MODIFICATION OF VENTRICULAR REPOLARIZATION
IN SILENT LONG QT SYNDROME MUTATION CARRIERS

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Academic dissertation

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in Auditorium 3, Meilahti Hospital, on June 10th, 2011, at 12 noon.

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ABSTRACT

Congenital long QT syndrome (LQTS) is a familial disorder characterized by ventricular repolarization that makes carriers vulnerable to malignant ventricular tachycardia, torsade de pointes, and sudden cardiac death. The most common subtypes, LQT1 and LQT2, are caused by loss-of-function mutations in slow (I_{Ks}) and fast (I_{Kr}) cardiac potassium channels, whereas subtype LQT3 is caused by gain-of-function mutations in cardiac sodium channel (I_{Na}). The disorder is characterized by a prolonged QT interval in electrocardiograms (ECG), but a considerable portion are silent carriers presenting normal (QT_c <440 ms) or borderline (QT_c <470 ms) QT interval, making the diagnosis challenging. Genetic testing is available for 60-70% of patients, leaving the rest without definite diagnosis. However, identifying affected asymptomatic relatives before their first attack may have a life-saving outcome. A number of pharmaceutical compounds also have affinity to the cardiac I_{Kr} channel, causing a clinically similar disorder called acquired long QT syndrome. LQTS carriers - who already have impaired ventricular repolarization - are especially vulnerable. For example, they are usually not included in clinical safety studies. Our aim was to create methods to improve the diagnosing of silent LQTS carriers. A method for accurate QT interval measurement was developed and the effects of antihistamine cetirizine on ventricular repolarization was studied.

We studied a total of 49 subjects with LQTS: 22 in the LQT1 group, 17 in LQT2 and 10 in LQT3. All were asymptomatic genotyped carriers of different LQTS-causing mutations, and all had a normal or only marginally prolonged QT interval in resting ECG. In addition, 32 healthy controls were studied. The body surface potential mapping (BSPM) system was utilized for ECG recording, and signals were analyzed with an automated analysis program. Resting QT intervals were adjusted for heart rate by Bazett’s formula (QT_c), otherwise intervals were compared without correction formulas. In addition to QT interval length, the end part of the T wave, the Tpe interval, was studied during exercise stress testing and an epinephrine provocation test. In the latter, T wave morphology was also
analyzed. The effect of cetirizine on ventricular repolarization was studied in LQT1 and LQT2 carriers and also with supra-therapeutic dose in healthy volunteers.

LQTS mutation carriers had slightly longer QTc intervals than healthy subjects (427 ± 31 ms and 379 ± 26 ms; p<0.001), but significant overlapping existed. LQT2 mutation carriers had a conspicuously long Tpe-interval (113 ± 24 ms; compared to 79 ± 11 ms in LQT1, 81 ± 17 ms in LQT3 and 78 ± 10 ms in controls; p<0.001). In repeated exercise tests, the method for QT and Tpe interval measurements was accurate and highly repeatable. In exercise stress tests, LQT1 mutation carriers exhibit a long QT interval at high heart rates and recovery, whereas LQT2 mutation carriers have a long Tpe interval at the beginning of exercise and at the end of recovery at low heart rates. LQT3 mutation carriers exhibit prominent shortening of both QT and Tpe intervals in exercise stress tests. A small epinephrine bolus revealed disturbed repolarization, especially in LQT2 mutation carriers, who developed prolonged Tpe intervals. A higher epinephrine bolus caused abnormal T waves with a different T wave profile in LQTS mutation carriers compared to healthy controls (p=0.027). These effects were seen in LQT3 as well, a group that may easily escape other provocative tests. In the cetirizine test, the QT and Tpe intervals were not prolonged in LQTS mutation carriers or in healthy controls, even at supra-therapeutic doses. The Tpe interval was slightly shortened in the LQT1 group.

LQTS mutation carriers with normal or non-diagnostic QT interval duration exhibit abnormalities during exercise stress tests and epinephrine provocation. These subtype-specific findings help to diagnose silent LQTS mutation carriers and to guide subtype-specific treatments. The Tpe interval, which signifies the repolarization process, seems to be a sensitive marker of disturbed repolarization along with the QT interval, which signifies the end of repolarization. The method is useful in studying compounds that are suspected to affect repolarization. Cetirizine did not adversely alter ventricular repolarization and would not be pro-arrhythmic in common LQT1 and LQT2 subtypes when used at its recommended doses.
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## ABBREVIATIONS

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<th>Description</th>
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<tr>
<td>APD</td>
<td>action potential duration</td>
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<tr>
<td>bpm</td>
<td>beats per minute</td>
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<td>BSPM</td>
<td>body surface potential mapping</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>HR</td>
<td>heart rate</td>
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<td>LQT1</td>
<td>subtype 1 of long QT syndrome</td>
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<td>LQTS</td>
<td>long QT Syndrome</td>
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<td>QT&lt;sub&gt;c&lt;/sub&gt;</td>
<td>heart rate corrected QT end interval, by Bazett’s formula</td>
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<td>QT&lt;sub&gt;fcd&lt;/sub&gt;</td>
<td>heart rate corrected QT end interval, by Fridericia’s formula</td>
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<tr>
<td>QT peak</td>
<td>the interval from the onset of the Q wave to the peak of the T wave, QT apex interval</td>
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<tr>
<td>QT end</td>
<td>the interval from the onset of the Q wave to the end of the T wave, QT interval</td>
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<tr>
<td>TdP</td>
<td>torsade de pointes ventricular tachycardia</td>
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<tr>
<td>TDR</td>
<td>transmural dispersion of repolarization</td>
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<td>Tpe</td>
<td>T wave peak to T wave end interval</td>
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1. INTRODUCTION

Congenital long QT syndrome (LQTS) is a familial disorder caused by mutations in genes that encode cardiac ion-channel proteins. Defects in these ion channels lead to impaired ventricular repolarization, which may provoke a polymorphic ventricular tachycardia called torsade de pointes (TdP) and lead to syncope—and occasionally—to ventricular fibrillation and sudden cardiac death. The same features characterize acquired LQTS, in which ventricular repolarization is impaired by, for example, drugs or other ion channel blocking agents.

Several subtypes of congenital LQTS have been described, but the three most common (LQT1, LQT2, and LQT3) constitute 95% of the cases (Zareba 2008). Although the disorder is characterized by QT interval prolongation in surface electrocardiograms, in 25-35% of carriers the resting QT interval is normal or only slightly lengthened (Priori et al. 1999, Johnson and Ackerman 2009). These silent carriers are also at risk of developing TdP tachycardia, and sudden death (Schwarz 2006), especially when exposed to agents that may further impair ventricular repolarization.

Genetic testing has become an important tool for diagnosing carriers of congenital LQTS mutations. Still, a large proportion of carriers escape molecular diagnosis. Additional tests that would reveal abnormal ventricular repolarization are needed, especially for recognizing LQTS subtypes, and for helping in choosing subtype specific treatments.
2. REVIEW OF THE LITERATURE

2.1. Action potential

Electrical signalling in cells involves the passage of ions though ionic channels. The major charge carriers are Na\(^+\), K\(^+\), Ca\(^{2+}\) and Cl\(^-\) ions. Each ion moves primarily through its own ion-specific channel. Their movements across the cell membrane generate excitation, which spreads from one cell to the next through gap junctions.

Cardiac action potential (AP) consists of five phases. These phases are a result of passive ion fluxes moving according to electrochemical gradients established by active ion pumps and exchange mechanisms. The intracellular resting potential during diastole is -50 to -95 mV. Phase 0 – rapid depolarization - evokes an action potential. It is caused by sudden increase in membrane conductance to Na\(^+\) ions (the I\(_{Na}\) channels). Phase 1 – early rapid repolarization - is caused by inactivation of the I\(_{Na}\) channels and concomitant activation of several transient outward K\(^+\) currents (I\(_{to}\)). Phase 2 – plateau – is maintained by competition between the outward movement of K\(^+\) through rapid (I\(_{Kr}\)) and slow (I\(_{Ks}\)) outward rectifying channels, the inflow of Ca\(^{2+}\) ions through open L-type Ca\(^+\) channels, and the inflow of Na\(^+\) by Na\(^+\)/Ca\(^+\) exchanger. Phase 3 – final rapid repolarization - proceeds owing to inactivation of I\(_{Ca-L}\) channels and activation of repolarizing K\(^+\) currents (I\(_{Ks}\), I\(_{Kr}\)) and inwardly rectifying K\(^+\) currents (I\(_{K1}\), I\(_{k,Ach}\)). All of this causes an increase in the movement of positive charges out of the cell and the membrane potential shifts to the resting potential. Phase 4 – resting membrane potential – the negative intracellular potential is maintained before the next AP (Rubart and Zipes 2005). Phase 0 produces cardiac depolarization, and repolarization consists of phases 1 to 3.

2.1.1. Action potential duration

Myocardial electrical activity is initiated in the pacemaker cells in the sinoatrial node, then propagated though the atria to the atrioventricular node and, after a brief pause in the conducting Purkinje fibers, further into the myocardium. Each of these regions has
slightly different AP waveforms, because of differences in ion channel expression levels (Nerbonne and Kass 2005).

Prolongation of action potential duration (APD) can be achieved by a reduction of the outward currents, particularly the $I_{Ks}$ or $I_{Kr}$ potassium currents and/or enhancement of the inward currents ($I_{Na}$) during phases 2 and 3 of the action potential (Ackerman and Clapham 1997). The different APD lengths between layers of cells or areas of the heart is named dispersion of repolarization. This is studied by recording the signals of monophasic action potential (MAP) in a variety of experimental mammalian heart models and in intact animal hearts (Killeen et al. 2008). An experimental model based on arterially perfused, left ventricular free-wall wedge preparations suggests that, among the three prominent cell-types within the ventricular wall, the APD is briefest in epicardial cells, and longest in mid-myocardial M cells, and of middle duration in endocardial cells. Thus, the difference in APD between the epicardium and the M region defines the transmural dispersion of repolarization (TDR) (Yan et al. 1998). Although some have shown differences in $I_{Ks}$ density between the right and the left ventricle contributing to interventricular dispersion (Volders et al. 1999), others have claimed that it is increased transmural rather than interventricular repolarization that generates the vulnerable window for the development of arrhythmias (Milberg et al. 2005). On the other hand, recently the TDR from transseptal region was shown to be markedly long compared to TDR from the left ventricular free wall, highlighting the non-homogenous nature of ventricular repolarization (Sicouri et al. 2010).

