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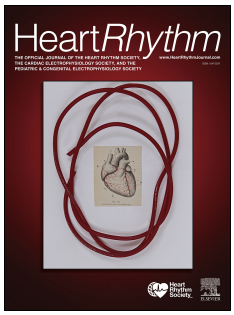

Novel risk predictor of arrhythmias for patients with potassium channel related congenital Long-QT Syndrome

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Novel risk predictor of arrhythmias for patients with potassium channel related congenital Long-QT Syndrome

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Abstract

Background: Congenital long-QT syndrome (LQTS) is characterized by delayed ventricular repolarization, predisposing to potentially lethal ventricular arrhythmias. The variability in disease severity among patients remains largely unexplored, underscoring the limitations of current risk stratification methods.

Objective: We aimed to evaluate the potential utility of exercise stress test (EST) electrocardiographic markers in identifying high-risk LQTS patients.

Methods: The study, which considered LQT1 and LQT2 patients, comprised a discovery cohort of 695 and a validation cohort of 635 patients.

Results: The change in QTc between rest and recovery (between rest and 3-4 minutes into recovery period, called Recovery-Rest ΔQTc) was consistently greater in symptomatic patients. Sensitivity analyses conducted on EST data obtained on and off BB as well as upon distinguishing between patients with a baseline QTC below or above 470 milliseconds (ms), demonstrated consistent findings. The association of Recovery-Rest ΔQTc with cardiac events remained significant in a sub-analysis focussing on future events (i.e. occurring after EST). An optimal Recovery-Rest ΔQTc cut-off was determined for LQT1 (35 ms) and LQT2 (16 ms) separately and was shown to be significantly associated with cardiac events.

Conclusion: Our findings suggest that in LQTS patients, dynamic QT interval measures obtained on EST are associated with lifetime arrhythmic events, and events following EST. Such measures can be helpful in identifying a higher-risk subset of LQTS patients in order to optimize their management. Further research may confirm these findings in larger cohorts, and explore the potential benefit of combining genetic and EST data for more precise risk stratification.

Abbreviations

AUROC	Area under ROC curve
BB	Beta-blocker
bpm	Beat per minute
EAD	Early afterdepolarization
ECG	Electrocardiogram
EST	Exercise stress test
FU	Follow up
HR	Hazard ratio
ICD	Implantable cardioverter defibrillator
LQTS	Long QT Syndrome
ms	Millisecond
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
ROC	Receiver operating characteristic
SCD	Sudden cardiac death
TdP	Torsade de Pointes
ΔHR	Delta heart rate
ΔQTc	Delta QTc

Introduction

Congenital long-QT syndrome (LQTS) is an inherited cardiac channelopathy characterized by delayed ventricular repolarization, resulting in a propensity for early afterdepolarisations (EAD) which in turn promote the occurrence of potentially lethal ventricular arrhythmias such as Torsade de pointes (TdP).(1, 2)

The discovery of the causal ion channel genes and reappraisal of gene-disease associations had a large impact on the diagnostic procedures and clinical management of patients with LQTS.(3, 4) However, the low disease penetrance and variability in disease severity, even among related individuals harbouring the same causal genetic variant, varies significantly and remains largely unexplained. Historically, besides symptom status, age and sex, the QTc on the baseline ECG has so far been the only clinical marker for risk stratification. Breakthroughs in refinement of risk prediction for cardiac events in more recent years comprised the consideration of the genetic subtype, and the location of the causal genetic variant within the ion channel structure.(5-7) Yet, the quest for additional risk factors continues to be fundamental for enhancing the personalized treatment of each LQTS patient.(1, 8) Notably, outcomes from exercise testing, which are used in clinical practice for risk stratification, have so far only been considered for diagnostic purposes in current guidelines for LQTS.(9, 10)

In LQTS, cardiac events often occur during increased sympathetic activity, such as physical exertion or emotional distress.(11) Under these circumstances, alpha- and beta-receptor activation enhances potassium currents, and subsequently shortens the repolarization phase of the cardiomyocyte. LQTS-causing genetic variants that impair potassium currents make the heart particularly susceptible to the effects of adrenergic activation and increased heart rates.(12, 13) Such conditions can lead to pronounced repolarization disparities across the myocardium, and when combined with increased calcium currents, in this setting, create a

risk for arrhythmias.(14) Crotti et al.(15) showed that vagal reflexes, translated as heart rate reduction during EST recovery, can contribute to identify patients -in a gene specific fashion- at high or low risk for life-threatening arrhythmias. More recently, Rieder et al.(16) demonstrated in a small LQTS patient cohort that post-exercise QTc may be a valuable marker for distinguishing symptomatic from asymptomatic patients, also in a gene-specific manner.

The exercise stress test (EST) evokes sympathetic activation and may thus enhance impaired repolarization, thereby accentuating or unmasking QT and T-wave changes, and mimic pro-arrhythmic conditions(17). This is based on the paradoxical prolongation or altered dynamics of QT intervals in response to sympathetic neural stimulation.(18)

We built on previous work describing the phenomenon of QT-RR hysteresis during exercise and recovery, comprising delayed adaptation of the QT interval to changes in heart rate,(19, 20) with effects being more pronounced in LQTS patients.(21)

Through large independent cohorts of LQT1 and LQT2 patients, we tested the hypothesis that not only heart rate dynamics but also measurements of QTc dynamics during EST may hold the potential for identifying patients at high-risk of cardiac events.

Methods

Study Population

Genotyped LQTS patients from the Amsterdam University Medical Center (Netherlands), University Medical Center Groningen (Netherlands), Istituto Auxologico Italiano (Italy), L'institut du Thorax (France), Rigshospitalet (Denmark), Mayo Clinic (United States) and Münster University Hospital (Germany) were retrospectively included in the study. Here, only probands and family members diagnosed with LQTS and who carried a rare genetic variant in

KCNQ1 (LQT1) or *KCNH2* (LQT2), reflecting the two major LQTS subtypes, were included. The study was approved by the Medical Ethics Committee of Amsterdam University Medical Center (ID W19_153 # 19.188). The patients sourced from the Netherlands, Italy, France and Denmark constituted the discovery cohort, while the patients from the United States (USA) and Germany comprised the validation cohort. Genetic variant curation was conducted as per American College of Medical Genetics and Genomics and Association of Molecular Pathology guidelines.(22)

Clinical definitions

Symptomatic patients were defined as LQTS patients who experienced a (presumed) arrhythmic syncope, documented sustained ventricular tachycardia (susVT) or (aborted) sudden cardiac death. These were defined as explained in **Table S1 in the Data Supplement.**

ECG Analysis

Twelve-lead ECGs were digitally acquired at clinical presentation for analysis of the "baseline QTc". EST was performed with the modified or standard Bruce protocol treadmill test or bicycle ergometry in upright position, during which a digital ECG was acquired. The EST was generally (but not always) performed at first presentation in the clinic, regardless of BB usage. During EST, digital ECG was acquired during exercise testing. Heart rate (in beats per minute, bpm) and QT (in milliseconds, ms) measurements were determined uniformly at specific time points of interest selected and measured in: (1) supine resting position, referred to as 'Rest'; (2) at peak exercise, referred to as 'Peak'; and (3) at 3-4 minutes after cessation of exercise, referred to as 'Recovery'. ECG analysis (i.e., QT measurements at the pre-specified time points) was performed by experienced physicians. The tangent method was used to measure

the QT interval. The longest QT interval, occurring in any of the 12 leads was considered, but generally measured in leads II and V5. The mean QT interval of 3 consecutive beats was used. When the isoelectric baseline was not a consistent straight line during certain heart rates, the closest heart rate was chosen to measure the QT interval to obtain correct measurements (**Figure S1-A in the Data Supplement**). Hereafter, the QT interval was corrected for heart rate (QTc) according to Bazett's formula.(23) The change in the QTc between (1) Rest and Recovery (Recovery-Rest ΔQTc), (2) Rest and Peak (Peak-Rest ΔQTc), and (3) Peak and Recovery (Recovery-Peak ΔQTc) was calculated for each patient and used in association analyses. An example of an ECG from patients with a large and small (negative) delta between rest and recovery QTc can be observed in **Figure S1-B and C in the Data Supplement**. Differences in heart rate (ΔHR) were also calculated for each patient at the same time points, providing Recovery-Rest ΔHR, Peak-Rest ΔHR and Recovery-Peak ΔHR for analyses. An overview of all analyses performed in discovery and validation cohorts can be seen in **Figure 1**.

Statistical Analysis

Baseline continuous variables are presented as mean ± SD and compared between discovery and validation sets using a t-test. Baseline categorical variables are presented as count and percentage and compared using a Pearson's chi-square test.

Lifetime cardiac events

All analyses related to lifetime cardiac events (i.e. follow up starting at birth) were carried out in LQT1 and LQT2 patients separately. To investigate associations between the above mentioned six ECG parameters and lifetime cardiac events, we 1) compared the ECG parameters between symptomatic and asymptomatic individuals using a t-test, 2) performed logistic regression with lifetime cardiac event yes/no as outcome and 3)

performed Cox proportional hazard regression with time to the first lifetime cardiac event as outcome. This was done separately in the discovery and validation set. For all regression models, the ECG parameters were (initially) entered as continuous variables, resulting in effects per one millisecond (ms) or one bpm increase. Quantitative covariates that were used in all models were age and QTc at baseline 12-lead ECG. Categorical variables were proband or family member status, sex, BB use during EST and clinical centre. We additionally corrected for the LQT1 and LQT2 specific type of genetic alteration (missense versus non-missense) and the location in the channel protein of the amino acid residue affected by missense variants. This was based on previous publications correlating location of amino acid change with arrhythmia risk (see **Table S2 in the Data Supplement)**.(24, 25). To evaluate the added value of including the ECG parameter, the fit of two nested logistic/Cox regression models (i.e. ECG parameter plus covariates vs covariates only) were compared using a likelihood ratio test. In case of multiple significantly associated ΔQTc (or ΔHR) variables, the variable with the best fit was selected, resulting in maximal one ΔQTc and/or ΔHR to be evaluated in further analyses. Using ROC curves based on a predicted survival at 30 years in the discovery set, the optimal cut off for the selected ΔQTc and/or ΔHR variables were determined based on the maximal Youden's index. Calibration curves were created for the models with the dichotomized ΔQTc and/or ΔHR in the discovery set.

