Original articles

# Gene-specific differences in the circadian variation of ventricular repolarization in the long QT syndrome: a key to sudden death during sleep?

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*Key words:*
**Genetics; Long QT syndrome; Sleep; Sudden cardiac death.**

*Background.* **In the long QT syndrome (LQTS) most life-threatening cardiac events occur in association with physical or emotional stress. However, a minority of patients dies suddenly during sleep; intriguingly, these sleep-related sudden deaths tend to cluster in families. The mechanism(s) underlying this phenomenon and the reason why it occurs in few selected families are unknown. Recently, some of the LQTS genes have been identified leading to three main subgroups (LQT1, LQT2, LQT3) associated respectively with mutations affecting the following ionic currents involved in the control of ventricular repolarization: $I_{Ks}$, $I_{Kr}$, $I_{Na}$. We have recently observed that cardiac events nighttime are rare in LQT1 and frequent in LQT3 patients.**

*Methods.* **We studied 26 LQTS patients all genotyped (11 LQT1, 9 LQT2, 6 LQT3) and 26 healthy controls matched by age and gender. Using a specific software, 24-hour ambulatory ECG recordings were performed and the QT interval was measured in order to allow comparison between QTc nighttime and daytime.**

*Results.* **The main finding is that while LQT1 patients show a trend for modest QTc shortening and LQT2 patients a trend for modest lengthening nighttime versus daytime, LQT3 patients show clear lengthening of the QTc nighttime. These changes are not explained by heart rate changes or by the use of β-blockers.**

*Conclusions.* **The marked tendency for further QT prolongation nighttime, which clearly increases arrhythmic risk, present among LQT3 patients and absent among LQT1 patients, provides an explanation for the gene-specific higher risk for sudden death during sleep for LQT3 compared to LQT1 patients.**

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## Introduction

The circumstances associated with sudden death in the congenital long QT syndrome (LQTS) are usually those involving stress, either physical or emotional[1-3]. However, it is well known that a few patients have their cardiac events (syncope, cardiac arrest, sudden death) at rest or during sleep. The reason by which certain LQTS patients are at higher risk under very different conditions, some during exercise and others while asleep, has remained a puzzle for many years. The fact that sleep is a state associated with significant arrhythmic risk[4] was not providing *per se* a direct explanation.

The realization that all genes responsible for LQTS identified so far encode ion channels involved in the control of ventricular repolarization[5] has provided new opportunities for understanding the correlation between genotype and phenotype. Two observations were central for subsequent investigations.

One came from the first study to examine gene-specific physiological and behavioral differences in LQTS[6] in which it was noted that LQT3 patients, those with mutations on the cardiac sodium channel gene *SCN5A*, had a propensity to have life-threatening cardiac events during sleep or at rest.

This preliminary observation had been made in a very small population, as only a handful of patients had been genotyped in 1995. When a truly large number of genotyped and symptomatic patients could be studied, it was observed that while LQT1 patients (those with mutations affecting the repolarizing current $I_{Ks}$) only exceptionally (4%) were at risk while at rest or during sleep, this happened in 50% of LQT3 patients[7].

The other observation resulted from a cellular model developed to mimic the ionic abnormalities present in LQT2 (those with mutations affecting the repolarizing current $I_{Kr}$) and in LQT3 patients[8]. These experiments disclosed the unexpected finding that the prolongation of action potential duration caused by the persistence of a late inward $Na^+$ current, the electrophysiologic consequence of most mutations on *SCN5A*[9], was quite sensitive to rapid pacing suggesting that increases in heart rate could shorten the QT interval of LQT3 patients more than that of other patients and even of normal individuals. This experimental hypothesis was soon confirmed in preliminary clinical observations[6,10].

It was on this basis that we decided to test the possibility that LQT3 patients might have had a tendency for further prolongation of the QT interval nighttime and that the behavior of LQT1 might have been quite different. Accordingly, in this preliminary study we analyzed the Holter recordings of genotyped LQTS patients (LQT1, LQT2, and LQT3) and compared their QTc nighttime versus daytime. This analysis was carried out in healthy controls as well.

## Methods

**Study population.** The study was performed in 26 patients affected by LQTS and in 26 healthy controls matched by age and gender.

