Utility of Provocative Testing in the Diagnosis and Genotyping of Congenital Long QT Syndrome: A Systematic Review and Meta-Analysis

Ying Yang, PhD*; Ting-ting Lv, PhD*; Si-yuan Li, PhD; Peng Liu, PhD; Qing-gele Gao, PhD; Ping Zhang, MD, PhD

BACKGROUND: Diagnosis is particularly challenging in concealed or asymptomatic long QT syndrome (LQTS). Provocative testing, unmasking the characterization of LQTS, is a promising alternative method for the diagnosis of LQTS, but without uniform standards.

METHODS AND RESULTS: A comprehensive search was conducted in PubMed, Embase, and the Cochrane Library through October 14, 2021. The fixed effects model was used to assess the effect of the provocative testing on QTc interval. A total of 22 studies with 1137 patients with LQTS were included. At baseline, QTc interval was 40 ms longer in patients with LQTS than in controls (mean difference [MD], 40.54 [95% CI, 37.43–43.65]; \( P < 0.001 \)). Compared with the control group, patients with LQTS had 28 ms longer \( \Delta \)QTc upon standing (MD, 28.82 [95% CI, 23.05–34.58]; \( P < 0.001 \)), nearly 30 ms longer both at peak exercise (MD, 27.31 [95% CI, 21.51–33.11]; \( P < 0.001 \)) and recovery 4 to 5 minutes (MD, 29.85 [95% CI, 24.36–35.35]; \( P < 0.001 \)). With epinephrine infusion, QTc interval was prolonged both in controls and patients with QTS, most obviously in LQT1 (MD, 68.26 [95% CI, 58.91–77.60]; \( P < 0.001 \)) and LQT2 (MD, 60.17 [95% CI, 50.18–70.16]; \( P < 0.001 \)). Subgroup analysis showed QTc interval response to abrupt stand testing and exercise testing varied between LQT1, LQT2, and LQT3, named Type I, Type II, and Type III.

CONCLUSIONS: QTc trend Type I and Type III during abrupt stand testing and exercise testing can be used to propose a prospective evaluation of LQT1 and LQT3, respectively. Type II QTc trend combined epinephrine infusion testing could distinguish LQT2 from control. A preliminary diagnostic workflow was proposed but deserves further evaluation.

Key Words: bicycle ■ diagnosis ■ epinephrine ■ genotyping ■ long QT syndrome ■ stand ■ treadmill

Congenital long QT syndrome (LQTS) is a potentially lethal cardiac channelopathy, characterized by prolonged QT interval on electrocardiography and predisposition to ventricular arrhythmia and sudden cardiac death.¹ To date, >17 genes have been identified as LQTS-causing genes, but recent expert opinion disputes the validity of several of them. The most common subtypes are caused by mutations in KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3), accounting for ≈95% of genotype-positive LQTS cases, whereas 75% to 80% account for clinically definite LQTS cases.² ³ Schwartz score, combining characterizations of electrocardiography abnormalities, clinical parameters, and family history, is widely used in the diagnosis of LQTS.⁴ However, the diagnosis is particularly challenging in patients who were asymptomatic (asymptomatic LQTS) or had normal to borderline QTc interval at rest (concealed LQTS), but were still...
at significant risk of ventricular arrhythmia and sudden cardiac death. Consequently, there is an urgent need for an alternative method for the diagnosis of LQTS. Provocative testing, including exercise stress testing (treadmill test, bicycle test), abrupt stand testing, and epinephrine QT stress testing, has been explored as a means of unmasking the characterization of patients with LQTS. QTc interval changes in provocative testing have been proposed as meaningful parameters to facilitate diagnosis and genotyping of LQTS but without uniform standards between studies. Therefore, we performed a systematic review and meta-analysis of currently available medical literature, for 3 objectives: (1) to validate the differences between patients with LQTS and healthy population in provocative testing; (2) to explore the characteristics of patients with LQT1, LQT2, and LQT3 in provocative testing; and (3) to propose a diagnostic workflow for diagnosis and genotyping of LQTS.

METHODS
Search Criteria
All authors declare that the supporting data are available upon reasonable request. Our systematic review and meta-analysis were performed in accordance with the guidelines from the Preferred Reporting Items of Systematic Review and Meta-Analysis27 and Meta-analysis of Observational Studies in Epidemiology.28 A comprehensive search was conducted by using the electronic database PubMed, Embase, and the Cochrane Library without language restriction through October 14, 2021. The electronic search terms were as follows: provocative test, exercise, treadmill, bicycle, stand, epinephrine, and long QT syndrome. The reference lists of trials, systematic reviews, and meta-analyses were also manually screened for additional studies that fit our inclusion criteria. All citations were initially identified at the level of title and abstract, and the candidate list of articles was confirmed by reviewing the full text. Two authors (Y.Y. and L.T.T.) checked the citations separately, and had a discussion with the corresponding author (Z.P.) to achieve consensus if controversies existed.

Inclusion Criteria and Exclusion Criteria
Studies were included in this meta-analysis if (1) patients with LQTS and control (healthy population or low probability of LQTS) were both included in the study; (2) LQTS was diagnosed by clinical features and/or cardiac channel gene screening; (3) provocative testing was performed in both patients with LQTS and control; and (4) provocative testing included abrupt stand testing, treadmill test, bicycle test, or epinephrine infusion testing. Studies were excluded if (1) <10 patients with LQTS enrolled; (2) studies included either patients with LQTS or control; (3) the most appropriate studies were included, then duplicate groups were excluded; (4) studies in which QTc interval or ΔQTc could not be extracted, and conference abstracts were excluded; and (5) provocative testing including adenosine exposure, mental stress, Valsalva maneuver, and cold pressor test were excluded because fewer studies were insufficient for analysis.