Cardiac events after EST

Next, we examined the association with the incidence of cardiac events occurring after EST assessment. For this, we conducted Cox regression analyses focusing on the ΔQTc and/or ΔHR marker that demonstrated the most robust and consistent association with lifetime cardiac events across both the discovery and validation cohorts (see above). Kaplan-Meier

curves were created based on the dichotomized variables. In these analyses, patients with LQT1 and LQT2 were combined to increase statistical power.

Sensitivity analyses

Several sensitivity analyses were carried out to evaluate the utility in clinically relevant scenarios: 1) Since both patients with- and without beta blocker (BB) use during the EST were included (to increase statistical power), sub-analyses were conducted in these two patient groups separately. We also compared the association between EST and events within patients that had both an EST on and off BB; 2) To reveal any potential differences in risk for cardiac events depending on the baseline QTc, and to account for concealed LQTS patients, sub-analyses were performed in two subsets of patients with a baseline QTc above or below 470 ms, respectively; 3) In patients not having suffered from a previous cardiac event only and 4) in relatives only.

All statistical analyses were performed using R software (version 4.3.2) and rms, survival and survival ROC packages. P-values < 0.05 were regarded as statistically significant.

Results

Lifetime cardiac events

Discovery Study

We collected 695 LQTS patients (LQT1, 60.6%; LQT2, 39.4%) with available EST from The Netherlands, France, Italy and Denmark. Baseline characteristics and demographics can be seen in **Table 1**. They comprised 239 probands (34.4 %) and 456 family members (65.6 %).

LQT1

There were 421 LQT1 patients who had both a baseline 12-lead ECG and an EST. The results of the direct comparison between symptomatic and asymptomatic patients, the logistic and Cox regression for association analyses of baseline QTc and ΔQTc with cardiac events are shown in **Table 2.**

Association analyses of Peak-Rest ΔHR, Recovery-Peak ΔHR and Recovery-Rest ΔHR with cardiac events in LQT1 patients demonstrated a statistically significant association for Peak-Rest ΔHR (**Table S3 in the Data Supplement**). We observed that symptomatic patients had a lesser degree of heart rate increase between rest and peak exercise (Peak-Rest ΔHR) compared to asymptomatic patients (59 ± 25 bpm vs 67 ± 18 bpm, p = 0.008). Logistic regression showed similar results, demonstrating an OR per bpm increase of 0.984 (95% CI: 0.970 – 0.999). However, Cox regression did not reveal significant association (HR = 0.989, 95% CI: 0.977 – 1.001).

As expected, symptomatic patients had a higher baseline QTc compared to asymptomatic patients (482 ± 45 ms vs 460 ± 38 ms, p<0.001). Logistic regression showed an odds ratio (OR) per ms increase in QTc of 1.016 (95% CI: 1.010 – 1.023) and survival analysis demonstrated a hazard ratio (HR) per ms increase in QTc of 1.006 (95% CI: 1.001 – 1.011).

Association analyses of Peak-Rest ΔQTc, Recovery-Peak ΔQTc and Recovery-Rest ΔQTc with cardiac events in LQT1 patients demonstrated a statistically significant association for Recovery-Rest ΔQTc (**Table 2**). The mean Recovery-Rest ΔQTc was 28 ± 37 ms. The Recovery-Rest ΔQTc was significantly higher in symptomatic individuals compared to asymptomatic (47 ± 39 ms vs 24 ± 35 ms, p < 0.001), see **Figure 2-A**. Logistic regression and Cox regression demonstrated an association between Recovery-Rest ΔQTc and occurrence

of lifetime cardiac events (OR per ms Recovery-Rest ΔQTc: 1.034, 95% CI: 1.023 – 1.047, C-statistic = 88.4%; HR per ms Recovery-Rest ΔQTc: 1.022, 95% CI: 1.015 – 1.030, Concordance = 0.87).

A Recovery-Rest ΔQTc cut-off of 35 ms was calculated for the optimal ROC (0.84) in the Cox regression at 30 years follow up from birth. The cut-off was determined based on logistic regression for cardiac events occurring by age 30 (i.e., cardiac events at this age may be reported later during the first evaluation). This gave a hazard ratio of 3.3 for patients above this cut-off compared to below (95% CI: 1.96 – 5.57). The positive predictive value (PPV) was 0.288 (95%CI: 0.217 – 0.360), and the negative predictive value (NPV) 0.911 (95% CI: 0.875 – 0.946). Moreover, the inclusion of this Recovery-Rest ΔQTc cut-off to the Cox proportional hazards model with baseline QTc and the aforementioned covariables revealed a significant enhancement in model fit compared to the basic model ($P < 0.001$), see **Figure 2-B**.

LQT2

Recovery-Rest ΔHR was significantly higher in symptomatic patients and associated with events in both logistic (OR = 1.076 per bpm) and Cox regression analyses (HR = 1.040), but adding it to a model with Recovery-Rest ΔQTc did not improve predictive power, and no association was found for Peak ΔHR or Recovery-Peak ΔHR.

The mean QTc at baseline was higher in symptomatic patients compared to asymptomatic (494 ± 55 ms vs 460 ± 39 ms, p = 0.002). Similar findings were observed for the logistic (OR per ms: 1.017, 1.004 - 1.030 95%CI) and Cox regression (HR per ms: 1.011, 1.001 – 1.021 95% CI), as reported in **Table 2**.

The mean Recovery-Rest ΔQTc was 10 ± 35 ms. Similar to patients with LQT1, we observed that the Recovery-Rest ΔQTc was significantly higher in symptomatic patients compared to

asymptomatic (43 ± 39 ms vs 6 ± 32 ms, p < 0.001), see also **Figure 2-D**. Also similarly, logistic regression and Cox regression demonstrated an association between Recovery-Rest ΔQTc and occurrence of lifetime cardiac events (OR per ms: 1.040, 95% CI: 1.023 – 1.059, C-Statistic = 92.1%; HR per ms: 1.011, 95% CI: 1.001– 1.024, Concordance=0.96).

A Recovery-Rest ΔQTc cut-off of 16 ms was calculated for the optimal ROC (0.87) in the cox regression at 30 years follow up from birth. This gave a hazard ratio of 4.8 for patients above this cut-off compared to below (95% CI: 2.00– 11.72). The PPV was 0.288 (95%CI: 0.217 – 0.360), and the negative predictive value (NPV) 0.911 (95% CI: 0.875 – 0.946). Moreover, the inclusion of this Recovery-Rest ΔQTc cut-off to the Cox proportional hazards model with baseline QTc and the aforementioned covariables revealed a significant enhancement in model fit compared to the basic model (P = 0.004), see **Figure 2-E**.

Peak-Rest ΔQTc was associated with cardiac events in both logistic (OR per ms: 1.018, 95%CI: 1.001 – 1.038) and cox regression (HR per ms: 1.017, 95% CI: 1.000 – 1.035). A direct comparison however did not show significant differences between symptomatic and asymptomatic patients for this parameter (-13 ± 45 ms vs -12 ± 38 ms, p = 0.933). Peak - Recovery ΔQTc did not display any association (**Table 2**).

Validation Study

Heart rate dynamics

The association between Peak-Rest ΔHR and cardiac events observed in LQT1 patients was replicated, with symptomatic patients from the validation set exhibiting a smaller increase in heart rate from rest to peak exercise, as detailed in **Table S3 in the Data Supplement**. However, this analysis did not show a significant difference in BB use between symptomatic

and asymptomatic patients (P = 0.13). The association between Recovery-Rest ΔHR in LQT2 patients was not replicated.

QTc dynamics

Given the consistent association of Rest-Recovery ΔQTc with cardiac events in the LQT1 and LQT2 discovery sets, we aimed to validate this in independent LQT1 (n=403) and LQT2 (n=232) patients with available baseline ECG and EST (**Table 1)**.

In both LQT1 and LQT2, mean Recovery-Rest ΔQTc was higher in symptomatic compared to asymptomatic patients (LQT1: symptomatic, 47 ± 43 ms vs. asymptomatic, 34 ± 42 ms, p = 0.018; LQT2: symptomatic, 10 ± 34 ms vs. asymptomatic, -7 ± 47 ms; p = 0.008). Recovery-Rest ΔQTc was significantly associated with lifetime events in both LQT1 (HR per ms: 1.009, 95% CI: 1.003 – 1.015, Concordance=0.77) and LQT2 patients (HR per ms: 1.008, 95% CI: 1.000 – 1.016, Concordance=0.83), thus validating the findings in the discovery set. The association of Peak-Rest ΔQTc observed in LQT2 patients did not replicate (See also **Table 2**). For LQT1 and LQT2 together, when applying their gene-specific Recovery-Rest ΔQTc cut-off (LQT1 = 35 ms, LQT2 = 16ms) we were able to validate the significant association with lifetime events in both a single predictor and a multivariable and Cox regression model (**Table S4 in the Data Supplement**).

