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# Sex influences on ventricular repolarization duration in normal subjects and in type 1, 2 and 3 long QT syndrome patients: Different effect in acquired and congenital type 2 LQTS

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ABSTRACT

*Aim:* To evaluate the interaction between sex and rate corrected QT interval (QTc) duration in normal subjects after drug-induced QT prolongation and in LQTS patients.
*Methods:* Semi-automated measurements were performed on 875 digital ECGs (200 normal subjects off drugs (100 females), 200 normal subjects on Moxifloxacin (100 females), 259 LQT1 patients (161 females), 183 LQT2 patients (100 females) and 33 LQT3 patients (15 females)). A sex specific coefficient was calculated in each group and was used to calculate group specific corrected QT intervals (QTci).
*Results:* The mean sex difference (female minus male) in QTci interval duration was 17 ms 95%CI(12.7; 21.3) in normal subjects, 19 ms (14.5; 23.5) on Moxifloxacin, and 13 ms (4.8; 21.2) in LQT1 patients. The mean difference was 2 ms (−7.9; 11.9) in LQT2 and − 5 ms (−32.2; 22.2) in LQT3 patients ($p = 0.0067$ for the group and sex interaction).
In the subgroup of patients above 15 years and without beta blocker treatment, the sex effect (female minus male) on QTci interval duration was 17 ms (4.1; 29.9) in LQT1 patients. QTc duration was not different between sex in LQT2 and in LQT3 patients (mean difference − 3 ms (−21.6; 15.6) and 12 ms (−28.4; 52.4), respectively) ($p = 0.0191$ for group and sex interaction).
*Conclusions:* The interaction between sex and QTc interval is preserved in type 1 LQTS and drug-induced QTc prolongation but blurred in type 2 LQTS. Further experimental studies are warranted to better understand the interaction of sexual hormones with malfunctioning KCNH2 encoded repolarizing potassium channel.


## Introduction

Heart rate corrected QT interval duration (QTc) is longer in adult females than in adult males. Because this sex-related difference in QTc is evidenced only after puberty, this effect is believed to be related to sex hormones [1–3]. Indeed, testosterone shortens ventricular action potential duration (and thereby the QT interval duration) while female sexual hormones have more complex effect on action potential duration with lesser net effect on QT duration [4,5].

Sex hormones effects on action potential duration are the consequence of their interaction with voltage gated ionic channels implicated in the repolarization process [4,5]. The affected channels and currents (mainly IKr and IKs) are those which malfunctioning causes the most frequent types of congenital long QT syndrome (LQT1 and 2) [6,7]. This raises the question of the impact of LQTS mutations on sex differences in QT interval duration.

Different studies, with different methodologies, all describe genotype specific differences in sex effects on QT interval but the interaction between sex, age, LQTS genotype and QTc duration appears complex and sometimes inconsistent between studies [8–11].

Kligfield et al. studied differences in ECG intervals measured by digital electrocardiographs from 4 manufacturers including 200 digital ECGs from our LQTS patient database (pooling LQT1 and LQT2 genotypes) [12]. A second ECG algorithm project was conducted with the previous 4 algorithm developers, and 3 additional manufacturers using a new dataset from the CSRC ECG Warehouse. A total of 800 ECGs were equally divided among normal, moxifloxacin and genotyped congenital long QT type 1 (LQT1) and type 2 (LQT2). Similarly, the mean

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value of paired differences among algorithms was higher in LQTS patients compared to the normal groups with QT differences up to 10.5 and up to 12.8 ms in the LQT1 and LQT2 groups, respectively [13].

In the more recent study [13], unpublished data showed that, as expected, automated uncorrected QT interval duration was longer in females than in males in normal subjects, after Moxifloxacin administration, and in LQT1 patients, but preliminary examination suggested that QT differences according to sex might be different in different QT genotypes. A sex related difference in rate corrected QT interval is well established, evidenced only after puberty, linked to direct sex hormones effects [2]. It is known that in normal subjects, testosterone shortens ventricular action potential duration (and thereby the QT interval duration) while female sex hormones have more complex effects on action potential duration and QT interval [5].

Therefore, we examined the relationship of sex to QT intervals in an expanded population of genotyped LQT subjects compared with normal subjects and normal subjects given moxifloxacin, using semi-automated ECG intervals measurements and population specific QT rate correction.

