Diagnostics and modulators in long QT syndrome

Towards a better assessment in the young

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CHAPTER 10

Effect of Age and Sex on QTc in Children and Adolescents with Type 1 and 2 Long QT Syndrome

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ABSTRACT

Background

In congenital long QT syndrome (LQTS) age, sex and genotype have been associated with cardiac events, but their effect on the QTc has never been established. We therefore aimed to assess the effect of age and sex on QTc in children and adolescents with type 1 (LQT1) and type 2 (LQT2) LQTS.

Methods and Results

QTc of 12-lead rest electrocardiograms were determined and trends over time were analyzed using a linear mixed-effects model. The study included 278 patients with a median follow-up of 4 years (IQR 1-9) and a median number of 6 (IQR 2-10) electrocardiograms per patient. LQT1 and LQT2-males both showed QTc shortening after the onset of puberty. In LQT2-males this was preceded by a progressive QTc-prolongation. In LQT1, after the age of 12 years, males had a significantly shorter QTc than females. In LQT2, during the first years of life and from 14-26 years, males had a significantly shorter QTc than females. On the contrary, between 5-14 years, LQT2-males had significantly longer QTc than LQT2-females.

Conclusions

There is a significant effect of age and sex on QTc in LQTS, with a unique pattern per genotype. The age of 12-14 years is an important transitional period. In the risk-stratification and management of LQTS-patients, clinicians should be aware of these age-, sex- and genotype-related trends in QTc, and especially the important role of the onset of puberty.

INTRODUCTION

Congenital long QT syndrome (LQTS) is a heterogeneous group of inheritable cardiac repolarization disorders, with a predisposition to malignant ventricular arrhythmias that can precipitate syncope, sudden cardiac arrest (SCA) or sudden cardiac death (SCD).[1-3] The delayed repolarization results in a prolongation of the QT-interval corrected for the heart rate (QTc) on the electrocardiogram (ECG). The most common types of LQTS are type 1-3 (LQT1-3), which are a result of mutations in potassium channel genes KCNQ1 (I_{Ks}) and KCNH2 (I_{Kr}), or sodium channel gene SCN5A (I_{Na}),[4] respectively.

The genesis of LQTS-related cardiac events is not only dependent on the degree of QTc-prolongation. A complex influence of a variety of factors including age, sex and genotype is described. Children and adolescents constitute an especially important risk group.[5] In children, LQT1-males have a higher risk and an earlier onset of cardiac events compared to LQT1-females,[6-8] but no sex-related differences in LQT2- and LQT3-children have been described.[6, 7, 9] After the onset of puberty changes occur in the risk for cardiac events, with an increased risk in LQT2-females compared to LQT2-males, and no sex-related differences in LQT1- and LQT3-patients.[6-9] Unfortunately, there are no data on the influences of age, sex and genotype on QTc other than comparisons of baseline QTc between categorical age groups.[7, 8, 10, 11]

The onset of puberty plays an important role in sex-related differences in the risk for cardiac events in especially LQT1- and LQT2-patients, which is not seen in LQT3-patients. We hypothesize that the onset of puberty also effects sex-related differences in QTc. Therefore our objective was to assess sequential QTc data from a large cohort of children and adolescents with LQT1 and LQT2, in order to gain insight in the trend of QTc in LQTS.

METHODS

Study population

A multicenter retrospective cohort study was performed including LQT1- and LQT2-patients born after January 1st 1985. LQTS type was defined as a confirmed pathogenic mutation in either KCNQ1 or KCNH2, detected using conventional methods. Consecutive patients from five medical centers in The Netherlands were included until June 2015. Patients were excluded if they were double mutation carriers or a known compound heterozygote. The study was approved by the Academic Medical Center Review Board.

Data collection and management

Patients characteristics were collected and all 12-lead rest ECGs were digitalized, blinded and manually analyzed by one investigator (SV) using the open source image processing program Image J 1.50i [National Institutes of Health, USA]. The QT-intervals of three consecutive complexes and their preceding RR-intervals were measured in a period without a marked sinus

arrhythmia. The duration of the QT-interval was determined from the beginning of the QRS-complex to the end of the T wave using the tangent method in lead II or V5. If the QT-interval could not be determined using lead II and V5, one of the remaining leads was preferably used in all ECGs of that individual patient. QTc was calculated with Bazett's correction formula[12] averaging the three consecutive complexes.

Baseline ECGs were excluded from analysis if they were made during the first month after birth, during hospital admission or in the presence of QT-prolonging drugs as registered on CredibleMeds®, as were ECGs with ventricular pacing or atrial- and ventricular arrhythmias.

Follow-up duration was defined as the time period in years from the date of the first ECG until the date of the latest.