Sensitivity analysis

Symptomatic patients were more likely to be on BB therapy at the time of the EST compared to asymptomatic patients, with 76% of symptomatic patients and 38% of asymptomatic patients taking BB (P < 0.001). In a sensitivity analysis, we combined LQT1 patients from the Discovery and Validation cohorts, as well as for LQT2 patients. Cox regression showed significant association for Recovery-Rest ΔQTc in both the group on BB as well as those

without (**Table S5 in the Data Supplement**). In a sub-population of 122 LQTS patients for whom an EST on as well as off BB therapy was available, we found that Recovery-Rest ΔQTc was higher in symptomatic patients in both analyses (Off BB: 56 ms vs 14 ms, $p < 0.001$; On BB (49 ms vs 19 ms, $p = 0.047$). See also **Figure S2 in the Data Supplement**.

Similarly, an analysis conducted separately in patients with a baseline QTc lower than 470 ms and in those with a QTc higher than 470 ms also showed significant association for Recovery-Rest ΔQTc in both groups (**Table S5 in the Data Supplement**).

A sensitivity analysis of the Rest-Peak ΔHR confirmed that the association with cardiac events remained significant when stratifying the cohorts based on beta-blocker use at the time of EST (**Table S6 in the Data Supplement**).

In addition to baseline QTc, we also analysed the 4 minute recovery QTc for LQT1 and LQt2 in both the discovery and validation cohort, demonstrating similar associations to that of the baseline QTc (**Table S7 in the Data Supplement**).

Cardiac events after EST

The above analyses demonstrated the relationship between the Recovery-Rest ΔQTc and lifetime cardiac events, not distinguishing between events before or after the assessment of the EST. We thus performed an analysis, focussing specifically on events after EST evaluation until the date of last follow-up, to gauge the potential utility of incorporating Recovery-Rest ΔQTc into a prediction model for future risk.

The LQT1 and LQT2 discovery patient groups were combined to ensure adequate statistical power (N=448 patients; N=18 cardiac events). Mean follow up time was 5.1 years. Multivariable Cox regression analysis, accounting for all aforementioned covariates

demonstrated an association between Recovery-Rest ΔQTc and future cardiac events (**Table 3**). Specifically, The HR for a cardiac event post-EST was 1.025 per ms (95% CI: 1.010–1.041, Concordance=0.90). To prevent overfitting due to limited events, a Cox model with only Recovery-Rest ΔQTc showed an HR of 1.017 per ms (95% CI: 1.005–1.028). Similar findings were made in the LQT1 and LQT2 validation sets combined (N=330 patients; N=15 cardiac events), where the mean follow up time was 4.3 years. Recovery-Rest ΔQTc was associated with prospective occurrence of a cardiac event, both in a Cox regression model with and without inclusion of covariates, with hazard ratios similar to the discovery set (Covariate model: HR of 1.020, 95% CI: 1.003 – 1.038, Concordance=0.88).

The previously calculated Recovery-Rest ΔQTc cut-off for the optimal ROC in LQT1 and LQT2 was also significantly associated with future events (**Table 3**). Although both groups were analyzed together, each patient group retained its LQT type-specific cut-off values for the Cox regression analysis.

To better determine potential clinical applicability, we next restricted the analysis (combining LQT1 and LQT2 from the discovery and validation cohorts) to the family members of probands, patients (probands and relatives) that had never suffered from cardiac events before, patients with and without BB therapy at time of EST and patients with a QTc higher and lower than 470 ms (**Table S8 in the Data Supplement**). A significant association between Recovery-Rest ΔQTc and future cardiac events was observed in all these sub-categories.

Finally, we assessed the association between the previously calculated Recovery-Rest ΔQTc cut-off for the optimal ROC in LQT1 and LQT2, and occurrence of future cardiac events

(**Figure 3**). This demonstrated a significant association for future events in patients above the QTc cut-off compared to patients below (**Table 3**).

Discussion

We investigated the association between dynamic changes of the QTc and heart rate during exercise stress testing (EST) and the occurrence of cardiac events in patients with LQT1 and LQT2. We demonstrated that the change in QTc between rest and the 3-4th minute post-exercise recovery (Recovery-Rest ΔQTc) was independently associated with lifetime cardiac events in both LQT1 and LQT2 patients, a finding that was replicated in independent patient sets sourced from different clinical centres. Similarly, Recovery-Rest ΔQTc was associated with prospective cardiac events, despite management by clinical experts, occurring after EST assessment. This underscores its potential for clinical utility, particularly in the sub-categories that were assessed, as well as the potential use of a cut-off value for recommendations of therapy adjustment.

According to our findings, LQT1 and LQT2 patients have an increased relative risk of approximately 1-2% to develop cardiac events over time for every ms increase in Recovery-Rest ΔQTc. Previous research has demonstrated that repolarization markers on the baseline ECG, such as baseline QTc and T wave morphology, are closely linked to cardiac events, making them crucial for risk stratification.(5, 26) Our findings build upon this foundation, suggesting that dynamic QTc responses to exercise stress offer a window into the arrhythmic potential not visible under resting conditions.

Until now, the QTc at the 3-4th minute recovery period has been used exclusively for diagnostic purposes and is included in the LQTS diagnostic criteria (Schwartz score).(27) However, to date, no studies have focused on the actual change in QTc (ΔQTc) in LQTS patients as a predictor of risk for cardiac events, although one study demonstrated a longer QTc at 4 minutes post exercise in four symptomatic LQT1 patients compared to four asymptomatic ones.(16) In our study, a likelihood ratio test indicated that adding Recovery-Rest ΔQTc to both the logistic and Cox regression model with baseline QTc, improved the model significantly. This improvement in model performance can be applied to more accurately stratify patient risk, enabling targeted interventions and monitoring for individuals at higher risk.

An approach that incorporates novel markers of risk could potentially lead to a re-evaluation of current treatment paradigms, such as the indications for BB therapy (adjustment). For example, patients exhibiting marked ΔQTc changes during EST might benefit from more aggressive management strategies, even if their resting QTc does not indicate high risk (**Table S5**). Such a shift towards dynamic risk assessment could optimize treatment efficacy, minimize unnecessary interventions, and ultimately improve patient outcomes. Our focused sub-analysis, particularly among family members of probands, emphasizes the predictive value of Recovery-Rest ΔQTc, reinforcing its utility in risk stratification. This finding is particularly relevant for family screening and management in LQTS, where genetic predisposition plays a crucial role.

In our analysis of heart rate dynamics, we accounted for the presence or absence of BB usage among the patients. Our analysis revealed some associations, symptomatic patients in

the discovery cohort were more likely to be prescribed BB compared to asymptomatic patients (P<0.001). This was not observed in the validation cohort (P = 0.130). There were no consistent findings across the discovery and replication sets, with the exception of Peak-Rest ΔHR in LQT1. These findings suggest some nuanced interplay between autonomic regulation and arrhythmic risk. This is consistent with the broader literature, where Crotti et al. identified the autonomic nervous system's role in modulating arrhythmic risk in LQTS. In this study, they identified that a greater heart rate reduction in the first minute of recovery from exercise was able to stratify arrhythmia risk for LQT1 patients, independently of BB therapy.(15) Such insights into the autonomic underpinnings of LQTS provide a compelling rationale for incorporating heart rate dynamics into risk assessment models, alongside traditional marker.

Recent literature indicates that LQT1 patients may exhibit chronotropic insufficiency, highlighting the importance of adjusting for beta-blocker use in risk assessment.(28) Due to data availability, especially for patients that had not been seen in the last years, we were able to retrieve more exercise tests on BB usage in the discovery cohort compared to the validation cohort. To address this, a sub-analysis was conducted to assess the association between ΔQTc and cardiac events in ESTs conducted on and off BB, revealing a consistent effect in both groups.

Our findings reveal a clear link between the extent of QTc prolongation following exercise and an increased risk for cardiac events. Rare large-effect genetic variants that disrupt repolarizing potassium currents render the heart especially vulnerable to the effects of adrenergic activation. It is well-established that β-adrenergic receptor activation enhances

I_{Ks}, leading to action-potential shortening.(29) The impairment of I_{Ks} in patients with LQT1 undermines the effect of β-adrenergic stimulation, preventing proper shortening of repolarization with increasing heart rate. This leads to exaggerated regional dispersion of repolarization and premature ventricular beats which may precipitate torsade de pointes ventricular tachycardia.(30-32) We propose that compromised augmentation of I_{Ks}, and the resultant QTc prolongation, remains significant into the 3rd to 4th minute of recovery. Patients with a more pronounced QTc prolongation post-exercise may harbour either more severe mutations (associated with a greater loss of channel function) or a constellation of modifiers, including genetic modifiers that act to delay repolarization (33-36). Future studies should aim to correlate the extent of QTc prolongation in LQTS patients following exercise with ion channel functionality, or to identify distinct adrenergic response variations within these channels.

Prior research has shown that unlike I_{Ks}, the I_{Kr} current, which is compromised in LQT2 patients, predominates at rest. However, it experiences a moderate reduction during adrenergic stimulation.(37) Our results identify a significant association between the Recovery-Rest ΔQTc and cardiac events in LQT2 patients as well, suggesting that 3 to 4 minutes post-exercise, the I_{Kr} current fails to regain its dominance, resulting in QTc prolongation. Again, this observation could imply the presence of more severe mutations affecting ion channel functionality or the influence of other factors exacerbating this condition.

