Electrophysiologic Substrate in Congenital Long QT Syndrome:
Noninvasive Mapping with Electrocardiographic Imaging (ECGI)

Running title: Vijayakumar et al.; ECG Imaging of EP substrate in human LQTS

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Abstract

Background—Congenital Long QT syndrome (LQTS) is an arrhythmogenic disorder that causes syncope and sudden death. While its genetic basis has become well-understood, the mechanisms whereby mutations translate to arrhythmia susceptibility in the in situ human heart have not been fully defined. We used noninvasive ECG imaging (ECGI) to map the cardiac electrophysiologic substrate and examine whether LQTS patients display regional heterogeneities in repolarization, a substrate which promotes arrhythmogenesis.

Methods and Results—25 subjects (9 LQT1, 9 LQT2, 5 LQT3 and 2 LQT5) with genotype and phenotype positive LQTS underwent ECGI. Seven normal subjects provided control. Epicardial maps of activation, recovery times (RT), Activation-recovery intervals (ARI) and repolarization dispersion were constructed. Activation was normal in all patients. However, RT and ARI were prolonged relative to control, indicating delayed repolarization and abnormally long APD (312 ± 30 ms vs. 235 ± 21 ms in control). ARI prolongation was spatially heterogeneous, with repolarization gradients much steeper than control (119 ± 19 ms/cm vs. 2.0 ± 2.0 ms/cm). There was variability in steepness and distribution of repolarization gradients between and within LQTS types. Repolarization gradients were steeper in symptomatic patients (130 ± 27 ms/cm in 12 symptomatic patients vs. 98 ± 19 ms/cm in 13 asymptomatic patients; P < 0.05).

Conclusions—LQTS patients display regions with steep repolarization dispersion caused by localized APD prolongation. This defines a substrate for reentrant arrhythmias, not detectable by surface ECG. Steeper dispersion in symptomatic patients suggests a possible role for ECGI in risk stratification.

Key words: Electrocardiographic Imaging, Electrocardiographic Mapping, long QT syndrome, electrophysiology, imaging
Introduction

Congenital Long QT syndrome (LQTS) is an inherited cardiac channelopathy which affects 1 in 2000\(^1\) patients worldwide, predisposing healthy young adults with structurally normal hearts to syncope and sudden cardiac death due to polymorphic ventricular tachycardia (torsades de pointes).

Molecular and genetic studies in humans have uncovered many of the genes\(^2\) associated with LQTS, thus providing molecular insight into the pathogenesis of the disease. LQT1 is caused by loss of function mutations in KCNQ1\(^3\), which encodes the \(\alpha\) subunit of the slowly activating potassium channel \(I_{\text{Ks}}\), leading to reduced \(I_{\text{Ks}}\) current. Similarly, LQT2 is associated with loss of function mutations in KCNH2 (\(hERG\))\(^3\) and a reduced rapidly activating potassium current \(I_{\text{Kr}}\). LQT3 is caused by gain of function mutations in SCN5A\(^4\) and increased \(I_{\text{Na}}\) current during the plateau and late phase of the action potential (AP). The rare LQT5 is associated with mutations in the KCNE1 (\(mink\))\(^4\), which encodes the auxiliary \(\beta\) subunit of the \(I_{\text{K}}\) channel, causing loss of current. The loss of repolarizing current (LQT1, LQT2 and LQT5) or the increase in depolarizing current (LQT3) leads to prolongation of the ventricular AP duration (APD) and delayed ventricular repolarization, which is reflected in a long QT interval on the ECG (the LQTS phenotype).

The diagnostic characteristic for a positive phenotype is prolongation of the QT interval (corrected for heart rate using Bazett’s equation; QT\(_c\)) on the 12-lead ECG, with values over 450 ms in male and 460 ms in female\(^5\). QT interval longer than 500 ms indicates increased arrhythmia risk\(^6\). Diagnosis can also be based on the Schwartz score\(^2\) which includes patient’s age, medical and family history, symptoms and QT\(_c\). Genotype-specific changes in T- wave morphology\(^7,8\) that are suggestive but not diagnostic (broad-based in LQT1, notched in LQT2,
late-appearing in LQT3) and occasional alternans are present on the body-surface ECG. Clinical arrhythmia triggers are distinct for each subtype. Importantly, while genetic testing is increasingly used in the diagnosis, it is not useful for risk stratification among mutation carriers since the disease is characterized by incomplete penetrance and variable expressivity.

Experiments\(^{10,11,12}\) and modeling studies\(^{7,13-16}\) have shed valuable light on the cellular and tissue-level mechanisms of arrhythmia in LQTS. Prolongation of the AP leading to the development of early after depolarizations (EADs)\(^{17}\) has been proposed as a trigger, and amplification of spatial dispersion of repolarization has been proposed as the substrate for development of reentry. Body surface potential mapping in LQTS patients has demonstrated multiple distributions and high nondipolar content, suggestive of regional electrical disparities in the heart\(^{18}\). However, the arrhythmogenic substrate in the intact human heart of patients with congenital LQTS has not been characterized due to lack of noninvasive, high resolution, panoramic mapping techniques. In the absence of such methods, attempts have been made to infer dispersion of repolarization in LQTS patients from body-surface ECG characteristics\(^{19}\) such as QT\(_c\) dispersion (difference between the maximum and minimum QT\(_c\) on the 12-lead ECG). Because the signal in each ECG lead is generated by the integrated activity over the entire heart, geometrical relationships of repolarization in the heart are not preserved in the body surface ECG. Hence, the surface ECG cannot distinguish between regional and global repolarization abnormalities, and global ECG-based measures such as QT\(_c\) dispersion are not well defined and indeed have not proven useful.