It has been suggested that $I_{Ks}$ is the dominant determinant of the physiological heart rate dependent shortening of APD. With increasing heart rate $I_{Ks}$ channels remain open, leading to faster rate of repolarization (Tamargo et al. 2004). APD of the ventricle shortens with adrenergic stimulation. M cells are distinguished by the ability of their AP to be more prolonged than the AP of epicardial and endocardial cells in response to β-adrenergic stimulation. This may be due to the relative scarcity of $I_{Ks}$ currents in M cells (Liu and Antzelevitch 1995, Shimizu and Antzelevitch 2000a).
2.1.2. Torsade de pointes

Afterdepolarizations are oscillations of the transmembrane potential that are capable of generating a premature AP (a triggered beat). Afterdepolarizations may occur before (early afterdepolarizations; EADs) or after (delayed afterdepolarizations; DADs) full repolarization. EADs occur during the course of an AP, and their appearance strongly depends on the AP duration and dispersion of repolarization within the ventricular wall (Yan et al. 2001). EAD can induce a propagated response and an extra beat, potentially launching torsade de pointes (TdP) ventricular tachycardia. Usually a “short-long-short” sequence, or “pause dependent” phenomenon (oscillations in preceding RR intervals), is seen before the initiating extra beat (Viswanathan and Rudy 1999, Vos et al. 2000, Noda et al. 2004).

In re-entry, anatomically defined separate pathways (anatomical re-entry) or a group of fibers (functional re-entry) serve as a link to re-excite areas that were just discharged. El-Sherif et al. presented evidence that EAD-induced activity initiates TdP, but the arrhythmia is maintained by a re-entrant mechanism (El-Sherif et al. 1996). Heterogeneous ventricular repolarization (dispersion of repolarization) may serve as substrate for re-entry under various conditions (Patel et al. 2009), and decreasing transmural dispersion of repolarization suppresses TdP (Shimizu and Antzelevitch 1997, Shimizu and Antzelevitch 1998, Shimizu and Antzelevitch 2000b). It has been suggested that mid-myocardial M cells play an important role in the genesis of TdP, because EADs may develop preferentially in M cells (Viswanathan and Rudy 1999) and M-cell zones may produce the discrete refractory borders responsible for the conduction block that gives rise to re-entry (Akar et al. 2002). A Dutch group has proposed interventricular dispersion of repolarization as a factor that predisposes to TdP (Verduyn et al. 1997, Vos et al. 2000).
2.2. Ventricular repolarization in electrocardiogram

2.2.1. QT interval

The QT interval represents the duration from the onset of depolarization to the completion of repolarization. In ECG, it is defined as the interval from the earliest onset of the QRS complex to the end of the T wave (Rautaharju et al. 2009). The onset of the QRS complex is usually easily identified, but the end of T wave may be more difficult to determine. In the most frequently used method, a tangent line to the steepest part of the descending limb of the T wave is drawn; the intersection between this and the isoelectric line is defined as the end of T wave (Lepechkin and Surawitch 1952). The shape of the T wave is variable. Problems arise when the T wave is complex, or when the T and U waves cannot be distinguished. QT interval length has been shown in several studies to be an independent risk factor for arrhythmias in LQTS patients (Moss et al. 1991, Priori et al. 2003, Sauer et al. 2007).

Traditionally, lead II of a standard 12-lead ECG has been used for QT interval measurement, because in this lead the vectors of repolarization usually result in a long single wave. This lead has been recommended for measuring the QT interval in LQTS patients, and when not measureable, then a left precordial lead (preferable V5) (Mönnig et al. 2006). The maximum QT interval duration, however, frequently occurs in different leads. If one wishes to report the longest QT interval duration, each lead should be measured separately. This is usually not possible in large studies. Cowan et al. have stated that leads V2 and V3 provided the closest approximation to the maximum QT interval and therefore recommended that they be used for the measurement (Cowan et al. 1988). The average, or median, QT interval duration as measured by a number of different leads has, for simple mathematical reasons, much superior stability (Camm at al. 2004). In general, it is not recommended to measure a single beat; at least three separate measurements over time should be considered (Malik et al. 2002, Hinterseer et al. 2009).
QT interval is strongly correlated to heart rate. It adapts to heart rate changes, which makes QT intervals recorded at different heart rates difficult to compare. To allow such a comparison, several formulas have been proposed. Most of these formulas are expressed as $\text{QT}_c = \text{QT}/\text{RR}^\alpha$, where $\text{RR}$ is the preceding cycle length in seconds. The most used are Bazett’s square-root formula where $\alpha$ is 0.5 (Bazett 1920) and Fridericia’s cubic-root formula where $\alpha$ is 0.33 (Fridericia 1920). However, the QT/heart-rate relation exhibits high inter-subject variability, and therefore the use of general heart-rate correction formulas may lead to inaccurate conclusions (Malik et al. 2002). If heart-rate adjusting formulas are used, the representative ECG trace should be selected carefully since general heart-rate correction formulas can only be used for approximate clinical assessments over a narrow band of resting heart rates (Malik et al. 2002, Napolitano et al. 2006) and the QT interval does not immediately adapt to rapid heart rate changes (Lau et al. 1988, Toivonen et al. 1997).

In repeated studies, e.g. in examining drug-induced QT interval changes, the variability of QT interval measurements over time should be taken into account (Sarapa et al. 2004). QT interval lengths also vary in patients with congenital LQTS. In serial ECG measurements of 375 paediatric LQTS patients, Goldenberg et al demonstrated considerable variability in $\text{QT}_c$ interval measures, the mean ± SD difference between minimum and maximum $\text{QT}_c$ values being $47 \pm 40$ ms. This highlights the need for serial ECG measures over time, especially in risk stratification (Goldenberg et al. 2006). In studies involving drug effects, it has been proposed that the maximum change from baseline should be reported. It is difficult to determine, however, whether the value obtained is a result of the drug intervention, the inherent variability of the QT interval over time, or of other confounding factors such as circadian changes. The QT interval has been shown to be approximately 20 ms longer during sleep (Bexton et al. 1986, Viitasalo and Karjalainen 1992). The U.S. Food and Drug Administration (FDA) suggests that $\Delta\text{QT}_c$ intervals between 30-60 ms are likely to represent drug effects, while changes of greater than 60 ms should raise concern about a potential proarrhythmic risk (Rautaharju et al. 2009).
2.2.2. Dispersion of repolarization

In 1990, the interlead difference in QT interval duration, termed “QT dispersion”, was proposed as an index of the spatial dispersion of ventricular repolarization (Day et al. 1990). This is calculated simply by the difference between maximum and minimum QT intervals in a 12 –lead ECG. Later, this concept has been criticised by several studies. It seems that it does not reflect in a quantifiable way the heterogeneity of ventricular recovery times, and the methodology of the measurement is questionable. The measurement is poorly reproducible, and there are no reference values (Gang et al. 1998, Malik and Batchvarov 2000, Liang et al. 2005). It has not been adequately validated as a risk indicator of proarrhythmia and therefore is not recommended as a parameter in assessing drug-induced alteration in ventricular repolarization (Haverkamp et al. 2000, Zareba 2007). Probably only very extreme values (e.g. > 100 ms) are of clinical significance (Priori et al. 1994, Malik and Batchvarov 2000, Dabrowski et al. 2000).

Since the concept of M cells and TDR were introduced by Antzelevitch and co-workers, there has been debate about its equivalent on electrocardiogram. In an experimental model, the TDR could be estimated from the ECG as the interval between the peak and the end of the T wave (Yan and Antzelevitch 1998, Shimizu and Antzelevitch 1998). Later, prolonged Tpe interval has been shown to be an index of arrhythmias. Tpe intervals were prolonged in a heterogeneous group of patients who had suffered ventricular arrhythmias (Watanabe et al. 2004), and the dynamic behaviour of Tpe intervals has been shown in ambulatory electrocardiographic recordings in LQTS patients (Viitasalo et al. 2002a).

Recent studies with animal models suggest, however, that Tpe intervals do not correlate with transmural dispersion of repolarization, but are rather an index of the total dispersion of repolarization (Ophthof et al. 2007). Although the genesis of the Tpe interval still remains controversial, it has been shown to be a predictor of arrhythmias under a variety of clinical conditions (Patel et al. 2009).
2.2.3. Morphology of T waves

Experimental models have provided evidence for a cellular basis for the T wave. The opposing voltage gradients between three myocardial cell -types (epi-, M-, and endocardial cells) have been shown to contribute to the deflection of the T wave in ECG. The end of repolarization of epicardial cells coincides with the peak of the T wave, and that of the M cells coincides with the end of T wave. The interplay between these opposite regions determines the height of the T wave and the degree to which the ascending or descending limb of T wave is interrupted, leading to bifurcated or notched appearance. The so-called T-U complex would then actually be an interrupted T wave, because the forces that give rise to U wave appear no different than those responsible for the T wave (Yan and Antzelevitch 1998). Notching of the T wave may be difficult to discriminate from a U wave, and it is suggested that the interval between a monophasic T wave and the U wave usually exceeds 150 ms at heart rates of 50 to 100 bpm (Lepechkin and Surawitz 1957, Lehmann et al. 1994).