Limitations and Future Directions

Despite the strengths of our study, we acknowledge several limitations. The focus on LQT1 and LQT2 subtypes, while clinically relevant, leaves unanswered questions regarding the applicability of our findings to other LQTS subtypes. Additionally, the relatively low event rate observed in our cohort underscores the need for larger, multicentre studies to validate our findings and explore their applicability to broader LQTS populations.

The exercise tests were conducted using either a treadmill or a bicycle. While the majority of patients concluded the test due to exhaustion, there were instances where the test was stopped upon reaching the target heart rate.

The results for LQT2, although the association is statistically significant, are less compelling than for LQT1. A smaller delta QTc may be difficult for non-experts to detect, potentially resulting in difficulties in achieving reliable or accurate findings.

Moreover, it has been previously suggested that in children, later evaluation in recovery predicts better LQTS,(38) and it is known that children have a more gradual heart rate deceleration.(39) In our study we measured QTc at the 4th minute of recovery. Future studies, focussing on children may investigate the value of measuring Qtc in the 7th minute of recovery in this age group.

The integration of novel markers, such as genetic modifiers, serum biomarkers, and imaging parameters, could further refine risk stratification models. Longitudinal studies assessing the predictive value of dynamic EST parameters over time would also provide valuable insights into their role in guiding therapeutic interventions and monitoring disease progression, according to the concept of yearly therapeutic optimization.(40)

The implementation of dynamic QTc measurements in clinical practice will require standardized EST protocols and clinician training to ensure accurate and consistent

interpretation of results. Investigating the integration of these parameters into existing clinical workflows and guidelines will be an essential step forward. Moreover, with the expanding use of AI in healthcare,(41, 42) ΔQTc could be leveraged in AI-driven risk stratification models, providing a valuable tool for enhancing risk assessment and decision-making in the near future.

Conclusion

In summary, our study highlights for the first time the clinical utility of dynamic QTc during EST for potential risk stratification of lifetime and future cardiac events in LQT1 and LQT2. The validation of these findings in an external cohort strengthens the case for their integration into clinical practice. By moving beyond traditional static measurements to embrace the dynamic responses of the heart to exercise stress, we can achieve a more comprehensive and personalized approach to managing LQTS. As we advance towards this goal, continued research and collaboration across the field will be essential in translating these insights into improved clinical outcomes for patients with LQTS.

Funding

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Figure Legends

Figure 1. Flowchart of analyses performed in discovery and validation cohorts. Flowchart demonstrates which parameters were assessed for association with lifetime and future events.

Figure 2. Analyses in LQT1 and LQT2 discovery and validation cohorts uncovers an association of ΔQTc with lifetime cardiac events. A: Comparison of Recovery-Rest ΔQTc between asymptomatic and symptomatic LQT1 patients. B: Blue line = Receiver Operating Characteristic (ROC) curve from predicted survival at 30 year (model1) with baseline QTc + covariates as described in the methods. Red line = ROC curve model 1 with addition of Rest-Recover ΔQTc, increasing the area under the ROC curve (AUROC) from 80% to 85% for LQT1 patients. C: Calibration plot for LQT1 patients on the optimal ΔQTc cut-off value (35 ms) D: Comparison of Recovery-Rest ΔQTc in LQT2 patients. E: Blue line = ROC curve from model (1) with baseline QTc + covariates. Red line = ROC curve from model 1 with addition of Rest-Recover ΔQTc, increasing the AUROC from 75% to 88% in LQT2 patients. F: Calibration plot for LQT2 patients on the optimal ΔQTc cut-off value (16 ms).

Figure 3. Analysis of LQT1 and LQT2 patients combined in the Discovery and Validation cohorts uncovers and association with future cardiac events. This figure illustrates our findings demonstrating the relationship between ΔQTc and future (i.e. post EST) arrhythmic events for LQT1 and LQT2 patients together. A Kaplan Meier curve shows the difference in future cardiac events between patients classified as high and low Rest-Recover ΔQTc (LQT1 > 35 ms, LQT2 > 16 ms).

Table 1.Characteristics of the studied discovery and validation LQT1 and LQT2 patient sets. BB = beta blocker, EST = Exercise stress test, SD = standard deviation.

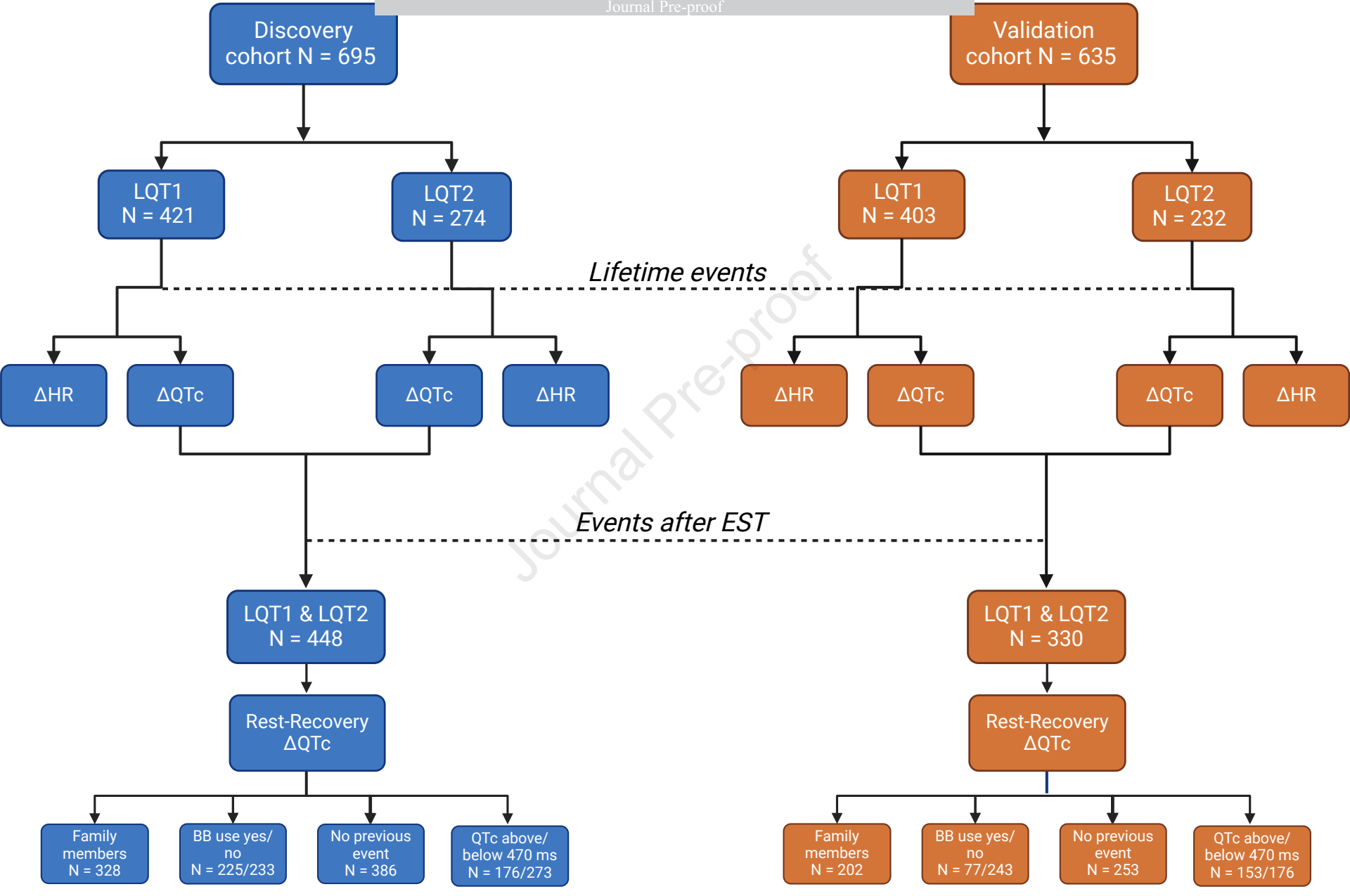
	LQT1			LQT2		
	Discovery (N = 421)	Validation (N = 403)	p-value	Discovery (N = 274)	Validation (N = 232)	p-value
Female, n (%)	253 (60)	270 (67)	0.204	147 (54)	145 (63)	0.039
Age during EST, years (mean ± SD)	30 ± 18	35 ± 17	0.002	29 ± 18	35 ± 18	0.565
QTc at baseline, ms (mean ± SD)	461 ± 40	458 ± 42	0.312	463 ± 43	464 ± 37	0.872
Heart rate at rest, bpm (mean ± SD)	73 ± 15	72 ± 14	0.423	76 ± 15	74 ± 15	0.817
BB use at EST n (%)	230 (57)	53 (13)	0.001	131 (48)	40 (17)	0.003
Lifetime cardiac events (%)	68 (16)	74 (18)	0.495	31 (11)	38 (16)	0.032

Table 2. Association analyses of Rest-Peak ΔQTc, Peak-Recovery ΔQTc and Rest-Recovery ΔQTc with occurrence of lifetime cardiac events in patients with LQT1 and LQT2. All QTc and ΔQTc values are expressed as milliseconds (ms). Odds ratio (OR) and Hazard ratio (HR) are per one ms increase. Direct comparison between symptomatic and asymptomatic. * $p < 0.05$