## Patients and methods

### *Normal subjects and LQTS patient selection*

The Cardiac Safety Research Consortium (CSRC) is a public-private partnership established as part of the FDA's Critical Path Initiative through a memo of understanding between the FDA and Duke Clinical Research Institute (DCRI) which attempts to bring together members from FDA, academia and industry in order to promote scientific knowledge relevant to cardiovascular safety of new drugs and devices. To facilitate the CSRC's mission, a centralized ECG repository was established for the upload of annotated ECG waveform XML data from thorough QT studies (TQT) and related studies submitted to the FDA during drug development by industry sponsors. A Scientific Oversight Committee (SOC) was established within the CSRC to assess research applications from investigators requesting CSRC ECG data to guarantee scientific credibility consistent with the CSRC's mission [14].

CSRC ECG data are currently comprised of digital XML waveforms only collected from healthy volunteers at baseline, under placebo and Moxifloxacin (a fluoroquinolone blocker of the human cardiac potassium channel hERG used as positive control) in Thorough QT (TQT) studies (excluding ECG waveforms from the sponsor's proprietary drug arm). Currently, 20 TQT studies have been released by sponsors for incorporation into the CSRC ECG Data Warehouse, encompassing 123,824 ECGs from 1370 subjects aged at least 18 (average age 35.1 years) and 51% male [14]. More challenging data submitted by our group are now available including >1000 digital resting ECGs and Holter recording from patients with genotyped variants of congenital long QT syndrome (LQTS).

The ECGs from healthy volunteers in the present study were those used in the previous CSRC algorithm comparison study [13]. They had been randomly selected from available digital XML ECGs, consisting of 2 sub-groups: ECG collected under placebo or at baseline (200 subjects, 100 females), and a separate group of ECG collected at expected peak Moxifloxacin concentration (another 200 subjects, 100 females).

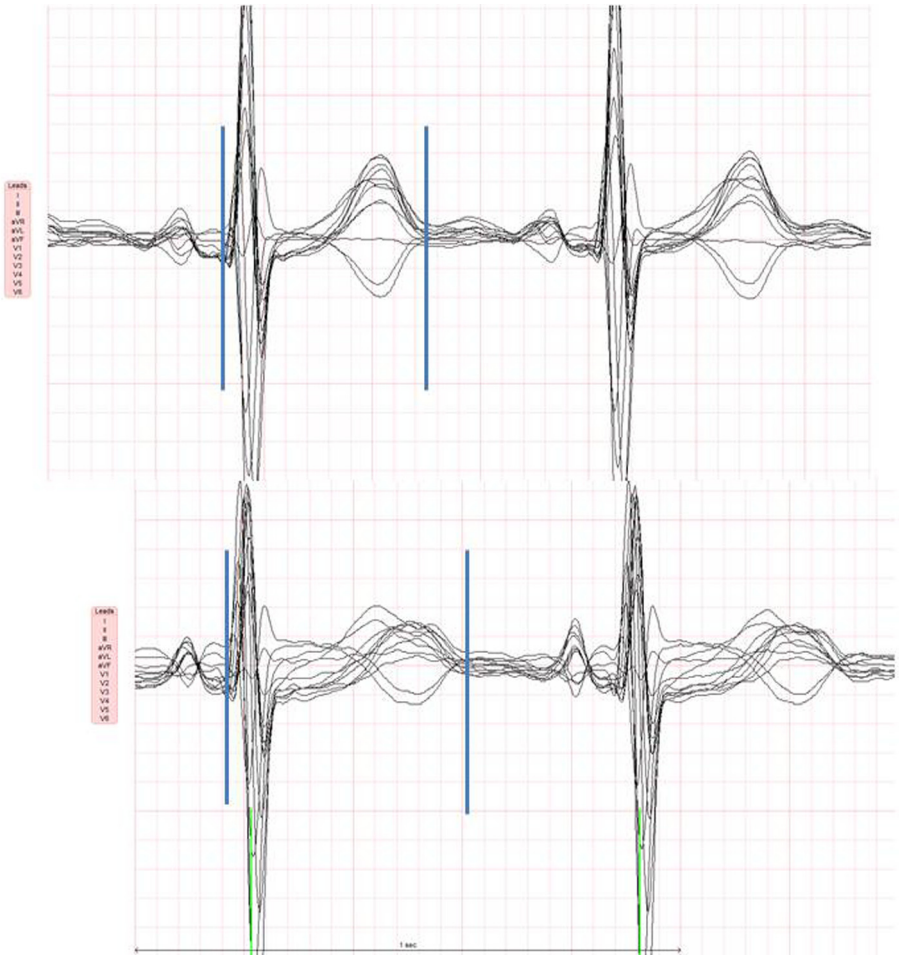

**Fig. 1.** 12-lead butterfly ECG display in a normal subject (upper panel) and in a LQT2 patient (lower panel).

For comparison, a new set of digital LQTS ECGs was extracted from our database. The database contains more than 1000 digital ECGs from 506 genotype positive LQTS patients. ECGs from patients with double mutations (homozygous KCNQ1 mutations or heterozygous KCNQ1 and KCNE2 mutations) were excluded. For a given LQTS patient, we selected the first ECG collected in chronological order. Accordingly, age of recording ranged from birth to above 80 year-old.