All patients had been genotyped: 11 carried a mutation on the *KVLQT1* gene, encoding the $I_{Ks}$ current (LQT1), 9 had a mutation on the *HERG* gene, encoding the $I_{Kr}$ current (LQT2), and 6 had a mutation on the *SCN5A* gene, encoding the $I_{Na}$ current (LQT3).

Among the LQTS patients there were 8 males and 18 females (69%), and median age was 25 years (range 1-58). All of them had a family history of LQTS and 19 had either syncope or cardiac arrest, whereas 7 were asymptomatic. Seventeen patients were on b-blocker therapy (82% of LQT1, 78% LQT2, and none of LQT3), in 6 a left cardiac sympathetic denervation had been performed and 1 patient had an implantable cardiac defibrillator.

Among the symptomatic patients, major cardiac events during sleep occurred in 2 LQT3 and in 2 LQT2 patients; no cardiac event during sleep occurred in LQT1 patients.

**Automatic analysis of Holter recordings.** All recordings were obtained by portable battery-operated two-channel Holter recorders (ELA Medical recorder model Synesis or Marquette Electronics Inc., recorder model 8500). All patients (or their parents, when appropriate) provided a detailed diary of their activities, included time of sleep and wakefulness. All tapes were analyzed by an automatic analysis system, performing a 200 Hz A/D conversion with an 8 bit resolution. The digitized two-channel ECG signals were processed by the ELATEC Holter analysis software (ELA Medical, Mountrouge, France), which classified all beats as sinusal, ventricular or artifacts, and allowed a manual verification of the automatic classification by a trained operator (MSB). The verified digitized data were then processed by a dedicated QT analysis software developed by ELA Medical, which sampled the 24-hour recording into 2880 templates obtained by 30-s time intervals. To improve the signal-to-noise ratio, one median complex was computed every 6 s from the consecutive sinus beats, then the 5 median beats within each 30-s template were averaged in order to obtain a single representative PQRST complex for each of the 2880 templates. QT interval was automatically measured from each template. The T wave end was determined by the intersection of the tangent to the downslope of the T wave with the isoelectric baseline. For each hour the mean and the standard deviation of RR interval, QT interval and QT interval corrected for heart rate according to the Bazett's formula were calculated. The 24-hour mean QTc was calculated.

The long-term QT interval variability was expressed as the standard deviation of 24-hour mean QTc and as the coefficient of variation of QTc (standard deviation/mean QTc u 100). The difference between the highest and the lowest value of hourly QTc (QTc max-min) was also considered.

The circadian variation of QTc was expressed as the difference between QTc measured during sleep and during wakefulness in absolute and percent values.

**Statistical analysis.** Differences between wakefulness and sleep in the whole group or within the three groups were assessed by Wilcoxon test. Mean comparisons among groups were performed with ANOVA and Bonferroni's correction. Data are presented as mean ± SD. A p value of $< 0.05$ was considered the limit for significance.

## Results

**Long QT syndrome patients.** *QT interval variability*. The 24-hour QT interval variability did not differ between groups despite a trend for a lower value among LQT1 patients when compared with the other two groups. The difference between the highest and the lowest value of hourly QTc was 58 ± 16 ms in LQT1 patients, 97 ± 49 ms in LQT2 and 82 ± 24 ms in LQT3 patients (p = NS). The standard deviation of the mean QTc was 15 ± 4 ms in LQT1 patients, 30 ± 19 ms in LQT2 and 24 ± 7 ms

in LQT3 patients. Also the coefficient of variation of QTc did not differ among the three groups. Importantly, the standard deviation of the RR interval did not differ in the three groups.

*Comparison between sleep and wakefulness.* When considered as a whole group the 26 LQTS patients showed a significant circadian variation of heart rate but not of ventricular repolarization. Mean heart rate was lower during sleep than during wakefulness (64 ± 13 vs 73 ± 15 b/min, $p < 0.001$) whereas QTc duration did not differ (497 ± 44 vs 493 ± 38 ms, p = NS). However, when we analyzed the circadian variation of QTc in the three genotypes a specific pattern emerged. As an example, figure 1 shows the circadian variation of QTc of 3 patients belonging to the different LQTS groups. In the LQT2 patient, despite the large variability, QTc did not differ between waking and sleeping hours, while in the LQT1 patient QTc tended to decrease during sleep, when compared to wakefulness. By contrast, in the LQT3 patient there is a marked prolongation of QTc during sleep. These examples reflect what happens in the three groups (Fig. 2), but also point to the importance of individual variability (Fig. 3).