	Discovery Cohort				**Validation Cohort**			
	LQT1 patients							
	Peak-Rest ΔQTc	*Recovery-Peak ΔQTc*	*Recovery-Rest ΔQTc*	*Baseline QTc*	*Peak-Rest ΔQTc*	*Recovery-Peak*	*Rest-Recover ΔQTc*	*Baseline QTc*
Symptomatic vs asymptomatic	20 ± 55 vs 18 ± 38, p = 0.804	25 ± 55 vs 11 ± 38, p = 0.058	47 ± 39 vs 24 ± 35, **p < 0.001***	482 ± 45 vs 460 ± 38, **p < 0.001***	34 ± 45 vs 23 ± 47, p = 0.074	-3 ± 53 vs -4 ± 45, p = 0.852	47 ± 43 vs 34 ± 42, **p = 0.018***	472 ± 43 vs 455 ± 32, **p = 0.002***
Logistic regression	OR: 1.005 (95%CI: 0.996-1.014)	OR: 1.007 (95%CI: 0.998 – 1.016).	OR: 1.034 (**95%CI: 1.023 – 1.047**)*	OR: 1.016 (**95%CI: 1.010 - 1.023**)*	OR: 1.010 (**95%CI: 1.003-1.017**)*	OR: 1.001 (95%CI: 0.994 – 1.008).	OR: 1.010 (**95%CI: 1.002 – 1.018**)*	OR: 1.011 (**95%CI: 1.002 - 1.020**)*
Survival analysis	HR: 1.005, 95%CI: 0.998 – 1.013)	HR: 1.005, 95%CI: 0.999 – 1.011)	HR: 1.022 (**95%CI: 1.015 – 1.030**)*	HR: 1.006 (**95%CI: 1.001 – 1.011**)*	HR: 1.007 (**95%CI: 1.002 – 1.013**)*	HR: 0.998 (95%CI: 0.994 – 1.005)	HR: 1.009 (**95%CI: 1.003 – 1.015**)*	HR: 1.015 (**95%CI: 1.007 – 1.023**)*
	LQT2 patients							
	Peak-Rest ΔQTc	*Recovery-Peak ΔQTc*	*Recovery-Rest ΔQTc*	*Baseline QTc*	*Peak-Rest ΔQTc*	*Recovery-Peak*	*Rest-Recover ΔQTc*	*Baseline QTc*
Symptomatic vs asymptomatic	-13 ± 45 vs -12 ± 38, p = 0.933	39 ± 46 vs 20 ± 37, p = 0.082	43 ± 39 vs 6 ± 32, **p < 0.001***	494 ± 55 vs 460 ± 39, **p = 0.002***	-13 ± 50 vs -20 ± 51, p = 0.461	1 ± 58 vs 5 ± 55, p = 0.734	10 ± 34 vs -7 ± 47, **p = 0.008***	497 ± 52 vs 461 ± 35, **p < 0.001***
Logistic regression	OR: 1.018 (**95%CI: 1.001 – 1.038**)*	OR: 0.998 (95%CI: 0.983 – 1.013).	OR: 1.040 (**95%CI: 1.023 – 1.059**)*	OR: 1.017 (**95%CI: 1.004 - 1.030**)*	OR: 1.004 (95%CI: 0.948-1.014)	OR: 0.997 (95%CI: 0.988 – 1.005).	OR: 1.009 (95%CI: 0.998 – 1.021)	OR: 1.022 (**95%CI: 1.010 - 1.035**)*
Survival analysis	HR: 1.017 (**95%CI: 1.000 - 1.035**)*	HR: 0.987 (95%CI: 0.974 – 0.9996)	HR: 1.011 (**95%CI: 1.001– 1.024**)*	HR: 1.011 (**95%CI: 1.001 – 1.021**)*	HR: 1.003, (95%CI: 0.996 – 1.009)	HR: 0.998 (95%CI: 0.994 – 1.006)	HR: 1.008 (**95%CI: 1.000 – 1.016**)*	HR: 1.017 (**95%CI: 1.010 – 1.025**)*

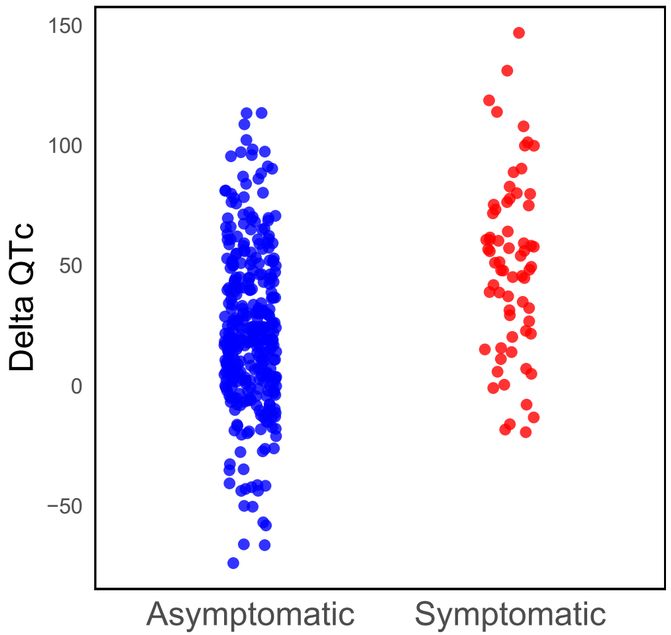
Table 3. Association of Rest-Recovery ΔQTc with occurrence of future events. HRs shown for cox regression models testing Rest-Recovery ΔQTc as single predictor as well as with other covariates (multivariable model) as described in the methods. HRs are per one ms increase, or above vs below the ΔQTc cut-off previously calculated (LQT1 = 35 ms, LQT2 = 16ms). N = number of patients, followed by number of events in that specific sub-population. * p < 0.05

	Discovery cohort		**Validation cohort**		**Discovery & Validation together**	
All patients (LQT1 & LQT2)	*Single predictor*	*Multivariable model*	*Single predictor*	*Multivariable model*	*Single predictor*	*Multivariable model*
Per 1 ms increase	1.017 (**95%CI: 1.005 – 1.028**)*	1.025 (**95%CI: 1.010 – 1.041)***	1.010 (**95%CI: 1.000 – 1.022**)*	1.020 (**95%CI: 1.003 – 1.038**)*	1.014 (95% CI: **1.005 – 1.022**)*	1.021 (95% CI: **1.010 – 1.032**)*
	N=448, 18 events		N=330, 15 events		N=778, 33 events	
Above vs below optimal ΔQTc cut-off	4.11 (**95%CI: 1.46 – 11.52**)*	5.47 (**95%CI: 1.69 – 17.69)***	4.21 **(95%CI: 1.18 – 15.00)***	4.45 **(95%CI: 1.13 – 17.81)***	3.47 **(95%CI: 1.61 – 7.47***	3.13 **(95%CI: 1.36 – 7.23***
	N=448, 18 events		N=330, 15 events		N=778, 33 events	



LQT1

A



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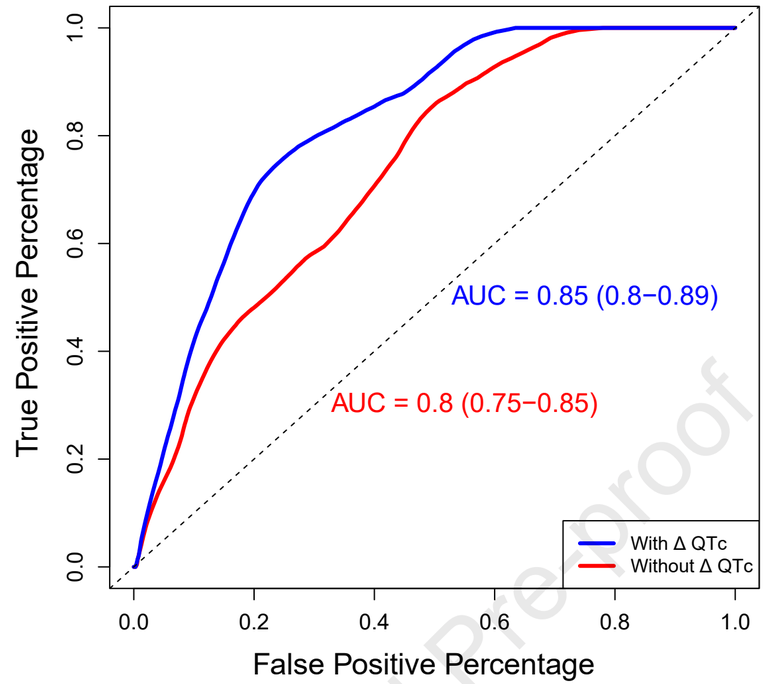

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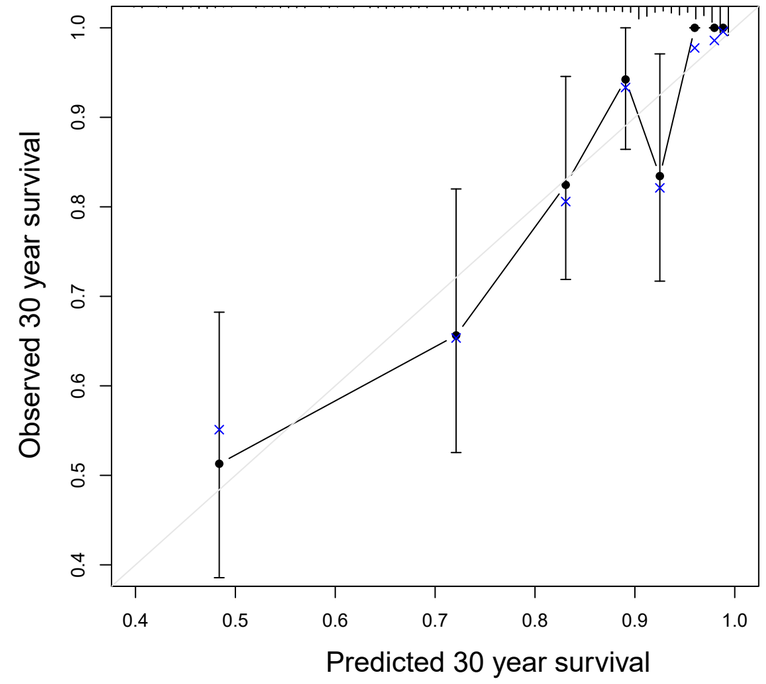