Control population

The trends in QTc for LQTS-patients were compared to the trends for healthy controls. To gather information on trends in healthy controls, we used two different sources. Firstly, we compared the trends in QTc for LQTS-patients to the normal median values reported in Dutch children[13] and adolescents.[14] Age- and sex-dependent normal non-serial values were obtained from population-based prospective cohorts and medical students. Details on the exact measurements are described elsewhere,[13, 14] but in short, the QT-interval was measured by the Modular ECG Analysis System (MEANS)[15] and QTc was calculated using the Bazett's correction formula.[12]

Secondly, we used the original data from a previous study on the cut-off values for QT-intervals in Japanese children and adolescents.[16] In this study, serial QT- and RR-intervals were obtained at the age of 6, 12 and 15 years in 1240 males and 1338 females. Three consecutive QT- and RR-intervals were measured using the tangent method and averaged. QTc was calculated using the Bazett's correction formula.[12] Further details of this study are described elsewhere.[16]

Statistical analysis

All data were manually entered into a SPSS statistics database version 20.0 and analyzed with R version 3.1.3. We considered four subgroups: LQT1-males, LQT1-females, LQT2-males and LQT2-females. Characteristics of the study population were presented as frequencies (percentage) for categorical variables, mean (± standard deviation; SD) for continuous variables with an approximately symmetric distribution and median (Interquartile range; IQR) for continuous data with a skewed distribution. Binary data between two groups were evaluated using a Chi-square test.

We estimated average age trends in QT-interval, heart rate (HR) and QTc using a linear mixed-effects model. A mixed-effects model takes account of repeated measurements per patient over time; the number and timing of measurements may vary per patient. To avoid selection bias, all patients with at least one ECG were included in the analysis.[17] QTc was allowed to vary

smoothly by age via restricted cubic splines. Trends were allowed to differ by sex. The onset of puberty was set at 11.5 years in males and 10.7 years in females.[18] We compared changes between birth an onset of puberty, as well as between onset of puberty and 20 years. Sampling uncertainty was quantified via 95% confidence intervals (CI) and p-values. A p-value < 0.05 was considered to be statistically significant.

A sensitivity analysis for the trend in QTc in the presence of constant medication conditions was performed for (I) beta-blocker therapy, (II) propranolol treatment and (III) both QT-prolonging[19] and QT-shortening therapy. A more detailed description of the mixed-effects model and the sensitivity analysis is provided in the **Supplemental Material**.

The trends in QTc for both control populations were differentiated by sex. The Dutch data published by Rijnbeek et al.,[13, 14] was plotted as absolute median values and the Japanese data was analyzed using a linear mixed-effects model with age as a factor.

RESULTS

Population characteristics

A total of 343 patients were eligible for the study. Sixty-five patients (19%) were excluded, either because they had no ECG (n=63), or only one ECG that was made within 28 days after birth (n=2). These 65 patients were generally referred for genetic testing alone and follow-up was done in another hospital. The resulting cohort comprised 278 patients from 147 families, which were all included in the analysis. These patients had a total of 2367 ECGs of which 251 ECGs were excluded from analysis, mainly because they were made during hospital admission (61%), leaving a total of 2116 ECGs for analysis.

Baseline clinical characteristics of all four groups are shown in **Table 1**. The median age at presentation for the total cohort was 8 years (IQR 3-14 years), and most patients were diagnosed as a consequence of family screening (80%). Five percent was symptomatic before presentation, but these patients did not differ in baseline QTc duration from asymptomatic patients (p=0.997).

Table 2 | Baseline characteristics.

	All patients n=278	LQT1-males n=60	LQT1-females n=70	LQT2-males n=67	LQT2-females n=81
Presentation					
Family screening	220 (80%)	46 (77%)	50 (71%)	59 (89%)	65 (81%)
Syncope	26 (9%)	4 (7%)	11 (16%)	3 (5%)	8 (10%)
Near-drowning	3 (1%)	2 (3%)	1 (1%)	0 (0%)	0 (0%)
SCA	7 (3%)	1 (2%)	2 (3%)	1 (2%)	3 (4%)
Incidental*	9 (3%)	3 (5%)	4 (6%)	1 (2%)	1 (1%)
Other†	11 (4%)	4 (7%)	2 (9%)	2 (3%)	3 (4%)
Median age at presentation in years (IQR)	8 (3-14)	7.5 (3-12)	8 (3-14)	9 (4-14)	8 (4-15)
Symptomatic before or at presentation‡					
Near-drowning	7 (2%)	3 (5%)	4 (6%)	0 (0%)	0 (0%)
SCA	7 (3%)	1 (2%)	2 (3%)	1 (2%)	3 (4%)
Ventricular arrhythmias	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)
Median age first event in years (IQR)	11 (6.0-15.0)	11 (10.5-12.5)	7 (6.0-15.0)	0 (0-0)	16.5 (10.0-20.0)
Family history					
Negative	74 (28%)	11 (19%)	22 (33%)	16 (25%)	24 (33%)
Positive but not malignant	28 (11%)	13 (22%)	8 (12%)	3 (5%)	4 (5%)
Malignant‡	162 (62%)	35 (59%)	37 (55%)	45 (70%)	45 (62%)
Number of families	147	48	52	45	50
Probands	55 (20%)	14 (23%)	20 (29%)	7 (10%)	14 (17%)
Mean QTc (±SD)	451 (±39)	444 (±33)	451 (±38)	453 (±48)	454 (±35)

SCA = sudden cardiac arrest, IQR = interquartile range, QTc = QT-interval corrected for heart rate, SD = standard deviation
*Incidental = Due to pre-operative screening or regular health exam.
†Other = During the evaluation of specific symptoms i.e. palpitations, murmur, dyspnea, chest pain, near-syncope, dizziness or prenatal symptoms.
‡i.e. near-drowning, SCA or documented ventricular arrhythmias.

