysfunction in LQT2 rabbits, we next tested whether MRI can provide sufficient information to discern differences in the extent of APD prolongation by using random forest machine learning algorithms. LQT2 rabbits were divided into 2 groups: (1) rabbits with "very long" APD and "pronounced" dispersion (longest APD ≥ 155 ms,