A number of descriptive terms are used for T waves, including peaked, flat, biphasic, bifid, asymmetrical and inverted. In study by Lehmann et al., three configurations of bifid T waves were designated and graded depending on the place and height of T wave protuberances (just beyond the peak or on the descending limb of the T wave) (Lehmann et al. 1994).

T wave alternans – beat-to-beat alternation of the morphology, amplitude and/or polarity of the T wave – has also been explained as an alternation in the M-cell APD (Shimizu and Antzelevitch 1999) indicating instability of repolarization (Rautaharju et al. 2009). Catecholamine-provoked microvoltage T -wave alternans was discovered at low heart rates in genotyped LQTS patients, but it failed to identify high-risk patients (Nemec et al. 2003).
2.3. Congenital long QT syndrome

In 1957 Jervell and Lange-Nielsen described a syndrome with prolonged QT intervals and congenital deafness, and in the early 1960s Romano and Ward independently described a similar disease with normal hearing. Thus, the Romano-Ward (RW) syndrome is an autosomal dominant disorder without congenital deafness while Jervell-Lange-Nielsen (JLN) syndrome has been viewed as an autosomal recessive disease with congenital deafness. Since those days, it has been learned that the congenital LQTS is a genetic cardiac chanelopathy characterized by delayed ventricular repolarization, which exposes the affected to TdP ventricular tachycardia, possibly leading to syncope and sudden cardiac death (Moss and Robinson 1992, Schwarz et al. 1993, Camm et al. 2000, Chiang and Roden 2000).

In 1995 and 1996, the three main genes for LQTS were identified. The LQT1 form involves mutations on the \textit{KCNQ1} (previously \textit{KvLQT1}) gene, which encodes the slow delayed rectifier potassium repolarization channel, resulting in a reduction in \(I_{Ks}\) current; the LQT2 form involves mutations on the \textit{KCNH2} (\textit{HERG}) gene, which encodes the rapid delayed rectifier potassium repolarization channel, resulting in a reduction in \(I_{Kr}\) current; and the LQT3 form involves mutations on \textit{SCN5A}, the cardiac sodium channel gene, resulting in an increase in late \(I_{Na}\) current. LQT1 and LQT2 account for about 90% of positively genotyped LQTS cases, whereas LQT3 probably accounts for 5-8%. In a North American/European population, LQT1 constituted 42%, LQT2 45% and LQT3 8% of LQTS cases, whereas in Finland the corresponding figures were 71%, 23% and 6% (Splawski et al. 2000, Fodstad et al. 2004). As of today, 12 LQTS genes have been identified, the remaining types being extremely rare (Schwarz 2006, Ruan et al. 2008, Zareba and Cygankiewicz 2008). The prevalence of congenital LQTS has been estimated to be close to 1:2500 (Lehnart et al. 2007, Schwarz et al. 2009).
2.3.1. Diagnosing LQTS

Until now, genetic testing has yielded from research laboratories to commercially available. Among patients with definitive clinical evidence of LQTS, the portion diagnosed by genetic testing is about 75% (Tester et al. 2006). In Finland, two \textit{KCNQ1} and two \textit{HERG} mutations account for 73% of the Finnish LQTS cases (Fodstad et al. 2004). As many patients still escape molecular diagnosis, genetic testing and clinical evaluation should be combined in the diagnosis and management of LQTS (Schwarz 2006, Lehnart et al. 2007).

Clinical diagnostic criteria were first proposed in 1985, and were updated in 1993 (Schwarz et al. 1993). The main revision concerned QT interval length. Merri et al. had discovered that, among healthy subjects average QTc values were significantly longer in women than in men (Merri et al. 1989). Vincent et al. confirmed the sex difference among LQTS carriers and described a large overlap of QTc values between carriers and non-carriers of LQTS. The highest overall accuracy for the diagnosis of gene-carrier status occurred at QTc \(\geq 460\) ms, yet 9% of male LQTS gene carriers had QTc values <440 ms (Vincent et al. 1992). These findings emphasized the limitations of resting QTc interval measurements for LQTS diagnosis and highlighted the need for additional criteria. See Table 1 for details.

The typical cases –syncope or cardiac arrest, often during physical or emotional stress, and QT interval prolongation on the ECG- present no diagnostic difficulty. However, borderline cases are more complex (Moss et al. 1985, Schwarz et al. 1993, Schwarz 2006). Because LQTS may appear with a low penetrance, the family members considered to be normal may be silent mutation carriers and unexpectedly at risk of developing TdP, especially if exposed to either cardiac or non-cardiac potassium channels blockers.
Table 1. *LQTS diagnostic criteria*

<table>
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<tr>
<th>ECG findings&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Points</th>
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<tr>
<td>A. QT&lt;sub&gt;c&lt;/sub&gt; &lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>≥ 480 ms</td>
<td>3</td>
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<tr>
<td>460-470 ms</td>
<td>2</td>
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<tr>
<td>450 ms (in males)</td>
<td>1</td>
</tr>
<tr>
<td>B. Torsade de pointes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>C. T wave alternans</td>
<td>1</td>
</tr>
<tr>
<td>D. Notched T wave in three limb leads</td>
<td>1</td>
</tr>
<tr>
<td>E. Low heart rate for age&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.5</td>
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Clinical history

<table>
<thead>
<tr>
<th>A. Syncope&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td>With stress</td>
<td>2</td>
</tr>
<tr>
<td>Without stress</td>
<td>1</td>
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</tbody>
</table>

| B. Congenital deafness | 0.5 |

Family history (either A or B)

| A. Family member with definite LQTS<sup>e</sup> | 1 |
| B. Unexplained sudden cardiac death below age 30 among immediate family members | 0.5 |

<sup>a</sup>In the absence of medications or disorders known to affect these electrocardiographic features

<sup>b</sup>QT<sub>c</sub> calculated by Bazett’s formula.

<sup>c</sup>Mutually exclusive

<sup>d</sup>Resting heart rate below the second percentile for age.

<sup>e</sup>Definite LQTS is defined by an LQTS score ≥4.

Zero or one point indicate a low probability of LQTS, 2 or 3 points an intermediate and 4 or more points a high probability of LQTS.
Although the disorder is characterized by QT interval prolongation, in nearly 40% of LQTS patients, resting QTc interval is normal or only slightly lengthened. In other words, the average QTc penetrance – i.e. those individuals presenting a QTc longer than 440 ms for men and 460 ms for women- has been shown to be 60% among genetically affected family members. Patients with LQT2 and LQT3 present higher QTc penetrance (70-79%) compared to LQT1 (55%)(Vincent et al. 1992, Priori et al. 1999, Priori et al. 2003, Napolitano et al. 2005). On the other hand, fully 15% of the general population may have a QTc in the borderline range, so there is substantial overlap in the distribution of QTc between otherwise healthy subjects and patients with genetically confirmed LQTS (Johnson and Ackerman 2009).

Silent mutation carriers are potentially at risk for sudden cardiac death (Schwarz 2006, Priori et al. 2003). Many attempts have been made to facilitate the diagnosis of LQTS. The most useful tools for diagnosing LQTS as well as the main subgroups are clinical presentation, special ECG features, exercise stress tests, ambulatory electrocardiographic recordings, and epinephrine infusion tests.

Clinical presentation
The conditions (triggers) associated with cardiac events suggest certain LQTS subtypes. In LQT1, most events occur during exercise and only a minority during rest or sleep. Swimming as a trigger is particularly frequent in LQT1 patients. In contrast, in LQT3 patients only a minority of the events occur during exercise, and most occur during rest or sleep. LQT2 patients have an intermediate pattern with most of the events occurring during emotional stress. Auditory stimuli occur as a specific trigger in LQT2 (Schwarz et al. 2001).

Typical ST-T–wave patterns
Already in 1995, Moss et al. described LQTS-subtype-specific T wave patterns (Moss et al. 1995). In a study by Zhang et al., four typical LQT1 patterns (infantile, broad-based T wave, normal -appearing, and late –onset normal -appearing); four LQT2 patterns (4 subtypes of bifid T –waves); and two LQT3 patterns (late –onset peaked/biphasic T wave, and asymmetrical peaked T –wave) were described. These
patterns were present in 88% of LQT1 and LQT2 carriers and in 65% of LQT3 carriers. Family-grouped ECG analysis improved genotype identification accuracy. In that study, however, only 6/387 patients (1.6%) had a normal ST-T-wave pattern with a normal QTc interval (Zhang et al. 2000).

**Exercise stress test**

The LQT1 form of LQTS is characterized by an inadequate sinus rate response to exercise and an exacerbation of QT interval prolongation after physical effort. In contrast, in LQT2 the QT interval shortens more than in LQT1 and the sinus node response is normal. LQT3 patients shorten their QT intervals in response to heart rate even more than LQT2 patients (Vincent et al. 1991, Schwarz et al. 1995, Swan et al. 1999). Takenaka et al. studied Tpe intervals during exercise tests in LQT1 and LQT2 patients. They reported increased heart rate adjusted Tpe –intervals (Tpec) in LQT1, but not LQT2 patients. The majority of patients were symptomatic with markedly prolonged QT intervals (Takenaka et al. 2003).

**Ambulatory electrocardiographic recordings**

Holter monitoring may reveal periods of profound bradycardia, runs of TdP, transient QTc prolongation, or episodes of T wave alternation that may help in revealing the syndrome (Moss and Robinson 1992). Measures of QT interval dynamics and Tpe intervals may help in differentiating between LQT1, LQT2 and healthy subjects, since, LQT1 patients exhibit increasing Tpe intervals at elevated heart rates, whereas LQT2 patients increase their Tpe intervals at a much wider range of heart rates (Nemec et al. 2004, Viitasalo et al. 2002a, Viitasalo et al. 2002b).