LQT2

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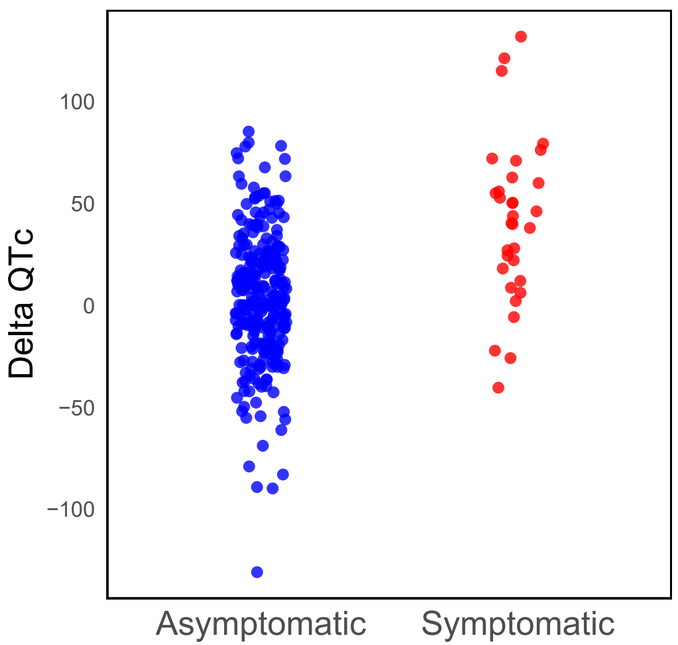

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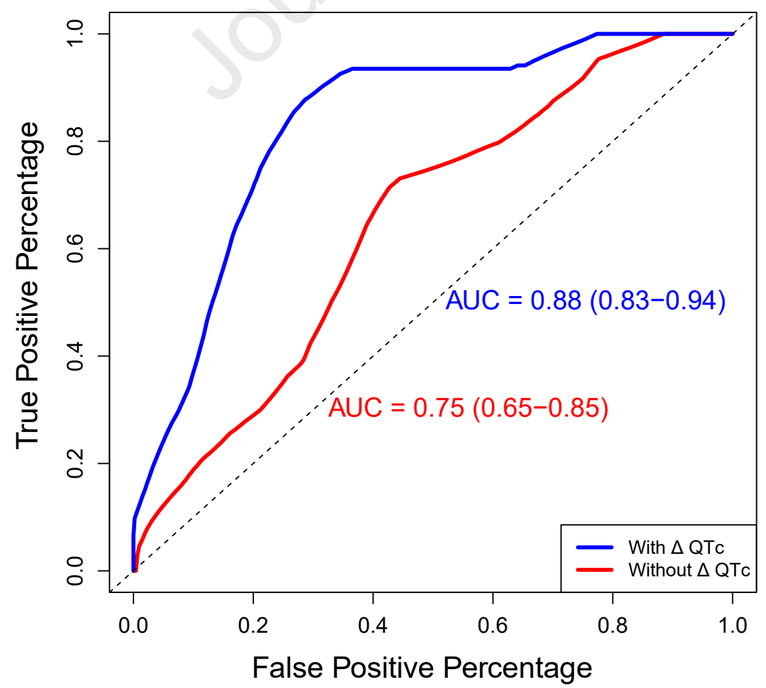

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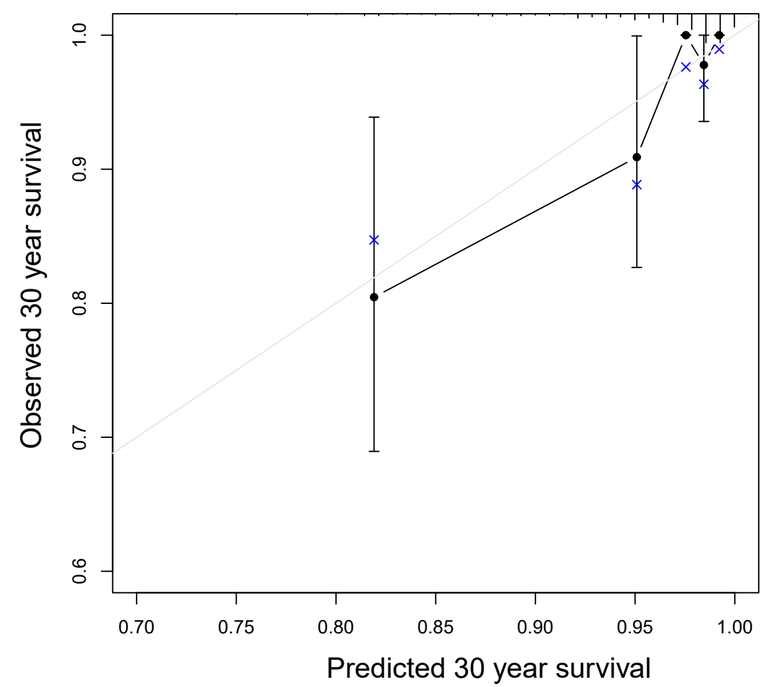

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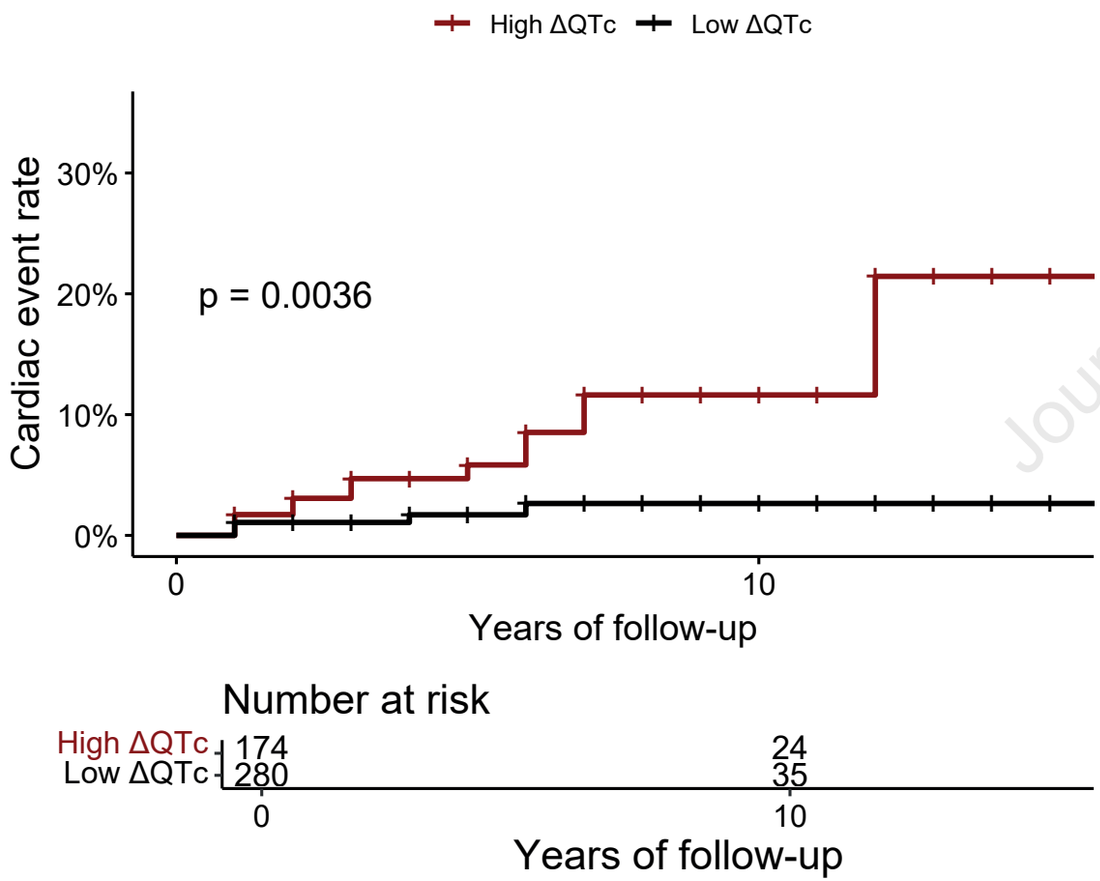

