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ORIGINAL RESEARCH

Diagnostic Accuracy of the Standing Test in Adults Suspected for Congenital Long-QT Syndrome

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BACKGROUND: An elegant bedside provocation test has been shown to aid the diagnosis of long-QT syndrome (LQTS) in a retrospective cohort by evaluation of QT intervals and T-wave morphology changes resulting from the brief tachycardia provoked by standing. We aimed to prospectively determine the potential diagnostic value of the standing test for LQTS.

METHODS AND RESULTS: In adults suspected for LQTS who had a standing test, the QT interval was assessed manually and automated. In addition, T-wave morphology changes were determined. A total of 167 controls and 131 genetically confirmed patients with LQTS were included. A prolonged heart rate–corrected QT interval (QTc) (men ≥430 ms, women ≥450 ms) at baseline before standing yielded a sensitivity of 61% (95% CI, 47–74) in men and 54% (95% CI, 42–66) in women, with a specificity of 90% (95% CI, 80–96) and 89% (95% CI, 81–95), respectively. In both men and women, QTc≥460 ms after standing increased sensitivity (89% [95% CI, 83–94]) but decreased specificity (49% [95% CI, 41–57]). Sensitivity further increased (P<0.01) when a prolonged baseline QTc was accompanied by a QTc≥460 ms after standing in both men (93% [95% CI, 84–98]) and women (90% [95% CI, 81–96]). However, the area under the curve did not improve. T-wave abnormalities after standing did not further increase the sensitivity or the area under the curve significantly.

CONCLUSIONS: Despite earlier retrospective studies, a baseline ECG and the standing test in a prospective evaluation displayed a different diagnostic profile for congenital LQTS but no unequivocal synergism or advantage. This suggests that there is markedly reduced penetrance and incomplete expression in genetically confirmed LQTS with retention of repolarization reserve in response to the brief tachycardia provoked by standing.

Key Words: ECG ■ LQTS ■ QTc ■ QT interval

Congenital long QT-syndrome (LQTS) is an inherited cardiac arrhythmia disorder that can be lethal attributable to malignant ventricular arrhythmias. Treatments such as lifestyle advice, beta-blocker therapy, and invasive therapy (eg, left cardiac sympathetic denervation and implantable defibrillators) are effective in preventing cardiac events and mortality.[1] Given the potentially lethal consequences of LQTS and its treatability, an early and reliable diagnosis is crucial.

Prolongation of the QT interval corrected for heart rate (QTc) on a 12-lead resting ECG[2] is the cornerstone for an LQTS diagnosis but meets clinical challenges. A considerable overlap in QTc exists between patients with LQTS and healthy individuals[3] because a significant number of patients with LQTS do not always show a prolonged QTc.[4] This feature in Mendelian disorders such as LQTS is known as reduced penetrance and incomplete expression. Besides a prolonged QTc, an

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CLINICAL PERSPECTIVE

What Is New?

- Despite an additional value of the standing test for long-QT syndrome (LQTS) diagnosis in earlier retrospective studies, our prospective evaluation in 167 controls and 131 patients with LQTS displayed a different diagnostic profile compared with a baseline ECG but no unequivocal synergism or advantage.
- This finding mirrors the concept that there is markedly reduced penetrance and incomplete expression in genetically confirmed LQTS and that repolarization reserve in response to the brief tachycardia provoked by standing can be variably preserved in genotype-positive LQTS.

What Are the Clinical Implications?

- Diagnosing LQTS thus remains a challenge and requires an extensive and detailed history-taking that emphasizes specific triggers and symptoms together with meticulous and repeated ECG evaluations in individuals under evaluation and his/her family members.
- The standing test could be of additional value, especially when including beat-to-beat dynamics.
- We advocate the use of the standing test in expert centers to be able to gain more insights before more widespread use.

Nonstandard Abbreviations and Acronyms

LQT-2	long-QT syndrome type-2
LQTS	long-QT syndrome

LQTS diagnosis can also be made in the presence of LQTS-associated clinical and other electrocardiographic features or in the presence of a pathogenic genetic variant.[2] However, these elements in LQTS diagnoses are also hampered by clinical challenges. The interpretation of symptoms as either benign or malignant can be difficult,[5,6] and distinguishing pathogenic variants from innocuous rare variants can be complex.[7] In addition, in ≈11% to 25% of the clinically diagnosed patients with LQTS, no pathogenic variant is found in one of the known pathogenic LQTS genes.[8,9]

Because diagnosing LQTS remains challenging, additional tests to enhance diagnostic capacity have been developed, such as QTc adaption to epinephrine infusion[10,11] and QTc measurements during the recovery phase of exercise.[12,13] In addition, an elegant bedside "standing test" was developed that exploits the sudden heart rate acceleration produced by standing to reveal insufficient QT-interval shortening in patients with LQTS.[14] Furthermore, in patients with LQTS, QTc-prolongation often remains present after the heart rate returned to baseline values,[15] and abnormal T-waves can be observed after standing, both with added value for diagnosing LQTS.[16]

This standing test was developed in a retrospective case-control cohort, including data of our own, and was further detailed in cumulative larger cohorts.[14,15] As the pretest probability and performance of any test is influenced by the prevalence of the disease tested for, here we aimed to prospectively evaluate the diagnostic value of the standing test for an LQTS diagnosis in adults. Particularly, we evaluated all parameters derived from the previous studies[14–16] to establish the most coherent prospective evaluation of the standing test in patients with a suspicion of LQTS.

METHODS

Study Design, Setting, and Population

All individuals aged >18 years who received a standing test between December 2008 until September 2018 in our tertiary referral cardiogenetic and cardiology clinic, as part of regular care, were included. These standing tests were performed because of (I) family screening in case of familial-LQTS or sudden cardiac death in the family, or (II) because of symptoms often in combination with a prolonged or high-normal QTc.

The study was approved by the Academic Medical Center Review Board, and informed consent of the individuals was waived as this study used data from regular care. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Data Collection and Measurements

ECGs and Additional Data

The standing test was performed as described previously[14–16]: individuals rested supine for several minutes before a continuous 5-minute ECG-recording was started in which patients remained supine for 2 minutes and were then asked to stand up and stay standing for the remaining minutes. As the standing test was part of the prospective initial evaluation of an individual, aside from a standard ECG, medical history, etc, many individuals did not receive a final LQTS diagnosis. Standing-test analyses were performed blinded to the final classification of the patients. For the main analyses, only healthy individuals after evaluation or individuals who, after genetic testing, appeared to be genotype-negative family members of patients with genotype-positive LQTS were included as controls. Confirmed congenital patients with LQTS (pathogenic variant in KCNQ1, KCNH2, or SCN5A according to the

American College of Medical Genetics and Genomic criteria[17]) were included as cases. Other diagnoses were excluded from the main analyses, and included *possible LQTS* (prolonged QTc or positive epinephrine/isoprenaline test without a confirmed pathogenic variant or in family members of patients with genotype-elusive LQTS or in the presence of a suspected family history for LQTS) and o*ther patients* (eg, idiopathic ventricular fibrillation, cardiomyopathies, polymorphisms [ie, variant of uncertain significance], Brugada syndrome, acquired-LQTS, Andersen-Tawil syndrome).

Manual Measurements

All standing test ECGs, be it on paper or digitally recorded, were manually analyzed as described in previous studies,[14–16] while the investigators were blinded for the final diagnosis. RR interval and QT interval were determined at 4 different time instances: (I) at *baseline*; in supine position before standing, in which the RR interval corresponds with the longest RR interval after standing, (II) at *maximal tachycardia*; at maximal sinus rate after standing up, (III) at *maximal QT stretching*; after standing up, in which the end of the T-wave gets nearest to the next P-wave, and (IV) at *return to baseline*; at slowest sinus rate while standing. To find matching RR intervals in supine and standing position, the longest RR interval within 30 s *after* standing was chosen for the "return to baseline" measurements, and a corresponding RR interval (±40 ms) in supine position was used for the baseline measurements. This procedure avoids excluding tests where the heart rate after standing did not return to the initially selected baseline conditions and was a slight deviation of protocol compared with previous studies.[14,15]

At all stages, the QT interval was measured in 1 lead, preferably lead II or V5, using the tangent method and corrected for heart rate using Bazett formula.[18] In addition, the T-wave morphology was assessed at all stages in 4 lead groups: (I) *inferior leads* (ie, II, III, and aVF); (II) *right-precordial leads* (ie, V1–V3); (III) *left-precordial leads* (ie, V4–V6); and (IV) *lateral leads* (ie, I and aVL) and classified as described previously[16] (Figure S1).

Automated Measurements

To study the dynamic response of the QT interval to the abrupt change in heart rate in more detail, all digitally available standing tests were analyzed beat-to-beat using custom-made software in MATLAB (2018a, Mathworks, Natick, MA). We used our previously described QT-interval algorithm modified for use with a single ECG lead (ie, lead II or V5) to mirror the manual measurements.[22] To compare automated measurements to manual measurements, single complexes were chosen based on the same definitions as the manual measurements: (I) baseline, (II) maximal tachycardia, (III) maximal QT stretching, and (IV) return to baseline. To analyze the complete dynamic behavior of the standing test, a moving average filter with a 15-s window and 5-s overlap was applied to all individuals' beat-to-beat QT and RR intervals individually. Thereafter, the overall dynamic behavior for every subgroup was calculated as the median (as well as the first and third quartiles) of the moving average filtered beat-to-beat intervals.

Statistical Analysis

All data were analyzed with R version 3.4.3 (The Foundation for Statistical Computing, Vienna, Austria). For the main analyses only patients with LQTS and controls were included. For the analyses on interreader, intrareader, and intermethod reliability, all individuals with a standing test were included.

Baseline and ECG characteristics are presented as numbers (percentage, %) for categorical variables and mean (±SD) or median (interquartile ranges) for continuous variables, stratified by group. Differences between groups were tested using a χ^2-test for categorical variables, and a *t* test or Mann–Whitney *U* test for continuous variables as appropriate. A Bonferroni adjustment was performed to correct for multiple testing.

To test the *diagnostic value* of the standing test for diagnosing LQTS, receiver-operating characteristic curve analyses were used to calculate the area under the curve (AUC) and to evaluate the specificity at a predefined sensitivity of 90% (ie, similar to earlier studies).[14,15] No substantial departures from the assumption of linearity on the log-odds scale were observed. DeLong method[23] was used to calculate the 95% CI around the AUC and to compare receiver-operating characteristic curves. Stratification by sex was performed using the 95th percentile of a previous large control cohort, resulting in a baseline QTc cut-off value of 430 ms in men and 450 ms in women.[3] Logistic regression analyses were used to analyze single dichotomous QTc predictors and to determine whether T-wave morphology changes added to the diagnostic value by establishing odds ratios (diagnostic OR). A given grouping of predictor variables were handled as separate independent dichotomized variables in the model. Potential outliers and influential points were analyzed using Cook distances.

Reliability of Measurements

To determine interreader and intrareader measurement reliability for continuous variables, a random sample of 10% was measured by an additional reader (B.H.) and remeasured by the same reader. Interreader, intrareader, and intermethod reliability were expressed

as the intraclass correlation coefficient for single measurements for continuous variables based on a 2-way agreement (interreader reliability) and consistency (intrareader reliability) model according to Cicchetti[19] and Fleiss.[20] Bland–Altman analyses[21] were then performed to assess bias and 95% limits of agreement.

Interreader and intrareader reliability for T-wave morphology was expressed as Cohen kappa statistic from a random sample of 10% that was measured by an additional reader (B.H.) and remeasured by that same reader.

Sensitivity analyses were performed by (I) excluding all individuals using beta-blocker therapy, (II) excluding all patients with LQTS with obvious QTc-prolongation above the 99th percentile of a previous large control cohort (>450 ms in men and >460 ms in women)[3] at baseline, (III) including only individuals with a pretest probability for LQTS of 50% (eg, family screening in case of familial-LQTS), and (IV) associations between family members. In the latter sensitivity analyses, we used a generalized estimating equations model to account for clustering using robust standard errors.