Data Extraction and Quality Assessment
We used a predefined standard data-extraction form to collect information by 1 author (Y.Y.) and verified by another author (L.T.T.). The information for each trial included author, study design, enrolled population, sex, age, resting QTc, QTc formula, subtypes of LQTS, patients LQTS with cardiac events (CEs) and β-blocker, and types of provocative testing and specific protocols. QTc intervals at baseline, abrupt stand, peak exercise and recovery 4 to 5 minutes were extracted; recovery 4 to 5 minutes represented late recovery in exercise testing. Data in the scatter plots were extracted by GetData Graph Digitizer software. Of the included studies, 4 analyzed overt or concealed patients with LQTS separately, and the rest enrolled both
overt and concealed LQTS. To explore the pooled effect of QTc at different time points during provocative testing, ΔQTc was used to balance the differences of baseline QTc interval. ΔQTc was calculated by QTc interval at any time point minus QTc interval at supine before the test. The NIH Quality Assessment Tool for Case Series Studies was used to assess study quality.

Statistical Analysis
Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) was used for all data analyses. We used the fixed-effects model to pool data, assuming that the underlying effects in contributing studies are identical. Differences of QTc or ΔQTc between groups were expressed as mean difference (MD) with a 95% CI. Heterogeneity across each meta-analysis was evaluated by I², and values >50% were considered as a high degree of between-study statistical heterogeneity. P<0.05 was considered statistically significant.

RESULTS
Study Characteristics
Two authors independently screened titles and abstracts of 2253 citations yielded from electronic search. After removing the duplicate and irrelevant articles, 22 studies met the predefined inclusion criteria and were included in our meta-analysis (Figure 1A). Finally, a total of 1137 patients with LQTS were included; 42% were asymptomatic LQTS, 18% were symptomatic, and clinical symptoms were not mentioned in 40% of patients (Figure 1B). In terms of LQTS subtypes, the percentage of LQT1, LQT2, and LQT3 were 50%, 39%, and 7%, respectively (Figure 1C). The characteristics of all included studies are summarized in Table 1. Among these studies, 9 studies evaluated the effect of treadmill exercise testing, 3 of which completed abrupt stand testing at the same time. 5,6,8 5 focused on bicycle test, 14-18 4 performed abrupt stand testing, 18-22 and 4 concerned epinephrine QT stress testing. 23-26 Of enrolled patients with LQTS, the age ranged from 0 to 64 years old, <20% with CEs, and >21% with β-blocker treatment. Details of another provocative testing (adenosine exposure, 38 mental stress, 18,39 Valsalva maneuver, 40,41 and cold pressor test 41,45), which is excluded from our meta-analysis, have been presented in Table 2.

Effect of Exercise Stress Testing and Abrupt Stand Testing on QTc Interval
To determine the pooled effect of exercise stress testing and abrupt stand testing on QTc interval, we performed forest plots at baseline, peak exercise, recovery 4 to 5 minutes, and abrupt stand. As shown in Figure 2A, at baseline, the QTc interval was 40 ms longer in patients with LQTS than in control (MD, 40.54 [95% CI, 37.43–43.65]; I²=94%, P<0.001). Compared with control group, patients with LQTS had nearly 30 ms longer ΔQTc both at peak exercise (MD, 27.31 [95% CI, 21.51–33.11]; I²=89%, P<0.001) and recovery 4 to 5 minutes (MD, 29.85 [95% CI, 24.36–35.35]; I²=92%, P<0.001), as presented in Figure 2B and 2C. As shown in Figure 2D, patients with LQTS had >29 ms longer ΔQTc at the abrupt stand (MD, 28.82 [95% CI, 23.05–34.58]; I²=89%, P<0.001) than the control group.

Effect of Exercise Stress Testing on QTc Interval in Different Subtypes of LQTS
To facilitate genotyping of LQTS, we performed a subgroup analysis of the 3 most common LQTS (LQT1, LQT2, and LQT3). As shown in Figure 3A and 3B, ΔQTc was nearly 40 ms longer both at peak exercise (MD, 36.72 [95% CI, 33.08–40.36]; P<0.001) and recovery 4 to 5 minutes (MD, 39.72 [95% CI, 35.06–44.38]; P<0.001) in patients with LQT1 than control. In contrast, ΔQTc were shorter at peak exercise (MD, −8.84 [95% CI, −12.62 to −5.07]; P<0.001), but longer at recovery 4 to 5 minutes (MD, 11.69 [95% CI, 6.48–16.91]; P<0.001) in patients with LQT2 (Figure 3C and 3D). Unlike subjects with LQT1 or LQT2, ΔQTc in patients with LQT3 was both shorter at peak exercise (MD, −14.06 [95% CI, −29.02 to 0.89]; I²=8%, P=0.07), and recovery at 4 to 5 minutes (MD, −15.84 [95% CI, −28.10 to −3.58]; I²=90%, P<0.001), which was confined to a limited population. Results are shown in Figure 3E and 3F.

