DIAGNOSTIC EVALUATION OF CONGENITAL LONG QT SYNDROMES

HEIKKI SWAN

Department of Medicine, Division of Cardiology

University of Helsinki

Finland

Academic dissertation

To be publicly discussed with the permission of

the Medical Faculty of the University of Helsinki,

in Auditorium 1, Meilahti Hospital, on May 26th, 2000, at 12 noon

HELSINKI 2000

Supervised by

Docent Lauri Toivonen, M.D.
Department of Medicine
University of Helsinki
Helsinki, Finland

Docent Matti Viitasalo, M.D.
Department of Medicine
University of Helsinki
Helsinki, Finland

Reviewed by

Professor Michael H. Lehmann, M.D.
Electrocardiography Laboratory
University of Michigan Medical Center
Ann Arbor, Michigan, USA

Professor Heikki Huikuri, M.D.
Department of Medicine
University of Oulu
Oulu, Finland

ISBN 952-91-2124-5 (nid)
ISBN 952-91-2204-7 (PDF version)
Yliopistopaino, Helsinki 2000
http://ethesis.helsinki.fi

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I Swan H, Toivonen L, Viitasalo M. Rate adaptation of QT intervals during and after exercise in children with congenital long QT syndrome. European Heart Journal 1998; 19: 508-13.

II Swan H, Saarinen K, Kontula K, Toivonen L, Viitasalo M. Evaluation of QT interval duration and dispersion and proposed clinical criteria in diagnosis of long QT syndrome in patients with a genetically uniform type of LQT1. Journal of The American College of Cardiology 1998; 32: 486-91

III Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L. Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects. Journal of The American College of Cardiology 1999; 34: 823-9.

IV Swan H, Piippo K, Viitasalo M, Heikkilä P, Paavonen T, Kainulainen K, Kere J, Keto P, Kontula K, Toivonen L. Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. Journal of The American College of Cardiology 1999; 34: 2035-42.

ABBREVIATIONS

ECG	Electrocardiogram, electrocardiographic
LQT1	LQT1 type of long QT syndrome
LQT2	LQT2 type of long QT syndrome
LQT3	LQT3 type of long QT syndrome
LQTS	Long QT syndrome
MAP90	Duration of 90 % repolarization of monophasic action potential
QTc	Rate adjusted QT interval (Bazett's square root formula)
QTfc	Rate adjusted QT interval (Friedericia's cubic root formula)

1. INTRODUCTION

Unraveling the cause of syncopal spell in an young individual with apparently normal heart has turned into a challenge of clinical cardiology and molecular genetics at the dawn of the 21st century. The stakes are the risk of death of the symptomatic individual and, ever since the recognition of the genetic nature of these diseases, even his or her relatives.

In adult population, the majority of sudden deaths is related to coronary heart disease and its consequences. Although the cardiac deaths make up only a small proportion of all causes of deaths in children, adolescents and young adults under the age of 35 years, sudden cardiac deaths occur in the absence of any apparent heart disease, sometimes in families with a history of a previous sudden death at young age.

From the 1950's, it has been recognized that there are cardiac disorders for which the only symptoms are disturbances of heart rhythm without any signs of structural alterations of the heart whatsoever. The presence of a prolonged repolarization time on an electrocardiogram, the QT interval, was first related to propensity to sudden cardiac death in a mute individual (Jervell and Lange-Nielsen 1957). A few years later, it became apparent that stress could provoke syncopal spells even in people with long QT interval but with normal hearing (Romano et al. 1963, Ward 1964).

While the number of identified individuals with this novel long QT syndrome, LQTS, was increasing, a claim on separating this congenital disorder from the acquired form was presented in 1975 (Schwartz et al. 1975). Indeed, there had been reports presenting cases with similar manifestations with syncopal spells, even sudden death and long QT interval upon some concomitant pharmacological agents and interestingly, without any known stress factor provoking those arrhythmias.

Since the triggering factor for the cardiac events often was adrenergic stimulus, theories about the pathogenesis of the LQTS started to be based on abnormalities in the sympathetic nervous system (Schwartz 1985). Until 1994, the sympathetic imbalance theory was frequently quoted and even used as the rationale for therapy of LQTS.