Sampling uncertainty was quantified with 95% CI and a level of significance of 0.05.

RESULTS

In total, 361 individuals were evaluated (Table S1). Baseline characteristics of the included 167 controls and 131 patients with LQTS (n=71 LQTS type-1, n=48 LQTS type-2 [LQT-2], n=12 LQTS type-3) are shown in Table 1. Both groups were of similar age and showed no statistical difference as to sex (P=0.171), or beta-blocker therapy (P=0.500). Beta blockers were prescribed for non-LQTS indications such as hypertension, hyperthyroidism, migraine, and atrial fibrillation. Four patients with LQTS had an out-of-hospital cardiac arrest or aborted cardiac arrest before diagnosis and received an implantable cardioverter-defibrillator and beta-blocker treatment for that indication.

As expected, the reason for genetic testing (P<0.001) and symptoms at presentation (P=0.003) differed between controls and patients with LQTS.

Table 1. Baseline Characteristics and Manual ECG Measurements

	Controls	LQTS	
	n=167	n=131	*P* value
Age, y	44(33–54)	41(29–50)	0.024
Women	106(63%)	72(55%)	0.171
Presentation			<0.001
Family screening	133(80%)	103(79%)	
Family SCD	24(14%)	4(3%)	
Near-drowning/OHCA/ACA	0(0%)	4(3%)	
Other	10(6%)	19(15%)	
Symptomatic at presentation	0(0%)	7(5%)	0.003
BB-therapy	11(7%)	11(8%)	0.500
Supine position			
$HR_{baseline}$, bpm	68(±13)	67(±13)	0.261
$QT_{baseline}$, ms	382(±34)	428(±51)	<0.001
$QTc_{baseline}$	404(±32)	447(±43)	<0.001
Standing position			
HR_{maxHR}, bpm	93(±14)	89(±14)	0.039
QT_{maxHR}, ms	383(±42)	431(±58)	<0.001
QTc_{maxHR}	473(±45)	521(±56)	<0.001
$HR_{stretch}$, bpm	92(±14)	87(±14)	0.013
$QT_{stretch}$, ms	383(±40)	434(±58)	<0.001
$QTc_{stretch}$	470(±50)	519(±55)	<0.001
QT_{return}, ms	377(±40)	443(±64)	<0.001
QTc_{return}	404(±42)	464(±60)	<0.001
Response to standing			
Time to maximal tachycardia, s	12(10–14)	12(10–14)	0.413
Time to maximal QT stretching, s	11(9–14)	11(9–13)	0.200
Time to return to baseline, s	31(24–55)	28(21–45)	0.030
ΔHR during maximal tachycardia, bpm	25(±11)	23(±11)	0.163
ΔQT during maximal tachycardia, ms	1(±29)	2(±38)	0.670
ΔQTc during maximal tachycardia	69(±44)	74(±46)	0.276
ΔHR during maximal QT stretching, bpm	23(±11)	21(±11)	0.074
ΔQT during maximal QT stretching, ms	2(±27)	5(±37)	0.441
ΔQTc during maximal QT stretching	67(±44)	72(±46)	0.321
ΔQT upon return to baseline HR, ms	−4(±28)	14(±43)	<0.001
ΔQTc upon return to baseline HR	0(±32)	17(±46)	0.001

P value <0.002 is statistically significant based on Bonferroni correction. ACA indicates aborted cardiac arrest; BB, betablocker; HR, heart rate; QTc, heart rate–corrected QT interval; OHCA, out-of-hospital cardiac arrest; and SCD, sudden cardiac death.

Manual Measurements

QT Interval/QTc Changes in Response to Standing

The manual measurements are shown in Table 1 and Figure S2. As expected, patients with LQTS had a longer QT interval and QTc at baseline compared with controls (both P<0.001). This difference remained during standing *without* an important difference in the response to standing between the groups. Consequently, the receiver-operating characteristic curves demonstrate an AUC that did not differ significantly between baseline, during maximal tachycardia, QT stretching, or return to baseline (Table 2 and Figure S3). A prolonged QTc during QT stretching improves sensitivity but not the AUC (Table 2).

Table 2. Diagnostic Value of the QT Interval and QTc With or Without Accompanied T-Wave Abnormalities During the Standing Test

	AUC	95% CI	Cut-off at 90% sensitivity	Specificity
$QT_{baseline}$	0.79	0.73–0.84	365	28%
$QTc_{baseline}$	0.79	0.74–0.84	396	38%
QT_{maxHR}	0.76	0.70–0.81	365	34%
QTc_{maxHR}	0.75	0.70–0.81	456	41%
$QT_{stretch}$	0.77	0.71–0.82	365	31%
$QTc_{stretch}$	0.76	0.70–0.81	457	45%
QT_{return}	0.82	0.77–0.87	365	36%
QTc_{return}	0.80	0.75–0.85	393	44%

	LQTS (%)	Controls (%)	AUC	95% CI	Sensitivity (95% CI)	Specificity (95% CI)
$QTc_{baseline}$ ≥390 ms	120 (92%)	117 (70%)	0.61	0.57–0.65	92% (85%–96%)	30% (23%–37%)
$QTc_{baseline}$ ≥450 ms*	62 (47%)	15 (9%)	0.69	0.64–0.74	47% (39%–56%)	91% (86%–95%)
Men $QTc_{baseline}$ ≥430 ms	36 (61%)	6 (9%)	0.76	0.68–0.83	61% (47%–74%)	90% (80%–96%)
Women $QTc_{baseline}$ ≥450 ms	39 (54%)	12 (11%)	0.71	0.65–0.78	54% (42%–66%)	89% (81%–94%)
$QTc_{baseline}$ ≥390 ms with abnormal T-waves†	120 (92%)	118 (71%)	0.61	0.56–0.65	92% (85%–96%)	29% (23%–37%)
$QTc_{baseline}$ ≥450 ms* with abnormal T-waves†	65 (50%)	17 (10%)	0.70	0.65–0.75	50% (41%–59%)	90% (84%–94%)
Men $QTc_{baseline}$ ≥430 ms with abnormal T-waves†	36 (61%)	7 (11%)	0.75	0.67–0.82	61% (47%–74%)	89% (78%–95%)
Women $QTc_{baseline}$ ≥450 ms with abnormal T-waves†	41 (57%)	13 (12%)	0.72	0.66–0.79	57% (45%–69%)	88% (80%–93%)
$QTc_{stretch}$ ≥460 ms	117 (89%)	82 (51%)	0.69	0.65–0.74	89% (83%–94%)	49% (41%–57%)
$QTc_{stretch}$ ≥490 ms*	91 (69%)	51 (32%)	0.69	0.64–0.74	70% (61%–77%)	68% (61%–75%)
$QTc_{stretch}$ ≥460 ms with abnormal T-waves‡	124 (95%)	93 (58%)	0.68	0.64–0.73	95% (89%–98%)	42% (35%–50%)
$QTc_{stretch}$≥490 ms* with abnormal T-waves‡	103 (79%)	68 (42%)	0.68	0.62–0.73	79% (71%–85%)	58% (50%–66%)
$QTc_{baseline}$≥390 ms and $QTc_{stretch}$≥460 ms	129 (98%)	136 (81%)	0.59	0.55–0.62	98% (95%–100%)	19% (13%–25%)
$QTc_{baseline}$≥450 ms* and $QTc_{stretch}$≥460 ms	120 (92%)	82 (51%)	0.70	0.66–0.75	92% (86%–96%)	49% (41%–57%)
Men $QTc_{baseline}$≥430 ms and $QTc_{stretch}$≥460 ms	55 (93%)	27 (45%)	0.74	0.67–0.81	93% (84%–98%)	55% (42%–68%)
Women $QTc_{baseline}$≥450 ms and $QTc_{stretch}$≥460 ms	65 (90%)	57 (56%)	0.67	0.61–0.73	90% (81%–96%)	44% (34%–54%)
$QTc_{baseline}$≥450 ms and $QTc_{stretch}$≥490 ms	99 (76%)	52 (32%)	0.72	0.67–0.77	76% (67%–83%)	68% (60%–75%)
$QTc_{baseline}$≥390 ms and $QTc_{stretch}$≥460 ms with abnormal T-waves‡	103 (99%)	139 (83%)	0.58	0.55–0.61	99% (96%–100%)	17% (11%–23%)
$QTc_{baseline}$≥450 ms* and $QTc_{stretch}$≥460 ms with abnormal T-waves‡	126 (96%)	93 (58%)	0.69	0.65–0.73	96% (91%–99%)	42% (35%–50%)
Men $QTc_{baseline}$≥430 ms and $QTc_{stretch}$≥460 ms with abnormal T-waves‡	57 (97%)	31 (52%)	0.73	0.66–0.79	97% (88%–100%)	48% (35%–62%)
Women $QTc_{baseline}$≥450 ms and $QTc_{stretch}$≥460 ms with abnormal T-waves‡	69 (96%)	64 (63%)	0.67	0.61–0.72	96% (88%–99%)	37% (28%–47%)
$QTc_{baseline}$≥450 ms and $QTc_{stretch}$≥490 ms* with abnormal T-waves‡	107 (82%)	69 (43%)	0.69	0.64–0.75	82% (74%–88%)	57% (49%–65%)

AUC indicates area under the curve; LQTS, long QT-syndrome; and QTc, heart rate–corrected QT interval.
*Previously reported cut-off values.[14–16]
†Abnormal T-waves include broad, notched, and late-onset T-waves in the right-precordial leads (V1–V3).
‡Abnormal T-waves include notched, biphasic, and flat T-waves in the left-precordial leads (V4–V6).

Of the patients with LQTS, 7 (5%) had ventricular extrasystoles during the standing test, while this was only seen in 4 controls (2%, *P*=0.222; Table S2). Six of the 7 patients with LQTS with ventricular extrasystoles were asymptomatic, and 1 had aborted cardiac arrest before diagnosis. Ventricular extrasystoles were mostly present <30 s after standing (71%). In 43% (3 out of 7) of the patients with LQTS, these ventricular extrasystoles were QT-related extrasystoles originating from the terminal part of the obviously prolonged QT interval and were only present <30 s after standing.

There were no statistic genotype differences in the QT interval/QTc response to standing (data not shown), and the interreader and intrareader reliability of the manual QT-interval measurements was moderate to good (Table S3).

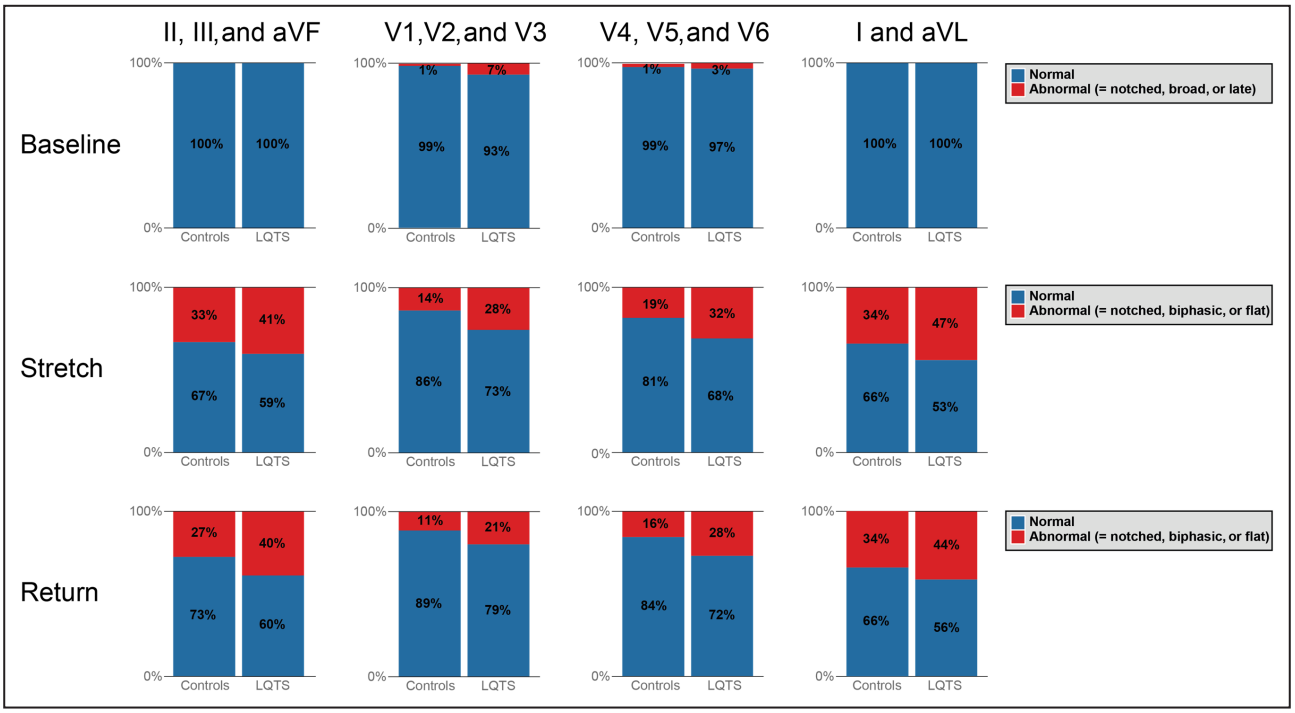

Figure 1. Partition of normal and abnormal T-waves at baseline, during maximal QT stretching and return to baseline in 4 lead groups.
LQTS indicates long QT-syndrome.