Effect of Epinephrine Infusion on QTc Interval
With epinephrine infusion, the QTc interval was prolonged both in control and patients with LQTS, while patients with LQTS had 70 ms longer ΔQTc than control (MD, 70.16 [95% CI, 58.80–81.52]; I²=3%, P<0.001), as shown in Figure 4A. In terms of the most common LQTS subtypes, patients with both LQT1 and LQT2 had longer ΔQTc than control, more obviously in LQT1 (MD, 68.26 [95% CI, 58.91–77.60]; P<0.001) than LQT2 (MD, 60.17 [95% CI, 50.18–70.16]; P<0.001). On the contrary, in patients with LQT3 with epinephrine infusion, ΔQTc were remarkably shorter than control (MD, −17.36 [95% CI, −30.01 to −4.63]; I²=35%, P<0.001), which was consistent with exercise stress testing, as shown in Figure 4B through 4D.

Trend of ΔQTc in Provocative Testing
As Figure 5A shows, in the control population, abrupt stand prolonged QTc interval by 40 ms; in the
Yang et al Provocative Testing in LQTS

subsequent exercise testing, either at peak exercise or recovery 4 to 5 minutes, QTc interval was consistent with baseline. As Figure 5B shows, in the population with LQTS, abrupt stand prolonged QTc interval by nearly 70 ms, while QTc interval at peak exercise or recovery 4 to 5 minutes were prolonged mildly.

To clarify the manifestations of LQTS subtypes, we analyzed LQT1, LQT2, and LQT3, separately. In patients with LQT1, abrupt stand prolonged QTc interval by 40 ms, the QTc interval was also prolonged by 40 ms at peak exercise, and 30 ms at recovery 4 to 5 minutes (Figure 5C). In patients with LQT2, abrupt stand prolonged QTc interval by nearly 50 ms, and QTc interval was nearly consistent with baseline at peak exercise and recovery 4 to 5 minutes (Figure 5D). In patients with LQT3, QTc interval was both shortened at peak exercise and recovery 4 to 5 minutes (Figure 5E). Unfortunately, no data were available on QTc interval by abrupt stand in patients with LQT3. Moreover, QTc interval was prolonged in both control and patients

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**Figure 1.** Flowchart of literature search and distribution of enrolled LQTS population. A, Flowchart of literature search; (B) Distribution of symptoms of patients with LQTS; (C) Distribution of subtypes of LQTS, others included other subtypes, unclassified and LQTS subtype not applicable. LQTS indicates long QT syndrome; and NA, not applicable.
Table 1. Characteristics and Quality Analysis of Included Studies

| Author, y Design | Population, n | Male, n | Age, y | QTc formula | Subtype | CEs | β-blocker | Testing Protocols | Quality assessment |
|------------------|---------------|---------|--------|-------------|---------|-----|---------|-------------------|-------------------|
| Roston, 20216 Re | Asymptomatic LQT 14 | 7 | 1 | <19 | Bazett's | 475 (434–500) | LQT1: 9 | LQT2: 4 | LQT8: 1 | 0 | 0 | Treadmill exercise test QT-stand test | BOCH or Bruce | Good |
| Takahashi, 2020 | Asymptomatic LQT 20 | 9 | 8 | 11.2±3.4 | Fridericia's | 510±43 | LQT1: 3 | LQT2: 3 | LQT3: 12 | LQT7: 1 | LQT8: 1 | 0 | 0 | Treadmill exercise test Cold-water face immersion | Bruce | Good |
| Patel, 2020 | Concealed LQT 21 | 9 | 44 | ≤21 | Bazett's | 446±23 | LQT1: 13 | LQT2: 5 | LQT3: 3 | Syncope: 5 | VA: 2 | 15 | Treadmill exercise test | Bruce | Good |
| Wu, 2013 | LQT 18 | 5 | 19 | 8–47 | 8–41 | Bazett's | 521±65 | LQT1: 1 | LQT2: 15 | NA | NA | Treadmill exercise test | Bruce | Fair |
| Sy, 2011 | Asymptomatic LQT 48 | 26 | 35±18 | Bazett's | 47.7±38 | LQT1: 28 | LQT2: 20 | 0 | 23 | Treadmill exercise test or bicycle ergometry test | Bruce | Good |
| Homier, 2011 | LQT 155 | 59 | 38 | 25±14 | 21±13 | Bazett's | 468±31 | LQT1: 82 | LQT2: 55 | LQT3: 18 | NA | 71 | Treadmill exercise test | Bruce | Good |
| Wong, 2010 | LQT 95 | 33 | 25 | Median 26 | Bazett's | 471±36 | LQT1: 461 (43) | LQT2: 482 (99) | LQT1: 50 | LQT2: 45 | NA | NA | Treadmill exercise test, bicycle ergometry test | Bruce | Good |
| Takenaka, 2003 | LQT 82 | 27 | 17 | Mean 28–32 | Bazett's | 510±68 | LQT1: 510±68 | LQT2: 520±61 | LQT1: 51 | LQT2: 31 | 47 | 0 | Treadmill exercise test | Bruce | Good |
| Shimizu, 1991 | LQT 11 | 3 | 5 | 10–45 | Bazett's | 555±60 | NA | 10 | NA | Treadmill exercise test Isoproterenol infusion | Bruce | Fair |
| Vink, 2021 | LQT 47 | 18 | 47 | 12 (8–15) | 10 (7–14) | Bazett's | 466±36 | LQT1: 26 | LQT2: 19 | LQT3: 2 | 3 | 9 | Abrupt standing test | Viskin protocol | Good |
| Muñoz-Esparza, 2017 | LQT 36 | 18 | 19 | 36±17 | 39±15 | Bazett's | 450±31 | LQT1: 6 | LQT2: 20 | LQT7: 3 | Unclassified: 7 | N/A | 26 | Abrupt standing test | Viskin protocol | Fair |
| Ben Bassat, 2013 | LQT 69 | 18 | 46 | 31±14 | 35±10 | Bazett's | 471±39 | LQT1: 31 | LQT2: 28 | LQT7: 3 | Unclassified: 6 | N/A | N/A | Abrupt standing test | Viskin protocol | Good |