Mean 24-hour QTc was longer in LQT3 than in

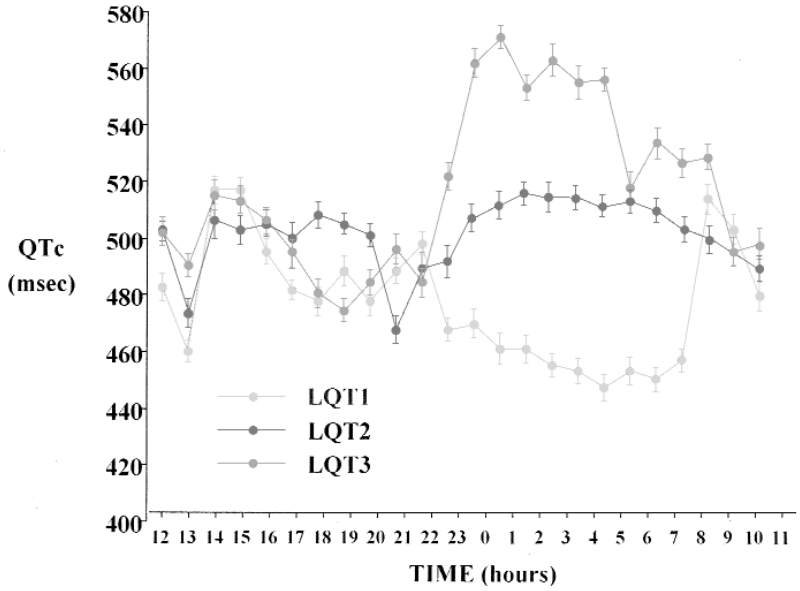


**Figure 1.** *Circadian variation of QTc of 3 patients representative of the three different LQTS subgroups. In the LQT2 patient, despite large variability, the QTc does not differ between waking and sleeping hours, while in the LQT1 patient the QTc tends to decrease during sleep, when compared to wakefulness. By contrast, in the LQT3 patient there is a marked prolongation of QTc during sleep.*

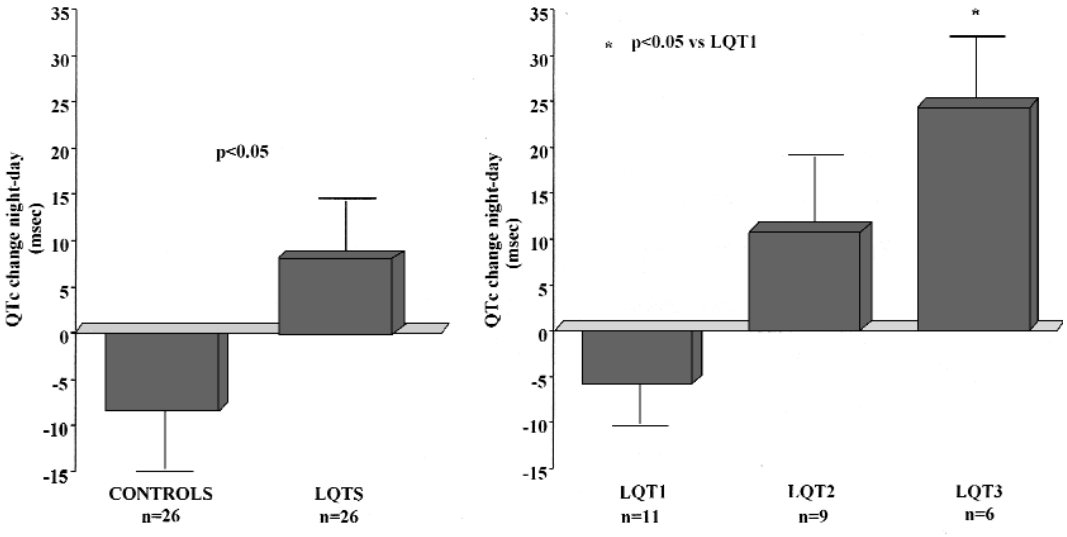


**Figure 2.** *Circadian variation of QTc.* Left panel: *difference between QTc during sleep and QTc during wakefulness in healthy controls and in LQTS patients.* Right panel: *difference between QTc during sleep and QTc during wakefulness in LQT1, LQT2 and LQT3 patients. Data are presented as mean – 1 SE.*