*ECG measurements*

All digital ECG were obtained in XML format. ECG waveforms were first automatically analyzed the CalECG software (AMPS LLC, NY, NY).

The position of automated cursors (R wave detection, QRS onset and T offset) was subsequently visually reviewed by two independent readers (FE & PMB) using a 10-s display for R-wave detection and a 12-lead butterfly display for QRS onset and T offset and electronic calipers were moved if needed (Fig. 1).

When the absolute value of QT difference between the 2 readers was <10 ms, the mean of the 2 values was retained as the final QT interval. In case of absolute difference in QT duration ≥10 ms, a single reconciliation session was performed by the 2 readers and the resulting QT interval duration was retained.

Semi-automated measured QT interval durations and corresponding mean RR intervals (mean of all RR intervals over 10 s) were used to calculate heart rate corrected QT interval (QTc with QTc = measured QT / (RR interval in seconds)$^{\alpha}$). The α coefficient was 0.5 and 0.33 for the Bazett's correction (QTcB) and the Fridericia's correction (QTcF), respectively. In addition, a sex specific α coefficient was calculated in each group using log transformed RR and QT intervals values (α coefficient = slope of the linear LogQT/LogRR relationship). This sex- and group-specific α coefficient was used to calculate the sex- and group-specific corrected QT interval named QTci.

The QT1000 value was calculated from the linear relationship of the LogQT/LogRR plots and corresponds to QT duration at RR = 1000 ms in the sub-population.

*Statistical analysis*

ECG Data are presented as mean ± SD or mean change with 95% confident interval (95% CI) bounds. The mean differences were evaluated by a two-way analysis of variance. The two independent variables were sex and patient group (normal, Moxifloxacin, LQT1, LQT2, and LQT3). The two-way ANOVA was carried out to understand if there is an interaction between the two independent variables.

In LQTS patients, a subgroup analysis was undertaken because the effects of beta blocker treatment on QTc duration are gene dependent [15]. Hence, we examined separately the sex effect in two LQTS subgroups, those with and without beta blocker treatment at the time of the ECG recording. Given the repeatedly documented QTc shortening in males throughout puberty, whereas the QTc of females remains unchanged [2], we evaluated the sex effect on ventricular repolarization excluding patients before puberty.

Finally, the relationship between QTc duration and age was explored by using single regression analysis.

A $p$ value <0.05 was considered statistically significant.

## Results

*ECG database*

Out of the 478 LQTS ECGs included in this study, 3 ECG were of too low quality for interval measurements.

Manual measurements were performed on 875 ECGs, 200 from normal subjects off drugs (100 females), 200 from normal subjects on Moxifloxacin (100 females), 259 LQT1 patients (161 females), 183 LQT2 patients (100 females) and 33 LQT3 patients (15 females).

Automatic cardiac beat (R-wave) detection was accurate in all ECGs as assessed by both readers (FE & PMB).

The mean difference in QT interval duration between the two readers was 2.3 ± 8.7 ms and the mean absolute difference was 5.2 ± 7.4 ms (with a maximum difference up to 112 ms).

The absolute QT difference was smaller than 10 ms in 87% of ECGs, 10 to 19 ms in 9.5%, and 20 to 29 ms in 2.2% and ≥ 30 ms in 1.2% of ECGs.

Accordingly, the QT "reconciliation" session was performed on 117 ECG (13%).

*Overall population ECG measurements*

Table 1 shows RR, QT, QTc (QTcB, QTcF and QTci) and α coefficient in males and females in the 5 groups.

Mean RR intervals were shorter in females than in males, except in LQT1 patients. Mean RR intervals were also shorter in LQTS patients than in normal subjects (both off drugs and under Moxifloxacin).

Rate corrected QT interval duration was longer under Moxifloxacin and in LQTS patients when compared to untreated normal subjects.

Sex effect (female minus male) on QTc interval duration is different depending on the group ($p = 0.0067$ for QTci). The values in parentheses (in the following text) are the 95%CI. QTc are longer in females for 3 out of the 5 groups. The mean difference was 17 ms (12.7; 21.3) in normal subjects, 19 ms (14.5; 23.5) on Moxifloxacin, and 13 ms (4.8; 21.2) in LQT1 patients. Conversely, QTc duration was not significantly different according to sex in the 2 other groups. In LQT2 the mean difference is 2 ms (−7.9; 11.9)) and it is −5 ms in LQT3 patients (−32.2; 22.2).