In this study, we used noninvasive Electrocardiographic Imaging (ECGI; also called Electrocardiographic Mapping, ECM) to map the electrophysiologic (EP) substrate in patients with genotype positive, phenotype positive LQTS. It has been established, through canine
experiments, that ECGI can noninvasively reconstruct repolarization properties accurately and localize areas of increased dispersion of repolarization in the heart\textsuperscript{20}. In the intact human heart, ECGI has been used to map normal repolarization\textsuperscript{21} and conditions associated with altered repolarization, including WPW syndrome\textsuperscript{22} and ventricular pacing\textsuperscript{23}. We hypothesized that the EP substrate in patients with hereditary LQTS comprises of regions with delayed repolarization and steep spatial dispersion of repolarization on the ventricular epicardium, providing the substrate for reentrant arrhythmias and torsades de pointes.

**Methods**

**Subject enrollment**

25 patients participated in the study (9 LQT1, 9 LQT2, 5 LQT3 and 2 LQT5). Twenty four of them underwent ECGI at Washington University in St. Louis (six were referred by Vanderbilt University School of Medicine and drove to St. Louis). One patient underwent ECGI at Bordeaux University Hospital and raw data was forwarded electronically to Washington University for processing, epicardial reconstruction and analysis. All patients were genotype positive (class I mutation in \textit{KCNQ1}, \textit{KCNH2}, \textit{SCN5A} and \textit{KCNE1}) and phenotype positive for LQTS (\(QT_c \geq 450\) ms male, \(\geq 460\) ms female). Subjects with (symptomatic) or without (asymptomatic) history of syncope or sudden cardiac arrest and appropriate defibrillation were included. Table 1 summarizes the clinical characteristics of the LQTS group. The control group\textsuperscript{21} consisted of seven healthy volunteers with normal hearts (4 male, 3 female; mean \(QT_c: 384 \pm 12\) ms) and no history of cardiac events. All subjects signed a written informed consent and all protocols were reviewed and approved by the Human Research Protection Office at Washington University in Saint Louis and Bordeaux University Hospital.
Electrocardiographic Imaging

The ECGI methodology has been described previously. Briefly, 256 body-surface ECGs were recorded in sinus rhythm. Following this, the patients underwent a thoracic CT scan gated at 70% of the R-R interval while wearing the electrodes. The patient-specific heart-torso geometry, digitized from the CT images, was combined with the body surface potentials using custom-developed mathematical algorithms to noninvasively reconstruct electric potentials and 502 ventricular unipolar electrograms (EGMs) on the epicardial surface of the heart.

Analysis

For each subject, the epicardial EGMs were analyzed as described below. Local activation time (AT) was determined from the time of steepest negative deflection (dV/dt) during the QRS complex. Local recovery time (RT) was determined from the time of maximum dV/dt during the upstroke of the T wave. As shown, this determination is independent of T wave polarity and morphology. The local activation-recovery interval (ARI), shown to be a surrogate for APD, was computed as the difference between local AT and local RT for each epicardial site. The ARI values were corrected for heart rate using Bazett's formula ($\text{ARI}_{c} = \text{ARI} / (R R^{0.5})$; $\text{RR} = \text{R-R interval}$). From the RT map and ARI map, epicardial dispersion of repolarization was computed as the maximum differences $\Delta \text{RT}$ and $\Delta \text{ARI}_{c}$ between two adjacent EGM sites on the epicardium. Their gradients ($\Delta \text{RT}/\Delta x$, $\Delta \text{ARI}_{c}/\Delta x$) were computed through division by the distance $\Delta x$ between the two adjacent sites.

Statistics

Continuous variables (AT, RT, $\text{ARI}_{c}$, $\Delta \text{RT}$, $\Delta \text{ARI}_{c}$, $\Delta \text{RT}/\Delta x$, $\Delta \text{ARI}_{c}/\Delta x$) were plotted as mean ± standard deviation. A one-way ANOVA was used to identify group differences. All pairwise comparisons between LQT groups and control were conducted (4 tests) with Bonferroni
correction to control the type I error. An unpaired two-tailed Satterthwaite’s modified t-test was used to quantify the differences in mean ECGI-derived steepness of repolarization gradients and differences in mean body-surface QTc between symptomatic and asymptomatic LQTS patients. All tests were considered statistically significant if P < 0.05.

Results

The results section focuses on the more common types of LQTS (LQT1, 2 and 3); results from two patients with the rare LQT5 are also presented. Figures 1-3 below provide representative examples; Supplemental Figures 2-5 provide repolarization (ARI) maps for all individual patients in each LQTS group. Supplemental Tables 1, 2 and 3 contain results for all LQT1, LQT2 and LQT3 patients, respectively, and Supplemental Table 4 (Section 3) relates ECGI derived parameters to cardiac events history for family members with the same mutation. Online Supplement Section 4 provides results from statistical analysis. Graphs from multiple comparison tests between control and the four LQTS groups are provided for each variable being compared. A sensitivity analysis was conducted using the natural logarithm for all continuous variables due to concern of variance heterogeneity between groups. The results of all pairwise comparisons, after Bonferroni correction, were consistent with the unadjusted (non-logarithmic) analyses. These results are also included in Section 4 of the Online Supplement.