**Epinephrine infusions**

From the clinical presentation of the syndrome, exogenous adrenergic stimulation might be expected to prolong QT intervals in affected subjects. In an experimental LQT1 model, β -adrenergic stimulation prolonged the QT and APD of mid-myocardial M cells, but abbreviated the same in epi- and endocardial myocytes, causing a persistent increase in TDR. In a LQT2 model, adrenergic stimulation initially prolonged and then reduced the APD of M cells, but always reduced the APD of epicardial cells, thus
transiently increasing TDR. In a LQT3 model, the APD of all three cell–types was reduced, causing a persistent decrease in TDR (Shimizu and Antzelevitch 1998, Shimizu and Antzelevitch 2000).

Two protocols with epinephrine infusion and adequate sensitivity and specificity values have been published: the Shimizu protocol and the Mayo protocol. In Shimizu, the test begins with an epinephrine bolus of 0.1 µg/kg followed by immediate infusion of 0.1 µg/kg/min. Shimizu et al have reported a lengthening of heart rate adjusted QT and Tpe intervals, with more pronounced effects in the LQT1 group compared to LQT2. The protocol may distinguish latent LQT1 mutation carriers, and also help to distinguish LQT2 patients with transient QTc prolongation during the peak epinephrine–effect (Noda et al. 2002, Shimizu et al. 2003, Shimizu et al. 2004). A larger increase in spatial dispersion of repolarization (measured as time between the longest and shortest QTc intervals) in LQT1 patients compared to LQT2 patients has been reported. In the same study, there was no difference between LQT1 and LQT2 in terms of spatial dispersion of heart rate adjusted QT peak interval (Tanabe et al. 2001).

In the Mayo protocol, an epinephrine infusion without a bolus is used, and QT intervals are not adjusted for heart rate. A low-dose epinephrine infusion (0.05 µg/kg/min) caused marked prolongation in absolute QT interval in LQT1 patients and shortening in both the LQT2 and the LQT3 group. At higher doses, infusion caused paradoxical QT prolongation in some controls. The QTc interval showed no discriminative value (Ackerman et al. 2002, Vyas and Ackerman 2006, Vyas et al. 2006).

2.3.2. Management of LQTS

β -blockers have remained the first choice of therapy for LQTS irrespective of the genotype, although their benefit in LQT3 has not been demonstrated (Lehnart et al. 2007, Patel and Antzelevitch 2008). β -blockers have been shown to provide significant reduction in event rates in LQT1 and LQT2 patients (Moss et al. 2000, Sauer et al. 2007, Vincent et al. 2009), whereas there was no evident effect on events in LQT3
patients (Moss et al. 2000). The rate-adjusted QTc intervals at rest were not changed (Moss et al. 2000), but abrupt lengthening of QT and Tpe intervals at elevated heart rates were decreased in LQT1 patients (Viitasalo et al. 2006). The limitations of β-blocker treatment are due to side-effects, which may result in noncompliance (Patel and Antzelevitch 2008, Vincent et al. 2009).

Sodium channel blockade represents a rational approach as gene-specific therapy for LQT3, since mutations causing LQT3 induce excess sodium to enter into the myocytes. Preliminary studies have shown that mexiletine effectively shortens the QT interval in LQT3 (Schwarz et al. 1995), but there is no long-term data that mexiletine improves survival in LQT3 (Napolitano et al. 2006). Another sodium channel blockade, flecainide, should be used with caution in LQT3 patients since it induced an ST-segment elevation resembling Brugada syndrome in about 50% of cases in unselected LQT3 patients (Napolitano et al. 2006).

Oral potassium supplements are effective in reducing repolarization abnormalities in LQT2, but no data demonstrate that they can also reduce the risk of cardiac events (Ruan et al. 2008, Napolitano et al. 2006, Patel and Antzelevitch 2008). LQT2 patients are vulnerable when the potassium level is low, so maintaining adequate levels is advised (Schwarz 2006).

Implantable cardioverter-defibrillators (ICD) are indicated for patients who are at high risk for ventricular arrhythmias, who have recurrent arrhythmogenic syncope or who have ventricular fibrillation during adequate β-blocker therapy (Priori et al. 2002, Lehnart et al. 2007, Hayes and Zipes 2005, Zareba and Cybankiewicz 2008); as well as for primary prevention in patients with markedly prolonged QT intervals (QTc >550 ms) and signs of high electrical instability (Schwarz et al. 2010). Since LQT3 patients are at highest risk of TdP when the heart rate is slow, pacemaker therapy is thought to be most beneficial for them. Pause-dependent TdP is most prevalent in LQT2, and hence pacemaker therapy may also be therapeutic for this group by suppressing pauses (Patel and Antzelevitch 2008). For patients who develop severe bradycardia during β-blocker therapy, concomitant pacemaker therapy is indicated (Chiang and Roden 2000).
Although *left cardiac sympathetic denervation* is very effective in reducing the risk of repeated events in LQTS, this treatment should be limited to a subset of the patients who continue to experience cardiac events despite β-blocker treatment and interventional management in terms of pacemakers and defibrillators (Patel and Antzelevite 2008, Zareba and Cygankiewicz 2008, Schwarz 2006).

All LQTS patients (symptomatic and asymptomatic) *should be provided with a list of the potentially harmful drugs* that may affect ventricular repolarization. There is a consensus that individuals with LQTS *should not be considered eligible to perform competitive sports*, except low-dynamic and low-static sports (golf, billiards, bowling etc.). The recommendations for recreational physical activity are much less clear; high-intensity sports are “not advised” or “discouraged”, whereas low-intensity activities are “probably permitted”. The recommendations can be individualized based on genotype: LQT1 patients should avoid physical stress; in LQT2, limits can be more flexible; and in LQT3, recreational activity does not have to be restricted at all. Based on the arrhythmia triggers, *swimming and diving are contraindicated in LQT1 patients* (Priori et al. 2002, Napolitano et al. 2006, Schwarz 2006, Kapetanopoulos et al. 2006).

### 2.3.3. Risk stratification

Contrary to previous knowledge, LQTS appears to carry a high risk of life-threatening cardiac events throughout adulthood. This risk is associated with clinical and genetic factors. Genotype is an independent risk factor: patients with LQT2 mutations are at a greater risk for cardiac events than patients with LQT1 or LQT3. However, the lethality of cardiac events is highest in LQT3. Women have a higher risk of cardiac events, as do patients who experienced their first event before the age of 18. Long QTc intervals (≥470 ms) predisposes to arrhythmias; patients with borderline (440-469 ms) or normal (<440 ms) QTc are at lower risk (Priori et al. 2003, Zareba et al. 2003, Sauer et al. 2007, Goldenberg et al. 2008).
2.4. Acquired long QT syndrome

2.4.1. Drug–induced long QT

Syncope related to the use of quinidine was described already in the 1920s, but the cause of syncope was not understood until the 1960s. The QT prolonging effect of the drug leads to TdP tachycardia. Non-cardiac drugs may also have this property, and assessing the QT prolonging effects of compounds is currently a major concern in drug development. The risk of possible repolarization prolongation must be weighed against the possible benefits. If a drug is effective in treating oncological disorders, then some degree of QT prolongation may be acceptable. On the other hand, if the drug is marketed to treat allergic rhinitis in otherwise healthy people, then even minor QT prolongation may be unacceptable (Haverkamp et al. 2000, Heist and Ruskin 2010).

Drug-induced QT prolongation is a complicated phenomenon related not only to the ion channel blocking properties of a given drug but also to drug-drug interactions and a variety of patient-specific factors (Heist and Ruskin 2010). Most clinically relevant ion-channel blocking occurs via inhibition of $I_{Kr}$ potassium current, encoded by HERG. Both direct block of the HERG channel and disruption of HERG trafficking to the cardiac cell membrane have been described (Roy et al. 1996, Taglialatela et al. 1998, Heist and Ruskin 2010). Concomitant use of several drugs that share the same metabolic pathway, such as the major cytochrome P450 (CYP3A4) enzyme system, is likely to augment individual drug levels (Honig et al. 1992, Honig et al. 1993, Zareba 2007).

Most patients subjected to drug-induced TdP have at least one recognizable risk factor (Table 2). Women account for 70% of drug-induced LQTS cases (Makkar et al. 1993). Metabolic factors like low magnesium (Kay et al. 1983), low calcium (Akiyama et al. 1989) and especially low extracellular potassium (Kay et al. 1983, Yang and Roden 1996) predispose to TdP. Spontaneous or drug-induced bradycardia also increase the risk (Topilski et al. 2007). In cohorts of acquired LQTS patients, 10-15% carry variants of the known LQTS genes (Yang et al. 2002, Paulussen et al. 2004). Of
disease–causing mutations, the most common occurs in \textit{KCNQ1} (LQT1), where a subclinical loss of I_{Ks} function may become apparent when superimposed to I_{Kr} block (Roden 2006). Common variants (polymorphism) within the human genome may also impair repolarization reserves and predispose to drug-induced TdP (Splawski et al. 2002, Kannankeril 2005, Marjamaa et al. 2008).

Most cases of drug-induced TdP occur in the context of a substantial prolongation of the QT interval. However, QT interval alone seems to be relatively poor predictor of arrhythmic risk because not all drugs that prolong QT intervals also induce TdP (van Opstal et al. 2001, Heist and Ruskin 2010). In experimental models, drugs that cause homogenous prolongation of APD within the three predominant myocardial cell types do not initiate TdP, whereas drugs that cause heterogeneous prolonging of APD (increase TDR) frequently do (Weissenburger et al. 1999, Antzelevitch 2004).