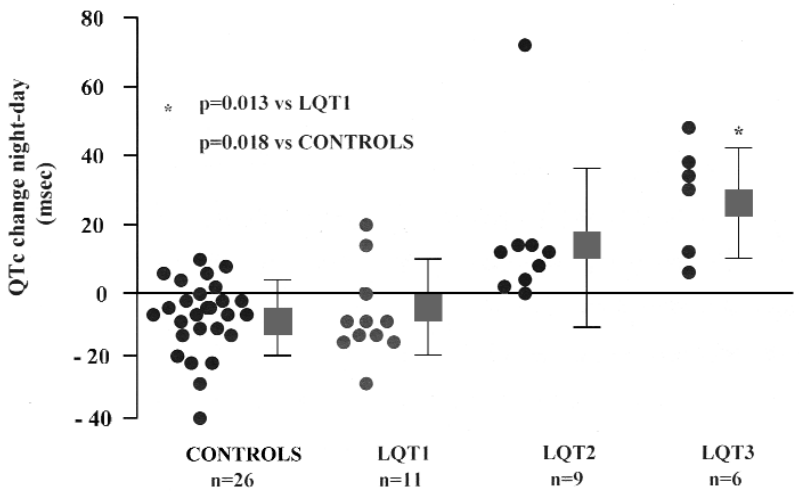


**Figure 3.** *Difference between QTc during sleep and QTc during wakefulness in healthy controls, LQT1, LQT2 and LQT3 patients. Individual values* (open circles) *and mean – 1 SD of QTc changes in each group are shown.*

LQT1 (534 ± 26 vs 477 ± 35 ms, $p < 0.01$) and than in LQT2 (484 ± 32 ms, $p < 0.05$). This finding is partly dependent on what happens nighttime. Indeed, during the waking hours the difference between the three groups is not so marked while it is during sleep that the QTc of LQT3 patients becomes markedly longer than that of LQT1 patients (549 ± 23 vs 475 ± 35 ms, $p < 0.005$), with intermediate values for LQT2 patients (490 ± 39 ms).

The circadian variation of ventricular repolarization, expressed as the difference between QTc during sleep and during wakefulness is of opposite sign and significantly greater in LQT3 than in LQT1 (+27 ± 18 vs -5 ± 15 ms, $p < 0.05$; Fig. 2). This difference remains present also when the percent changes of QTc between sleeping and waking hours are considered (+5 ± 3 vs -1 ± 3%, $p < 0.05$). Importantly, the difference in the circadian variation of QTc between LQT1 and LQT3 cannot be explained by a different circadian variation in cardiac cycle, as the heart rate decrease during sleep was similar. The data with LQT2 patients show an intermediate pattern, largely because of a single outlier.

The longest hourly QTc was found during sleep in all 6 LQT3 patients, in contrast to only 3 of 9 LQT2 and 2 of 11 LQT1 patients.

**Healthy controls.** Among the controls, QTc had only minor circadian variations and the overall trend was actually opposite to that of the LQTS patients (Fig. 2).

When the healthy controls are compared to the LQT1 or LQT2 patients (matched by age and gender) there is no difference in circadian variation; by contrast there is a significant difference between the controls and LQT3 patients (-11 ± 15 vs +27 ± 18, $p < 0.05$).

## Discussion

The present study provides a first glimpse on the mechanisms involved in the differential distribution of risk for sudden death during sleep among the different genetic subgroups forming the LQTS. It also indicates that the relative immunity from major cardiac events at rest/sleep among LQT1 patients and the markedly increased risk for LQT3 patients under the same circumstances do not reflect the play of chance but, rather, a genetically-controlled predisposition for further sleep-related QT prolongation in the latter group.

**Circadian variation of the QT interval.** When all the LQTS patients were considered together, irrespective of their genotype, QTc duration was similar during wakefulness and sleep. This pattern did not differ from that of the healthy controls matched by age and gender. At first glance this is in contrast with previous reports indicating the presence of a circadian variation in the QT interval of healthy subjects[11,12]. These studies reported that the QT interval was longer during sleep than in the waking hours; a critical point, however, is that the duration of the QT interval during sleep was compared to that measured during the awake state at the same RR interval. This methodology excluded the QT intervals measured at heart rates that did not overlap in the two states. We considered important to assess QT changes in truly physiological conditions without excluding changes in heart rate because otherwise transient increases in risk related to non-sustained QT prolongation would be missed. When QT intervals at increased heart rate (i.e. exercise or early morning after awakening) are taken into account, such a circadian variation is blunted or even reversed[13].