Epicardial Activation Patterns

Figure 1 shows representative ECGI epicardial activation isochrone maps for control, LQT1 (patient 21), LQT2 (patient 3) and LQT3 (patient 7). The epicardial activation pattern in LQTS during sinus rhythm was characterized by normal right ventricular (RV) breakthrough (marked by *) followed by rapid activation of the ventricles, with the excitation spreading uniformly
without lines of block, regions of delayed activation, or regions of slow conduction (isochrone crowding). The latest region to activate was the left ventricular (LV) base. This sequence of activation was the same as that of the control group. The total ventricular activation time, measured as the difference between latest and earliest AT, was around 50 ms - comparable to control (Figure 1). The mean ventricular activation times (Supplemental Tables 1, 2 and 3) were 54 ± 5 ms, 49 ± 9 ms and 55 ± 11 ms for LQT1, LQT2 and LQT3 groups, respectively; control was 47 ± 9 ms. Similarly, the mean ventricular activation time for LQT5 was 53 ± 8 ms. These results indicate that ventricular activation in all LQTS types was normal.

Epicardial Recovery Pattern

Figure 2 shows representative epicardial RT maps for control, LQT1 (patient 15), LQT2 (patient 16) and LQT3 (patient 8) in superior and inferior views. There were marked local changes in recovery pattern in all LQTS types compared to control; the LQTS subjects showed regions of delayed ventricular recovery on the epicardium. For instance, maximum RT in the LQT1 subject was 443 ms compared to 328 ms in control. This led to recovery time differences (ΔRT) that were abnormally large compared to control. For instance, maximum ΔRT in LQT1 was 100 ms (Figure 2) compared to only 28 ms in control. This resulted in a steep gradient of recovery in LQTS (shown by black arrows in figure 2) which was much greater than that of control (Table 2). The mean RT values in the three LQT groups were 372 ± 30 ms in LQT1, 369 ± 42 ms in LQT2 and 404 ± 35 ms in LQT3 compared to control value of 257 ± 15 ms (P < 0.05 for each LQTS type versus control). The mean ΔRT values were 95 ± 19 ms in LQT1, 134 ± 37 ms in LQT2 and 112 ± 9 ms in LQT3 compared to 16 ± 12 ms in control (P < 0.05). The mean ΔRT/Δx values were 89 ± 18 ms/cm in LQT1, 115 ± 25 ms/cm in LQT2 and 133 ± 6 ms/cm in LQT3 compared to only 5.0 ± 3.0 ms/cm in control (P < 0.05). The mean RT, ΔRT and ΔRT/Δx for the
two LQT5 patients were \(343 \pm 24\) ms, \(136 \pm 23\) ms and \(134 \pm 13\) ms/cm respectively, as shown in Table 2. Online Supplement Section 4 provides graphs of pairwise comparison of mean RT, \(\Delta RT\) and \(\Delta RT/\Delta x\) between control and the four LQTS groups. Note that the morphologies of EGMs from locations across the region of steep dispersion differ markedly (Figure 2, panel B). In particular, EGMs in region 1 of delayed repolarization exhibit a predominantly negative T wave compared to EGMs from region 2. This reflects a change in the direction of the voltage gradient across the region of steep dispersion. As shown\(^{24}\), the maximum \(dV/dt\) determines recovery time independent of T-wave polarity.

**Activation-Recovery Intervals**

Figure 3 shows the ARI maps for the patients of Figure 2 in two views. ARI was computed at each epicardial site by subtracting local AT from local RT and was corrected for heart rate. ARI reflects local repolarization and was shown to be a surrogate for local APD\(^{24,25,26}\). Note that the RT maps (Figure 2) and the ARI maps (Figure 3) are very similar. RT is determined by both the activation sequence and local repolarization, while ARI is determined by local repolarization only (independent of the activation sequence). Therefore, the close similarity of the maps indicates that local repolarization is the major determinant of the repolarization sequence in LQTS. A similar property was observed in the normal human heart\(^{21}\). However, as seen from Figure 3, all LQTS subtypes had regions with significant prolongation of ARI compared to control. For instance, the maximum \(ARI_c\) in LQT3 (Figure 3) was \(430\) ms compared to control value of \(320\) ms. The maximum ARI dispersion in each LQTS group (shown by black arrows) was much greater than that of control (Table 2). The location of maximum ARI gradient varied within and among the LQTS types as shown in Supplemental Figures 2, 3, 4 and 5. The mean \(ARI_c\) values in the three LQT groups were \(316 \pm 28\) ms in LQT1, \(307 \pm 36\) ms in LQT2 and 335
\[ \pm 18 \text{ ms in LQT3 compared to } 235 \pm 21 \text{ ms in the control group (P < 0.05 for each LQTS type versus control)}. \text{ The mean } \Delta \text{ARI}_c \text{ values were } 99 \pm 20 \text{ ms in LQT1, } 136 \pm 36 \text{ ms in LQT2, } 110 \pm 14 \text{ ms in LQT3 compared to } 19 \pm 13 \text{ ms in control (P < 0.05). The mean } \Delta \text{ARI}_c/\Delta x \text{ values were } 92 \pm 18 \text{ ms/cm in LQT1, } 117 \pm 29 \text{ ms/cm in LQT2, } 129 \pm 14 \text{ ms/cm in LQT3 compared to only } 2.0 \pm 2.0 \text{ ms/cm in control (P < 0.05). The mean ARI, } \Delta \text{ARI} \text{ and } \Delta \text{ARI}/\Delta x \text{ for the two LQT5 patients were } 288 \pm 36 \text{ ms, } 139 \pm 28 \text{ ms and } 137 \pm 17 \text{ ms/cm, respectively (Table 2). Online Supplement Section 4 provides graphs of pairwise comparison of mean ARI, } \Delta \text{ARI} \text{ and } \Delta \text{ARI}/\Delta x \text{ between control and the four LQTS groups.}

\textbf{Discussion}

This is the first study of its kind, characterizing the electrophysiologic substrate in patients with hereditary LQTS. Noninvasive ECGI made it possible to map with high resolution the entire ventricular epicardium of the intact heart in unanaesthetized patients. The panoramic mapping was essential for the characterization of the substrate.