Table 2. Risk factors for drug-induced TdP

<table>
<thead>
<tr>
<th>Risk Factor</th>
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<td></td>
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<tr>
<td>Female gender</td>
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<tr>
<td>Hypokalaemia</td>
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<tr>
<td>Hypomagnesaemia</td>
</tr>
<tr>
<td>Hypocalcaemia</td>
</tr>
<tr>
<td>Bradycardia</td>
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<tr>
<td>Age (polypharmacy)</td>
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<tr>
<td>Congestive heart failure (prolonged APD)</td>
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<tr>
<td>Left ventricular hypertrophy (prolonged APD)</td>
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<tr>
<td>High drug concentration (overdose or impaired elimination)</td>
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<tr>
<td>Concealed congenital LQTS</td>
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<tr>
<td>Predisposing DNA polymorphism</td>
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27
2.4.1.1. Cardiac drugs

The drugs that carry the greatest risk for QTc prolongation and TdP are class III antiarrhythmics (sotalol, ibutilide, dofetilide and azimilide), for which HERG inhibition and QTc prolongation are part of the therapeutic mechanism of action (Heist and Ruskin 2010). Amiodarone, on the other hand, is rarely associated with TdP. This is possible because amiodarone causes homogenous APD lengthening by blocking several ion currents and suppressing EADs (van Opstal et al. 2001). In contrast, sotalol, which is a rather selective IKr current blocker, prolongs the QT interval and sometimes produces TdP (Funck-Breantano 1993, Hohnloser 1997). The effect is dose- and concentration dependent.

Quinidine, a class Ia antiarrhythmic, produces marked prolongation of APD in M cells at relatively low therapeutic concentrations. High concentrations cause prolongation of APD in epicardial and endocardial cells, but shortening in M cells by suppressing late INa current. Thus, low concentrations prolong TDR and cause TdP whereas high concentrations do not (Jenzer and Hagemeijer 1976).

2.4.1.2. Non-cardiac drugs

The main non-cardiac drugs that prolong the QT interval and are associated with TdP include certain psychiatric drugs (antipsychotics and antidepressants), antimicrobial drugs (macrolides and quinolones), and antihistamines (terfenadine and astemitzole) (Haverkamp et al. 2000).

Second-generation antihistamines were widely used in the 1980s because they lack the sedative properties displayed by first generation antihistamines. Soon, several reports about TdP after administration of terfenadine and astemitzole were published. These drugs were discovered to be potent HERG channel blockers, thus prolonging APD and the QT interval. Although the effect was evident mainly at supra-therapeutic concentrations (overdose or concomitant use of CYP3A4 blockers), these drugs were withdrawn from the market (Honig et al. 1992, Honig et al. 1993, Benton et al. 1996,
Ducic et al. 1997, Pratt et al. 1996). Another second-generation antihistamine, cetirizine, was shown to lack HERG blocking properties in experimental studies (Carmeliet 1998, Taglialatela et al. 1998) and to avoid the QT prolonging effects in healthy subjects (Sale et al. 1994). Thus, HERG block is not a class effect of second-generation antihistamines.

2.4.2. Other QT prolonging conditions

QT interval is prolonged in many cardiac diseases, e.g. myocardial infarction, dilated cardiomyopathy, congestive heart failure and hypertrophic cardiomyopathy (Ahnve 1985, Berger et al. 1997, Atiga et al. 2000). Bradycardia increases the amplitude of EADs and produces abnormal repolarization, thus increasing the risk of TdP (Brachmann et al. 1983).

Insulin secretion and glucose intolerance are independently associated with the QT_c interval length, which may predict ventricular electrical instability in these diabetic patients (Dekker et al. 1996). Other metabolic causes are hypokalaemia, hypomagnesaemia, and hypocalcaemia (Curry et al. 1976, Loeb et al. 1968, Akiyama et al. 1989).
3. AIMS OF THE STUDY

The overall purpose of these studies was to improve the detection of long QT syndrome mutation carriers who have indeterminate phenotype.

First, a multichannel BSPM electrocardiogram with a computerized system was tested for repeatability and accuracy of QT interval analysis (Substudy I). In addition to the QT interval duration, T wave peak to T wave end intervals (Substudies I-III, V) and T wave shapes (Substudy IV) were studied.

In order to find differences in ventricular repolarization between LQTS mutation carriers and healthy subjects, the method was applied in interventions such as a physical exercise test (Substudy II) and an epinephrine injection test (Substudies III and IV). A drug exposure with the antihistamine cetirizine was also conducted (Substudy V).
4. SUBJECTS AND METHODS

4.1. Subjects

A total of 81 subjects participated in the studies. We included 49 LQTS family members (27 females), all asymptomatic mutation carriers of KCNQ1, HERG or SCN5A genes. They were otherwise healthy and had normal or only marginally prolonged QT end intervals. No one had β-blocker therapy prior to or during the studies. Subjects with LQT1 (n=22) had four different KCNQ1 mutations (G589D, R366W, IVS7-2A, R518X), with LQT2 (n=17) four HERG mutations (del453C, L552S, R176W, G584S), and with LQT3 (n=10) four SCN5A mutations (V1667I, I239V, A691T, E1784K). In addition, 32 healthy volunteers (14 females) were studied. They had no history of syncope, or any clinical evidence of cardiovascular disease, and had normal baseline QT intervals. None took any regular medication during the studies. All participants were 18-60 years old.

4.2. Methods

4.2.1. Physical exercise stress test

At the beginning of the test, ECG was recorded in supine position for 3 minutes. Thereafter an exercise stress test was performed using a bicycle ergometer. The initial load was set at 30 W, increased each minute by increments of 15 W for women and 20 W for men until exhaustion. After cessation of exercise, subjects immediately lay down, and an ECG was recorded for 10-15 min (the recovery period). Blood pressure was measured manually at rest, and every 3 minutes during exercise and recovery.
4.2.2. Epinephrine test

Subjects were lying on a bed in a quiet and dim room, in order to achieve restful and comfortable conditions. An antecubital vein was cannulated, and physiological saline solution infused. After an adequate period of relaxation, the test was started with a one-minute ECG recording. A rapid bolus dose of epinephrine was administrated intravenously. Five doses (0.005 µg/kg, 0.01 µg/kg, 0.02 µg/kg, 0.04 µg/kg, and 0.1 µg/kg) were given consecutively; however, no more than 10 µg was administered at a time. ECG recording was started at the moment of injection, and registered continuously for six minutes. A short pause was always observed before the next dose was given. Blood pressure was measured at 1, 2, 4 and 6 minutes after injection. Measurement was done non-invasively from standard position of the arm by using an automated blood pressure meter (Welch-Ally Inc., NY, USA).

4.2.3. Electrocardiogram

4.2.3.1. Signal acquisition

A body surface potential mapping (BSPM, BioSemi Mark-6) system was used for recording potentials from the anterior chest. The BSPM system utilizes 120 electrodes in 18 flexible plastic strips, and three limb leads with electrodes attached to the right and left shoulders and to the left hip area. Twelve pre-cordial leads located on the anterior chest were selected for the final analysis (Fig.1). These leads usually showed positive T waves, and were not notably disturbed while cycling. In the antihistamine study (Substudy V), a reduced layout with six pre-cordial leads was used. Recordings were stored on a computer disk.
Figure 1. Electrode layout of the 12 unipolar BSPM chest leads. White circles indicate standard chest leads.

4.2.3.2. Analysis of electrocardiograms

An automatic algorithm was applied to analyze the ECG data (Oikarinen et al. 1998). All selected channels were visualised simultaneously on the computer screen, and noisy channels were rejected. All 12 channels were included in the analysis of the resting data, whereas on average 9.4 channels were utilized for exercise test data analysis. In the antihistamine study, analyses were carried out utilizing signals of the six selected channels. Each ECG lead was first pre-processed by detecting the QRS complexes by an amplitude trigger, by determining the baseline, and creating a QRS template. Atrial and ventricular premature complexes were excluded. Each normal QRS-T deflection was replaced by an averaged QRS-T deflection comprised of the two previous and two following normal heartbeats, using a moving window. After pre-processing, the QRS onset was determined by going towards the QRS from the PR interval until a certain threshold limit was reached.
The QT peak was determined as the peak of the parabola fitted to the highest amplitude change after the QRS. In cases of monophasic T waves, or at high heart rates, the QT end was defined as the intersection of the baseline with the steepest tangent after the QT peak. In cases of non-monophasic T waves, the second derivative was also used to detect discontinuities after the peak, and the U waves were excluded using the previously published guidelines (Lepechkin and Surawicz 1952). Bifid T waves exhibiting a time interval $\leq 0.15$ s between the first and second components were regarded as T waves. Otherwise, the second component was considered as a U wave (Lehman et al. 1994). The Tpe interval was defined as the QT peak interval subtracted from the QT end interval. All measurements were visually inspected, and clearly misinterpreted time points were excluded.

4.2.3.3. Determination of the intervals

After pre-processing, the values for the QT peak, QT end, and Tpe intervals for each heartbeat and lead were obtained. The mean values over the selected leads and intervals were calculated for each heartbeat. These beat-by-beat values were then averaged over certain time periods.

At rest before the exercise test, the QT peak, QT end, and Tpe intervals were averaged for a 30 second period at the end of the recording. Resting QT end intervals were corrected for heart rate by using Bazett’s and/or Fridericia’s formulas (Bazett 1920, Fridericia 1920; QT_c and QT_f, respectively). Intervals were analysed at specified heart rates from 90 to 150 bpm during workload, and from 140 to 100 bpm during recovery by steps of 10 beats/min, allowing a tolerance of $\pm 2$ bpm. Averages of 10-20 consecutive heartbeats were used. Intervals were then compared without heart-rate correction formulas. QT interval/heart-rate (QT/HR) and Tpe interval/heart-rate (Tpe/HR) slopes were also calculated during exercise and recovery for each group. To examine whether QT intervals differed between channels, the intervals were averaged in each lead separately for the last 30 s at rest, and the standard deviation (SD) of the 12 leads was then calculated.
In the epinephrine test, time intervals were determined as an average from three consecutive beats at rest. After administration of epinephrine, QT end and Tpe intervals were examined at their longest, and QT peak at its shortest. The interlead variation was studied by calculating SD of the channels. At rest and at the highest HR, the QT end interval was also corrected for HR by Bazett’s formula. In cases of remarkable T wave changes, when interval values from all twelve channels could not be obtained, T wave morphology was determined. T wave patterns were classified as follows: 1) Normal – appearing, when epinephrine did not cause any remarkable changes despite slight flattening of the T wave; 2) Biphasic, consisting of positive and negative oscillations, followed by a slight third positive component; 3) Inverted, in which the T wave lay completely under the baseline with only minor or no positive oscillations in the vicinity; 4) Obvious bifid; 5) Combined pattern, with a biphasic or inverted component in some, and a bifid component in others of the 12 pre-cordial channels. The T wave pattern was evaluated at the time of its maximal change.