Sex and group specific α coefficients were close to the Fridericia's coefficient (0.33) in normal subjects including in subjects on Moxifloxacin but higher and closer to Bazett's (0.5) in LQTS patients (Table 1 and Fig. 2).

*Beta blocker treatment subgroups*

The proportion of LQTS patients receiving beta blockers at the time of the first available digital ECG is 46% (41%, 53% and 41% in LQT1, LQT2 and LQT3, respectively). The QTc patterns across LQTS genotypes were not affected by beta blocker treatment. A QTci longer in females than in males was observed in LQT1 patients both on and off beta blocker. In LQT2 patients the lack of sex effect holds true for the 2 beta blocker subgroups. Due to a low sample size in LQT3 group, confidence intervals are too wide to be useful (Table 2).

*Puberty criterion*

All normal subjects included in the study were older than 18 years (healthy volunteers enrolled in clinical trials). Overall, the mean age was 29.5 ± 19 years in LQTS patients without statistically significant difference between females and males (31 ± 18 vs. 28 ± 20 years, $p = 0.11$), and across LQTS types (28 ± 18, 31 ± 19 and 33 ± 19 years in LQT 1, 2, 3 groups, respectively, $p = 0.22$) and without group*sex interaction ($p = 0.87$).

QTci was not significantly correlated with age (QTci = 0.15*age + 454, $R^2 = 0.007$, $p = 0.07$) in the whole LQTS population. A significant but very weak positive correlation was found in LQT1 patients (QTci = 0.22*age + 451, $R^2 = 0.015$, $p = 0.0499$) but not in LQT2 (QTci = 0.11*age + 458, $R^2 = 0.004$, $p = 0.40$) or LQT3 patients (QTci = −0.13*age + 456, $R^2 = 0.004$, $p = 0.72$).

The proportion of LQTS patients below 12 years old was 18% in females and 28% in males. And 25% of LQTS females and 35% of LQTS males and were below 15 years old.

After excluding patients aged less than 12 years, the effect on QTc interval duration observed in the whole study population remained visible (group and sex interaction for QTci, $p = 0.0275$) (Supplemental Table 1). QTci according in both sex before and after 12 yo and 15 yo are shown in Supplemental Table 2 and in Table 3 respectively).

**Table 1**
All subjects.

| | RR (ms) | QT (ms) | QTcB (ms) | QTcF (ms) | QTci (ms) | α coefficient |
|---|---|---|---|---|---|---|
| Normal Female *N* = 100 | 924 ± 140 | 396 ± 26 | 414 ± 18 | 407 ± 16 | 408 ± 16 | 0.3557 |
| Normal Male N = 100 | 1030 ± 142 | 394 ± 26 | 390 ± 17 | 391 ± 16 | 391 ± 15 | 0.3733 |
| Moxi Female N = 100 | 945 ± 144 | 410 ± 28 | 424 ± 19 | 419 ± 17 | 420 ± 17 | 0.3692 |
| Moxi Male N = 100 | 1031 ± 145 | 404 ± 25 | 400 ± 17 | 401 ± 15 | 401 ± 15 | 0.3464 |
| LQT1 Female *N* = 161 | 917 ± 204 | 439 ± 60 | 461 ± 30 | 453 ± 36 | 462 ± 30 | 0.5327 |
| LQT1 Male *N* = 98 | 912 ± 205 | 428 ± 57 | 451 ± 36 | 443 ± 38 | 449 ± 36 | 0.4581 |
| LQT2 Female N = 100 | 925 ± 192 | 444 ± 50 | 465 ± 30 | 458 ± 31 | 462 ± 30 | 0.4427 |
| LQT2 Male *N* = 83 | 955 ± 219 | 446 ± 62 | 460 ± 38 | 455 ± 42 | 460 ± 38 | 0.5079 |
| LQT3 Female *N* = 15 | 894 ± 80 | 425 ± 36 | 450 ± 34 | 441 ± 33 | 449 ± 33 | 0.4777 |
| LQT3 Male *N* = 18 | 985 ± 217 | 447 ± 71 | 452 ± 43 | 450 ± 50 | 454 ± 42 | 0.6121 |
| Group effect | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| Sex effect | 0.0001 | 0.7866 | <0.0001 | 0.0036 | 0.0001 | |
| Group*Sex effect | 0.0079 | 0.2921 | 0.0003 | 0.0270 | 0.0067 | |