The results indicate that there is significant prolongation of the action potential on the ventricular epicardium of congenital LQTS patients compared to normal control. The prolongation is consistent with the clinical phenotype of long QT interval on the body-surface ECG. While the epicardial activation was normal in all types of LQTS studied (LQT1, 2, 3 and 5), there was a marked increase in heterogeneity of ventricular recovery on the epicardium which caused significant delay in repolarization in certain regions. These regions were located in close proximity (< 10 mm) to regions with earlier recovery, resulting in abnormally large differences (> 100 ms) in recovery time and ARIs on the epicardium. This is in marked contrast with the normal heart\textsuperscript{21}, where the mean LV apex-to-base ARI dispersion was only 42 ms and average LV
ARI exceeded RV ARI by only 32 ms. With ARI being the surrogate for local APD, these findings reflect spatially heterogeneous prolongation of the action potential, causing the formation of regions with steep dispersion of repolarization.

The epicardial regions with delayed recovery and long ARIs exhibited marked changes in the T-wave morphology (negative or bi-phasic) of the epicardial EGMs compared to the neighboring regions with normal recovery and ARIs (Figures 2 and 3). Such changes in the T-wave over a short distance (< 10 mm) reflected the large spatial dispersion of repolarization and differences in APD.

The location and magnitude of the steep gradients varied from patient to patient. Interestingly, patients with the same genetic mutation had different epicardial repolarization patterns with different regions of prolonged APD. For instance, in Supplemental Figure 3 (LQT2), patient 1 and patient 5 are family members who have the same mutation (KCNH2-Q376sp) but markedly different ARI maps. Patient 1 has long ARIs throughout the RV and a steep ARI gradient across the entire septum. Patient 5 shows long ARIs and a steep repolarization gradient in the basal and free walls of the RV. In the same figure, patient 3 and patient 6 are family members with the same mutation (KCNH2-H70R). Patient 3 has long ARIs and steep gradient in the basal and free walls of the RV. Patient 6 has long ARIs throughout the anterior RV and a steep ARI gradient across the anterior aspect of the septum. In the same figure, patient 16 and patient 17 are family members with the same mutation (KCNH2-Y652X) with markedly different ARI maps. In Supplemental Figure 2 (LQT1), patient 14 and patient 15 are family members with the same mutation (KCNQ1-340delF). Patient 14 has long ARIs in the anterior aspect of the septum and inferior RV base, but patient 15 has long ARIs only in the anterior RV. Similar observations were made for LQT1 patients 20, 21 and 23 (KCNQ1-T312I).
in Supplemental Figure 2, LQT3 patients 8 and 22 (SCN5A-R1644H) and patients 7 and 12 (SCN5A-E1784K) in Supplemental Figure 4, and LQT5 patients 9 and 10 (KCNE1-D76N) in Supplemental Figure 5. This finding is consistent with earlier studies27,28 which demonstrated considerable variability in the ECG-based clinical phenotype of LQTS (measured with body-surface QTc intervals and T-wave characteristics) in family members with the same gene mutation. We suggest that such variation among patients can be attributed to the following factors: differences in the spatial distribution of ion channels expression levels (the LQTS mutant channel and/or other ion channels), differences in gap junction distribution causing spatial heterogeneity of electric loading on repolarizing cells29, spatial variations in neural inputs and hormonal effects, coronary blood flow, and effects of modifier genes that result in variable expressivity of LQTS. A seminal multistage genome-wide association study showed that common variations of NOS1AP, a regulator of neuronal nitric oxide synthase, is significantly associated with QT interval variations in a general population derived from three cohorts30, thereby establishing the important role of modifier genes. It should be clarified that heterogeneous distribution of the LQTS mutant ion channel itself is not a necessary condition for the resultant dispersion of repolarization. Action potential repolarization and APD are determined by a delicate balance among multiple ionic currents which are expressed heterogeneously in the heart. On this background of heterogeneous substrate, even uniform distribution of the mutant channel can shift the delicate balance of currents and cause large and heterogeneous changes in APD.