4.2.4. Statistical analyses

Continuous values are presented as mean ± SD. Parametric tests were used for data having a normal distribution, otherwise nonparametric tests were used. Student’s paired t –test was used for intra-individual comparison. Differences between groups were assessed by one-way analysis of variance and by Scheffe’s test, an independent sample T test, and with the Mann-Whitney U test when appropriate. For dichotomous variables, chi-square test was used. A two-tailed P value <0.05 was considered statistically significant. The repeatability of the measurements during exercise tests was assessed by coefficient of variation (CV) (Bland and Altman 1996a,b). CV was determined by analysis of variance, calculated as the square root of the mean square of within-subject variability divided by the mean of each variable. CV values are expressed as percents. Statistical analyses were carried out with the commercial software SPSS for Windows (SPSS Inc., Chicago, IL, USA; versions from 12.0. to PASW 18.0)
4.3. Study designs

4.3.1. Physical exercise stress tests

We studied the reproducibility of QT interval measurements from several ECG leads by an automated algorithm at rest and during a maximal exercise stress test (*Substudy I*). Ten LQTS mutation carriers (5 with *KCNQ1* and 5 with *HERG* mutations), and 11 healthy controls performed an exercise stress test twice. The time interval between these ranged from 1 to 31 months, being 19 months on average.

To improve the diagnosis of LQTS, the QT and Tp e intervals were compared at pre-specified heart rates during the exercise and recovery periods of the exercise tolerance test (*Substudy II*). Fifteen *KCNQ1* (8 females), 15 *HERG* (8 females) and 9 *SCN5A* mutation carriers (5 females) as well as 27 healthy controls (12 females) performed the test. The mean ages were 34 ± 11, 41 ±10, 35 ±15, and 34 ± 7 years, respectively.

4.3.2. Epinephrine bolus challenges

The QT peak, QT end and Tpe intervals were examined after epinephrine bolus injection (*Substudy III*). At the highest epinephrine dose, the morphology of T wave was evaluated (*Substudy IV*). The epinephrine test was performed for 10 *KCNQ1* (5 females), 10 *HERG* (5 females) and 10 *SCN5A* mutation carriers (5 females) as well as for 15 healthy controls (8 females). The mean ages during the test were 36 ± 13, 42 ± 11, 34 ± 14, and 35 ± 9 years, respectively.
4.3.3. Antihistamine provocation

The effect of antihistamine cetirizine on ventricular repolarization was studied during the physical exercise test (Substudy V). LQTS mutation carriers took placebo and cetirizine 10 mg, and in addition, healthy controls took cetirizine 50 mg daily. The study was single–blinded and randomized. Subjects took capsules at home 3 days before the test-day; the last (fourth) dose was given at the hospital. Fifteen \textit{KCNQ1} mutation carriers (8 females), 15 \textit{HERG} mutation carriers (8 females), and 15 healthy controls (7 females) were included. The mean ages were $34 \pm 11$ years in LQT1, $41 \pm 10$ years in LQT2, and $35 \pm 8$ years in the control group.
5. RESULTS

5.1. Baseline values of the population

There was no difference in resting heart rate or blood pressure (data not shown). QTc and Tpe intervals are presented in Table 3. LQTS mutation carriers had longer QTc intervals than healthy controls (427 ± 31 ms, and 379 ± 26 ms, respectively; p<0.001), but significant overlapping existed. Among LQTS carriers, the QTc interval was ≥ 440 ms in 4/22 males, and ≥ 460 ms in 6/27 females. LQTS subgroups did not differ from each other by QTc interval, but LQT2 mutation carriers had longer Tpe –intervals than other LQTS mutation carriers or healthy controls.

Table 3. Baseline QTc and Tpe interval values

<table>
<thead>
<tr>
<th></th>
<th>QTc (ms)</th>
<th>QTc (range, ms)</th>
<th>Tpe (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQT1</td>
<td>416 ± 25*</td>
<td>367 - 430</td>
<td>444 - 477</td>
</tr>
<tr>
<td>LQT2</td>
<td>438 ± 32*</td>
<td>411 - 472</td>
<td>393 - 492</td>
</tr>
<tr>
<td>LQT3</td>
<td>431 ± 38*</td>
<td>355 - 443</td>
<td>405 - 491</td>
</tr>
<tr>
<td>Controls</td>
<td>379 ± 26</td>
<td>334 - 430</td>
<td>352 - 462</td>
</tr>
</tbody>
</table>

*p<0.001, compared to controls. **p<0.001, compared to others.
5.2. Reproducibility of QT and Tpe interval measurements

5.2.1. Immediate reproducibility within the test

Measurements without HR correction showed the lowest variation, whereas HR correction with formulas produced larger CV values. These were smaller with Fridericia’s than Bazett’s formula. The CV was 1.6% for QTfc and 2.4% for QTc. This was influenced by variation in HR, as the CV for HR was 5.5%. Overall, the CV for QT end intervals was 0.6%, for QT peak intervals 0.8%, and for Tpe intervals 2.3%.

5.2.2. Reproducibility between separate tests

The QT end and QT peak interval measurements showed similar CV values (4.4% and 4.2%, respectively), but the Tpe interval showed slightly higher figures (10.2%) at rest. During the exercise stress test, the CV of the QT end and QT peak intervals determined at specified heart rates ranged from 2.0% to 3.4% in the whole study cohort. The range extended from 1.8% to 3.8% in healthy subjects, and from 1.9% to 3.4% in LQTS mutation carriers. The CVs of the Tpe intervals ranged from 5.9% to 11.8%. These were of the same magnitude between the workload and recovery phases, and in the subgroups. See Substudy I for details.

5.3. Ventricular repolarization dynamics during exercise and recovery

5.3.1. LQT1 carriers

LQT1 mutation carriers had a less steep QT/HR slope (-1.0 ± 0.5) than LQT2 (-1.5 ± 0.3; p<0.01) or LQT3 (-1.8 ± 0.4; p<0.001) carriers, indicating a weaker shortening of QT intervals during exercise. At high heart rates of 120-150 bpm, LQT1 mutation carriers had a longer QT interval than carriers of other LQTS types (p<0.01) or healthy controls (p<0.001). During the recovery period, LQT1 mutation carriers had a significantly longer QT interval than healthy controls or LQT3 carriers (p<0.01).
At the beginning of exercise stress test LQT1 carriers had similar Tpe intervals to LQT3 carriers or healthy controls, but at higher heart rates of 140-150 bpm they had longer Tpe intervals (p<0.05).

5.3.2. LQT2 carriers

LQT2 mutation carriers showed shortening of QT interval during exercise. The QT/HR slope was steeper than in LQT1 carriers (-1.5 ± 0.3 and -1.0 ± 0.5, respectively; p<0.01). During recovery, they had a longer QT interval than LQT3 carriers or controls at low heart rates (p<0.05).

At the beginning of exercise at low heart rates, LQT2 mutation carriers had a longer Tpe interval than other LQT S type carriers or controls (p<0.001). However, LQT2 carriers had a steeper Tpe/HR slope than LQT1 carriers (-0.54 ± 0.28 and -0.15 ± 0.43 respectively; p<0.01), so that above a heart rate of 110 bpm they did not differ from LQT1 carriers.

During recovery, LQT2 carriers had a steeper Tpe/HR slope (-0.90 ± 0.50) than LQT1 (-0.45 ± 0.38; p<0.05) or LQT3 (-0.31 ± 0.42; p<0.01) carriers. The Tpe interval at low heart rates of 100-110 bpm was longer in LQT2 carriers than in LQT1 carriers (p<0.05), or in LQT3 carriers or healthy controls (both p<0.001).

5.3.3. LQT3 carriers

During exercise, the QT/HR slope was steeper in LQT3 mutation carriers than in controls (-1.8 ± 0.4 and -1.3 ± 0.3, respectively; p<0.01) Nearly all LQT3 carriers exhibited an over 30 ms shortening in QT intervals at heart rates of 90 bpm to 100 bpm. At high heart rates they had a shorter Tpe interval compared to other subtypes (p<0.05), but not to controls.
5.3.4. Summary of notable findings

Figure 2 shows the dynamics of ventricular repolarization in the exercise stress tests. At the conclusion, certain cutoff values could be distinguished. At the beginning of exercise, at low heart rates, LQTS mutation carriers had longer QT intervals than healthy controls (p<0.01). At a heart rate of 90 bpm, most LQTS carriers had a QT interval above 370 ms, yielding a diagnostic sensitivity of 72% and specificity of 96%. In separating LQT3 carriers from healthy controls, this yielded a sensitivity of 86% and specificity of 96%. At a heart rate of 150 bpm, most of LQT1 carriers had QT interval above 300 ms. In separating LQT1 carriers from others, this limit had an 86% sensitivity and 96% specificity. In separating LQT1 carriers from other LQTS subtypes, sensitivity was 86% and specificity 91%. At a heart rate of 100 bpm during recovery, QT interval > 340 ms showed 96% sensitivity and 85% specificity for the combined LQT1 and LQT2 groups.