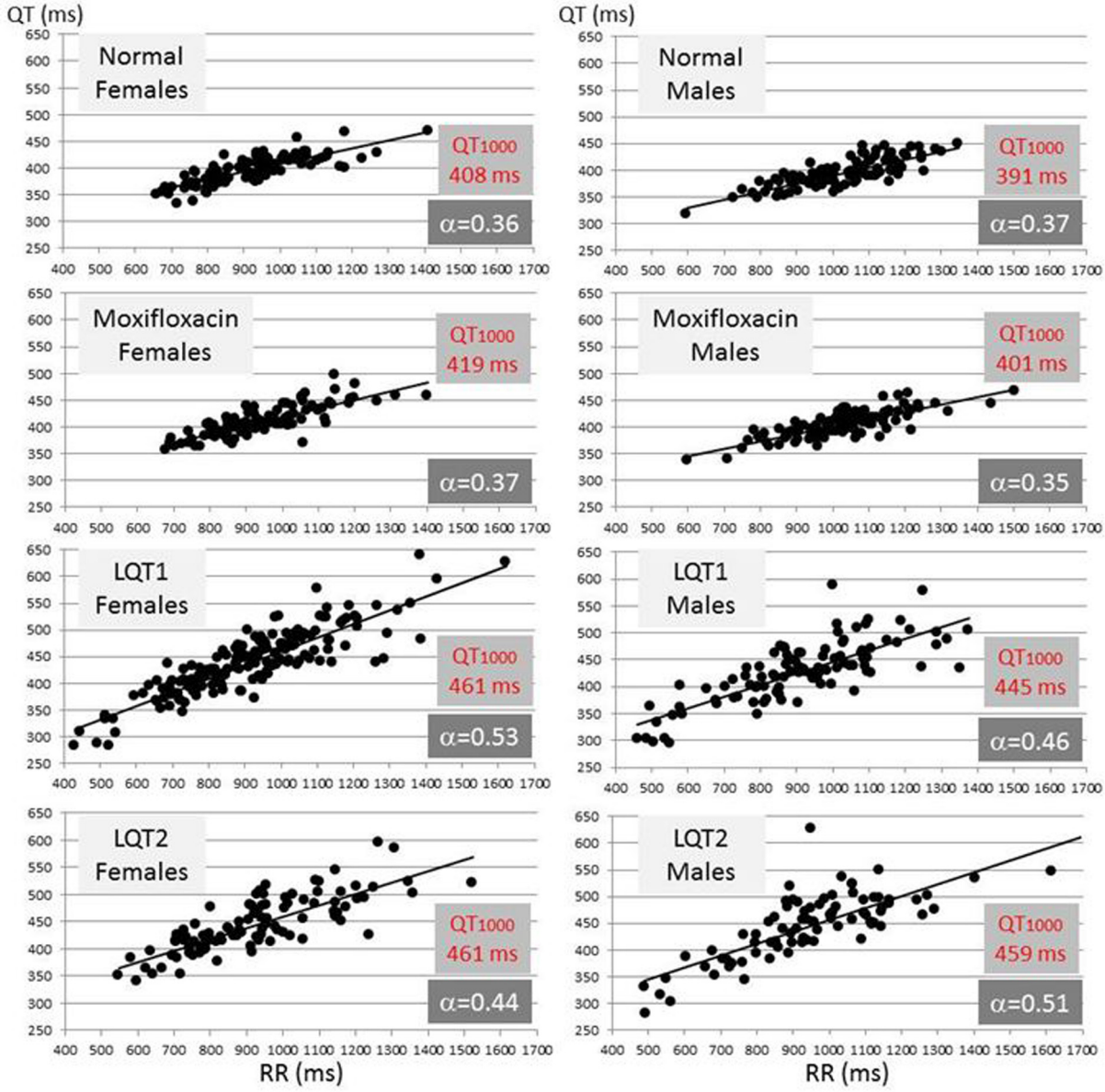


**Fig. 2.** QT/RR relationship in different clinical subgroups in females (left panels) and males (right panels). α coefficients are shown on QT/RR plots but have been calculated from LogQT/LogRR plots. The QT1000 value was calculated from the linear relationship of the LogQT/LogRR plots and corresponds to QT duration at RR = 1000 ms in the sub-population.