Follow-up

Fifty-four patients had only one ECG (19%). All were referred for genetic testing and follow-up was done elsewhere. Genetic testing was most often performed in the context of family screening (76%); four patients (7%) had a SCA. One of these patients had a severe postanoxic encephalopathy and follow-up was discontinued at the request of the parents. There was no difference with regards to the number of probands (22% versus 19%, p=0.77) and the percentage of symptomatic patients at presentation (8% versus 4%, p=0.46) between patients

without follow-up and patients with follow-up. Therefore, the patients without follow-up were considered to be a random sample of the total population and we assumed that data were missing at random.

Follow-up data of the 224 patients is shown in **Table 2**. Median follow-up duration was 5 years (IQR 2.5-10 years) with a median number of 8 ECGs (IQR 4-12 ECGs) per patient. Eleven patients (5%) had a cardiac event during follow-up. One of these patients (LQT2-female) had both symptoms at baseline and during follow-up. Most of the patients (88%) were on beta-blocker therapy during follow-up.

Thirty-seven patients had a set of ECGs during the pubertal period; from 8 years to 14 years of age (7 LQT1-males, 10 LQT1-females, 10 LQT2-males and 10 LQT2-females). These patients showed no differences in number of probands (8% versus 22%, p=0.09), symptomatology at presentation (5% versus 5%, p=1.00) or symptomatology during follow-up (8% versus 3%, p=0.37) compared to the other follow-up patients. Therefore, this group was considered to be a random sample of the total study population with respect to trends in QTc during puberty.

Table 3 | Follow-up.

	All patients n=224	LQT1-males n=50	LQT1-females n=58	LQT2-males n=52	LQT2-females n=64
New events during follow-up					
Near-drowning	1 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)
SCA	1 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)
Ventricular arrhythmias	9 (4%)	0 (0%)	4 (7%)	0 (0%)	5 (8%)
Beta-blocker therapy	188/213 (88%)	43/47 (91%)	53/58 (91%)	40/48 (83%)	52/60 (87%)
Device					
Pacemaker	3 (1%)	0 (0%)	0 (0%)	1 (2%)	2 (3%)
ICD	12 (5%)	1 (2%)	3 (5%)	2 (4%)	6 (9%)
Median age start beta-blocker in years (IQR)	8 (5.0-13.0)	7 (4.0-11.5)	8 (3.0-12.0)	9 (7.0-13.0)	9 (6.0-14.0)
Median number of ECGs per patient (IQR)	8 (4-12)	6 (4-10)	8 (5-11)	8.5 (5-16)	7 (4-13)
Median follow-up duration per patient in years (IQR)	5 (2.5-10.0)	5 (3.0-7.0)	5 (3.0-9.0)	8 (4.0-11.0)	5 (2.0-10.0)

SCA = sudden cardiac arrest, ICD = implantable cardioverter defibrillator, IQR = interquartile range

Age-related changes in QTc

Individual and average age-related changes in QTc for LQT1-males, LQT1-females, LQT2-males and LQT2-females are shown in **Figure 1**$_{A,C,B,D}$. The age-related changes in QTc were not statistically significantly different between symptomatic and asymptomatic patients for both LQT1 and LQT2 (p=0.52 and p=0.15, respectively), however note that the number of symptomatic patients was small. In addition, there was also no statistically different between probands and family members (LQT1 p=0.30 and LQT2 p=0.69).

Age-related changes differed by sex in LQT1-patients (p=0.01). In LQT1-males, age significantly influenced QTc (p=0.02). From birth to the onset of puberty, the QTc remained unchanged (p=0.57), after the onset of puberty the QTc shortened until the age of 20 years (p=0.02). In LQT1-females no influence of age on QTc was seen (p=0.30). LQT2-males showed an evident change in QTc over time (p<0.0001) with QTc-prolongation between birth and the onset of puberty (p<0.0001) and QTc-shortening thereafter (p<0.0001). In LQT2-females, there was also a significant influence of age on QTc (p=0.003), but with a different pattern than in LQT2-males (p<0.001). From birth to the onset of puberty the QTc tended to shorten (p=0.10), after which the QTc increased before it decreased again to the age of 20 years (p=0.29).

All four groups showed significant age-related changes in HR and QT-interval, with a higher HR before puberty compared to the HR after puberty and the opposite effect in QT-intervals (data provided in the **Supplemental Material**).