Continued
Table 2. Continued

| Author, y | Design | Population, n | Male, n | Age, y | QTc formula | Subtype | CEs | β-blocker | Testing | Protocols | Quality assessment |
|-----------|--------|---------------|---------|--------|-------------|---------|-----|-----------|---------|-----------|-------------------|
| Adler, 2012 | Re | LQT 108 Control 112 | 41 | 59 | 30±16 33±12 | Bazett’s | 469±40 | LQT1: 46 LQT2: 47 LQT3: 12 Unclassified: 3 | 28 | NA | Abrupt standing test | Viskin protocol | Good |
| Charisopoulou, 2019 | Re | LQT 47 Control 35 | 22 | 16 | 45±15 47±13 | Bazett’s | LQT 453±42 | LQT1: 36 LQT2: 11 | 20 | 25 | Bicycle ergometer test | Gradual increase | Good |
| Aziz, 2011 | NA | LQT 50 Control 108 | 20 | 45 | ≤21 | Bazett’s | LQT1 452±33 LQT2 47±35 | LQT1: 29 LQT2: 21 | 13 | 28 | Bicycle ergometer test | Gradual increase | Good |
| Chattha, 2010 | NA | LQT 50 Control 25 | 18 | 9 | 33±16 | Bazett’s | LQT1 367±32 LQT2 465±25 | LQT1: 25 LQT2: 25 | 14 | 28 | Bicycle ergometry test | Burst and gradual increase | Good |
| Walker, 2005 | NA | LQT 31 Control 31 | 8 | 9 | 16–45 | Bazett’s Concealed: 508±36 Overt: 496±69 | LQT1 413±14 LQT2 13±18 Unknown: 15 | 17 | 24 | Bicycle ergometer test | Burst | Good |
| Paavonen, 2001 | Re | Asymptomatic LQT 30 Control 14 | 13 | 8 | 13–59 22–58 | Fridericia’s | LQT1 429±31 LQT2 417±43 | LQT1: 3 LQT2: 13 Unknown: 15 | 0 | 0 | Bicycle ergometer test Mental stress | Burst and gradual increase | Good |
| Hekkala, 2012 | NA | Asymptomatic LQT 30 Control 15 | 15 | 7 | 20–53 | Bazett’s | LQT 413±25 | LQT1: 10 LQT2: 10 LQT3: 10 | 0 | 0 | Epinephrine Test Bolus infusion | Good |
| Vyas, 2006 | Re | LQT 81 Control 44 | 34 | 14 | 8–59 | Bazett’s | LQT1 456 (397–517) LQT2 486 (388–644) LQT3 473 (424–502) | LQT1: 40 LQT2: 30 LQT3: 11 | NA | 0 | Epinephrine test Incremental infusion | Good |
| Shimizu, 2004 | Pro | LQT 60 Control 30 | 24 | 14 | 4–64 | Bazett’s | LQT1 470±41 LQT2 503±33 LQT3 506±41 | LQT1: 31 LQT2: 23 LQT3: 6 | 28 | 0 | Epinephrine test | Bolus infusion+ continuous | Good |
| Shimizu, 2003 | NA | Overt LQT 19 Concealed LQT 15 Control 15 | 5 | 8 | Mean 22–31 | Bazett’s Overt 507±31 Concealed 427±21 | LQT1: 34 | 15 | 0 | Epinephrine test | Bolus+ continuous infusion | Good |

BCCH, British Columbia Children’s Hospital; CEs, cardiac events (syncope, sudden cardiac arrest, or ventricular arrhythmia); HR, heart rate; LQTS, long QT syndrome; NA, not applicable; Pro, prospective; and Re, retrospective.
### Table 2. Characteristics of Studies With Other Provocative Tests