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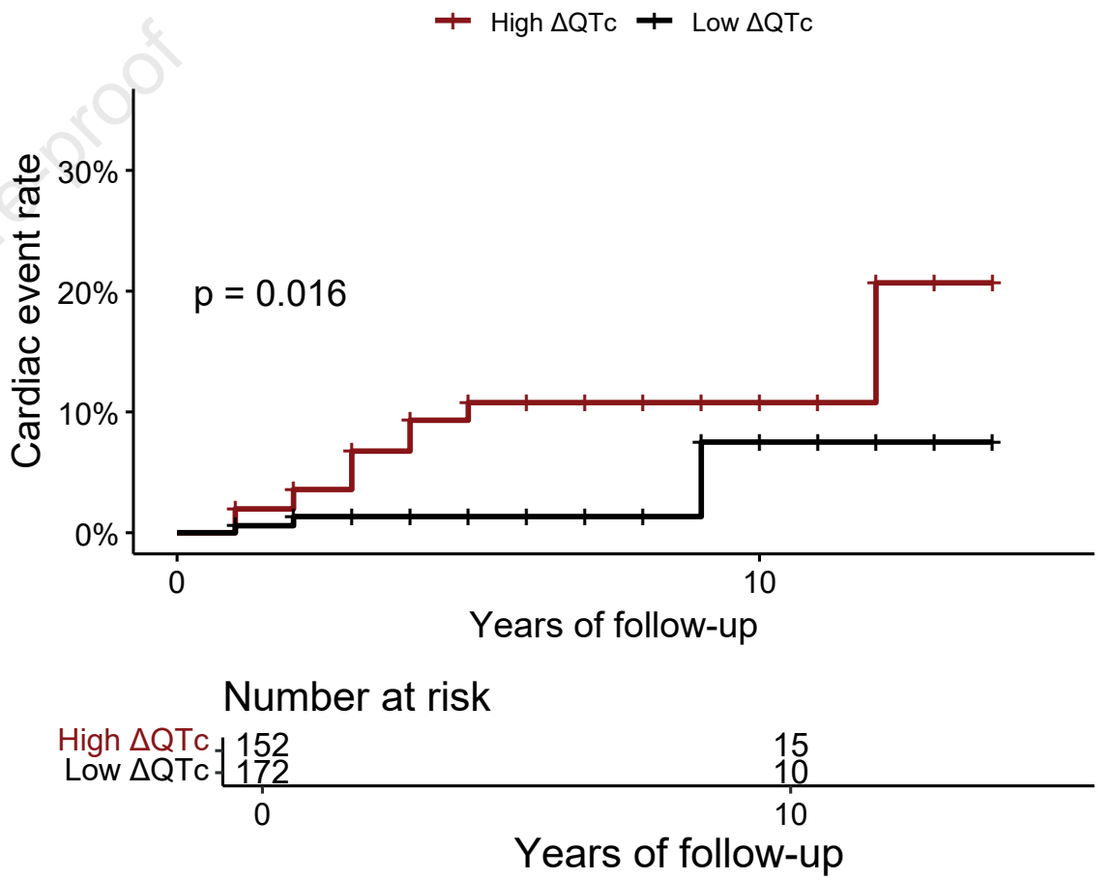

Supplementary data

Table S1. Clinical definitions of cardiac events.

Cardiac Event	Definition
Arrhythmic syncope	Transient loss of consciousness and postural tone with spontaneous recovery with arrhythmic mechanism likely at diagnosis. This thus excludes syncope of presumed vaso-vagal etiology.
Sustained ventricular tachycardia (susVT)	Ventricular tachycardia lasting ≥ 30 sec or with hemodynamic compromise at ≥ 100 beats per minute (bpm).
Aborted sudden cardiac arrest	A hemodynamic collapse that is reversed by cardiopulmonary resuscitation and/or defibrillation.
Sudden cardiac death	Death of cardiac origin that occurred unexpectedly within 1 hour of the onset of new symptoms or a death that was unwitnessed and unexpected within 24 hours of last seen alive.

Table S2. Classification of genetic variants.

Category	LQT1 (K_V7.1)	Category	LQT2 (K_V11.1)
1	Non-missense variants (i.e., splice sites, in-frame insertions, in-frame deletions, stop codons, and frameshift).	1	Non-missense variants (i.e., splice sites, in-frame insertions, in-frame deletions, stop codons, and frameshift).
2	Missense variants leading to amino acid substitution in the N-terminus region, defined as amino acid residues from 1 to 122.	2	Missense variants leading to amino acid substitution in the N-terminus, defined as amino acid residues from 1 to 403.
3	Missense variants leading to amino acid substitution in all transmembrane segments other than S6, including the extracellular loops (S1–S5, residues: 122–168, 197–248, and 262–327).	3	Missense variants leading to amino acid substitution in the transmembrane region (S1-S4), defined as amino acid residues 404 to 547.
4	Missense variants leading to amino acid substitution in the cytoplasmic loop (C-loop) (S2–S3 C-loop, residues: 169–196 and S4–S5 C-loop, residues: 249–261).	4	Missense variants leading to amino acid substitution in the transmembrane S5-loop-S6 region, defined as amino acid residues 548 to 659.
5	Specific variant p.Ala341Val (S6, residue: 341).	5	Missense variants leading to amino acid substitution in the C-terminus region, defined as amino acid residues 660 to 1159.
6	Missense variants leading to amino acid substitution neighbouring p.Ala341, i.e. in the S6 segment: 328–348 (other than p.A341V).		
7	Missense variants leading to amino acid substitution in the C-terminus, defined as amino acid residues 349 to 676.		

Table S3. Association analyses for ΔHR in LQTS patients with occurrence of lifetime cardiac events. All ΔHR values are expressed as beats per minute (bpm). Odds ratio (OR) and Hazard ratio (HR) are per one bpm increase. Direct comparison between symptomatic and asymptomatic. * $p < 0.05$

	Discovery Cohort			**Validation Cohort**		
	LQT1 patients					
	Peak-Rest ΔHR	*Recovery-Peak ΔHR*	*Recovery-Rest ΔHR*	*Peak-Rest ΔHR*	*Recovery-Peak ΔHR*	*Rest-Recover ΔHR*
Symptomatic vs asymptomatic	59 ± 25 vs 67 ± 18, **p = 0.008***	-48 ± 17 vs -50 ± 15, p = 0.221	16 ± 14 vs 16 ± 13, p = 0.681	61 ± 21 vs 71 ± 19, **p = 0.001***	-41 ± 13 vs -45 ± 12, **p = 0.046***	20 ± 15 vs 26 ± 16, **p = 0.005***
Logistic regression	OR: 0.984 (**95% CI: 0.970- 0.999**)*	OR: 1.011 (95%CI: 0.989 – 1.034).	OR: 1.008 (95%CI: 0.983 – 1.033)	OR: 0.963 (**95%CI: 0.944- 0.981**)*	OR: 1.037 (**95%CI: 1.011 – 1.065**)*	OR: 0.966 (**95%CI: 0.942 – 0.989**)*
Survival analysis	HR: 0.989, 95%CI: 0.977 – 1.001)	HR: 1.009 (95%CI: 0.992 – 1.027)	HR: 1.008 (95%: 0.988 – 1.029 CI)	HR: 0.976 (**95%CI: 0.963 – 0.990**)*	Hazard: 1.029 (**95%CI: 1.008 – 1.051**)*	HR: 0.980 (**95%CI: 0.962 – 0.999**)*
	LQT2 patients					
	Peak-Rest ΔHR	*Recovery-Peak ΔHR*	*Recovery-Rest ΔHR*	*Peak-Rest ΔHR*	*Recovery-Peak ΔHR*	*Rest-Recover ΔHR*
Symptomatic vs asymptomatic	74 ± 25 vs 77 ± 21 , p = 0.501	-51 ± 16 vs -58 ± 17, p = 0.081	30 ± 17 vs 20 ± 15, **p = 0.045***	72 ± 27 vs 76 ± 24 , p = 0.444	-45 ± 18 vs -50 ± 15, p = 0.167	27 ± 16 vs 26 ± 17, p = 0.719
Logistic regression	OR: 1.012 (95% CI: 0.989- 1.036)	OR: 1.015 (95%CI: 0.979 – 1.051).	OR: 1.076 (**95%CI: 1.033 – 1.126**)*	OR: 0.980 (95%CI: 0.958- 1.002)	OR: 1.041 (**95%CI: 1.008 – 1.079**)*	OR: 0.999 (95%CI: 0.967 – 1.030)
Survival analysis	HR: 1.019, 95%CI: 0.993 – 1.046)	HR: 1.004 (95%CI: 0.967 – 1.042)	HR: 1.040 (**95%CI: 1.000 – 1.081**)*	HR: 0.990 (95%CI: 0.974 – 1.009)	HR: 1.012 (95%CI: 0.987 – 1.037)	HR: 0.997 (95%CI: 0.976 – 1.018)

Table S4. Association of Recovery-Rest ΔQTc "optimal cut-off" with occurrence of lifetime events. HRs shown for cox regression models testing Recovery-Rest ΔQTc as single predictor as well as with other covariates (multivariable model) as described in the methods. HRs are above and below the optimal ΔQTc cut-off previously calculated (LQT1 = 35 ms, LQT2 = 16ms). N = number of patients, followed by number of events in that specific sub-population. * $p < 0.05$

	Discovery cohort		**Validation cohort**	
	Single predictor	*Multivariable model*	*Single predictor*	*Multivariable model*
LQT1 and LQT2	3.98 (**95%CI: 2.60 – 6.09**)*	4.69 (**95%CI: 2.94 – 7.50)***	1.88 **(95%CI: 1.28 – 2.75)***	1.88 **(95%CI: 1.25 – 2.83)***
	N=695, 99 events		N=635, 112 events	

Table S5. Cox regression for lifetime events distinguishing patients with BB therapy at time of ETT and those without, and distinguishing those with baseline QTc < 470 ms from those > 470ms. Analyses were conducted in patients from the discovery and validation cohorts combined (LQT1 and LQT2 separately). HRs are per one ms increase. BB = beta blocker. N = number of patients, followed by number of events in that specific sub-population. * p < 0.05