Sensitivity analysis

In the sensitivity analyses, ECGs excluded for LQT1 and LQT2 respectively were for (I) beta-blocker therapy 188 (20%) and 227 (20%), (II) propranolol treatment 166 (17%) and 110 (10%), and (III) both QTc-prolonging and QTc-shortening therapy 19 (2%) and 76 (7%). All sensitivity analyses showed no relevant changes in the results.

Sex differences in age-related changes in QTc

The age-related changes in QTc for males and females as described above were combined per genotype to evaluate the sex differences (**Figure 1**$_{E,F}$). LQT1-patients showed no sex-related differences in QTc during the first twelve years of life. After the age of 12 years, LQT1-males had a significantly shorter QTc compared to LQT1-females. There were no sex differences in age-related changes in HR and QT-interval. In LQT2-patients a significant sex-related difference in QTc was present during the first two years of life, during which LQT2-males had a shorter QTc compared to LQT2-females. Between the age of 5 to 14 years, the QTc in males becomes longer than in females and after the age of 14, again the QTc in males becomes shorter than in females. With regard to sex differences in trends of HR and QT-interval, no sex differences were observed in HR for both genotypes, but longer QT-intervals were seen in LQT2-males between the ages of 6-11 years compared to LQT2-females. There were no sex differences in QT-interval in LQT1.

Trends in QTc between healthy controls and patients with LQTS

The median QTc for multiple age groups as described by Rijnbeek et al.[13, 14] were shorter compared to the averaged QTc in both the LQT1- and LQT2-patients (**Figure 1**). In these Dutch controls there was a relatively constant trend in QTc with no sex-differences except between the ages of 1-3 months ($p<0.05$).[13]

Japanese controls showed a shorter averaged QTc compared to the median QTc of the Dutch controls, as well as for the averaged QTc of both LQT1- and LQT2-patients (**Figure 1**). Age significantly influenced QTc in these controls ($p<0.0001$), and the age-related change differed by sex ($p<0.0001$). At the age of 12 years, both males and females had a significant longer QTc than at 6 years ($p<0.0001$). At the age of 15 years, both males and females had a significant shorter QTc than at 12 years ($p<0.0001$). Males had a shorter QTc at the ages of 12 and 15 years compared to females.