Interestingly, in many of the pairs above, only one of the family members had a history of cardiac events (patient 1, patient 6, patient 16, patient 7, patient 8 and patient 10). These patients had steeper repolarization (ARI) gradients (patient 1 - 143 ms/cm, patient 6 - 159 ms/cm, patient
16 - 146 ms/cm, patient 7 - 134 ms/cm, patient 8 - 141 ms/cm, patient 10 - 149 ms/cm) compared to their respective family members with no events (patient 5 - 91 ms/cm, patient 3 - 106 ms/cm, patient 17 - 88 ms/cm, patient 12 - 110 ms/cm, patient 22 - 121 ms/cm, and patient 9 - 125 ms/cm). For the entire population of 25 LQT patients, 12 were symptomatic and 13 asymptomatic (Table 1). There was a statistically significant difference in the mean ARI between the two groups; it was 130 ± 27 ms/cm in symptomatic patients compared to 98 ± 19 ms/cm in asymptomatic patients (P = 0.002). In contrast, the difference in mean body-surface QTc was non-significant; it was 492 ± 25 ms in symptomatic patients compared to 508 ± 29 ms in asymptomatic patients (P = 0.1). The ECGI-derived steepness of repolarization gradients (ΔRT/Δx, ΔARI/Δx) did not correlate (R = 0.2) with the patient’s QTc measured from the body-surface ECG. This is not surprising because QTc, being a global marker, cannot adequately reflect the underlying cardiac EP substrate and its regional spatial properties. Thus the steepness of the repolarization gradients (EP substrate) better correlated with the patient’s history of cardiac events (syncope, ventricular fibrillation, ICD shock) than the body-surface QTc. While this observation is preliminary, it could potentially be of clinical relevance. If found to be consistent in a larger study, it could be the basis for noninvasive arrhythmia risk stratification, with ECGI adding to the already established risk factors2. Also, whether the patterns of repolarization dispersion persist and remain unchanged over time in a given patient remains to be explored in future studies.

Some clinical studies have suggested that there is a genotype specific T-wave morphology8 which can help to identify the LQTS subtypes based on the body-surface ECG. However, this possibility is limited because often family members with the same mutation have very different T waves27. This limitation is consistent with the ECGI finding that considerable
overlap exists in the locations of maximum ventricular repolarization gradients among the LQTS subtypes. Interestingly though, in many patients the region of maximum gradient involved the RV and septum (Supplemental Figure 2, 3 and 4). This finding is consistent with clinical EP studies in LQTS patients with severe syncope and arrhythmia, which recorded in-vivo human intracardiac monophasic action potentials from RV endocardium. These studies showed marked spatial differences in ventricular recovery, with abnormally long APD recorded in regions of the RV. Also of interest is a study that found that mERG (mouse ERG) protein expression in the developing embryonic mouse heart was not homogeneous but was greater in the RV and in the RV outflow tract, and that certain mutations in hERG caused developmental abnormalities that mainly affected the same regions. Taken together, these findings support the possible role of RV in creating spatial heterogeneities of repolarization in LQTS.

Prolonged APD and steep spatial dispersion of repolarization have long been known to form the substrate for EADs, unidirectional block and reentrant arrhythmias. The role of reentry in LQTS arrhythmias (torsades de pointes) has been established. However, the only noninvasive marker of dispersion of ventricular repolarization is the QT dispersion measured on the body-surface ECG. This marker lacks sensitivity and specificity and, more importantly, is not based on a solid principle. In fact, each body-surface ECG electrode records electrical signal generated by the integrated electrical activity over the entire heart. Therefore, spatial relationships in the heart are lost in the body-surface ECG and “spatial dispersion” cannot be defined in a meaningful way. ECGI overcomes this limitation by reconstructing the actual spatial properties and dispersion on the heart itself.

Although all 25 patients exhibited significantly longer ARI (APD) compared to the 7 controls, ARI dispersion was not clearly seen on the epicardium for some patients. Patient 4
(Supplemental Figure 3), patients 18, 20 and 21 (Supplemental Figure 2), patient 12
(Supplemental Figure 4) and patient 9 (Supplemental Figure 5) showed large ARI dispersion
only along the basal region of the ventricles. This does not rule out the possibility that steep
dispersion exists in the depth of the myocardium, as ECGI mapping is limited to the epicardium.
Being the first ECGI study of LQTS in human subjects, only small cohorts from the most
prevalent LQT types were included. As such, genotype-specific patterns of epicardial
repolarization could not be identified. Relationships between genotype and patterns of
repolarization need to be examined in a study with larger numbers of subjects from the diverse
LQTS types. In addition, genotype positive / phenotype negative patients should be included in
future studies.

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CardioInsight Technologies. Dr. Strom is a paid employee and stockholder of CardioInsight
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Table 1. Clinical Characteristics of the LQTS Subjects.

<table>
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<th>Gene</th>
<th>Mutation</th>
<th>LQTS Type</th>
<th>ID</th>
<th>Age (YRS.)</th>
<th>Sex</th>
<th>QTc (ms)</th>
<th>Cardiac Events (Symptoms)</th>
<th>Therapy</th>
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<td>F</td>
<td>500</td>
<td>syncope</td>
<td>ICD*</td>
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*The patients with ICD were not paced. The average duration of ICD implant was 5 ± 3 years at the time of the ECGI study
Table 2. Summary of repolarization parameters for all groups.