**Table 2**
QTci intervals according to beta blocker treatment.

| | LQTS group | Female | Male | Difference (95%CI) |
|---|---|---|---|---|
| Off beta blocker | LQT1 | 460 ± 30 | 445 ± 32 | 15 (4.6, 25.4) |
| | LQT2 | 453 ± 23 | 454 ± 42 | -1 (−15.7; 13.7) |
| | LQT3 | 451 ± 41 | 438 ± 30 | 13 (−24.6; 50.6) |
| On beta blocker | LQT1 | 468 ± 30 | 460 ± 40 | 8 (−6.4; 22.4) |
| | LQT2 | 468 ± 32 | 467 ± 33 | 1 (−12.4; 14.4) |
| | LQT3 | 442 ± 24 | 468 ± 53 | −26 (−89.1; 37.1) |

*Combined puberty and beta blockade criteria*

When considering the subgroup of patients above 15 years and without beta blocker treatment, the sex effect (female minus male) on QTc interval duration was genotype specific (Table 4). The difference was 17 ms (4.1; 29.9) in LQT1 patients. Conversely, QTc duration did not differ on sex in LQT2 and in LQT3 patients with a mean difference of −3 ms (−21.6; 15.6) and of 12 ms (−28.4; 52.4), respectively (group and sex interaction for QTci, $p = 0.0191$).

## Discussion

With a larger updated genotyped LQTS digital ECG database and using a robust ECG methodology, our study demonstrates that the normal sex effect on QTc interval duration (i.e. a longer QTc in females than in males) observed in normal subjects with and without pharmacological hERG potassium channel blockade as well as in type 1 LQTS patients is blunted in type 2 and 3 congenital long QT patients.

*ECG methodology*

Our findings are based on a robust ECG methodology. The ECGs were collected in digital format ($n = 875$). We could hence benefit from on-screen 12-lead overlap median "butterfly" displays for visual adjudication of automated measurements with moving electronic calipers. This approach has been proved to be particularly useful in TQT studies [16] and in LQTS ECGs which often show low amplitude T-waves on multiple leads and helped to differentiate notched T-waves from U-waves [18]. The differences in QT duration between the two readers were less than 10 ms in most ECGs. However, the maximum difference was above 100 ms and a third "reconciliation" session had to be performed for 13% of the ECGs. These figures illustrate the difficulties in QT duration measurements in LQTS patients. It is also important to underline that the evaluation of QT duration is dependent on the method used for measurement.

Another important characteristic of our database is inclusion of a substantial proportion of ECGs before beta blocker therapy initiation in LQTS patients (above 50%). This is not often the case in retrospective LQTS patient studies because of the risk of sudden death in this population and the efficacy of beta-blocker treatment in LQTS pushing for early initiation of the treatment. However, our group started to prospectively record digital ECGs in LQTS patients in the early nineties explaining why we were able to retrieve ECGs recorded before beta blocker initiation in a relatively large number of patients [19]. Beta blockers decrease heart rate and have genotype specific effects on QTc duration [15]. Evaluating sex effects in the subgroup of patients before treatment initiation was important to rule out a potential treatment bias.

An important issue is the mathematical model used for QT rate-correction. First, using a non-optimal rate-dependent correction with residual rate influences such as the Bazett's one may introduce a systematic bias in a population pooling both male and female normal volunteers, young and adult LQTS, with and without beta blockers. Second, previous studies have shown that the QT/RR relation is different in LQTS patients than in normal subjects [20]. In the present study, the alpha coefficient in normal subjects was near 0.33 which is the coefficient of Fridericia's formula but close to 0.5 in LQTS patients which is the Bazett's factor. Using a "universal" correction formula could have introduced a potential bias. Different numerical differences in QTc duration exist across study groups depending on the QT correction formula used; nonetheless a sex effect was identified regardless of the correction factor (Tables 1-4).

*Previous studies in LQTS patients*

Sex-related ECG differences in LQTS populations have been assessed in different studies based on different International LQTS databases [8–10,21]. However, comparisons with previous studies might be difficult because results can be quite different according to the ECG methodology used. In addition, because of the impact of puberty on QTc duration, the difference in age between LQTS cohorts may affect sex-related differences. Such divergence across studies has been highlighted by a review article by Vink et al. [11]. Nevertheless, it is always tempting to track down common findings between the studies.

In type 1 LQTS, most studies show longer QTc interval after puberty in females than in males [9,10,21]. Before puberty, a sex difference was not detected by Vink et al. [10] but reported by Lehmann [21]. The current study shows in LQT1 patients a statistically non-significant trend toward longer QTc for women than for men even before puberty.