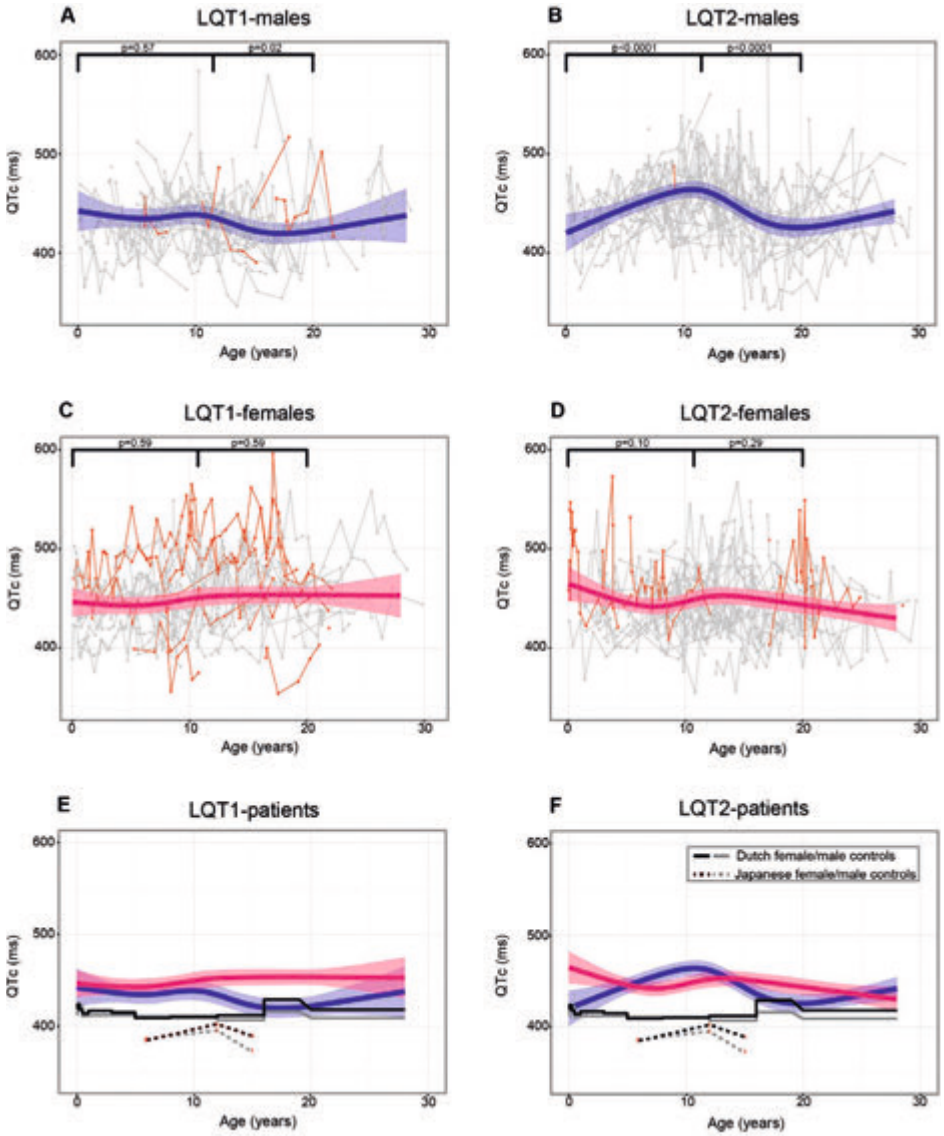

Figure 1 | Age-related changes in QTc in (**A**) LQT1-males, (**B**) LQT2-males, (**C**) LQT1-females, (**D**) LQT2-females, (**E**) LQT1-patients and controls and (**F**) LQT2-patients and controls.
A-D. Dots are individual measurements and lines are individual trends. Symptomatic patients are shown in red. The 95% confidence intervals for the fitted model are shown. **E-F**. Lines of LQTS-males and LQTS-females combined per genotype. Blue lines are LQTS-males and pink lines are LQTS-females. Both Dutch and Japanese control males and females are also shown. Grey lines are control males and black lines are control females. The 95% confidence interval for the Dutch controls is not shown. The 95% confidence interval for the Japanese controls is too narrow to be visible.

DISCUSSION

Main findings

The present study is the first to analyze unique data of serial QTc measurements in a large cohort of children and adolescents with LQT1 and LQT2, and has three major findings. Firstly, both LQT1- and LQT2-males show significant QTc-shortening after the onset of puberty. Secondly, in LQT2-males this is preceded by a progressive QTc-prolongation. Thirdly, the age of 12-14 years is an important transitional period where differences between males and females for both genotypes are seen, ages corresponding with the onset of puberty.

Age- and sex-related changes in QTc in LQTS

Prior studies reported age-related changes in QTc of both LQTS-males and LQTS-females by comparing children to adults. In LQT1-patients, male children showed a longer QTc compared to male adults, and conflicting results were described for LQT1-females.[7, 11] Zareba et al.[7] reported a longer QTc in LQT1-female children compared to LQT1-female adults, whereas Ozawa et al.[11] found no age-dependent changes. Our findings in LQT1-males are in line with these previous reports, and in agreement with the findings of Ozawa et al.,[11] observing no age-dependent changes in LQT1-females.

In LQT2-patients Zareba et al.[7] and Ozawa et al.[11] both showed no age-related changes in males and a shorter QTc during childhood compared to adulthood in females.[7, 11] This is in contrast to the results in this study, where LQT2-males have a shorter QTc after puberty and LQT2-females have no significant change in QTc during adolescence.

Differences in QTc between males and females have only been reported in patients above the age of 13-15 years.[7, 11] In LQT1-patients, females had either a longer QTc compared to males,[10, 11] or no sex-related differences were found.[7] In the present study we also found that LQT1-females have a longer QTc compared to LQT1-males after the age of 12 years. In LQT2-patients, consistent with previous studies,[7, 10, 11] we also demonstrated that after the second decade the QTc in females is longer than in males. However, in contrast to observations in these previous studies, we did find sex-related differences in LQT2-patients during childhood showing a significantly shorter QTc in males than females during the first years of life and opposite, between 5-14 years, a longer QTc in males than females.

This discrepancy between our findings and other studies may be explained by the methodology that was used. The previous studies compared a median baseline QTc between dichotomous age groups, and did not take individual age trends into account. These aspects may have masked age- and sex-related differences in QTc. The same holds for the trend in QTc in the Dutch controls. These controls showed a shorter QTc compared to LQTS-patients, but age and sex-related differences were not seen.

Sex hormones

Our findings on age- and sex-related changes in QTc for both LQTS and Japanese controls are most likely the result of changes in sex-specific hormones. Both clinical observational and animal studies have shown a QTc-shortening due to endogenous testosterone and progesterone.[20] Endogenous estrogen lengthens the QTc in animal models.[20] Concentrations of sex hormones in children are influenced by the activity of the hypothalamic-pituitary-gonadal (HPG) axis. The HPG axis is active during the (1) mid-gestational period in the fetus, (2) first months of life and (3) pubertal period,[21] and therefore higher concentrations of testosterone and estrogen are found during these periods.