| Author, y | Population, n | Age, y | LQTS subtype (n) | Testing | QTc in control (ms) | QTc in LQTS (ms) |
|-----------|----------------|--------|------------------|---------|---------------------|------------------|
|           |                |        |                  |         | Before | After | Before | After |
| Viskin, 2006\[16\] | LQT 18 Control 20 | LQT 32.7±15.8 Control 31.4±12.2 | NA | Adenosine test | 399±29 | 458±58 | 454±24 | 569±53 |
| Etienne, 2018\[17\] | LQT 36 Control 34 | Median 41±2.1 | LQT1: 20 LQT2: 16 | Mental stress test | ΔQTc 24 (10, 40) | ΔQTc 67 (44, 91) |
| Paavonen, 2001\[18\] | LQT 30 Control 14 | LQT1 35±13 LQT2 41±1 Control 43±10 | LQT1: 16 LQT2: 14 | Mental stress test | ΔQTc 24±26 | LQT1 ΔQT, 27±36 LQT2 ΔQT, 30±47 |
| Mitsutake, 1981\[19\] | LQT 8 Control 9 | LQT 39±7 Control 27±2 | NA | Valsalva strain | 500±30 | 570±60 | 450±20 | 480±20 |
| Eggeling, 1993\[20\] | LQT 14 Control 14 | NA | NA | Valsalva maneuver | 412±25 | 407±18 | 489±55 | 497±49 |
| Takahashi, 2020\[21\] | LQT 20 Control 22 | Mean 11.2±3.4 | LQT3: 12 Non-LQT3: 8 | Cold-water face immersion | / | Ratio of min/max HR 0.47 (0.43, 0.52) | / | Ratio of min/max HR 0.49 (0.45, 0.51) |
| Yoshinaga, 1999\[22\] | LQT 10 Control 48 | LQT 11.7±2.8 Control 12.6±2.3 | NA | Cold-water face immersion | 420±20 | 390±30 | 560±60 | 600±30 |
| Shimizu, 1991\[23\] | LQT 11 Control 12 | LQT 10–45 Control 15–28 | NA | Isoproterenol infusion | 399±10 | 436±13 | 466±50 | 556±33 |

HR, heart rate; LQTS, long QT syndrome; and NA, not applicable.
with LQTS with epinephrine infusion, especially in patients with LQT1 and LQT2 (Figure 5F).

**Diagnostic Workflow for Diagnosis and Genotyping of LQTS**

Based on the current meta-analysis, we combined baseline electrocardiogram (ECG) (QTc interval) as the first step, abrupt stand testing plus exercise testing (QTc trend) as the second step, and epinephrine testing (ΔQTc) as the third step to diagnosis and genotyping of LQTS (Figure 6A). First, based on QTc interval in baseline ECG, overt LQTS was distinguished from concealed LQTS or normal population, because QTc ≥480 ms in women or ≥470 ms in men was determined with 100% specificity for the diagnosis of LQTS (Figure 2).

**Figure 2. Effect of exercise stress testing and abrupt stand testing on QTc interval.**

A, QTc interval at baseline; B ΔQTc at peak exercise; C ΔQTc at recovery 4 to 5 minutes; D ΔQTc at abrupt stand. LQTS indicates long QT syndrome; and SD, standardized difference.
Figure 3. Effect of exercise stress testing on QTc interval in different subtypes of LQTS.

A. ΔQTc of LQT1 at peak exercise; (B) ΔQTc of LQT1 at recovery 4 to 5 minutes; (C) ΔQTc of LQT2 at peak exercise; (D) ΔQTc of LQT2 at recovery 4 to 5 minutes; (E) ΔQTc of LQT3 at peak exercise; (F) ΔQTc of LQT3 at recovery 4 to 5 minutes. LQTS indicates long QT syndrome; and SD, standardized difference.
LQTS. While in family members with LQTS, considering a priori likelihood, QTc ≥430 ms can be used as a cutoff to distinguish carriers from noncarriers. Then, QTc trend in abrupt stand testing plus exercise testing (treadmill or bicycle) were used to genotype LQTS, in which the QTc trend exhibited 3 types, named Type I, Type II, and Type III. In Type I (Figure 6B), the QTc interval increased markedly in abrupt stand and peak exercise, and persists despite HR recovery during the recovery period, seen in patients with LQT1; in type II (Figure 6C), the QTc interval increased markedly in the abrupt stand, shortened slightly in peak exercise, and lengthened mildly during the recovery period, seen in patients with LQT2 or normal population, while in type III (Figure 6D), the QTc interval shortened during the entire test, and persists during the recovery period despite HR recovery, seen in patients with LQT3. Diagnosis of LQTS was particularly challenging in patients with normal or borderline QTc interval in baseline ECG and presented type II QTc trend in abrupt stand testing plus exercise testing. Consequently, we propose that an epinephrine testing be performed in patients suspected to have LQTS, in whom larger ΔQTc would suggest LQT2, and smaller ΔQTc suggests unaffected individuals.

DISCUSSION

To the best of our knowledge, this is the first attempt to determine the pooled utility of provocative testing in the diagnosis and genotyping of LQTS. The main findings of the present meta-analysis are as follows: (1) Insufficient QT interval shortening in response to tachycardia, delayed QT interval recovery at the late recovery of exercise, is of great value for distinguishing LQTS.
from control. (2) QTc interval response to abrupt stand testing and exercise testing differed between LQTS subtypes (Type I, Type II, and Type III), which is of additional value for genotyping LQTS. (3) Epinephrine infusion testing prolonged QTc interval both in control and patients with LQTS, most obviously in patients with LQT1 and LQT2, which is a potential powerful test for diagnosis and genotyping of LQTS. (4) We first draft a diagnostic workflow of LQTS, which may be helpful for the diagnosis and genotyping of LQTS, but still deserves further evaluation.