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<th>Parameter (Mean ± SD)</th>
<th>NORMAL (n=7)</th>
<th>LQT1 (n=9)</th>
<th>LQT2 (n=9)</th>
<th>LQT3 (n=5)</th>
<th>LQT5 (n=2)</th>
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<tr>
<td>RT (ms)</td>
<td>257 ± 15</td>
<td>372 ± 30</td>
<td>369 ± 42</td>
<td>404 ± 35</td>
<td>343 ± 24</td>
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<tr>
<td>ΔRT (ms)</td>
<td>16 ± 12</td>
<td>95 ± 19</td>
<td>134 ± 37</td>
<td>112 ± 9</td>
<td>136 ± 23</td>
</tr>
<tr>
<td>ΔRT/Δx (ms/cm)</td>
<td>5.0 ± 3.0</td>
<td>89 ± 18</td>
<td>115 ± 25</td>
<td>133 ± 6</td>
<td>134 ± 13</td>
</tr>
<tr>
<td>ARIc (ms)</td>
<td>235 ± 21</td>
<td>316 ± 28</td>
<td>307 ± 36</td>
<td>335 ± 18</td>
<td>288 ± 36</td>
</tr>
<tr>
<td>ΔARIc (ms)</td>
<td>19 ± 13</td>
<td>99 ± 20</td>
<td>136 ± 36</td>
<td>110 ± 14</td>
<td>139 ± 28</td>
</tr>
<tr>
<td>ΔARIc/Δx (ms/cm)</td>
<td>2.0 ± 2.0</td>
<td>92 ± 18</td>
<td>117 ± 29</td>
<td>129 ± 14</td>
<td>137 ± 17</td>
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</tbody>
</table>

All results were statistically significant (P < 0.05 for each LQTS type versus control). However, LQT5 group had only two patients. The difference in mean ARIc between control and LQT5 group was not statistically significant. Online Supplement Section 4 provides graphs showing pairwise comparisons between control and each LQTS group.

Figure Legends:

**Figure 1.** Epicardial Activation Times (AT) Isochrone Maps. Examples of activation in (left to right) control, LQT1 (patient 21), LQT2 (patient 3), and LQT3 (patient 7). In all LQTS types as in normal control, epicardial activation starts from breakthrough at anterior RV (shown by white asterisk) near the RVOT region, 20-30 ms after the onset of QRS. It proceeds in a uniform fashion to activate the ventricles synchronously. The latest region to activate is the LV basal region (dark blue). The total ventricular activation time (TVAT) in all LQTS types was around 50 ms, comparable to normal control. RA = right atrium, LA = left atrium, RV = right ventricle, LV = left ventricle, AO = aorta, ms = milliseconds.

**Figure 2.** Epicardial Recovery Time (RT) Maps. A. Maps are shown in superior (top row) and inferior (bottom row) views for control, LQT1 (patient 15), LQT2 (patient 16), and LQT3 (patient 8). All three LQTS subjects had regions with abnormally long RT as shown by predominant magenta and white colors in the maps. The maximum RT value in LQTS was 470
ms. The maximum RT value in the normal heart (left most column) was 360 ms (predominant blue and green colors in the map). The heterogeneity in ventricular recovery resulted in large RT differences in all three LQTS types. The solid yellow line (top panels) connects two closest neighboring EGMs (from site 1 and site 2) with maximum ΔRT. In all three LQTS patients, ΔRT (RT(1)-RT(2)) exceeded 100 ms (compared to normal value of only 28 ms in the left most column). As a result, there was a steep gradient of repolarization ΔRT/Δx across this region (shown by black arrows); it was much steeper than control (Normal: 6 ms/cm, LQT1: 102 ms/cm, LQT2: 159 ms/cm, LQT3: 139 ms/cm). **B.** ECGI-reconstructed unipolar EGMs from the three LQT patients exhibited drastic changes in T-wave morphology across the yellow line. The T waves obtained from site 1 (red) were inverted or predominantly negative compared to those from site 2 (blue; upright or predominantly positive). Such T-wave changes over a short distance (<10 mm) were absent in the control group. RT (time of dV/dt max during upstroke of T wave) is indicated by the pink dot on the corresponding EGMs (site 1 red; site 2 blue). Corresponding 12-lead ECG tracings are provided in Supplemental Figure 1. mV = millivolts.

**Figure 3.** Activation-Recovery Interval (ARI) Maps. **A.** Maps are shown in superior (top row) and inferior (bottom row) views for the patients of figure 2. ARI (surrogate for local APD) values were abnormally long (magenta and white regions) in all three LQTS patients compared to control. The maximum ARI value in LQTS was 450 ms compared to only 340 ms (green in the left most column) in control. The localized prolongation of APD resulted in large ARI differences in all three LQTS types. The solid yellow line (top panels) connects two closest neighboring EGMs (from site 1 and site 2) with maximum ΔARIc. In all three LQTS patients, ΔARIc (ARIc(1)-ARIc(2)) exceeded 100 ms (compared to normal ΔARIc of only 30 ms in the left
most column). As a result, there was a steep gradient of repolarization $\Delta$ARI/$\Delta$x across this region (indicated by black arrows) which was two orders of magnitude greater than control (Normal: 7 ms/cm, LQT1: 104 ms/cm, LQT2: 146 ms/cm, LQT3: 140 ms/cm). B. The ECGI-reconstructed EGMs depict the time instances of AT (black dots) and RT (pink dots). The corresponding ARI values (RT – AT) are indicated below.
Figure 1
Figure 2
Figure 3
Electrophysiologic Substrate in Congenital Long QT Syndrome: Noninvasive Mapping with Electrocardiographic Imaging (ECGI)
Ramya Vijayakumar, Jennifer N. A. Silva, Kavit A. Desouza, Robert L. Abraham, Maria Strom, Frédéric Sacher, George F. Van Hare, Michel Haïssaguerre, Dan M. Roden and Yoram Rudy

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Electrophysiologic Substrate in Congenital Long QT Syndrome: Noninvasive Mapping with Electrocardiographic Imaging (ECGI)

Ramyaa Vijayakumar, MS; Jennifer N.A. Silva, MD; Kavit A. Desouza, MD; Robert L. Abraham, MD; Maria Strom, PhD; Frederic Sacher, MD; George F. Van Hare, MD; Michel Haïssaguerre, MD; Dan M. Roden, MD; Yoram Rudy, PhD
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Section 2: Results for each LQTS group

Section 3: ECGI derived parameters and cardiac events history for family members with the same mutation

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Section 1

This section contains Supplemental Figure 1 with 12-lead ECG tracings for the LQTS patients in figure 2 of the main text
Supplemental Figure 1: ECG tracings for the LQT patients in figure 2.