In type 2 LQTS, most studies similarly describe longer QTc interval in females than in males [8,9,21]. Vink et al. [10] specifically examined time trends in QTc intervals using a linear mixed-effects model in 278 LQT1 or 2 patients. They outline a more complex sex-age interaction

**Table 3**
QTci according to age group (before and after 15 years of age).

| Age | LQTS type | Female | Male | Difference (95%CI) |
|---|---|---|---|---|
| 0–15 | LQT1 | 460 ± 28 | 454 ± 37 | 6 (−8.7; 20.7) |
| | (n) | (41) | (37) | |
| | LQT2 | 457 ± 37 | 463 ± 35 | −6 (−25.3; 13.3) |
| | (n) | (26) | (30) | |
| | LQT3 | 431 ± 35 | 445 ± 12 | −14 (−79.2; 51.2) |
| | (n) | (2) | (3) | |
| > 15 | LQT1 | 463 ± 31 | 446 ± 35 | 17 (7.0; 27.0) |
| | (n) | (121) | (61) | |
| | LQT2 | 464 ± 27 | 458 ± 40 | 6 (−5.8; 17.8) |
| | (n) | (74) | (53) | |
| | LQT3 | 451 ± 34 | 456 ± 45 | −5 (−36.4; 26.4) |
| | (n) | (13) | (15) | |
| Change | LQT1 | +3 (−7.8; 13.8) | −8 (−22.8; 6.8) | |
| | LQT2 | +7 (−6.5; 20.5) | −5 (−22.4; 12.4) | |
| | LQT3 | +20 (−35.9; 75.9) | +11 (−45.7; 67.7) | |

**Table 4**
Patients above age 15 and without beta blocker treatment.

| | RR | QT | QTcB | QTcF | QTci |
|---|---|---|---|---|---|
| Normal Female N = 100 | 924 ± 140 | 396 ± 26 | 414 ± 18 | 407 ± 16 | 408 ± 16 |
| Normal Male N = 100 | 1030 ± 142 | 394 ± 26 | 390 ± 17 | 391 ± 16 | 391 ± 15 |
| Moxi Female N = 100 | 945 ± 144 | 410 ± 28 | 424 ± 19 | 419 ± 17 | 420 ± 17 |
| Moxi Male N = 100 | 1031 ± 145 | 404 ± 25 | 400 ± 17 | 401 ± 15 | 401 ± 15 |
| LQT1 Female *N* = 60 | 904 ± 175 | 436 ± 51 | 461 ± 31 | 452 ± 33 | 462 ± 31 |
| LQT1 Male *N* = 42 | 933 ± 157 | 430 ± 49 | 447 ± 34 | 440 ± 36 | 445 ± 34 |
| LQT2Female *N* = 30 | 848 ± 166 | 421 ± 41 | 460 ± 23 | 447 ± 24 | 456 ± 22 |
| LQT2 Male N = 30 | 954 ± 141 | 446 ± 51 | 459 ± 46 | 454 ± 46 | 459 ± 46 |
| LQT3 Female N = 9 | 868 ± 84 | 421 ± 42 | 453 ± 41 | 442 ± 40 | 451 ± 41 |
| LQT3 Male *N* = 7 | 954 ± 174 | 426 ± 60 | 436 ± 35 | 432 ± 42 | 439 ± 32 |
| Group effect | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Sex effect | <0.0001 | 0.4516 | <0.0001 | 0.0020 | <0.0001 |
| Group*Sex effect | 0.3125 | 0.0450 | 0.0146 | 0.0058 | 0.0191 |

in LQT2 than in LQT1 patients. In LQT2, females had longer QTc duration than males during childhood and between puberty and the age of 25 [10]. In contrast, they found longer QTc interval in males than in females between the age of 5 and 14 and similar QTc duration after the age of 25 [10].

In agreement with Vink, our data also show a trend of longer QTc in LQT2 males than in LQT2 females before puberty. But in contrast with other studies a blunted sex effect on QTc interval duration may extend after puberty in LQT2 population. Our data do not provide clear information on the reason for the discrepancy between our study and others. However, differences in QT interval measurement methods, QT heart rate correction formulae, accounting for beta-blocker treatment, inclusion of older LQTS patients as well as sampling fluctuation and differences in individual LQTS mutations might all have had an impact of the results.

### *Pharmacological hERG blockade versus congenital type 2 LQTS*

In 1995 Keating et al. [22] identified hERG (i.e. the gene coding for the channel protein supporting the IKr current) mutations as the basis for the type 2 congenital long QT syndrome. In parallel, multiple studies have made clear that the vast majority of drugs associated with Torsades de Pointes are also IKr blockers. This explains that in the following years similarity was intentionally created by scientific players between the congenital and drug-induced forms of the type 2 congenital LQTS. In this view, one would expect ECG similarities between LQT2 patients and normal subjects taking Moxifloxacin. This was however not the case in our study.