Based on the classic Schwartz score, diagnosis of LQTS is relatively straightforward in patients with overt QT prolongation or clinical symptoms. However, 25% to 50% of LQTS carriers have a normal or borderline resting QTc (concealed LQTS) because of a combination of variable penetrance, the effect of genes modification, and the dynamic nature of QT interval.5 Furthermore, up to 40% of individuals with congenital LQTS are “silent mutation carriers” (asymptomatic LQTS), who are exposed to a 10 times higher risk of CEs than unaffected family members.5,47 Thus, accurate identification of concealed LQTS and asymptomatic LQTS is of great importance, despite being particularly challenging. Genetic testing has been regarded as the criterion standard for diagnosis and genotyping of LQTS, but it is restricted by its cost and time delay. Consequently, several studies tried to evaluate additional readily applicable clinical tests, including abrupt stand testing,19 treadmill test,13 bicycle test,18 epinephrine QT stress testing,26 adenosine exposure,38 mental stress,18 Valsalva maneuver,49 and cold-water face immersion.7

Sympathetic activation has been considered an important trigger of malignant arrhythmia in patients with LQTS, and β-blockers or left cardiac sympathetic denervation have been recommended to reduce the occurrence of CEs,1 indicating that the autonomic nervous (sympathetic and parasympathetic nervous) system may influence the electrophysiological properties of the myocardium. Studies have shown that provocative testing, including the most common abrupt stand testing, exercise testing (treadmill and bicycle), and epinephrine infusion testing, plays an important role in diagnosis and risk stratification of LQTS.5,16,19,24,48 In terms of provocative tests, treadmill or bicycle tests are accessible in most pediatric and adult cardiology centers, both with a variety of protocols (gradual or burst increase), and can be recorded with ECG in supine position, upon standing, and any time during exercise.10 Studies showed that QTc upon standing, peak exercise, and late recovery are more sensitive markers of genotype-specific repolarization responses than other times during exercise.5,8

In our meta-analysis, we found that LQT1-3 corresponds to 3 types of QTc trend during abrupt stand testing plus exercise testing, LQT1 presented increased QTc upon standing and peak exercise, and persists in late recovery (type I); LQT3 presented shortened QTc.
during the entire test (Type III), while LQT2 was similar to control, QTc increased upon standing, and returned to baseline or mildly shortened at peak exercise, and gradually increased in late recovery (Type II). The patterns of Type I and Type II had been found in previous studies, but without graphic description. Despite patients with LQT2 having 9 ms shorter ΔQTc at peak exercise, and 12 ms longer ΔQTc at late recovery than control, these differences were not enough to distinguish patients with LQT2 from control, especially concealed LQT2. Furthermore, given the relative rarity of LQT3, our analysis enrolled only 74 patients with LQT3; thus the QTc trend of Type III deserves further evaluation.

In contrast, the epinephrine infusion testing, whether by bolus infusion (Shimizu protocol) or an incremental infusion (Mayo protocol), should be performed at LQTS specialty centers and requires a period of β-blockers washout, which were not needed in exercise testing. Magnano found that QTc was prolonged by epinephrine (79±40 ms) in healthy subjects. In our meta-analysis, epinephrine infusion testing prolonged QTc both in control and patients with LQTS, most obviously in LQT1 and LQT2, up to 60 ms difference of ΔQTc between patients with LQT1/LQT2 and control, which is a meaningful marker for distinguishing LQT1/LQT2 from control, covering the shortage of exercise testing.
Although all of these studies provided a meaningful theoretical basis, the appropriate cut-off of ΔQTc is still unknown.23–25 Furthermore, in the Shimizu protocol, electrocardiography data were collected under baseline and at steady-state conditions of epinephrine26; while in the Mayo protocol, ΔQTc were calculated by the difference between the maximal and minimal QTc.24 Whether the range of ΔQTc is consistent between different protocols is still unknown. Because of its arrhythmia risk and controversial evidence, guidelines recommend limited levels of epinephrine testing in the evaluation of a cardiac arrest survivor (class 2b).50 Therefore, in our diagnostic workflow, epinephrine testing is proposed for use in patients with normal or borderline QTc in baseline ECG and presented Type II QTc trend in exercise testing, in which patients with overt ΔQTc were probably concealed LQT2, otherwise, were normal population, which deserve further genetic testing validation.

Limitations
Given the nature of our study as a systematic review and meta-analysis, our analyses are limited by the reported data in the original reports. Limitations of our meta-analysis are as follows: (1) Given the relative rarity of LQTS, we only included 1137 patients with LQTS in our analysis; these mainly included patients with LQT1 and LQT2, and analysis enrolled only 74 patients with LQT3, so the pattern of Type III QTc trend warrants further evaluation. (2) The data were presented differently between studies, restricting analysis of optimal cut-off, and corresponding sensitivity and specificity, especially epinephrine infusion testing, making our diagnostic workflow of limited diagnostic value. (3) Fewer studies analyzed concealed or asymptomatic LQTS separately, restricting analysis of the diagnostic value of the provocative tests in concealed or asymptomatic LQTS. (4) Patients with LQTS have a changed risk of CEs as time goes on, so the different reactions of provocative testing between adolescents and children remain unknown. (5) Most of our included studies were retrospective cohort, only with derivation cohort, and lacked external validation, which inherently limited the quality of evidence. (6) Our meta-analysis has high heterogeneity between studies, mainly because studies enrolled different subtypes of LQTS, different types of provocative tests, and differences in QTc measurement. Given the above, there is an urgent need for prospective, multicenter, large-scale trials to validate the diagnostic value of our diagnostic workflow, especially in concealed or asymptomatic LQTS.

CONCLUSIONS
QTc trend Type I and Type III during abrupt stand testing and exercise testing can be used to propose a prospective evaluation of LQT1 and LQT3, respectively. Type II QTc trend combined epinephrine infusion testing could distinguish LQT2 from control. A preliminary diagnostic workflow was drawn up, but deserves further evaluation.