T-Wave Morphology Changes in Response to Standing

At baseline, there were no significant differences in T-wave morphology between patients with LQTS and controls. However, in response to standing, significant differences in T-wave patterns between patients with LQTS and controls arose, especially at the instance of QT stretching in the right- (V1–V3, $P<0.001$) and left-precordial leads (V4–V6, $P<0.001$; Figure S4). At QT stretching, T-wave morphologies that best discriminated patients with LQTS from controls included *notched*, *biphasic*, and *flat* T-waves. Therefore, we reanalyzed our results by grouping these morphologies into a single category named "*abnormal T-wave response to standing.*"

Diagnostic Value of T-Wave Morphology Changes in Response to Standing

Figure 1 shows the partition of T-waves at baseline and in response to standing into "normal" and "abnormal." At baseline, only in the right-precordial leads (V1-V3) was there a significant difference in abnormal T-waves (ie, *broad*, notched and *late* T-waves)[24–27] between patients with LQTS and controls (diagnostic OR, 6 [95% CI, 1–29] $P=0.02$). During QT stretching, the right-precordial leads still showed a difference in abnormal T-waves (ie, *notched*, *biphasic*, and *flat* T-waves) between patients with LQTS and controls but with a lower odds ratio (diagnostic OR, 2 [95% CI, 1–4] $P<0.01$) compared with baseline. Nevertheless, in the left-precordial leads (V4–V6) a significant difference in abnormal T-waves arose in response to standing between patients with LQTS and controls (diagnostic OR, 2 [95% CI, 1–4], $P<0.01$) that remained present when the heart rate returned to baseline conditions (diagnostic OR, 2 [95% CI, 1–4] $P<0.01$).

Added Diagnostic Value of T-Wave Morphology Changes to QT-Interval/QTc Changes

At baseline, there was no important incremental diagnostic value of the presence of T-wave abnormalities (ie, *broad*, *notched*, and *late* T-waves) to an abnormal baseline QTc.

After standing, during QT stretching, there was a significant improvement of sensitivity in a QTc≥460 ms accompanied with T-wave abnormalities in the left-precordial leads ($P=0.04$), as shown in Table 2. However, no improvement in AUC was seen because of the decrease in specificity.

If a prolonged QTc at baseline was present together with a QTc≥460 ms during QT stretching, there was no additional value of accompanied T-wave abnormalities.

Generally, for the assessment of T-wave morphology, there was a fair to moderate interreader reliability and a moderate intrareader reliability (Table S4).

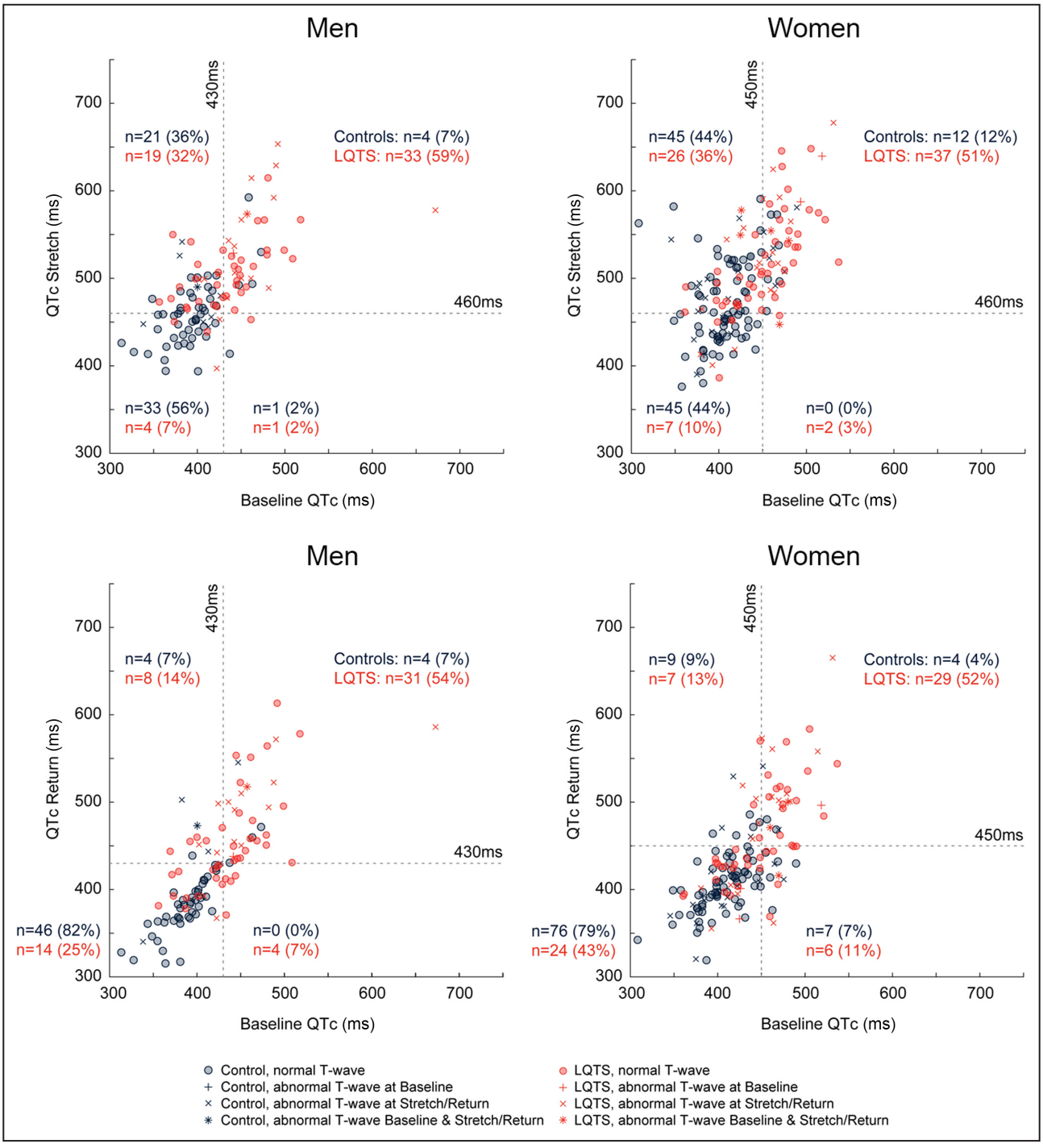

Figure 2. Distribution of patients with LQTS and controls according to QTc and T-wave morphology.
At baseline, abnormal QTc defined as ≥430 ms in men and ≥450 ms in women, and abnormal T-waves include broad, notched, and late-onset T-waves in V1 to V3. *During maximal QT stretching* and *at return to baseline*, the respective abnormal values are QTc≥460 ms for $QTc_{stretch}$, a QTc_{return} ≥430 ms in men and ≥450 ms in women, and notched, biphasic, and flat T-waves in V4 to V6. LQTS indicates long QT-syndrome; and QTc, heart rate–corrected QT interval.

Added Diagnostic Value of T-Wave Morphology Changes to QT-Interval/QTc Changes Stratified by Sex

The incremental value of T-wave morphology assessment during QT stretching and return to baseline values in the left-precordial leads for an LQTS diagnosis stratified by sex is appreciated from Figure 2. The percentage of patients with LQTS with an abnormal QTc increased in men from 61% at baseline to 92% during maximal QT stretching (absolute increment of 31%) and 54% to

88% in women (absolute increment of 34%). However, the percentage of patients with LQTS who had both abnormal QTc and abnormal T-wave morphology had an absolute increment of only 26% (from 3% at baseline to 29%) in men and 18% (from 7% at baseline to 25%) in women. Conversely, the percentage of controls with an abnormal QTc increased in men from 10% at baseline to 42% during maximal QT stretching (absolute increment of 32%) and 11% to 56% in women (absolute increment of 45%). Controls with abnormal results in both QTc and T-wave morphology increased in men from 0% at baseline to only 7% during maximal QT stretching and in women from 0% to 15%. When the heart rate returns to baseline conditions, there was a similar percentage of patients with LQTS and controls who had both abnormal QTc and abnormal T-wave morphology compared with QT stretching.

There was a significant incremental value of a QTc≥460 ms during QT stretching (ie, 90% sensitivity cut-off value in both men and women) to a baseline QTc≥430 ms^3 in men (P<0.001) and a QTc≥450 ms^3 in women (P=0.01) but, again, without improvement of the AUC (Table 2). Including the presence of T-wave abnormalities during QT stretching also yielded no additional value.

Added Diagnostic Value of T-Wave Morphology Changes to QT-Interval/QTc Changes Stratified by Genotype

T-wave morphology changes for different LQTS-genotypes are shown in Figure S5. T-wave abnormalities provoked by standing were most helpful for diagnosing LQT-2 in the inferior, right- and left-precordial leads during QT stretching. There were no significant differences in abnormal T-waves between LQTS type-1 and LQTS type-3. Between LQT-2 and controls, a QTc≥460 ms during QT stretching yielded a sensitivity of 88% (95% CI, 74–95) and specificity of 49% (95% CI, 41–57). Abnormal T-wave abnormalities had an additional value in the inferior (P=0.01), right- (P<0.001), and left-precordial leads (P<0.001) but without an improvement in sensitivity, specificity, or AUC.

Sensitivity Analyses Manual Measurements

The sensitivity analyses for QT interval/QTc changes in response to standing showed similar results as for the total cohort in (I) individuals not on beta-blocker therapy (n=150 controls, n=104 LQTS), (II) individuals with a baseline QTc ≤450 ms in men and ≤460 ms in women (n=157 controls, n=76 LQTS), and (III) individuals with a pretest probability for LQTS of 50% (n=133 controls, n=103 LQTS) (data not shown).

For the subgroups of individuals without obvious QTc-prolongation at baseline, there was no statistically significant difference for T-wave morphology assessment during QT stretching in the left-precordial leads (P=0.06). However, in the subgroup of individuals with a pretest probability of 50%, the results were similar to the total cohort, with a significant incremental value of abnormal T-waves to a QTc≥460 ms during QT stretching (P=0.02), whereas sensitivity improved from 88% (95% CI, 81–94; specificity 50%, 95% CI, 41–59) to 94% (95% CI, 88–98; specificity 45%, 95% CI, 36–54). There were no important differences in the diagnostic value of QTc accompanied by T-wave abnormalities when corrected for family correlations.

Automated Measurements

A total of 133 (37%) standing tests were digitally available, including 47 controls and 67 patients with LQTS (n=33 LQTS type-1, n=22 LQT-2, and n=12 LQTS type-3). Baseline characteristics, measurements at standing position, and responses to standing did not show any major differences with the total cohort (Table S5). The intermethod reliability between the automated and the manual measurements was good to excellent for almost all parameters (Table S6).