LQT1-patients have an impaired function of I_{Ks} channels, while LQT2-patients do not.[4] Since testosterone induces a dose-dependent shortening of the action potential duration through enhancement of I_{Ks} channels,[20] one could postulate that the shortening of QTc by testosterone is less pronounced in LQT1-males than in LQT2-males because the I_{Ks} channels may not be able to fully respond to the presence of testosterone. As a consequence, during periods of sudden changes in sex hormone concentrations (i.e. the first months of life and the onset of puberty), a marked QTc-shortening would be expected in LQT2-males in contrast to LQT1-males. This may explain our findings in LQT2-males, which showed a shorter QTc during the first year period and a more pronounced QTc-shortening after the onset of puberty compared to LQT1-males. This hypothesis is strengthened by the fact that male Japanese controls showed a similar pattern to the LQT2-males.

Our data on LQTS-females also indicates a differing sensitivity to changes in sex hormone concentrations between the genotypes studied, since an effect of age on QTc was only found in LQT2-females and Japanese female controls. Previous studies have shown that there is a significantly increased risk for cardiac events in LQT2-females compared to LQT1-females during periods of sudden changes in estrogen concentrations such as the postnatal period,[22, 23] onset of puberty,[6-9] puerperium period, first 9 months postpartum[24] and in the peri- and post-menopausal periods.[25] This data supports that LQT2-females may be more sensitive to changes in estrogen concentrations compared to LQT1-females, and this may be the underlying mechanism for our observations on the QTc-shortening in LQT2-females in the first months of life and the prolongation after the onset of puberty.

Cardiac events

In LQT1-patients, males have a higher risk during childhood and an earlier onset of cardiac events than females.[6-8] Our observations on sex-related differences in QTc could not explain this sex difference in cardiac events during childhood based on the length of the QTc, since this was similar in males and females. These study findings are in line with previous studies,[7, 10, 11] and therefore it has been suggested that the difference in risk for cardiac events between LQT1-males and LQT1-females is related to the increased physical activity in males compared to females during childhood. Since LQT1-patients experience malignant ventricular arrhythmias more frequently during physical effort,[26] a presumably higher level of physical activity in males

may contribute to a higher risk for cardiac events. We only measured the QTc on 12-lead rest EGCs, and could therefore have missed an impaired QTc response to a higher HR during physical effort in LQT1-males.

In LQT2-patients, a higher risk for cardiac events is found in LQT2-females after the onset of puberty compared to males.[6, 7, 9] Based on the findings in this study, this could be explained by a longer QTc in females.

Modulating factors

We have considered factors that may have influenced our findings on age and sex differences in QTc. Firstly, in this study a higher HR is observed in children compared to adolescents of both LQT1- and LQT2-patients. The Bazett correction formula has an optimal correction between 60 and 100 beats per minute (bpm), and correction at a slower or faster HR gives erroneous results with respectively over- and undercorrection.[27] A HR >100 bpm was observed from birth to an age of 3-4 years in both LQT1- and LQT2-patients. Therefore, the QTc calculated with the Bazett correction formula may be underestimated in this specific time period. Sex related differences in HR were not observed in this study, which is consistent with previous studies.[7, 10] Stramba-Badiale et al.[28] however, showed that females have a more steep QT/RR ratio than males. With the assumption that this also applies to children, the use of the Bazett formula may cause a correction induced difference between sexes.

Secondly, medication affecting QTc could have influenced the observed trends in QTc in our study. We therefore performed three sensitivity analyses with the assumption that the introduction of these medicaments only has a short-term effect on the QTc. Sensitivity analyses did not change the results with respect to age and sex related differences in QTc. However, we were not able to exclude possible effects of changes in the dose of the medication or the influences of specific types of medications on QTc.

LIMITATIONS OF THE STUDY

Although this is the largest multicenter study to date examining trends in QTc in LQT1 and LQT2 children and adolescents, it has the inherent limitation of being a retrospective study. A complete set of ECGs from 0-30 years was not available in all patients, and especially after the age of 25 years there are limited measurements. Missing ECGs are due to differences in the age of presentation, follow-up intervals, loss to follow-up and SCD. However, as long as those that were followed for a period can be seen as representative for the whole population of similar age, our linear mixed-effect model will provide unbiased estimated of the age trends. Our cohort seems to be a representative sample of the general LQT1 and LQT2 child population, since there were no indications that missing data were not at random. Finally, we were unable to investigate the trends in QTc for specific mutations.

CONCLUSIONS

There is a significant effect of age and sex on QTc in LQTS, with a unique pattern per genotype. The age of 12-14 years is an important transitional period. In the risk-stratification and management of LQTS-patients, clinicians should be aware of these age-, sex- and genotype-related trends in QTc, and especially the important role of the onset of puberty. LQT2-patients should be closely monitored during periods of changes in sex-hormone concentrations since they are more sensitive to these variations than LQT1-patients.