REFERENCES
1. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, Blom N, Brugada J, Chiang C-E, Huikuri H, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. Heart Rhythm. 2013;10:1932–1963. doi:10.1016/j.hrthm.2013.05.014
2. Adler A, Novelli V, Amin AS, Abiassi E, Care M, Nannenberg EA, Felicker H, Amenta S, Mazzia D, Bikker H, et al. An international, multicentered, evidence-based reappraisal of genes reported to cause congenital long QT syndrome. Circulation. 2020;141:418–426. doi:10.1161/CIRCULATIONAHA.119.031332
3. Schwartz PJ, Ackerman MJ, George AL Jr, Wilde AAM. Impact of genetics on the clinical management of channelopathies. J Am Coll Cardiol. 2013;62:169–180. doi:10.1016/j.jacc.2013.04.044
4. Schwartz PJ, Crotti L, Insolia R. Long QT syndrome: from genetics to management. Circ Arrhythm Electrophysiol. 2012;5:868–877. doi:10.1161/CIRCEP.111.962019
5. Sy RW, van der Werf C, Chatta IS, Chockalingam P, Adler A, Healey JS, Perrin M, Gollob MH, Skanes AC, Yee R, et al. Derivation and validation of a simple exercise-based algorithm for prediction of genetic testing in relatives of LGTS probands. Circulation. 2011;124:2187–2194. doi:10.1161/CIRCULATIONAHA.111.082858
6. Roston TM, De Souza AM, Romans HV, Franciosi S, Armstrong KR, Sanatani S. Potential overdiagnosis of long QT syndrome using exercise stress and QT stand testing in children and adolescents with a low probability of disease. J Cardiovasc Electrophysiol. 2021;32:500–506. doi:10.1111/jce.14865
7. Takahashi K, Shimizu W, Makita N, Nakayashiro M. Dynamic QT response to cold-water face immersion in long-QT syndrome type 3. Pediatr Int. 2020;62:899–906. doi:10.1111/ped.14319
8. Patel TM, Kamande SM, Jarosz E, Bost JE, Hanumanthaiah S, Berul CI, Sherwin ED, Moak JP. Treadmill exercise testing improves diagnostic accuracy in children with concealed congenital long QT syndrome. Pacing Clin Electrophysiol. 2020;43:1521–1528. doi:10.1111/pac.14085
9. Wu CC, Zhang P, Gao Y, Yang J, Li XB, Li D, Wang L, Guo JH. Utility of treadmill testing in diagnosis and genotype prediction in long QT syndrome. Chin J Heart & Heart Rhythm (Electronic Edition). 2013;11:30–34.
10. Horner JM, Horner MM, Ackerman MJ. The diagnostic utility of recovery phase QTc during treadmill exercise stress testing in the evaluation of long QT syndrome. Heart Rhythm. 2011;8:1698–1704. doi:10.1016/j.hrthm.2011.05.018

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Affiliations
School of Clinical Medicine, Tsinghua University, Beijing, China (Y.Y., P.L., S.L., P.Z.); and Department of Cardiology, School of Clinical Medicine, Beijing Tsinghua Changgung Hospital, Tsinghua University, Beijing, China (T.L., S.L., P.Z.).

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27. Moher D, Liberati A, Tetzlaff J, Altman DG; Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009;62:1006–1012. doi: 10.1016/j.cej.2009.06.005

28. Stroup DF, Berlin JA, Morton SC, Olin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283:2020–2022. doi: 10.1001/jama.283.24.2020

29. Takahashi K, Nabeshima T, Nakayashiro M, Ganaha H. QT dynamics during exercise in asymptomatic children with long QT syndrome type 3. Pediatr Cardiol. 2016;37:860–867. doi: 10.1007/s00246-016-1360-4

30. Viskin S, Postema PG, Bhuyan ZA, Rosso R, Kalman JM, Vohra JK, Guevara-Valldevia ME, Marquez MF, Kogan E, Belhassen B, et al. The response of the QT interval to the brief tachycardia provoked by standing: a bedside test for diagnosing long QT syndrome. J Am Coll Cardiol. 2010;55:1955–1961. doi: 10.1016/j.jacc.2009.12.015

31. Sy RW, Chatta IS, Klein GJ, Gula LJ, Skanes AC, Yee R, Bennett MT, Kranz AD. Repolarization dynamics during exercise discriminate between LQT1 and LQT2 genotypes. J Cardiovasc Electrophysiol. 2010;21:1242–1246. doi: 10.1111/j.1540-8167.2010.01788.x

32. Li CL, Hu DY, Shi XB, Yang H, Wang JY, Mei YQ, Liu WL, Li L, Xu Y. The characteristics during exercise test in long QT syndrome patients and the effects of left cardiac sympathetic denervation. Nat Med J China. 2005;85:2192–2195.

33. Noda T, Takaki H, Kurita T, Suyama K, Nagaya N, Taguchi A, Alhara N, Kamakura S, Sunagawa K, Nakamura K, et al. Gene-specific response of dynamic ventricular repolarization to sympathetic stimulation in LQT1, LQT2 and LQT3 forms of congenital long QT syndrome. Eur Heart J. 2002;23:975–983. doi: 10.1053/euhj.2001.3079.