Response to Standing

The beat-to-beat analyses are shown in Figures 3 to 5. Patients with LQTS had, on average, longer QT intervals and QTc as compared with controls during the entire test, while heart rates were similar (Figure 3 left column). Patients with LQTS showed a relative QT interval and QTc prolongation compared with baseline, which recovered after ≈30 s of standing (Figure 3 right column). This phenomenon was more apparent in women with LQTS (Figure 4, Figure S6) and in patients with LQT-2 (Figure 5, Figure S7). A sensitivity analysis including only individuals without beta-blocker therapy did not change these results (data not shown).

DISCUSSION

Data of this prospective cohort of individuals suspected for LQTS who had a standing test as an initial evaluation tool in addition to a regular cardiac work-up showed that an LQTS diagnosis can be based on a QTc≥460 ms during maximal QT stretching with a high sensitivity at the expense of a low specificity. An LQTS diagnosis can be made with more confidence when a QTc≥460 ms during maximal QT stretching is accompanied by abnormal T-waves (ie, *notched*, *biphasic*, or *flat*, especially in the left-precordial leads [V4-V6]) or in the presence of a prolonged QTc at baseline. Generally, however, these findings were of limited *additional* diagnostic value compared with a QTc on a standard resting ECG, as the AUC did not significantly

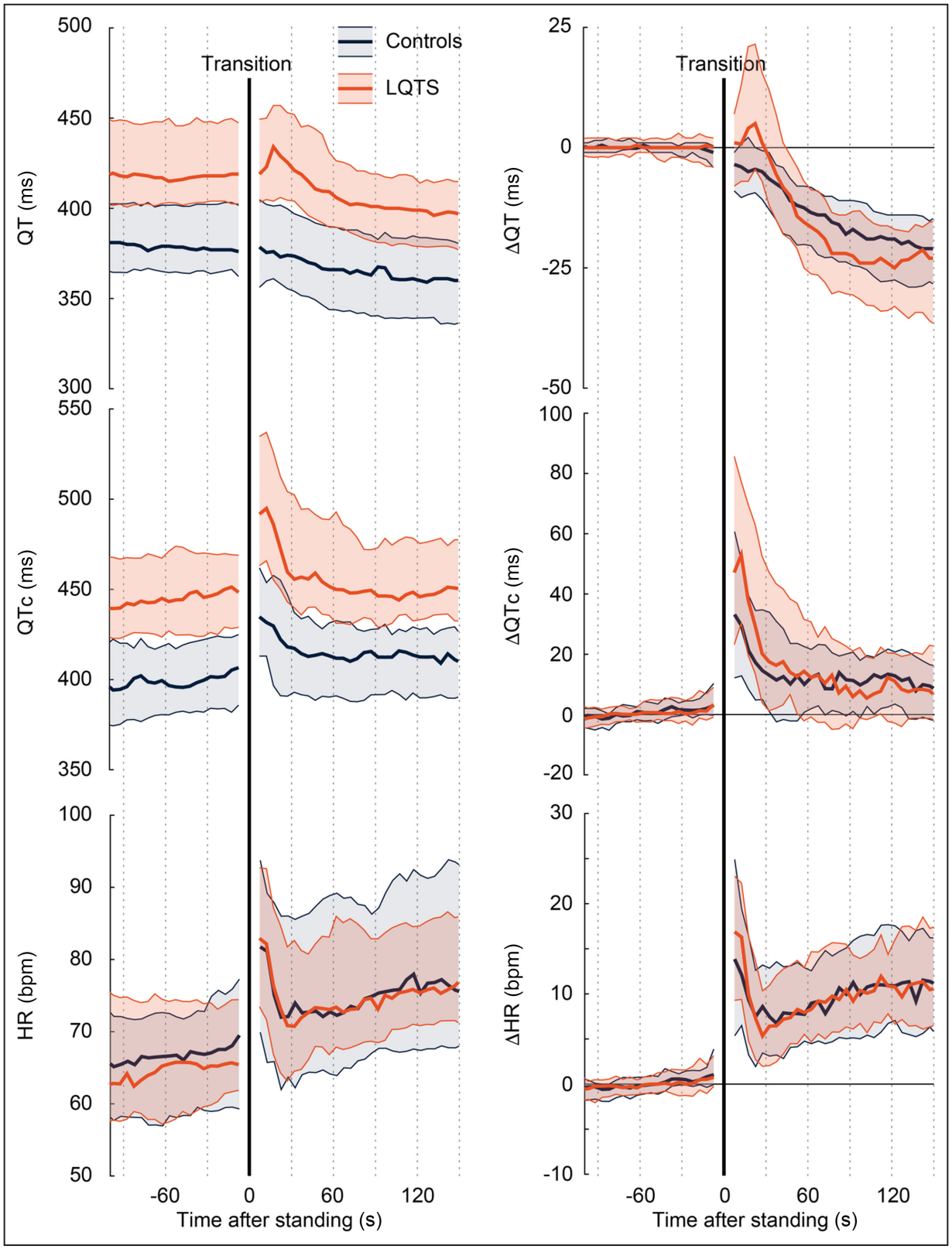

Figure 3. Standing test dynamics.
Left: median and interquartile range of absolute QT interval, QTc, and HR. Right: relative change of QT interval, QTc, and HR to baseline values. Transition from supine to standing is indicated by the black solid line. HR indicates heart rate; LQTS, long QT-syndrome; and QTc, heart rate–corrected QT interval.

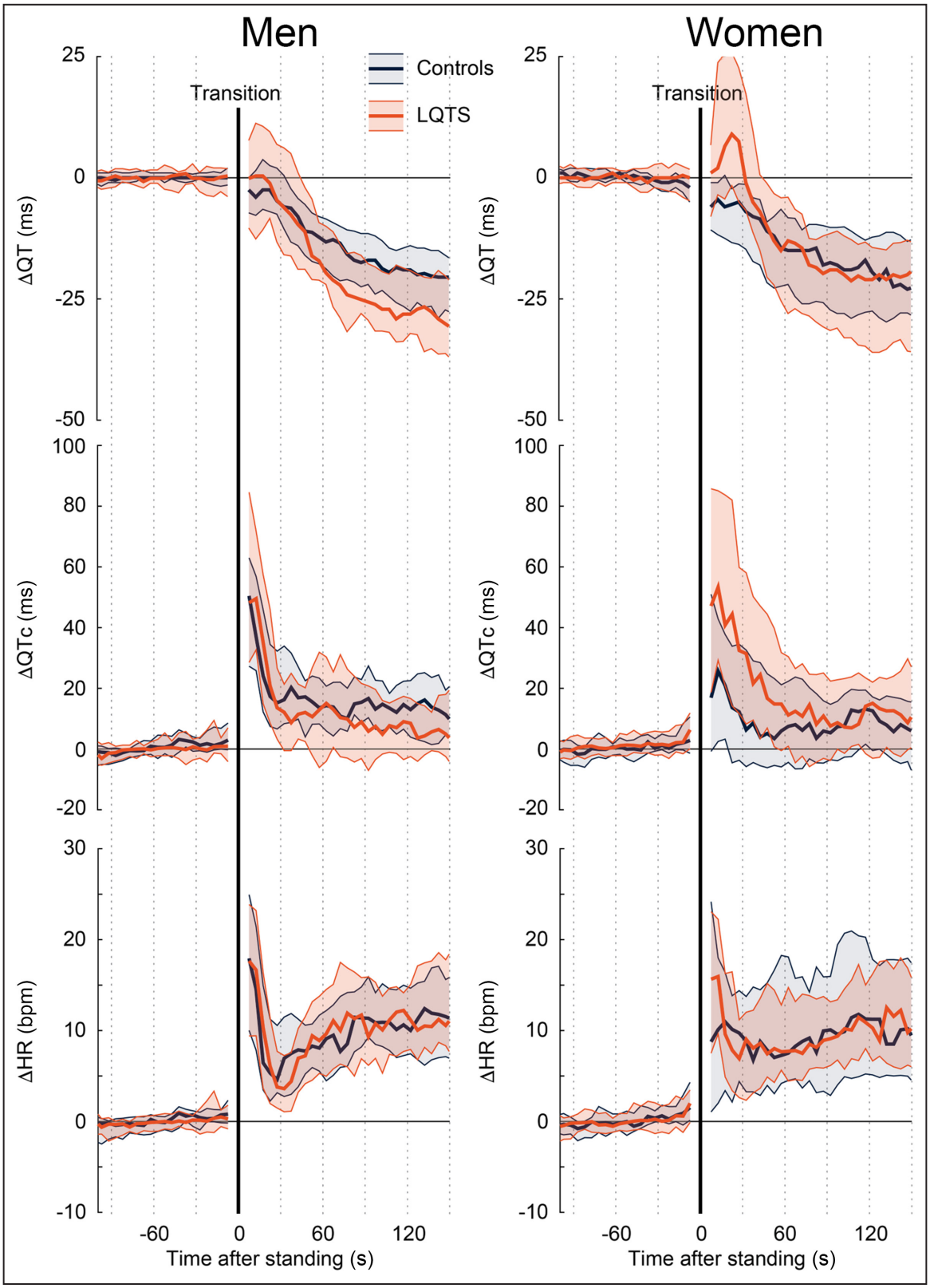

Figure 4. Sex-difference in standing test dynamics.
Including 48 men (n=20 controls, n=28 patients with LQTS) and 66 women (n=27 controls, n=39 patients with LQTS). Median and interquartile ranges of relative changes of QT interval, QTc, and HR to baseline. Transition from supine to standing is indicated by the black solid line. HR indicates heart rate; LQTS, long QT-syndrome; and QTc, heart rate–corrected QT interval.