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Statistical analysis

A repeated measurements analysis with a linear mixed-effects model was used to assess age trends in QT-interval, HR and QTc. Average age trends ('fixed effects') were allowed to differ by sex and were modeled via restricted cubic splines. The restricted cubic spline function allows to explore the effect of age without making restrictive assumptions about the shape of the time trends. Knots were placed at five fixed quantiles of the predictor's distribution as suggested by Stone.[1] Patients in the study population were considered to be a random sample of the total population. Therefore, we allowed the intercept (i.e. value at birth), slope and quadratic term over age to differ per patient, and assumed these parameters to follow a multivariate normal distribution (random effects). Hence, an unstructured 3x3 covariance matrix for the random effects was used. The correlation between the within-individual residuals as found to be negligible. Changes in QTc from birth to onset of puberty were compared testing for a difference in value between the ages of 0 year and 11.5 years for boys, or 0 years and 10.7 years for girls. These median ages for the onset of puberty were chosen based on observations in a Dutch cohort, where onset of puberty was defined as a testis volume of 4 milliliters in boys and a Tanner stage B2 in girls.[2]

Sensitivity analysis

We performed some sensitivity analyses in order to assess the influence of changes in therapy for (I) beta-blocker therapy, (II) specifically propranolol treatment and (III) the combination of both QT-prolonging and QT-shortening therapy. When a patient switched to beta-blocker therapy during follow-up, ECGs made in the absence of beta-blocker therapy were excluded with the assumption that the introduction of beta-blocker therapy only has a short-term effect on the QTc. Propranolol may have a stronger QTc-shortening effect compared to other beta-blockers[3] (Nadolol is not used in the Netherlands). Therefore, in patients that had varying types of beta-blocker therapy, ECGs made during propranolol therapy were excluded. A final sensitivity analysis was performed for the presence of QTc-prolonging drugs as registered on CredibleMeds®[4] or in the presence of 'QT-shortening' therapy i.e. mexiletine, potassium suppletion, potassium-sparing diuretics or left cardiac sympathetic denervation. ECGs made in the presence of these specific conditions were excluded from the analysis. The introduction of these medications were considered to have a short-term effect on the QTc and therefore they were excluded rather than to include them as a covariate in the model.