At a heart rate of 90 bpm, most LQT2 carriers had Tpe intervals over 90 ms, showing 83% sensitivity and 71% specificity between all others, and 83% and 70% among all LQTS carriers, respectively. Tpe values <70 ms showed 100% sensitivity and 75% specificity, thus identifying the LQT3 subtype among LQTS mutation carriers. During recovery at a heart rate of 100 bpm, all LQT2 carriers had Tpe intervals longer than 90 ms. This limit had 100% sensitivity and 60% specificity in separating LQT2 mutation carriers from other subtype carriers.
Figure 2. QT interval and Tpe interval dynamics during exercise and recovery phases of exercise test in phenotypically silent LQTS mutation carriers at resting ECG. Values are presented as mean ± SEM at specified heart rates.
5.4. Ventricular repolarization dynamics during epinephrine provocation

5.4.1. General results

All doses increased the heart rate and systolic blood pressure, the highest dose showing the biggest effect. With the highest dose, the heart rate increased significantly (p<0.001) in all groups and reached 97 ± 9 bpm in LQT1, 96 ± 13 bpm in LQT2, 95 ± 17 bpm in LQT3 and 95 ± 8 bpm in controls, with no difference between the groups. Systolic blood pressure rose similarly in all groups from 126 ± 14 mmHg to 132 ± 18 mmHg (p<0.001), and diastolic blood pressure decreased from 78 ± 9 mmHg to 74 ± 7 mmHg (p<0.001).

The first three doses showed little or no effect, whereas the fourth dose showed measureable effects on interval lengths. Therefore, the results of 0.04 µg/kg are presented (Substudy III). The fifth dose gave rise to grossly abnormal T waves, preventing interval measurement. The morphology of the T waves was thus evaluated (Substudy IV).

5.4.2. QT and Tpe intervals

Heart rate adjusted QTc intervals at maximal heart rates increased during an epinephrine 0.04 µg/kg bolus among all groups (Table 4). The QT peak interval was shortened, and Tpe interval lengthened. Interlead variation of QT end intervals (SD) between the 12 pre-cordial ECGs was slightly increased in all groups (for LQT2, p=0.055), while the SD of QT peak interval was increased only in the LQT1 and LQT2 groups.

The Tpe interval increased more in the LQTS population than in controls (32 ms and 18 ms, respectively; p<0.05) and the absolute change was largest in LQT2 (44 ms).

The interlead variation in T wave peak intervals was more pronounced in the LQTS population, especially in LQT1 and LQT2. Although LQT1 and LQT2 mutation carriers
tended to have a larger deviation in QT end intervals after epinephrine, this was not statistically significant compared to the others.

Table 4. $QT_c$, $Tpe$, SD of QT end and SD of QT peak at baseline and during epinephrine (epi) 0.04 μg/kg bolus.

<table>
<thead>
<tr>
<th></th>
<th>$QT_c$</th>
<th>$Tpe$</th>
<th>SD of QT end</th>
<th>SD of QT peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Epi</td>
<td>Baseline</td>
<td>Epi</td>
</tr>
<tr>
<td>$LQT1$</td>
<td>413±25</td>
<td>477±46§</td>
<td>74±10</td>
<td>96±9§</td>
</tr>
<tr>
<td>$LQT2$</td>
<td>425±33</td>
<td>510±64§</td>
<td>106±19</td>
<td>150±33+</td>
</tr>
<tr>
<td>$LQT3$</td>
<td>411±32</td>
<td>480±40§</td>
<td>75±13</td>
<td>105±25+</td>
</tr>
<tr>
<td>Controls</td>
<td>380±29</td>
<td>449±21§</td>
<td>79±8</td>
<td>97±15§</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD, in milliseconds. LQT1, LQT2 and LQT3 groups: n=10; control group: n=15. *p<0.05, +p<0.01, §p<0.001; compared to baseline.

5.4.3. T wave morphology

LQTS carriers and healthy controls had different T wave morphological profiles (p=0.027) (Figure 3). Eighty per cent of healthy controls showed no change or biphasic pattern after epinephrine bolus. One healthy control expressed a slight bifid pattern, but showed no accompanying negative T waves. LQTS mutation carriers had a variety of T wave patterns, the bifid or combined pattern being the most
frequent, present in 50% of cases. Normal appearing T waves were uncommon, present in 4/30 (13%); the biphasic pattern was more frequent, but still present in only 6/30 (20%) of LQTS subjects. It is noteworthy that LQT3 carriers had bifid or combined pattern in 6/10 (60%) of cases. The time from the injection to the maximal T wave changes was $52 \pm 8$ seconds (range 34–65 seconds).

Figure 3. *T* wave morphology after epinephrine bolus of 0.10 $\mu$g/kg. *LQTS* mutation carriers, $n=30$; healthy controls, $n=15$. “Combined” includes concomitant biphasic/inverted + bifid patterns.
5.5. Ventricular repolarization during antihistamine provocation

The antihistamine cetirizine did not affect resting QT interval in LQTS mutation carriers or in healthy subjects, even at supra-therapeutic 50 mg doses. The Tpe interval was slightly shortened in LQT1 (83 ± 11 ms with placebo and 76 ± 6 ms with cetirizine) and in LQT2 (114 ± 25 ms and 103 ± 20 ms, respectively) with a p value <0.05.

During the exercise test, LQT1 carriers had shorter QT intervals with cetirizine than with placebo at HR 110 bpm during workload, and at HR 140 bpm and 130 bpm during recovery. The Tpe interval was also shortened at HR 90, 120, 140, and 150 during workload, and at the highest heart rates of 140 -120 bpm during recovery. When the Tpe interval was shorter with cetirizine, the mean difference was 7.9 ± 2.3 ms. In LQT2 mutation carriers, cetirizine did not have an influence on QT interval or Tpe interval during exercise or recovery at any HR level.

In healthy controls, QT interval was longer with cetirizine 50 mg than with placebo during recovery at HR 140 bpm (267 ± 16 ms with cetirizine 50 mg, 267 ± 15 ms with cetirizine 10 mg and 259 ± 12 ms with placebo; p<0.05). No significant change was observed at other heart rates. The Tpe interval was longer with cetirizine than with placebo at HR 140 bpm during exercise, and at HRs 140, 130 bpm and 110 bpm during recovery (p<0.05). When Tpe interval was longer with cetirizine, the mean difference was 6.3 ± 1.5 ms between cetirizine 10 mg and placebo, and 5.0 ± 1.2 ms between cetirizine 50 mg and placebo. There was no difference between cetirizine 10 mg and 50 mg.

In summary, cetirizine did not lengthen the QT interval in LQTS mutation carriers or in healthy subjects at rest or during exercise tests. Even at supra-therapeutic doses of 50 mg, cetirizine did not lengthen QT intervals in healthy subjects. LQT1 carriers exhibit shorter Tpe intervals at rest and during exercise tests on cetirizine, whereas LQT2 carriers had a shorter Tpe at rest, but no effect was shown during the exercise tests. In healthy controls, the Tpe interval was slightly longer on cetirizine at one third of measuring points during the exercise tests.
6. DISCUSSION

6.1. Main observations

Resting QT interval durations overlapped considerably between silent LQTS mutation carriers and healthy volunteers, making the diagnosis challenging. The LQTS subgroups cannot be separated on the basis of QTc interval length alone. LQT2 carriers had the longest Tpe intervals, distinguishing them from the others. During the exercise stress test, certain features in repolarization parameters specific to subgroups could be identified, enabling separation into different LQTS subtypes. LQT1 mutation carriers had lack of normal shortening of QT and Tpe intervals during exercise and recovery. LQT2 mutation carriers had proper shortening of both, and exhibited remarkably long Tpe intervals at low heart rates at the beginning of exercise and again at the end of recovery. A small epinephrine bolus lengthened the Tpe intervals further. LQT3 mutation carriers had prominent QT and Tpe interval shortening in the exercise test, which distinguished them from the other LQTS subtypes. The highest epinephrine bolus separated LQTS mutation carriers (even LQT3 carriers) from controls. Along with QT interval measurement, evaluation of Tpe interval seems to add valuable diagnostic information about the different repolarization profiles of the LQTS subtypes. A combination of these tests may help to guide molecular genetic screening and treatments as well.

The effect of antihistamine cetirizine on ventricular repolarization was studied during exercise tests, which enabled intervals to be compared in certain physiological settings and at various heart rates. This eliminates the need for heart rate adjusting formulas, which should be avoided in studies involving drug effects. Cetirizine did not have adverse effects on ventricular repolarization in silent LQTS mutation carriers.
6.2. Relation to previous studies

6.2.1. Exercise tests

Some earlier observations of QT interval dynamics could be repeated in this population of silent mutation carriers with indeterminate ECG at baseline: QT interval does not shorten properly in LQT1 during exercise, whereas LQT2 does show proper shortening and LQT3 supra-normal shortening (Vincent et al. 1991, Schwarz et al. 1995, Swan et al. 1999). The Tpe interval discriminated between subgroups. Takenaka et al. reported that heart rate adjusted Tpe intervals increase during exercise in LQT1, but not in LQT2, highlighting the growing transmural dispersion that occurs during exercise in LQT1 (Takenaka et al. 2003). In their data, Tpe intervals shortened in LQT2 patients, as in our study. However, our study demonstrated a discriminating value for LQT2 in the Tpe interval at early exercise and in late recovery. There are three main differences between the present study and Takenaka et al. First, our interval comparisons were conducted at certain heart rates without using heart rate adjusting formulas. Second, our study population included silent LQTS carriers, whereas 57% of the patients in Takenaka et al. were symptomatic with markedly prolonged QTc intervals at baseline (mean QTc >500 ms). Finally, we included LQT3 mutation carriers. Repolarization abnormalities that were evident in symptomatic patients exhibiting prolonged QTc intervals can be provoked in silent carriers, and a combination of QT and Tpe interval evaluations helps to differentiate the subgroups.