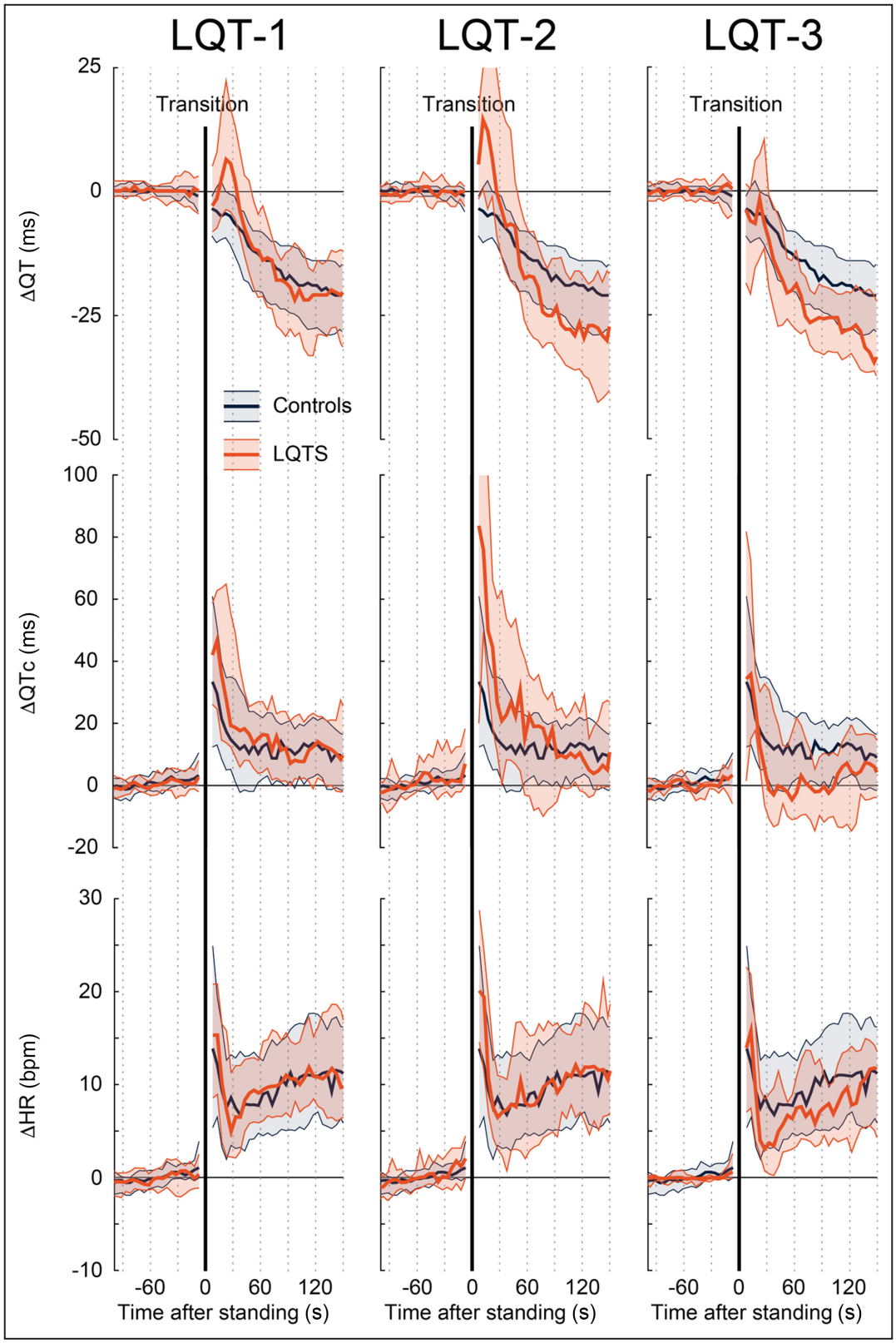

Figure 5. Genotype-differences in standing test dynamics.
Including n=33 controls, n=35 LQTS type-1, n=22 LQT-2, and n=12 LQTS type-3 patients. Median and interquartile ranges of relative changes of QT interval, QTc, and HR to baseline values. Transition from supine to standing is indicated by the black solid line. HR indicates heart rate; LQT-1, long QT-syndrome type 1; LQT-2, long QT-syndrome type 2; LQT-3, long QT-syndrome type 3; and QTc, heart rate–corrected QT interval.

improve. As such, from these data, the standing test appears not to be unequivocally superior to a baseline ECG as a prospective test to discriminate patients with genotype-positive LQTS from controls in individuals suspected for LQTS.

Dynamics of Standing Up

QT-interval dynamicity of the standing test is an interesting phenomenon, and insufficient repolarization reserve upon standing has often been clearly exemplified in patients with LQTS. The sudden heart rate acceleration within 3 s after standing is attributable to an inhibition of *parasympathetic* (*vagal*) activity as a reaction to the steep fall in blood pressure. Around 5 s after standing up, a more gradual secondary heart rate increase arises mainly because of further reflex inhibition of cardiac vagal tone and increased *sympathetic* activation of the sinus node. A normal response to this sudden acceleration of heart rate is a gradual QT-interval shortening. Because the QT-interval adaptation to sudden changes in heart rate is delayed (a phenomenon known as "QT-hysteresis"), the vagally mediated reflex tachycardia after standing results in a transient QTc-prolongation. This phenomenon was previously described in controls.[14,15] In contrast, patients with LQTS had an insufficient QT-interval shortening,[14] with a remaining QTc-prolongation after the heart rate returned to baseline values.[15] In the conception of this study, we were thus also rather confident that the previous retrospective data would be mirrored in our prospective cohort.

Why Didn't It Work?

There are several differences between the earlier described cohorts (which also included retrospective data of our group) and our current prospective data that contribute to our current conclusion that the standing test is not superior to a QTc measured on a resting ECG. First, it should be noted that the controls of earlier studies[14,15] consisted mainly of healthy volunteers. Our control group consisted mainly of genotype-negative family members of patients with genotype-positive LQTS. While the baseline QTc of our controls was similar to the previous controls (404±32 ms versus 405±25 ms[14] and 416±30 ms[15]), their response to standing was different (ΔQT during maximal QT stretching +2±27 ms versus –15±30[14] and –14±35 ms[15]). Equally important, the response to standing was similar between our controls (+2±27 ms) and patients with LQTS (+5±37 ms). This similarity between our controls and patients with LQTS might in part be attributable to modifier genes that occur within families independent of the disease-causing genotype, like in other genotype–phenotype relationships.[28,29]

Second, the patients with genotype-positive LQTS who were included in this study had a shorter baseline QTc (447±43 ms) compared with the patients with LQTS enrolled in the case-control study developing the standing test (465±44 ms[14] and 469±40 ms[15]). They also had a less pronounced QT-interval prolongation upon standing (+5±37 versus +13±37 ms[14] and +8±51 ms[15]). This implies that the patients with LQTS included in our prospective study had a (much) less pronounced phenotype, resulting in a lack of differentiation based upon the standing test. In the previous cohort studies, most patients with LQTS were genotype-positive, but there were also patients with gene-elusive LQTS. Because these latter patients generally require clear QT prolongation to get to a diagnosis, this discrepancy might further contribute to the current result.

These diverging characteristics of our prospective cohort compared with the initial case-control standing test studies resulted in the lack of added diagnostic value of the standing test.

Implications

The use of a standing test to screen for genotype-positive LQTS is thus not significantly better than a QTc on a resting ECG in a population suspected for LQTS. However, there are several remarks to be made to this statement. First, the key clinical aspect of genotype-positive LQTS is the evaluation of decreased repolarization reserve and the subsequent risk assessment for malignant arrhythmias. This study once again[30] shows that there is a markedly reduced penetrance and incomplete expression in LQTS (ie, there are many patients with genotype-positive LQTS without a clear LQTS-phenotype). Although the standing test can be regarded as a subtle provocation of the repolarization reserve, those patients with an excessive response will likely have a higher risk of cardiac events than those without and would thus warrant more aggressive therapy (primarily beta blockers). Whether the standing test can be used to defer further treatment or evaluation (eg, confirmation of adequate repolarization reserve, or further determination of a variant of unknown significant) is not yet so clear but should be regarded as grounds for further evaluation. Moreover, should stronger provocations be applied (eg, by using potent QT-prolonging drugs), there may well be a strong response in patients with LQTS who previously displayed no or only a minor LQTS-phenotype. Such responses will probably include aspects of modifier genes impacting the genotype.[9]

In addition, the clinical setting in which a differentiation in LQTS versus no-LQTS is made will also be important as this determines the pretest probability. For example, in a cardiogenetic outpatient clinic, there often is a near 50% chance of an

individual having LQTS, which might be different from other clinical settings. As the pretest probability and performance of any test is influenced by the prevalence of the disease tested for, the potential value of the standing test in the risk stratification for cardiac events should be further evaluated in patients suspected for LQTS with a pretest probability of ≈50%.

Additional Potential Value

In contrast to the previously established standing test parameters, the analysis of beat-to-beat dynamics did show a ΔQT interval and ΔQTc prolongation in patients with LQTS within 30 s after standing compared with controls. This difference in beat-to-beat dynamics between patients with LQTS and controls is not translated into a difference at QT stretching probably because controls had a short-term QT-interval increase that is measured at the previously established QT-stretching parameter, hampering its performance. In contrast, this short-term QT-interval prolongation in controls was reduced by the median filter of the beat-to-beat analysis.

Interestingly, especially patients with LQT-2 and women with LQTS showed a ΔQT interval and ΔQTc prolongation after standing. It is known that, in patients with LQT-2, cardiac events are characteristically triggered by situations involving sudden heart rate acceleration[1] and that women with LQTS have a higher risk for cardiac events during adulthood compared with men with LQTS.[31] Hence, this implies a potential role for the standing test in the risk stratification of patients with LQTS.

Study Limitations

In addition to the above-mentioned items, there are several limitations that should be considered. (I) Not all standing tests were performed by the same investigator under standardized conditions (eg, same time of the day or pretest physical activity), which could have affected heart rate and repolarization. However, all tests were performed in a calm environment after giving instructions and performing the necessary preparations. (II) We have no data on the intrasubject repeatability of the standing test, as the test was performed only once. (III) The standing test is accompanied by a substantial amount of muscle noise, which hampers QT-interval measurements for a short moment. We observed this more in adults than in children.[32] In automated analyses, short-term differences might be averaged out because of filtering. The measured ECG parameters, either manually or by algorithm, are single complexes and are therefore on the one hand more sensitive to short-term differences but on the other hand also more susceptible to outliers or erroneous measurements. (IV) The study is limited to patients with LQTS type-1, LQT-2, and LQTS type-3, although other LQTS-causing genes are much less common. (V) Although the number of included individuals is quite robust for a rare disorder, the number of individuals included in the extensive subanalyses was sometimes limited.

CONCLUSIONS

Despite earlier retrospective studies, a baseline ECG and the standing test in a prospective evaluation displayed a different diagnostic profile for LQTS but no unequivocal synergism or advantage. This finding mirrors the concept that there is markedly reduced penetrance and incomplete expression in genetically confirmed LQTS and that repolarization reserve in response to the brief tachycardia provoked by standing can be variably preserved in genotype-positive LQTS.

Diagnosing LQTS thus remains a challenge and requires an extensive and detailed history-taking that emphasizes specific triggers and symptoms together with meticulous and repeated ECG evaluations in individuals under evaluation and his/her family members. In this context, the standing test could be of additional value, especially when including beat-to-beat dynamics. Hence, we advocate the use of the standing test in expert centers to be able to gain more insights before more widespread use.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Table S1–S6
Figures S1–S7

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Supplemental Material

Table S1. **Baseline characteristics and manual ECG-measurements.**

	Controls n=167	*LQTS n=131*	*Possible LQTS n=35*	*Other n=28*
Age, years	44(33-54)	41(29-50)	44(28-55)	45(36-57)
Females	106(63%)	72(55%)	24(69%)	15(54%)
Presentation				
Family screening	133(80%)	103(79%)	17(49%)	4(14%)
Family SCD	24(14%)	4(3%)	6(17%)	3(11%)
Near-drowning/OHCA/ACA	0(0%)	4(3%)	0(0%)	15(54%)
Other	10(6%)	19(15%)	12(34%)	6(21%)
Symptomatic at presentation	0(0%)	7(5%)	0(0%)	16(57%)
BB-therapy	11(7%)	11(8%)	6(17%)	5(18%)
Supine position				
$HR_{baseline}$,*bpm*	68(±13)	67(±13)	68(±11)	65(±12)
$QT_{baseline}$,*ms*	382(±34)	428(±51)	399(±32)	397(±40)
$QTc_{baseline}$	404(±32)	447(±43)	421(±28)	410(±39)
Standing position				
$HR_{maxHR,}$*bpm*	93(±14)	89(±14)	91(±13)	89(±15)
QT_{maxHR},*ms*	383(±42)	431(±58)	395(±50)	390(±41)
QTc_{maxHR},*ms*	473(±45)	521(±56)	482(±55)	470(±44)
$HR_{stretch,}$*bpm*	92(±14)	87(±14)	90(±13)	86(±15)
$QT_{stretch}$,*ms*	383(±40)	434(±58)	393(±52)	393(±45)
$QTc_{stretch}$,*ms*	470(±50)	519(±55)	478(±59)	466(±44)
QT_{return},*ms*	377(±40)	443(±64)	393(±52)	383(±37)
QTc_{return},*ms*	404(±42)	464(±60)	416(±44)	398(±40)
Response to standing				
Time to maximal tachycardia,s	12(10-14)	12(10-14)	10(9-14)	13(10-15)
Time to maximal QT-stretching,s	11(9-14)	11(9-13)	11(9-13)	12(9-14)
Time to return to baseline,s	31(24-55)	28(21-45)	28(23-45)	34(24-77)
ΔHR during maximal tachycardia,bpm	25(±11)	23(±11)	23(±12)	24(±12)
ΔQT during maximal	1(±29)	2(±38)	-3(±33)	-6(±30)

tachycardia,ms				
ΔQTc during maximal tachycardia,ms	69(±44)	74(±46)	62(±53)	62(±37)
ΔHR during maximal QT-stretching,bpm	23(±11)	21(±11)	22(±11)	21(±11)
ΔQT during maximal QT-stretching,ms	2(±27)	5(±37)	-5(±31)	-3(±34)
ΔQTc during maximal QT-stretching,ms	67(±44)	72(±46)	57(±52)	58(±38)
ΔQT upon return to baseline HR,ms	-4(±28)	14(±43)	-7(±35)	-15(±38)
ΔQTc upon return to baseline HR,ms	0(±32)	17(±46)	-6(±36)	-10(±38)

SCD=Sudden Cardiac Death, OHCA=Out of Hospital Cardiac Arrest, ACA=Aborted Cardiac Arrest, BB=beta-blocker, HR=heart rate, QTc=QT-interval corrected for heart rate using Bazett's formula, bpm=beats per minute, (m)s=(milli)seconds.