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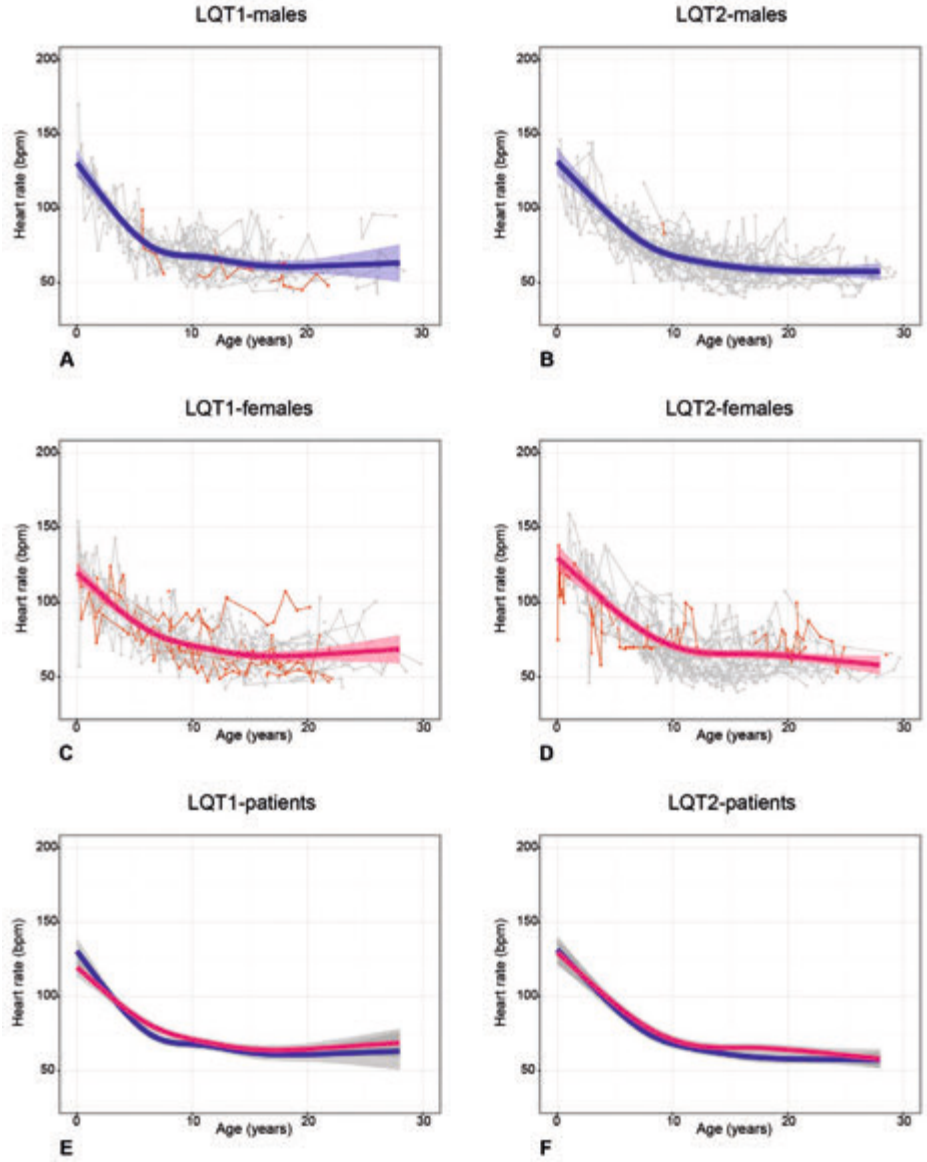
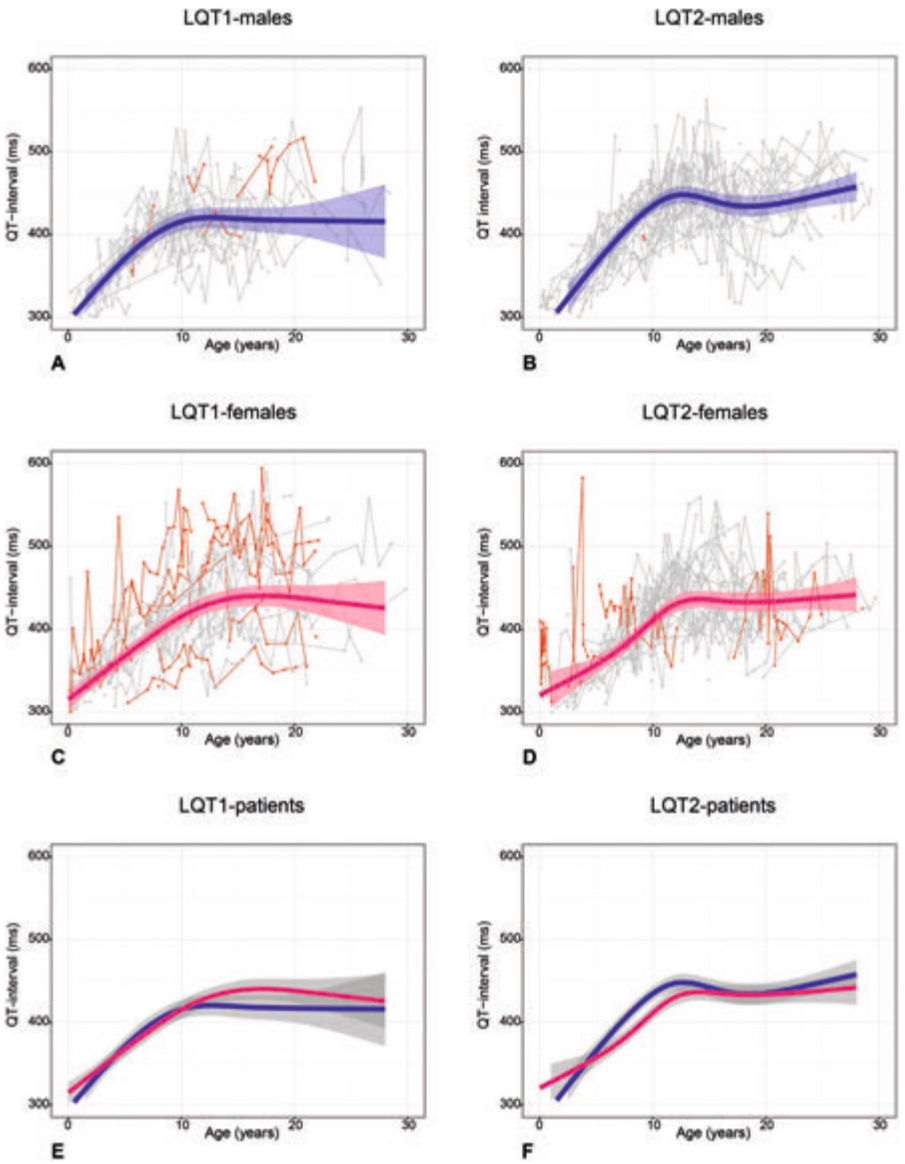
Supplemental Figures



Supplemental Figure S1 | Age-related changes in heart rate in (**A**) LQT1-males, (**B**) LQT2-males, (**C**) LQT1-females, (**D**) LQT2-females, (**E**) LQT1-patients and (**F**) LQT2-patients. **A-D.** Dots are individual measurements and lines are individual trends. Symptomatic patients are shown in red. The 95% confidence intervals for the fitted model are shown. **E-F.** Lines of males and females combined per genotype. Blue lines are males and pink lines are females.



Supplemental Figure S2 | Age-related changes in QT-interval in (**A**) LQT1-males, (**B**) LQT2-males, (**C**) LQT1-females, (**D**) LQT2-females, (**E**) LQT1-patients and (**F**) LQT2-patients.
A-D. Dots are individual measurements and lines are individual trends. Symptomatic patients are shown in red. The 95% confidence intervals for the fitted model are shown. **E-F.** Lines of males and females combined per genotype. Blue lines are males and pink lines are females.