	Single predictor	*Multivariable model*
	LQT1 patients (Discovery and Validation combined)	
On BB at EST	1.013 (**95%CI: 1.007 – 1.019**)*	1.018 (**95%CI: 1.011 – 1.025**)*
	N=296, 76 events	
No BB at EST	1.011 (**95%CI: 1.005 – 1.017**)*	1.012 (**95%CI: 1.005 – 1.018)***
	N=528, 63 events	
Baseline QTc < 470 ms	1.013 (**95%CI: 1.007 – 1.019**)*	1.017 (**95%CI: 1.011 – 1.023**)*
	N=514, 71 events	
Baseline QTc > 470 ms	1.009 (**95% CI: 1.003 – 1.015**)*	1.010 (**95% CI: 1.003 – 1.018**)*
	N=310, 68 events	
	LQT2 patients (Discovery and Validation)	
On BB at EST	1.010 (**95%CI: 1.001 – 1.019**)*	1.003 (95%CI: 0.994 – 1.013)
	N=174, 33 events	
No BB at EST	1.016 (**95%CI: 1.008 – 1.023**)*	1.014 (**95%CI: 1.007 – 1.023**)*
	N=332, 36 events	
Baseline QTc < 470 ms	1.020 (**95%CI: 1.008 – 1.033**)*	1.024 (**95%CI: 1.010 – 1.037**)*
	N=287, 20 events	
Baseline QTc > 470 ms	1.010 (**95%CI: 1.004 – 1.016**)*	1.007 (**95%CI: 1.000 – 1.014**)*
	N=219, 49 events	

Table S6. Cox regression for lifetime events Rest-Peak ΔHR, distinguishing patients with BB therapy at time of ETT and those without. Analyses were conducted in patients from the discovery and validation cohorts combined (LQT1). HRs are per one ms increase. BB = beta blocker. N = number of patients, followed by number of events in that specific sub-population. * $p < 0.05$

Rest-Peak ΔHR	*Single predictor*	*Multivariable model*
	LQT1 patients (Discovery and Validation combined)	
On BB at EST	0.984 (**95%CI: 0.976 – 0.993**)*	0.995 (95%CI: 0.984 – 1.006)
	N=296, 88 events	
No BB at EST	0.990 (**95%CI: 0.980– 0.999**)*	0.980 (**95%CI: 0.969 – 0.990**)*
	N=528, 95 events	

Table S7. Association analyses of QTc at the 4th minute recovery with occurrence of lifetime cardiac events in patients with LQT1 and LQT2. All QTc values are expressed as milliseconds (ms). Odds ratio (OR) and Hazard ratio (HR) are per one ms increase. Direct comparison between symptomatic and asymptomatic. * $p < 0.05$

	Discovery	Validation
LQT1		
Symptomatic vs asymptomatic	504 ± 49 vs 473 ± 39, **p < 0.001***	514 ± 47 vs 494 ± 43, **p = 0.001***
Logistic Regression	OR: 1.017 **(95%CI: 1.010 - 1.027)***	OR: 1.008 **(95%CI: 1.001 - 1.015)***
Survival analysis	HR: 1.016 **(95%CI: 1.009 – 1.023)***	HR: 1.009 **(95%CI: 1.003 – 1.016)***
LQT2		
Symptomatic vs asymptomatic	498 ± 42 vs 455 ± 39, **p < 0.001***	490 ± 42 vs 463 ± 45, **p < 0.001***
Logistic regression	OR: 1.020 **(95%CI: 1.008 - 1.035)***	OR: 1.012 **(95%CI: 1.002 - 1.024)***
Survival analysis	HR: 1.011 **(95%CI: 1.002 – 1.024)***	HR: 1.009 (95%CI: 0.999 – 1.018)

Table S8. Association analysis of Recovery-Rest ΔQTc with future events in specific patient sub-groups. HRs shown for different cox regression models, as for only Recovery-Rest ΔQTc (single predictor) as well as with other covariates as described in the methods (multivariable model). All ΔQTc values are expressed as milliseconds (ms). HRs are per one ms increase. BB = beta blocker. N = amount of patients, followed by amount of events in that specific sub-population. * $p < 0.05$

	LQT1 and LQT2, Discovery and Validation combined	
	Single predictor	*Multivariable model*
All patients	1.014 (95% CI: **1.005 – 1.022**)*	1.021 (95% CI: **1.010 – 1.032**)*
	N=778, 33 events	
Only family members	1.018 (95 % CI**: 1.006 – 1.030**)*	1.032 (95% CI**: 1.013 – 1.052**)*
	N=530, 16 events	
No BB at EST	1.020 (95% CI**: 1.006 – 1.035**)*	1.052 (95% CI**: 1.022 – 1.083**)*
	N=476, 13 events	
On BB at EST	1.009 (95% CI: 0.999 – 1.020)	1.014 (95% CI: **1.000 – 1.030**)*
	N=302, 20 events	
No previous event	1.010 (95% CI: 0.995 – 1.026)	1.025 (95% CI**: 1.003 – 1.047**)*
	N=639, 11 events	
QTc < 470 ms	1.024 (95% CI: **1.009 – 1.040**)*	1.052 (95% CI**: 1.019 – 1.085**)*
	N=449, 11 events	
QTc > 470	1.008 (95% CI: 0.998 – 1.019)	1.014 (95% CI**: 1.002 – 1.027**)*
	N=329, 22 events	

Figure S1. A: Example of a clear vs unclear ECG pattern for proper QT measurement of an Exercise Tolerance Test. B: Example of a large delta between recovery and rest QTc. C: Example of a small (negative) delta between recovery and rest QTc.

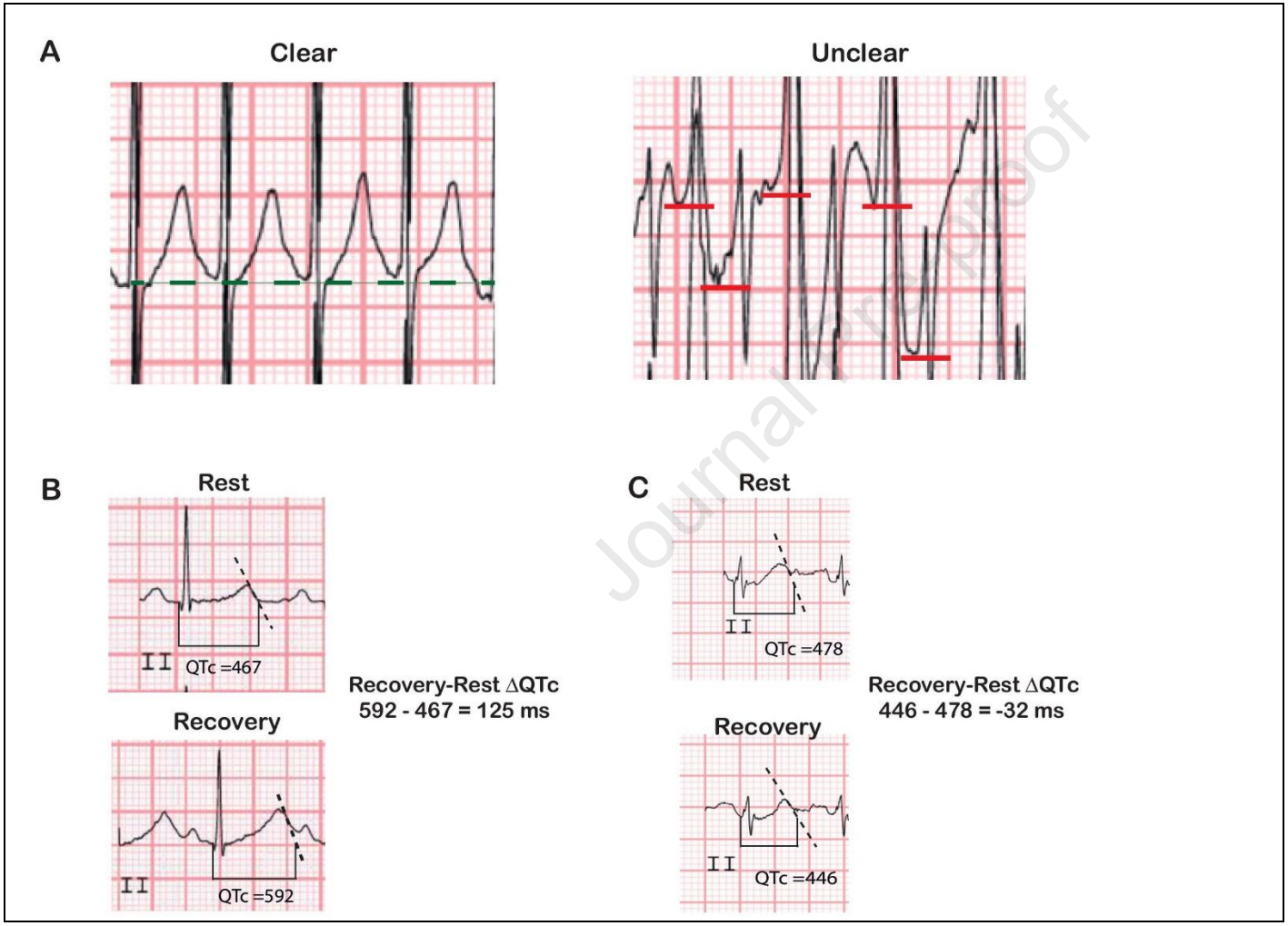

Figure S2. Sensitivity analysis conducted in patients for whom an EST on as well as off beta-blocker therapy was available. Left panel: Comparison of Recovery-Rest ΔQTc between symptomatic and asymptomatic LQTS patients with the EST conducted in the absence of BB therapy. Right panel: Comparison of Recovery-Rest ΔQTc between the same LQTS patients with the EST conducted in the presence of BB therapy. The Recovery-Rest ΔQTc was higher in symptomatic patients in both analyses. EST = exercise stress test, BB = beta blocker, N = amount of patients.