Table S2. Characteristics of the ventricular extrasystoles in both LQTS-patients and controls.

	Sex	Age	Symptomatic	PVCs supine	PVCs standing	HR *bpm*	QT *ms*	QTc *ms*	Couplings interval *ms*	Terminal part T-wave	QT-related	Remarks
LQTS												
1	Male	69	No	No	Yes	75	540	600	560	Possible	Possible	One PVC <30s after standing
2	Female	35	No	No	Yes	79	560	640	480	Yes	Clearly	>30s after standing isolated PVCs, No PVCs after post-extrasystolic pause.
3	Female	30	No	Yes	Yes	88	520	680	580	Yes	Clearly	Supine: PVCs in bigeminy with QT-related PVCs after post-extrasystolic pause. Standing: <30s isolated PVCs clearly QT-related
4	Male	70	Yes	Yes	Yes	73	440	485	400	No	No	Both in supine and standing position isolated PVCs. Was under metoprolol therapy.
5	Female	56	No	Yes	No	83	440	520	520	Yes	Clearly	In supine position PVCs in bigeminy with QT-related PVCs after post-extrasystolic pause.
6	Female	20	No	No	Yes	80	520	570	440	Yes	Clearly	<30s after standing isolated PVCs, No PVCs after post-extrasystolic pause
7	Female	23	No	No	Yes	80	460	400	400	Yes	Clearly	<30s after standing PVCs in bigeminy with QT-related PVCs after post-extrasystolic pause.
Controls												
1	Male	35	No	Yes	No	79	370	425	NA	No	No	In supine position isolated PVCs
2	Female	45	No	No	Yes	71	440	480	680	No	No	One PVC <30s after standing, >30s after standing isolated PVCs.
3	Female	50	No	Yes	Yes	79	440	500	660	No	No	Supine: isolated PVCs. Standing: >30s isolated PVCs
4	Female	43	No	No	Yes	79	560	640	480	Possible	Possible	<30s after standing isolated PVCs

Table S3. Inter- and intra-reader reliability.

	Inter-reader		*Intra-reader*	
	ICC (95% CI)	*Mean (±95% LoA)*	*ICC (95% CI)*	*Mean (±95% LoA)*
Supine position				
$HR_{baseline}$,*bpm*	0.96 (0.92-0.98)	2 (± 7)	0.97 (0.95-0.99)	1 (± 6)
$QT_{baseline}$,*ms*	0.90 (0.75-0.96)	12 (±41)	0.86 (0.73-0.93)	5 (±59)
Standing position				
HR_{maxHR},*bpm*	0.95 (0.90-0.97)	1 (±10)	0.96 (0.92-0.98)	2 (± 8)
QT_{maxHR},*ms*	0.70 (0.47-0.84)	14 (±82)	0.80 (0.63-0.89)	8 (±62)
$HR_{stretch}$,*bpm*	0.96 (0.93-0.98)	1 (± 8)	0.99 (0.98-0.99)	0 (± 5)
$QT_{stretch}$,*ms*	0.75 (0.45-0.88)	20 (±66)	0.78 (0.58-0.89)	12 (±60)
HR_{return},*bpm*	0.95 (0.88-0.98)	2 (±10)	0.98 (0.95-0.99)	1 (± 7)
QT_{return},*ms*	0.79 (0.52-0.90)	20 (±66)	0.90 (0.80-0.95)	5 (±46)

The differences in HR and QT-interval measurements were probably mainly driven by the differences in chosen P-QRS-T complexes that were measured and the differences in the leads that were chosen to measure these complexes in as previous data from our own group[3] showed that the inter- and intra-observer reliability is (very) high when complexes are marked to ensure measurement of the same P-QRS-T complexes.

HR=heart rate, QTc=QT-interval corrected for heart rate using Bazett's formula, bpm=beats per minute, ms=milliseconds, IC=confidence interval.

Table S4. Inter- and intra-reader reliability for T-wave morphology.

	Inter-reader		*Intra-reader*	
	Agreement	*Kappa*	*Agreement*	*Kappa*
Baseline				
Lead group I	79%	0.38	91%	0.47
Lead group II	88%	0.17	85%	0.33
Lead group III	82%	0.28	91%	0.67
Lead group IV	82%	0.54	79%	0.44
QT-stretch				
Lead group I	67%	0.38	67%	0.43
Lead group II	88%	0.67	82%	0.47
Lead group III	70%	0.40	76%	0.52
Lead group IV	67%	0.26	64%	0.36
Return				
Lead group I	64%	0.37	76%	0.46
Lead group II	73%	0.22	82%	0.40
Lead group III	85%	0.64	91%	0.72
Lead group IV	70%	0.28	70%	0.14

Lead group I = II, III, aVF, Lead group II = V1-V3, Lead group II = V4-V6, Lead group II = I and aVL.

Table S5. Baseline characteristics and automatic ECG-measurements of the digital available ECGs.

	Controls n=47	*LQTS n=67*	*Possible LQTS n=13*	*Other n=6*
Age, years	49(33-56)	40(27-48)	41(19-50)	46(44-48)
Females	27(57%)	39(58%)	8(61%)	3(50%)
Presentation				
Family screening	43(91%)	57(85%)	8(52%)	3(50%)
Family SCD	1(2%)	3(5%)	1(8%)	0(0%)
Near-drowning/OHCA/ACA	0(0%)	0(0%)	0(0%)	1(17%)
Other	3(6%)	7(9%)	4(31%)	2(33%)
Symptomatic at presentation	0(0%)	1(2%)	0(0%)	1(17%)
BB-therapy	8(17%)	4(6%)	1(8%)	0(0%)
Supine position				
$HR_{baseline}$,*bpm*	68(±15)	68(±13)	68(±11)	71(±13)
$QT_{baseline}$,*ms*	382(±31)	427(±44)	405(±45)	395(±22)
$QTc_{baseline}$	402(±33)	450(±36)	426(±33)	427(±33)
Standing position				
HR_{maxHR},*bpm*	93(±16)	93(±13)	95(±14)	96(±20)
QT_{maxHR},*ms*	375(±32)	423(±47)	401(±46)	396(±28)
QTc_{maxHR},*ms*	463(±34)	523(±60)	501(±47)	483(±35)
$HR_{stretch}$,*bpm*	91(±16)	90(±13)	93(±14)	94(±20)
$QT_{stretch}$,*ms*	384(±34)	441(±46)	412(±42)	399(±23)
$QTc_{stretch}$,*ms*	470(±38)	538(±56)	512(±52)	494(±37)
QT_{return},*ms*	383(±35)	439(±48)	410(±51)	381(±22)
QTc_{return},*ms*	410(±41)	464(±51)	432(±33)	430(±33)
Response to standing				
Time to maximal tachycardia,s	12(10-15)	12(10-14)	12(10-15)	14(11-16)
Time to maximal QT-stretching,s	11(9-15)	11(10-15)	11(9-15)	12(11-15)
Time to return to baseline,s	25(21-29)	22(20-27)	25(20-26)	25(23-26)
ΔHR during maximal tachycardia,bpm	25(±11)	24(±9)	27(±11)	25(±12)
ΔQT during maximal tachycardia,ms	-7(±20)	-4(±51)	-4(±18)	-9(±11)
ΔQTc during maximal tachycardia,ms	61(±37)	74(±56)	76(±41)	56(±26)
ΔHR during maximal QT-stretching,bpm	24(±11)	23(±10)	26(±12)	23(±14)
ΔQT during maximal QT-stretching,ms	1(±21)	14(±34)	8(±16)	4(±10)
ΔQTc during maximal QT-stretching,ms	68(±38)	89(±47)	91(±39)	67(±28)
ΔQT upon return to baseline HR,ms	0(±21)	11(±28)	5(±13)	-14(±7)
ΔQTc upon return to baseline HR,ms	7(±29)	14(±33)	7(±14)	3(±30)

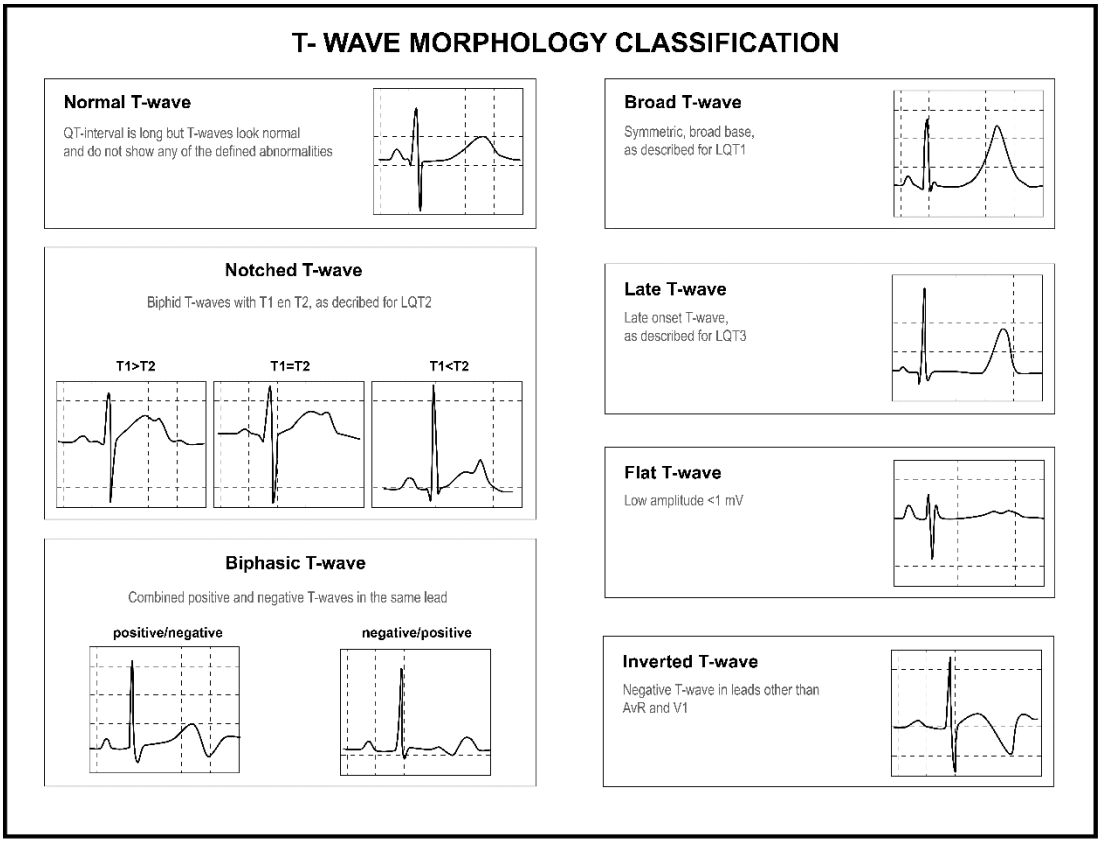
HR=heart rate, QTc=QT-interval corrected for heart rate using Bazett's formula, bpm=beats per minute, (m)s=(milli)seconds.

Table S6. Inter-method reliability.