Patient 15
LQT1

Patient 16
LQT2

Patient 8
LQT3
Section 2

A. Supplemental Figure 2 contains ARI maps for KCNQ1 mutations and Supplemental Table 1 contains the summary of results for all LQT1 patients.
Supplemental Figure 2: ARI Maps for KCNQ1 Mutations. Maps for 9 LQT1 patients are shown in two views (superior and inferior; atria removed). ARI map for a normal heart is shown in the bottom right panel for comparison. Although ARIs were prolonged in all LQT1 patients relative to normal control, there were considerable variations in ARI spatial distributions (hence in APD heterogeneity) among patients. There is considerable variation in spatial ARI distribution among the LQTS patients. Family members who had the same genetic mutation (Patients 13 and 24 in the first row (unknown); Patients 14 and 15 in the second row (340delF); Patients 20, 21 and 23 (T312I)) had different epicardial repolarization patterns with different regions of
prolonged ARIs and steep ARI gradients. Regions of steep repolarization dispersion are indicated by arrows.

Supplemental Table 1: Summary of results for LQT1 patients. Patients with the same mutation are in adjacent rows, shown in the same color.

<table>
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<tr>
<th>ID</th>
<th>QT&lt;sub&gt;c&lt;/sub&gt; (ms)</th>
<th>Mean Epicardial AT (ms)</th>
<th>Mean Epicardial RT (ms)</th>
<th>Mean Epicardial ARI&lt;sub&gt;c&lt;/sub&gt; (ms)</th>
<th>ΔRT (ms)</th>
<th>ΔRT/Δx (ms/cm)</th>
<th>ΔARI&lt;sub&gt;c&lt;/sub&gt; (ms)</th>
<th>ΔARI&lt;sub&gt;c&lt;/sub&gt;/Δx (ms/cm)</th>
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Section 2

B. Supplemental Figure 3 contains ARI maps for KCNH2 mutations and Supplemental Table 2 contains the summary of results for all LQT2 patients.
Supplemental Figure 3: ARI Maps for KCNH2 Mutations. Maps for 9 LQT2 patients are shown in two views (superior and inferior; atria removed). ARI map for a normal heart is shown in the bottom right panel for comparison. There is considerable variation in spatial ARI distribution among the LQTS patients. Overall, the RV has longer ARIs than the LV. Family members who had the same genetic mutation (Patients 1 and 5 in the first row (Q376sp); Patients 3 and 6 in the second row (H70R); Patients 16 and 17 in the fourth row (Y652X)) had different epicardial repolarization patterns with different regions of prolonged ARIs and steep ARI gradients. The maps for patients 1, 6 and 16, who had a history of cardiac events, exhibit more extensive and more distributed regions of long ARIs and steeper ARI gradients compared to their asymptomatic family members (patients 5, 3 and 17 respectively). Regions of steep repolarization dispersion are indicated by arrows.
<table>
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<th>ID</th>
<th>QT&lt;sub&gt;c&lt;/sub&gt; (ms)</th>
<th>Mean Epicardial AT (ms)</th>
<th>Mean Epicardial RT (ms)</th>
<th>Mean Epicardial ARI&lt;sub&gt;c&lt;/sub&gt; (ms)</th>
<th>ΔRT (ms)</th>
<th>ΔRT/Δx (ms/cm)</th>
<th>ΔARI&lt;sub&gt;c&lt;/sub&gt; (ms)</th>
<th>ΔARI&lt;sub&gt;c&lt;/sub&gt;/Δx (ms/cm)</th>
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**Supplemental Table 2: Summary of results for LQT2 patients.** Patients with the same mutation are in adjacent rows, shown in the same color.
Section 2

C. Supplemental Figure 4 contains ARI maps for SCN5A mutations and Supplemental Table 3 contains the summary of results for all LQT3 patients.
Supplemental Figure 4: ARI Maps for SCN5A Mutations. Maps for 5 LQT3 patients are shown in two views (superior and inferior; atria removed). ARI map for a normal heart is shown in the bottom right panel for comparison. Family members who had the same genetic mutation (Patients 8 and 22 in the top row (R1644H); Patients 7 and 12 in the middle row (E1784K)) had different epicardial repolarization patterns with different regions of prolonged ARIs and steep ARI gradients. The map for patient 7, who had a history of VF, exhibits more extensive and more distributed regions of long ARIs and steeper ARI gradients compared to patient 12, who was asymptomatic. Regions of steep repolarization dispersion are indicated by arrows.
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<th>Mean Epicardial RT (ms)</th>
<th>Mean Epicardial $ARI_c$ (ms)</th>
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<th>$\Delta RT/\Delta x$ (ms/cm)</th>
<th>$\Delta ARI_c$ (ms)</th>
<th>$\Delta ARI_c/\Delta x$ (ms/cm)</th>
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</table>

**Supplemental Table 3: Summary of results for LQT3 patients.** Patients with the same mutation are in adjacent rows, shown in the same color.
Section 2