The effects of Moxifloxacin on the QTc interval have been well characterized through its use in several hundred TQT studies as a positive control. A single oral dose of 400 mg induces a mean QTc prolongation of between 10 and 16 ms associated with a peak plasma concentration around 3 μg/ml [23] and consistent with our findings. Among quinolones, Moxifloxacin displays intermediate potency for inhibiting hERG channel current (IKr) with at peak free plasma concentration around 5% of the hERG IC50 values.

More specifically, Moxifloxacin has been shown to block the hERG channel (supporting IKr current) with a preference for the activated channel state [24]. On the other hand, dihydrotestosterone increases both activating and tail IKr currents [25] and there is general agreement that it explains the sex differences in QTc intervals. Based on our findings one could speculate that in normal subjects testosterone might increase IKr, and thereby shortening QT interval duration, and that remains true both with and without Moxifloxacin intake.

Pharmacological hERG channel (IKr current) blockade and loss of function in IKr observed in LQT2 patients could be expected to induce similar effects on ventricular repolarization. Indeed, the 2 mechanisms (acquired and congenital) of ventricular repolarization impairment do share common features as evidence by QT prolongation but also T-wave morphology changes [26]. However, the impact of LQT2 mutations on ventricular repolarization is quantitatively stronger (i.e. a 50 ms QTc prolongation together with steeper QT rate-dependency) than with Moxifloxacin. This could suggest that the degree of IKr impairment is important for sexual hormones influences on ventricular repolarization. Still, a "dose-response" pattern does not seem to explain our results.

The mechanisms leading to a decreased IKr are not identical with drugs and in LQT2 patients. In contrast with specific drug-channel interaction, it has been long recognized that type 2 LQTS could be the consequence of different mechanisms leading to a loss-of-function in IKr. It has been suggested that the majority of LQT2 mutations affect IKr through defective protein trafficking [27]. One could hypothesize that a decrease in transmembrane channel density would decrease the targets for testosterone effect of ventricular repolarization. This could be tested by comparing sex effect on QTc duration in LQT2 patients with 1) mutations leading to defective trafficking versus 2) patients with mutations altering the channel gating properties.

Finally, sex hormones have profound chronic impact on number of physiological parameters. For instance, sex differences in heart rate might be associated with a chronic electrophysiological remodeling and more complex chronic phenomenon cannot be ruled out. Nevertheless, our results support a pivotal role of IKr in sex differences in QTc interval duration.

### *Limitations*

Despite the large number of patients included in our database, statistically non-significant results in our study may reflect a lack of statistical power. In addition, QTc interval duration variability is about twice larger in LQTS patients than in control subjects and this could have participated to the wide confidence intervals observed in our study.

The evaluation of the effect of puberty on QTc interval duration would have been better addressed using sequential recordings before and after puberty in the same subjects and patients. Comparisons between different age groups from different patients might be biased by population sampling fluctuation. In normal subjects, QTc interval duration shortens with puberty in males while it tends to increase in females [2]. Similar trends have been described in LQTS patients (see reference 11 for review). Our study was not designed to evidence longitudinal changes. Still, we observe a trend toward a shorter QTc interval duration in LQT1 and 2 male patients after puberty but a trend toward longer QTc interval in LQT1 and 2 female patients.

QTc interval duration in a single patient might be variable over time. However, ECGs have been recorded in comparable condition (i.e. during a morning clinic visit) and there is no reason to believe that ECGs were not recorded randomly according to menstrual cycle.

Due to the recommendation to treat with beta blockers patients with a causative LQTS mutation, it is seldom possible to retrieve ECGs

off-treatment before and after puberty. Because beta blocker treatment has an effect on QTc interval duration in LQTS patients [5], treatment changes might bias a longitudinal evaluation of puberty consequences on ventricular repolarization duration.

Finally, beyond methodological differences, the different effect of sex-related QTc duration observed between studies might also be a consequence of mutation-specific channel interactions with sex hormones.

*Clinical implications and conclusion*

Interaction between ventricular repolarization duration and sex is a complex phenomenon because of the age dependent changes in sex hormones concentrations. This interaction seems further complicated by differential impact of sexual hormones on different mechanisms of malfunctioning KCNH2 encoded repolarizing potassium channel. Further experimental studies are warranted to better understand these interactions if we want to mitigate the potential proarrhythmic risk of hormonal and/or anti-hormonal treatments.

## Author statement

Individual contributions to the paper:

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Resources; Not applicable.

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Visualization; Not applicable.

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## Declaration of Competing Interest

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelectrocard.2020.08.016.

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