	Inter-method	
	ICC (95% CI)	*Mean (±95% LoA)*
Supine position		
$HR_{baseline}$,*bpm*	0.93 (0.90-0.95)	0 (±10)
$QT_{baseline}$,*ms*	0.85 (0.79-0.89)	3 (±48)
Standing position		
$HR_{maxHR,}$*bpm*	0.94 (0.92-0.96)	0 (± 9)
QT_{maxHR},*ms*	0.64 (0.53-0.73)	1 (±81)
$HR_{stretch,}$ *bpm*	0.92 (0.90-0.94)	1 (±11)
$QT_{stretch}$,*ms*	0.68 (0.58-0.76)	10 (±82)
$HR_{return,}$*bpm*	0.94 (0.92-0.96)	1 (± 9)
$QT_{return,}$*ms*	0.81 (0.74-0.86)	4 (±69)

HR=heart rate, QTc=QT-interval corrected for heart rate using Bazett's formula, bpm=beats per minute, ms=milliseconds, IC=confidence interval.

Figure S1. T-wave morphology classification adapted from Chorin et al.[16]



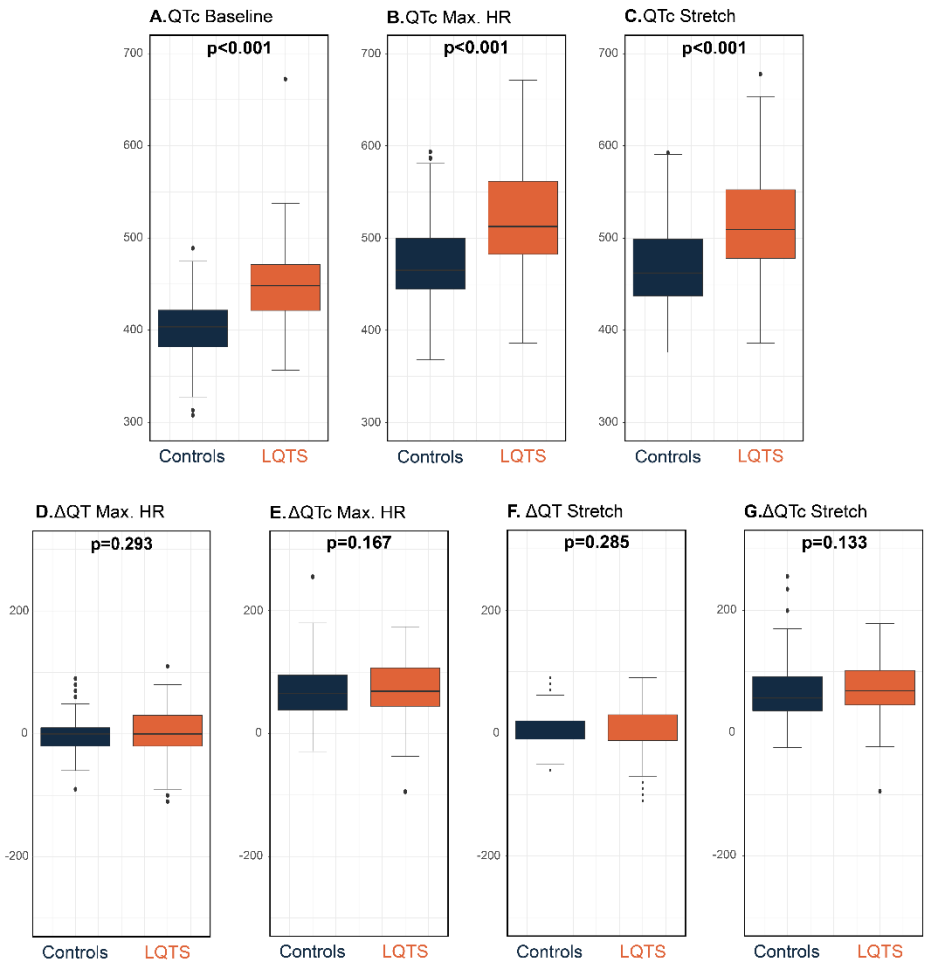


Figure S2. **The colored boxes represent the interquartile range (25th to 75th percentiles).** (A to G) The orange boxes represent LQTS- patients; the blue boxes rep- controls. The thick black line in the box is the 50th percentile, and the bars represent the range of results excluding outliers. Solid black circles indicate outliers. ΔQT = ΔQT-interval change from baseline, ΔQTc = Δ corrected QT-interval change from baseline.

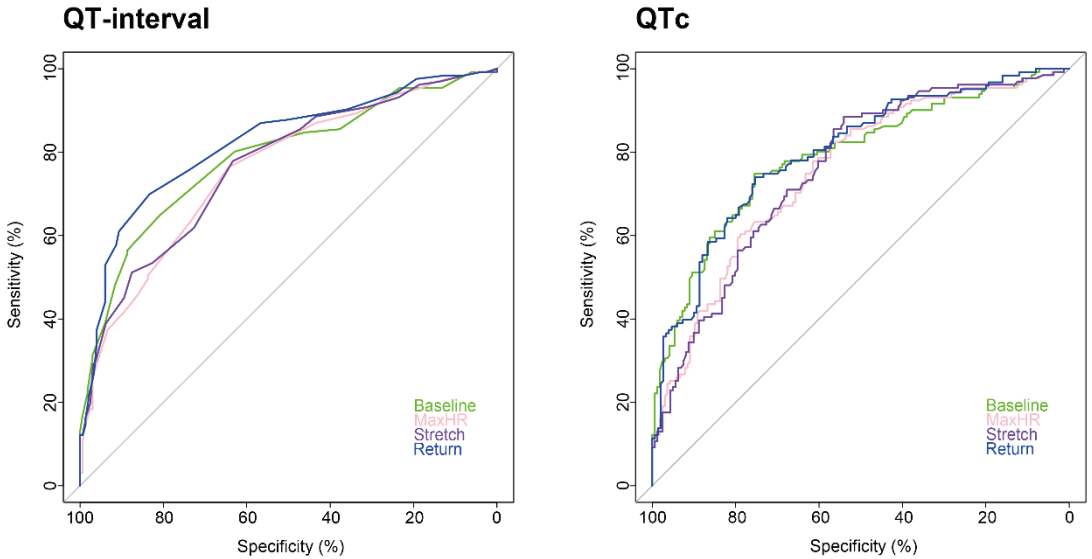


Figure S3. **Receiver-operating characteristic curves of the diagnosis LQTS for QT-interval and QTc at four different time instances: (I) at baseline; in supine position before standing, (II) at maximal tachycardia (MaxHR); at maximal sinus rate after standing-up, (III) at maximal QT-stretching (Stretch); after standing-up where the end of the T-wave gets nearest to the next P-wave, and (IV) at return to baseline (Return); at slowest sinus rate while standing.**

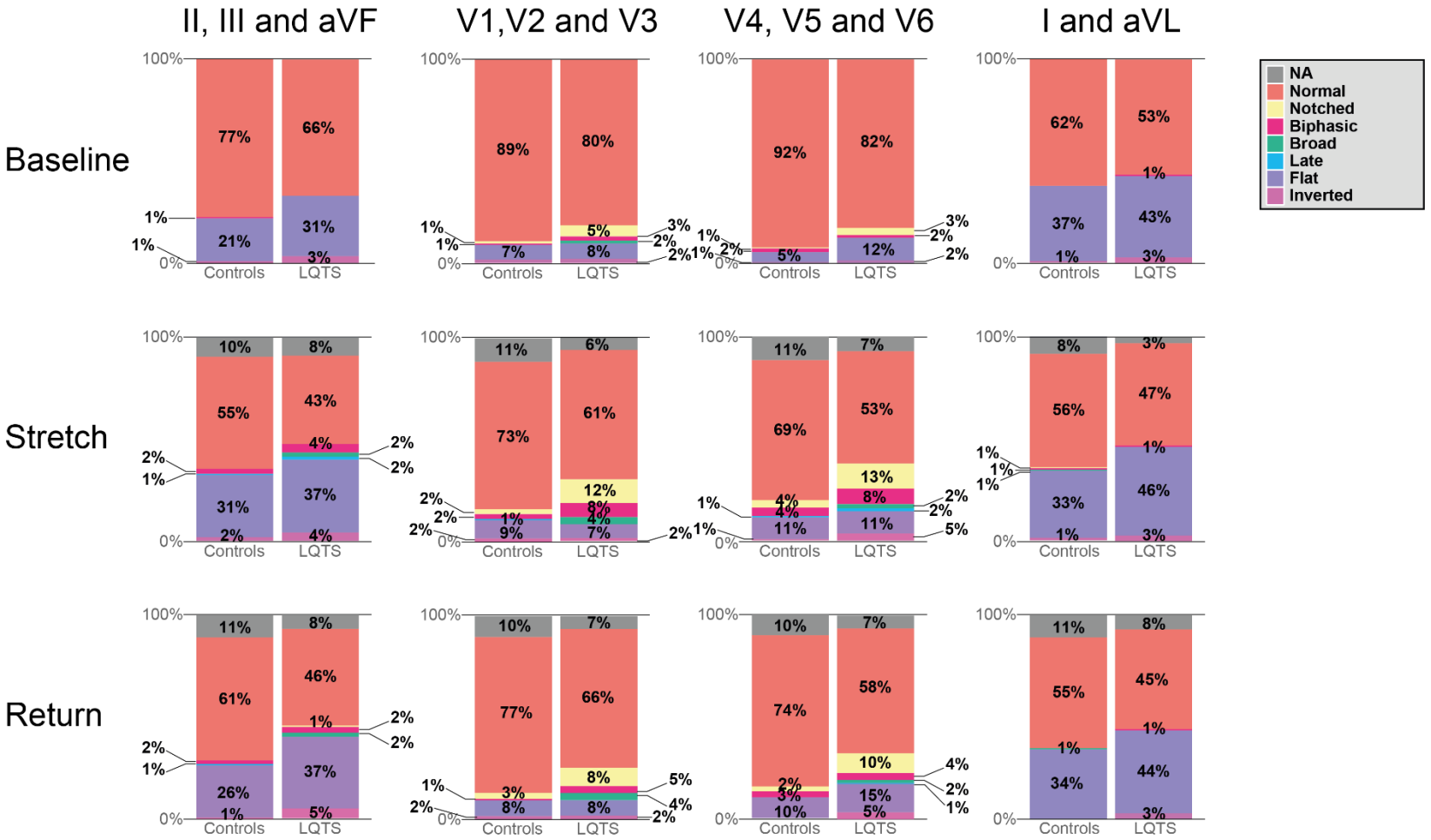


Figure S4. Percentage of present T-wave morphologies at three phases of the standing-test (i.e. at baseline, during maximal QT-stretching, and return to baseline) in controls and LQTS-patients for four different lead-groups: (I) *inferior leads* (i.e. II, III and aVF); (II) *right-precordial leads* (i.e. V1-V3); (III) *left-precordial leads* (i.e. V4-V6) and (IV) *lateral leads* (i.e. I and aVL).