34. Ackerman MJ, Khositseth A, Tester DJ, Heiljk JB, Shen WK, Porter CB. Epinephrine-induced QT interval prolongation: a gene-specific paradoxical response in congenital long QT syndrome. Mayo Clin Proc. 2002;77:413–421. doi: 10.4065/77.5.413

35. Tanabe Y, Inagaki M, Kurita T, Nagaya N, Taguchi A, Suyama K, Alhara N, Kamakura S, Sunagawa K, Nakamura K, et al. Sympathetic stimulation produces a greater increase in both transmural and spatial dispersion of repolarization in LQT1 than LQT2 forms of congenital long QT syndrome. J Am Coll Cardiol. 2001;37:911–919. doi: 10.1016/S0735-1097(00)02120-6

36. Shimizu W, Kamakura S, Kurita T, Suyama K, Alhara N, Shimomura K. Influence of epinephrine, propranolol, and atrial pacing on spatial distribution of recovery time measured by body surface mapping in congenital long QT syndrome. J Cardiovasc Electrophysiol. 1997;8:1102–1114. doi: 10.1111/j.1540-8167.1997.tb00996.x.

37. Chorin E, Havakuk O, Adler A, Steinvil A, Rozovski U, van der Werf C, Postema PG, Topaz G, Wilde AAM, Viskin S, et al. Diagnostic value of T-wave morphology changes during "QT stretching" in patients with long QT syndrome. Heart Rhythm. 2015;12:2263–2271. doi: 10.1016/j.hrthm.2015.06.040

38. Viskin S, Rosso R, Rogowski O, Belhassen B, Levitas A, Wagschal A, Katz A, Fourey D, Zeltser D, Oliva A, et al. Provocation of sudden heart rate oscillation with adenosine exposes abnormal QT responses in patients with long QT syndrome: a bedside test for diagnosing long QT syndrome. Eur Heart J. 2006;27:469–475. doi: 10.1093/eurheartj/ehi460

39. Etienne P, Huchet F, Gabort N, Barc J, Thollet A, Kyncht F, Gouymarch B, Le Marec H, Charpentier F, Schott J- P, et al. Mental stress test: a rapid, simple, and efficient test to unmask long QT syndrome. Europace. 2018;20:2014–2020. doi: 10.1093/europace/evy078.

40. Mitutake A, Takeshita A, Kuroiwa A, Nakamura M. Usefulness of the Valsalva maneuver in management of the long QT syndrome. Circulation. 1981;63:1029–1035. doi: 10.1161/01.CIR.63.5.1029

41. Eggeling T, Osterheus HH, Kochs M, Beyer M, Höher M, Hombach V. The diagnostic value of standard ECG methods, the cold-pressure test and Valsalva maneuver in idiopathic QT syndrome. Z Kardiol. 1993;82:1–7.

42. Yoshinaga M, Kamimura J, Fukushige T, Kusubae R, Shimago A, Nishi Y, Kono Y, Nomura Y, Miyata K. Face immersion in cold water induces QT interval prolongation: a bedside test for diagnosing long QT syndrome. J Electrocardiol. 2006;39:235–240. doi: 10.1016/j.jelecard.2005.03.011

43. The NIH Quality Assessment Tool for Case Series Studies. Available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools. Accessed November 1, 2018. doi: 10.1542/474X-2005.00077.x.
44. Rice K, Higgins JPT, Lumley T. A re-evaluation of fixed effect(s) meta-analysis. *J R Stat Soc*. 2018;181:205–227. doi: 10.1111/j.1540-8167.1991.tb01332.x

45. Vincent GM, Timothy KW, Leppert M, Keating M. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. *N Engl J Med*. 1992;327:846–852. doi: 10.1056/NEJM199209173271204

46. Hofman N, Wilde AA, Kaab S, van Langen IM, Tanck MW, Mannens MM, Hinterseer M, Bockmann BM, Tan HL. Diagnostic criteria for congenital long QT syndrome in the era of molecular genetics: do we need a scoring system? *Eur Heart J*. 2007;28:575–580. doi: 10.1093/eurheartj/ehm355

47. Goldenberg I, Horr S, Moss AJ, Lopes CM, Barsheshet A, McNitt S, Zareba W, Andrews ML, Robinson JL, Locati EH, et al. Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. *J Am Coll Cardiol*. 2011;57:51–59. doi: 10.1016/j.jacc.2010.07.038

48. Nemec J, Hejlik JB, Shen WK, Ackerman MJ. Catecholamine-induced T-wave lability in congenital long QT syndrome: a novel phenomenon associated with syncope and cardiac arrest. *Mayo Clin Proc*. 2003;78:40–50. doi: 10.4065/78.1.40

49. Magnano AR, Talathoti N, Halir R, Bloomfield DM, Garan H. Sympathomimetic infusions and cardiac repolarization: the normative effects of epinephrine and isoproterenol in healthy subjects. *J Cardiovasc Electrophysiol*. 2006;17:983–989. doi: 10.1111/j.1540-8167.2006.00555.x

50. Stiles MK, Wilde AAM, Abrams DJ, Ackerman MJ, Albert CM, Behr ER, Chugh SS, Cornel MC, Gardner K, Ingles J, et al. 2020 APHRS/HRS expert consensus statement on the investigation of decedents with sudden unexplained death and patients with sudden cardiac arrest, and of their families. *Heart Rhythm*. 2021;18:e1–e50. doi: 10.1016/j.hrthm.2020.10.010