6.2.2. Epinephrine tests

This is the first study in which epinephrine was administered as an intravenous bolus injection to reveal abnormal repolarization. The group by Shimizu et al. used a bolus injection followed by instant infusion of epinephrine (Noda et al. 2002, Shimizu et al. 2004). They used Bazett’s formula for adjusting the Tpe interval to the heart rate. This is not a validated method, as no studies for heart rate adjusting formulas for Tpe intervals exist. The Mayo group used only an infusion and compared QT intervals without heart rate correction formulas (Ackermann et al. 2002, Vyas and Ackermann
2006). The infusion protocols seem to be powerful in detecting carriers of LQT1, whereas our protocol with a small bolus helps to distinguish carriers of LQT2. Others studies have examined interval duration during “steady state” epinephrine infusion. The highest epinephrine bolus in our study caused marked, but rapidly dissolving, changes in T wave morphology. The T wave was also biphasic or inverted in many healthy controls, but the LQTS mutation carriers additionally exhibited bifid T waves. The phenomenon was evident in LQT3 mutation carriers too. Previous epinephrine infusion tests have failed to distinguish LQT3 carriers, whereas our bolus test may be used for this purpose.

6.2.3. Drug studies in congenital LQTS

Cardiovascular drugs that would be beneficial in preventing syncope and sudden cardiac death have been studied in congenital LQTS patients (Schwarz et al. 1995, Moss et al. 2000). However, no non-cardiac drug tolerability has ever been clinically tested in known carriers of congenital LQTS before. Pre-clinical screening includes in vitro and in vivo studies with various expression systems, disaggregated cells, isolated tissues and isolated intact hearts or animals. Clinical safety studies include healthy volunteers and/or the target population for which the drug is being developed (Haverkamp et al. 2000, Heist and Ruskin 2010). A commonly used antihistamine, cetirizine, had been carefully evaluated in previous studies, because some other previously marketed antihistamines (terfenadine and astemizole) had QT prolonging effects. This made it ethically acceptable to study cetirizine in LQTS mutation carriers with known defects in ventricular repolarization.

6.3. Methodological considerations

There are several critical issues in studying ventricular repolarization in repeated measurements. The first one concerns lead selection: generally, when comparing measurements, the intervals should be examined in the same leads. However, the intervals are not always measurable in the same leads, e.g. the T wave may be so flat that the measurements cannot be done. Furthermore, there may be intra-individual
variation in QT interval duration between lead sites. Therefore, in the present study the measurements of several pre-cordial leads were averaged. During exercise, signals in some of the leads became unreadable, but remained available for measurements in several other channels.

Manual measurements are prone to inter- and intra-observer variations. The computerized method is likely to diminish observer–dependent errors. Our method was not totally automate, since grossly misinterpreted measurements and channels with noisy recordings were eliminated manually. However, the examiner could not change the positions of measurement points, so that absolute interval values from interpretable data were fully determined by the computer.

The QT interval may have some natural variation over time. In our study, long-term repeatability of QT intervals was better during workload and recovery phases of exercise test compared to rest conditions. Using plain QT interval measurements at rest, one cannot be certain if the minor lengthening of QT interval is natural variation over time or the result of drug influence. The literature shows examples of drugs that were developed for generally benign conditions, but that led to death due to adverse effects on ventricular repolarization (Simons et al. 1988, Monahan et al. 1990, Woosley et al. 1993). Today the drugs under development are therefore examined carefully in terms of their QT prolonging properties. Examining QT and Tpe intervals in standardised physiological settings, e.g. during exercise stress tests, diminish the confounding factors that may modify QT intervals during plain resting measurements.

We examined and compared intervals during workload and recovery in the exercise stress test, which eliminated the need for heart rate corrections. In addition, there are no generally approved heart rate correction formulas for the Tpe interval, so comparison between different interventions, e.g. before and during drug therapy, should be done at the same heart rates.
6.4. Ventricular dispersion of repolarization in ECG

It is widely accepted that the QT interval in surface ECG is a presentation of action potential duration, and the end of the T wave signifies the end of ventricular repolarization. Factors that slow ventricular repolarization also prolong the QT interval. Depolarization (QRS interval) duration also affects the QT interval. However, factors that prolong QRS intervals do not necessarily affect repolarization time. Therefore, it is important to focus on repolarization, the last part of which is reflected as the Tpe interval on surface ECG recordings. The Tpe interval is likely to be less dependent on QRS duration. There is also growing evidence that the dispersion of repolarization serves as a substrate for TdP (Yan et al. 2001, Patel et al. 2009). Thus, measuring changes in repolarization demands delicate examination of the QT interval and, in particular, the Tpe interval.

Dispersion of repolarization, estimated as the difference between the longest and the shortest QT interval in a standard 12-lead ECG, has been criticised and partly abandoned for many reasons (Gang et al. 1998, Malik and Batchvarov 2000). The last years have seen a remarkable increase in knowledge regarding the electrical properties of cardiac cells. Ventricular myocardial tissue is not homogenous, but includes at least three electrophysiologically and functionally distinct cell types: endocardial, epicardial and mid-myocardial cells (M cells) (Yan and Antzelevitch 1998). M cells are not found in discrete bundles or islets, and the hallmark of the M cell is the ability of its APD to exceed epicardial or endocardial APD (Yan et al. 1998). Studies with arterially perfused left ventricular free wall wedge preparations suggested that the interval between T wave peak and T wave end, i.e. Tpe interval, may provide a measure for transmural dispersion of repolarization (Yan and Antzelevitch 1998). Later, this hypothesis has been challenged by whole-hearts models, suggesting that the Tpe interval is not an index of transmural dispersion, but rather, of total dispersion of repolarization (Opthof et al. 2007).

In our epinephrine study, a small intravenous dose caused deviation of the T wave peak and end intervals among the twelve precordial leads, signifying a more chaotic
repolarization pattern in LQTS mutation carriers. On the other hand, healthy controls had smaller deviation between the channels. Deviation of repolarization of the T wave peak and end intervals may be explained by the earlier observations that demonstrated a lack of distinct M cell layers in the ventricular myocardium. Repolarization finishes heterogeneously in different areas of the ventricles. The Tpe interval might not be a marker of transmural dispersion of repolarization, but it includes various kinds of non-homogeneities and reflects repolarization processes. Our method, which calculates the average of all selected precordial leads, may be a more adequate way to express the total dispersion of repolarization than measurement from a single lead.

An epinephrine bolus test distinguished LQT2 mutation carriers by eliciting prolonged Tpe intervals. The lengthened Tpe intervals may reflect early bifid T waves, which are more common in this subtype (Zhang et al. 2000). This is supported by the observation that LQT3 mutation carriers also exhibited lengthened Tpe intervals after a small epinephrine bolus, and after a higher epinephrine dose, exhibited obvious bifid T waves. The Tpe interval reflects dispersion of repolarization, as do the bifid T waves.

In our studies the Tpe interval seemed to be a more sensitive marker of disturbed repolarization than the plain QT interval. In exercise stress testing, the behaviour of the Tpe interval was specific for LQTS subtypes, separating not only the healthy controls, but also the different LQTS subtypes that were examined. In the epinephrine study, a small bolus injection affected the Tpe interval differently among the different subtypes. Besides the QT interval measurements, we propose measuring the Tpe interval, since it seems to contain additional information about the repolarization process and dispersion, whereas QT intervals reflect only the global end of repolarization.

6.5. Practical implications

Misdiagnosis of LQTS patients as healthy individuals poses a serious problem, since such individuals may be at risk for sudden cardiac death. Conversely, misclassifying non-carriers as affected can lead to substantial anxiety and inappropriate treatment (Vincent et al. 1992). Genetic testing reveals some of the carriers, but clinical
evaluation still has an important role as well. Exercise stress testing is a non-invasive and safe tool for evaluating ventricular repolarization, and it is currently recommended for all suspected cases. Although the Tpe interval has been recognized and studied for a number of years, to our knowledge it is not a commonly measured parameter in clinics. Nothing prevents it from being used in everyday clinical practice to assess congenital or acquired LQTS, in resting conditions and during exercise tests. This study suggests certain cut-off values for QT and Tpe intervals, although the small group size restricts their use as reference values.

Epinephrine tests can also be safely performed in cases of suspected LQTS, though it is somewhat more complicated to carry out due to its pharmacological nature. A standard 12-lead ECG may be used, provided that a continuous ECG recording is available, as the BSPM recordings are limited to research use only. We recommend that the test be performed in a hospital environment and interpreted by a cardiologist with experience in this particular field.

Regulatory authorities have expended much effort in diminishing the risks of acquired LQTS. Extensive preclinical studies on ventricular repolarization are of major importance in the preclinical safety assessments of new compounds. However, the effects on repolarization on congenital LQTS mutation carriers are never studied, although LQTS mutations may be prevalent in the general population and despite it could be possible in carefully observed conditions. Naturally, it would be unethical to expose symptomatic patients with long QT intervals to drugs that would potentially even further prolong the QT interval. However, silent carriers may be studied, while bearing in mind that this population may be subject to a further worsening of ventricular repolarization upon exposure to a new drug.
7. CONCLUSIONS

Examining multiple BSPM leads, calculating the average of these leads, and comparing interval values in a standardized physiological setting at the same heart rates produces highly reproducible and accurate measurements.

The Tpe interval is a more sensitive marker than QT interval duration. The Tpe interval signifies the repolarization process, whereas the QT interval signifies the global ending of ventricular repolarization.

Physical exercise stress tests and epinephrine tests are safe, non-invasive techniques that help in diagnosing LQTS mutation carriers and in making diagnostic distinctions between subtypes when resting ECG alone is not of sufficient diagnostic value.

Long QT interval durations during exercise and recovery from exercise stress testing may reveal subtype 1 of LQTS, with unusual lack of shortening of Tpe interval providing support for the diagnosis. Type 2 LQTS can be identified by having long Tpe intervals at low heart rates and proper shortening of both Tpe and QT intervals at elevated heart rates. Abrupt lengthening of Tpe intervals in epinephrine testing is typical for LQT2. Type 3 LQTS may be exposed by short Tpe intervals in exercise stress tests and bifid T waves in epinephrine challenge tests.

Finally, the validated QT and Tpe interval measurement method was shown to be useful in assessing the effects of pharmacological interventions on ventricular repolarization. Using this method, it was demonstrated that cetirizine at recommended doses is a safe antihistamine for silent LQTS mutation carriers.
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