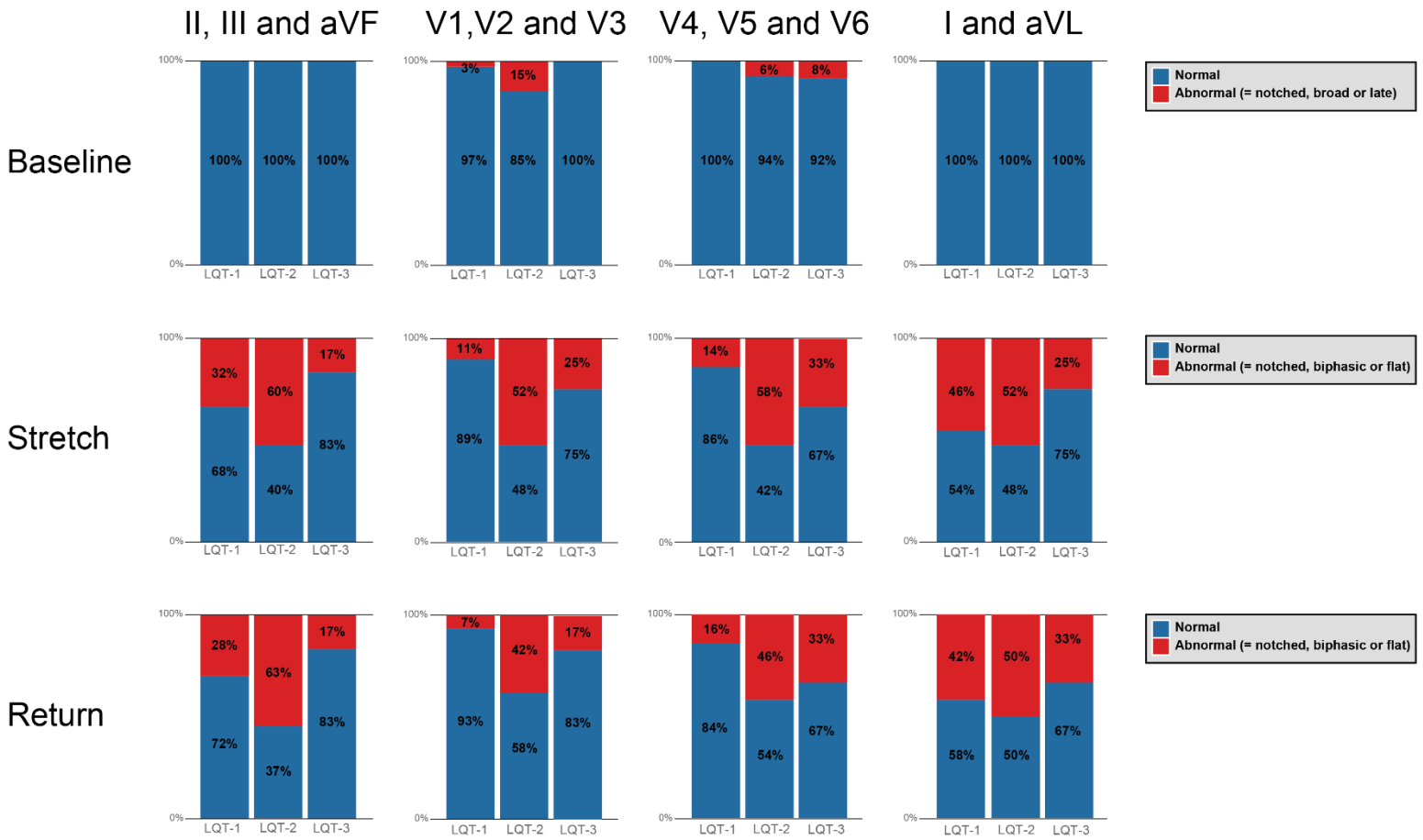


Figure S5. Partition of T-waves at baseline and in response to standing into "normal" and "abnormal" response in four different lead groups: (I) *inferior leads* (i.e. II, III and aVF); (II) *right-precordial leads* (i.e. V1-V3); (III) *left-precordial leads* (i.e. V4-V6) and (IV) *lateral leads* (i.e. I and aVL) including in LQTS-type 1 (LQT-1, n=71), LQTS-type 2 (LQT-2, n=48) and LQTS-type 3 patients (LQT-3, n=12).

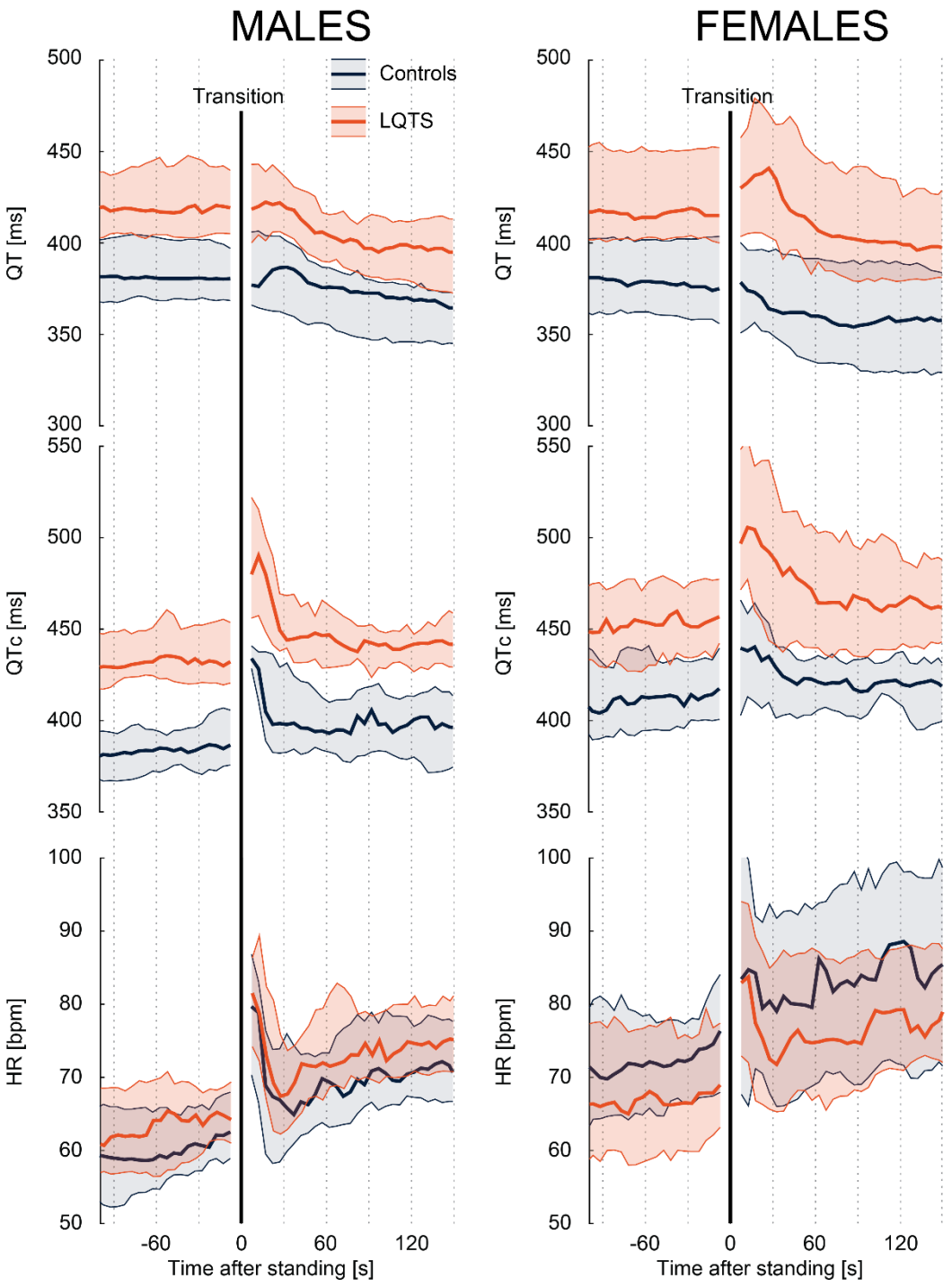


Figure S6. **Sex-difference in standing-test dynamics. Including 48 males (20 controls and 28 LQTS-patients) and 66 females (27 controls and 39 LQTS-females).** The median and interquartile range of the absolute QT-interval, QTc and heart rate of controls (blue) and LQTS-patients (orange), stratified for males (**left column**) and females (**right column**). Transition from supine to standing is indicated by the black solid line. HR=heart rate, LQTS=Long-QT syndrome, QTc=QT-interval corrected for heart rate using Bazett's formula.

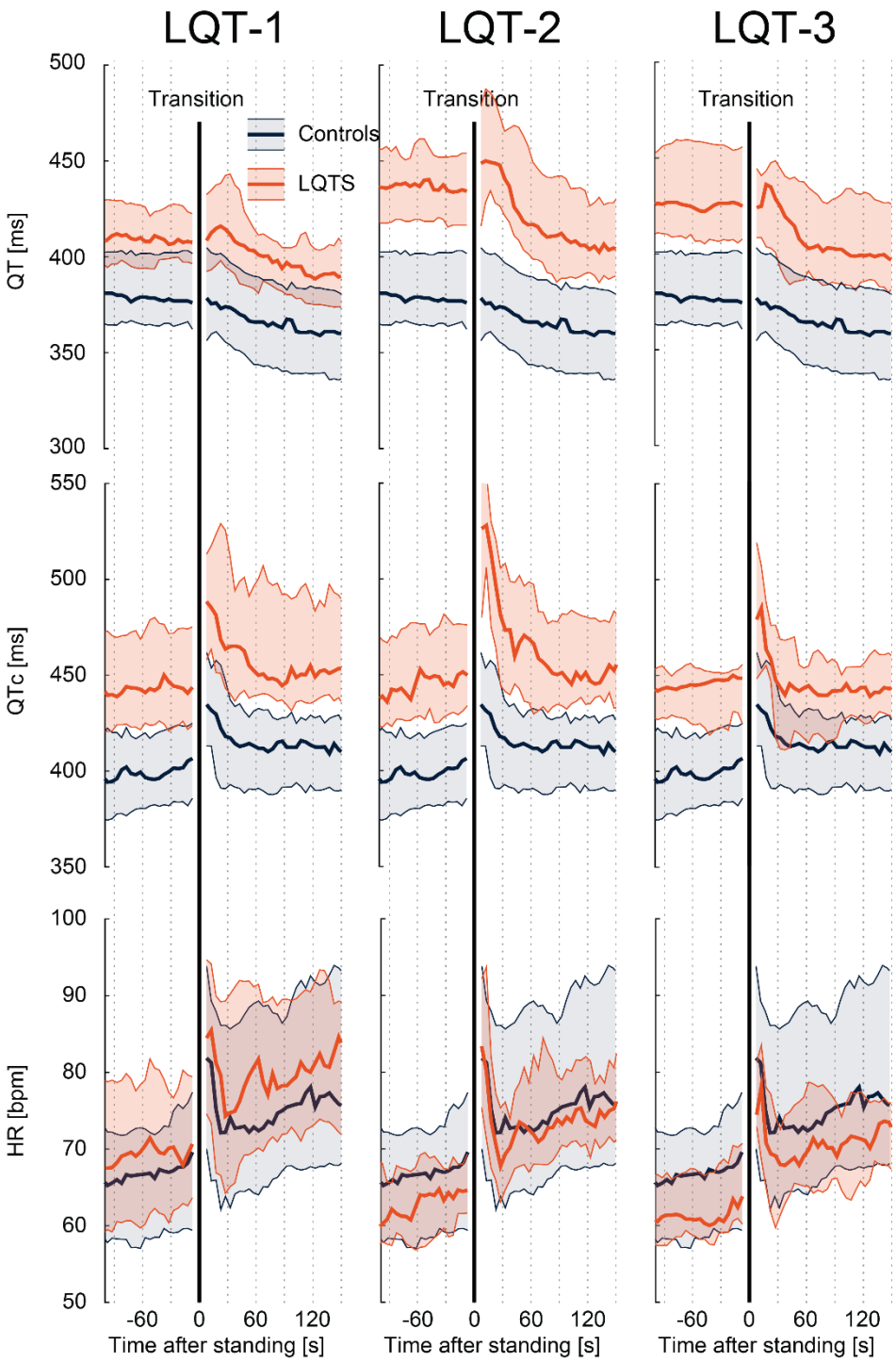


Figure S7. **Genotype-difference in standing-test dynamics. Including n=33 controls, n=35 LQT-1, n=22 LQT-2 and n=12 LQT-3 patients.** The median and interquartile range of the absolute QT-interval, QTc and heart rate of controls (blue) and LQTS-patients (orange), stratified for LQT-1 (**left column**), LQT-2 (**middle column)** and LQT-3 (**right column**). Transition from supine to standing is indicated by the black solid line. LQT-1= Long-QT syndrome type 1, LQT-2= Long-QT syndrome type 2, LQT-3= Long-QT syndrome type 3, HR=heart rate, QTc=QT-interval corrected for heart rate using Bazett's formula.