D. Supplemental Figure 5 contains ARI maps for \textit{KCNE1} mutations.
Supplemental Figure 5: ARI Maps for KCNE1 Mutations. Maps for 2 LQT5 patients are shown in two views (superior and inferior; atria removed). ARI map for a normal heart is shown in the bottom panel for comparison. Despite having the same mutation (D76N), the two LQT5 patients have markedly different ARI maps. The map for Patient 10, who had a history of syncope, exhibits an extensive region with long ARIs and steep ARI gradients along the anterior septum and in the LV. This substrate is not present in Patient 9, who was asymptomatic. Regions of steep repolarization dispersion are indicated by arrows.
Section 3

Supplemental Table 4 relates ECGI derived parameters to cardiac events history for family members with the same mutation.
<table>
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<th>Patient</th>
<th>Patient 1</th>
<th>Patient 5</th>
<th>Patient 3</th>
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<td>ARI gradient (ms/cm)</td>
<td>143</td>
<td>91</td>
<td>106</td>
<td>159</td>
<td>85</td>
<td>104</td>
<td>159</td>
<td>88</td>
</tr>
<tr>
<td>Location of steepest gradient</td>
<td>Septum and base</td>
<td>RV free wall and base</td>
<td>RV free wall and base</td>
<td>Anterior septum</td>
<td>Base and inferior RV</td>
<td>Anterior septum</td>
<td>Base, inferior septum</td>
<td>Base</td>
</tr>
<tr>
<td>History of events</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
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</tbody>
</table>

Supplemental Table 4: ECGI derived parameters and cardiac events history for family members with the same mutation (shown in the same color).
Supplemental Table 4 (continued): ECGI derived parameters and cardiac events history for family members with the same mutation (shown in the same color).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient 20</th>
<th>Patient 21</th>
<th>Patient 23</th>
<th>Patient 8</th>
<th>Patient 22</th>
<th>Patient 7</th>
<th>Patient 12</th>
<th>Patient 9</th>
<th>Patient 10</th>
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<tr>
<td>Gene mutation</td>
<td>KCNQ1 - T312I</td>
<td>KCNQ1 - T312I</td>
<td>KCNQ1 - T312I</td>
<td>SCN5A - R1644H</td>
<td>SCN5A - R1644H</td>
<td>SCN5A - E1784K</td>
<td>SCN5A - E1784K</td>
<td>KCNE1 - D76N</td>
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<td>496</td>
<td>543</td>
<td>478</td>
<td>533</td>
<td>486</td>
<td>500</td>
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<tr>
<td>AT (ms)</td>
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<td>48</td>
<td>52</td>
<td>40</td>
<td>69</td>
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<td>58</td>
<td>47</td>
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<tr>
<td>RT gradient (ms/cm)</td>
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<td>98</td>
<td>79</td>
<td>139</td>
<td>125</td>
<td>131</td>
<td>122</td>
<td>124</td>
<td>125</td>
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<tr>
<td>ARI gradient (ms/cm)</td>
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<td>99</td>
<td>81</td>
<td>141</td>
<td>121</td>
<td>134</td>
<td>110</td>
<td>143</td>
<td>149</td>
</tr>
<tr>
<td>Location of steepest gradient</td>
<td>Base</td>
<td>Base</td>
<td>Base and anterior septum</td>
<td>Base and anterior septum</td>
<td>Base and anterior septum</td>
<td>Base and RV free wall</td>
<td>Base</td>
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<td>Base and anterior septum</td>
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<tr>
<td>History of events</td>
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<td>yes</td>
<td>no</td>
<td>yes</td>
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<td>no</td>
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</tbody>
</table>
Section 4

Statistical Analysis

Results from pairwise comparisons of all continuous variables (RT, ΔRT, ΔRT/Δx, ARIc, ΔARIc and ΔARIc/Δx) between control and LQTS groups are provided (top panel).

To account for the heterogeneity of variance, we performed a sensitivity analysis using the natural logarithm for the above continuous variables (bottom panels). The graphs for all pairwise logarithmic comparisons were consistent with the unadjusted (non-logarithmic) analyses.
Supplemental Figure 6: Results from pairwise comparisons of RT (top) and the corresponding logarithmic values (bottom).
Supplemental Figure 7: Results from pairwise comparisons of RT dispersions (top) and their corresponding logarithmic values (bottom).
Supplemental Figure 8: Results from pairwise comparisons of RT gradients (top) and their corresponding logarithmic values (bottom).

Pairwise comparison of RT gradients

All LQTS groups have mean RT gradients significantly different from that of Control

Pairwise comparison of natural log values of RT gradient

All LQTS groups have means significantly different from that of Control

Supplemental Figure 8: Results from pairwise comparisons of RT gradients (top) and their corresponding logarithmic values (bottom).
Supplemental Figure 9: Results from pairwise comparisons of ARIc (top) and the corresponding logarithmic values (bottom).

**Pairwise comparison of ARIc values**

LQT1, LQT2 and LQT3 groups have mean ARI values significantly different from that of Control.

**Pairwise comparison of natural log values of ARIc**

Group means of LQT1, LQT2 and LQT3 are significantly different from that of control.
Supplemental Figure 10: Results from pairwise comparisons of ARI dispersions (top) and their corresponding logarithmic values (bottom).
Supplemental Figure 11: Results from pairwise comparisons of ARI gradients (top) and their corresponding logarithmic values (bottom).