A lack of circadian variation has been reported in patients with the LQTS[14]. This finding is in agreement with our observation when the LQTS patients are considered

as a whole group. However, when the circadian variation of QT interval is analyzed according to the genotype of LQTS patients, we found that LQT3 patients show a further prolongation of QTc during sleep, at variance with LQT1 and LQT2 patients.

**The clinical picture.** Only in the mid-nineties it was realized that LQTS is not a disease due to a single genetic disorder but that, despite a similar electrocardiographic phenotype and a similar propensity for life-threatening arrhythmias, it actually includes different abnormalities due to mutations on genes encoding quite different ion channels. Until then, the few families with a propensity for cardiac events at rest or during sleep were considered as an oddity[3,15]. The typical clinical picture of LQTS was that of individuals, usually young, developing syncope or cardiac arrest during physical or emotional stress. This was an important reason for focusing therapy, already early on, on b-blockers[1]. The identification of different subgroups, based on the ion channel involved, led to a reappraisal of the clinical picture.

In 1995, in a report based on a very small number of genotyped patients, we raised for the first time the concept that mutations on different LQTS genes might have favored the occurrence of cardiac events under different and specific circumstances[6]. That study was greatly expanded and it now includes data on almost 800 LQTS patients, all symptomatic and of known genotype, which indicate the existence of an opposite pattern between LQT1 and LQT3, with LQT2 appearing as more similar to LQT3 than to LQT1 possibly because both these groups have an intact $I_{Ks}$ current. It became evident that while less than 5% of LQT1 patients have their cardiac events during rest/sleep without arousal, this happens in 30% of LQT2 patients and in more than 50% of LQT3 patients. Even more striking are the data limited to lethal events: while only few of these events occur during sleep for LQT1 patients, this percentage rises sharply for LQT2 and reaches almost 80% for LQT3 patients.

The present data, by showing no change in QTc between day and night among LQT1 patients and – in striking contrast – a clear further prolongation of QTc nighttime among LQT3 patients, shed light on the sometime opposite clinical picture present in patients with mutations on different genes.

**Potential mechanisms.** At this time we can offer only hypotheses on the mechanisms underlying the marked QTc prolongation occurring nighttime among LQT3 patients. We consider unlikely for this prolongation to be related to sleep *per se*; it is instead more likely related to the prolongation in cardiac cycle (heart rate reduction) which accompanies most of the sleeping time.

A potential explanation is based on the fact that these *SCN5A* mutations appear to be associated with prolongation of the open state of $Na^+$ channels at plateau voltage, as it happens with a toxin, anthopleurin, that interferes with inactivation of the $Na^+$ current[8,9,16]. Thus, at slow heart rates, when the time spent at less negative potentials is increased, the excessive late inward flow of $Na^+$ would be accentuated and would thereby cause a greater prolongation of action potential duration and, hence, of the QT interval on the ECG.

The fact that none of the LQT3 patients was on b-blocker therapy compared to the high prevalence of such therapy between LQT1 and LQT2 raised the possibility that the nighttime QT prolongation of the LQT3 group might have been favored by the absence of b-blocker therapy. Figure 2 shows that the entire control group behaves very similarly to LQT1 patients and very differently compared to LQT3 patients. If the differences between LQT1 and LQT3 were due to the high incidence (82%) of b-blocker therapy among LQT1 patients, then the controls should have been similar to LQT3 and different from LQT1; the opposite was found, thus indicating that the difference between LQT1 and LQT3 was not due to b-blockers.

In conclusion, the findings of the present study allow us to propose a rational explanation for the striking association between sleep and sudden death among LQTS patients carriers of mutations on the cardiac sodium channel gene *SCN5A*. This high risk nighttime is even more striking considering that among the most common type of LQTS, LQT1 (with mutations on the potassium current $I_{Ks}$), it is very modest.

The evidence for a clear trend toward further QT prolongation during the sleeping hours for LQT3 patients compared to a decrease or no change for LQT1 patients indicates that the arrhythmogenic substrate, and thereby electrical instability, is enhanced nighttime in a gene-specific manner.

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