The discovery of the first three genes underlying the LQTS (Curran et al. 1995, Wang et al. 1996, Wang et al. 1996) solved the mystery of LQTS: it is a cardiac ion channel disorder. Its congenital form originates from ion channel gene defects and the acquired form from other factors disturbing the ion channel function. Knowledge on ion channel function, development of specific therapy and genetic testing would now become possible. After the identification of the LQTS caused by mutations in the potassium channel α-subunits KvLQT1 (also known as KCNQ1) and HERG, and the sodium channel gene SCN5A, additional ion channel genes underlying the syndrome have been discovered: those encoding for the cardiac potassium channel β-subunits minK (KCNE1) (Splawski et al. 1997) and MiRP1 (KCNE2) (Abbott et al. 1999). The number of genes is still likely to increase.

The spectrum of LQTS became much wider than thought as soon as the numerous carriers of ion channel defects were shown to exhibit normal ECG in families with an identified gene defect (Vincent et al. 1992). It thus became apparent that if genetic diagnosis was not yet available in a family, there could be members with normal ECG who were mutation carriers. Taken together with the existence of other life-threatening arrhythmogenic disorders in apparently normal hearts, the identification of these people and differential diagnosis remains a challenge for clinical electrophysiology. Its methods are now being developed in intimate collaboration with the discoveries of molecular genetics.

2. REVIEW OF LITERATURE

2.1. General features of congenital long QT syndrome

Congenital long QT syndrome is characterized by the prolongation of the QT interval associated with syncopal spells due to ventricular arrhythmias. Typically, these arrhythmias occur in conjunction of vigorous physical exercise or emotional stress. These arrhythmias are potentially fatal and high mortality figures have been presented. The initial figures of mortality were high due to the identification of most severely affected patients only. Mortality of untreated symptomatic patients first reported 71 %, and 20 % of patients with beta blocker treatment continue to have symptoms (Schwartz 1985). Reported high mortality in patients with other than antiadrenergic treatment has been likely to diminish the use of other antiarrhythmic drugs in treatment of LQTS patients (Schwartz 1985). Patients with Jervell and Lange-Nielsen syndrome, i.e. homozygous LQT1 patients possess the highest risk of cardiac events (Moss et al. 1985).

2.2. QT interval duration

QT interval prolongation is the main diagnostic feature of the LQTS, included as one of the major diagnostic criteria for the syndrome (Schwartz 1985, Schwartz et al. 1993). However, as soon as the first genetic linkage analyses were carried out, it was shown that a substantial proportion of LQTS (LQT1) patients had QTc intervals similar to their healthy relatives (Vincent et al. 1992). In that study it was shown that 5 % of LQT1 patients had QTc < 440 ms and that in 60 % of cases, the QTc interval was insufficient to separate the carriers from non-carriers.

Mutation site in each LQTS gene plays a role in the degree of QT interval prolongation; within KvLQT1 and HERG genes mutations in C-terminal end have been shown to prolong the QT interval less than mutations in pore region of these genes (Donger et al. 1997, Neyroud et al. 1998, Berthet et al. 1999). Despite the location of the mutation in

the pore region of a ion channel gene, there is still considerable overlap of QT intervals between mutation carriers and non-carriers (Saarinen et al. 1998).

2.3. Morphology of T -wave

Moss et al. (Moss et al. 1995) in 1995 showed genotype-specific T-wave patterns in LQT1, LQT2 and LQT3. LQT1 is characterized by the longest duration of the T -wave (from the beginning of T onset to the end of the T -wave), with a T-wave amplitude similar to that in LQT3. In LQT2, the typical T-wave pattern is of low amplitude and T-wave duration in leads II and V5 is shorter than in LQT1. Late-appearing T -waves of short duration are common in LQT3 whereas a T -wave usually is broad based and prolonged in LQT1 and LQT2 patients (Moss et al. 1995).

The mechanism of a late onset T -wave with short duration, similar to that encountered in LQT3 type of LQTS, has also been produced experimentally by ATX-II, an inhibitor of sodium channel SCN5A. It prolongs the action potential in epicardial and endocardial layers and is capable of delaying the onset of T -wave, as seen in the congenital LQT3 subtype of LQTS (Shimizu and Antzelevitch 1997). Thus, the ECG morphological features of repolarization may have specific underlying molecular mechanisms, but the clinical utility of these characteristics remains to be studied in large unselected LQTS populations.

Thus far, the prevalence of morphological T -wave abnormalities have been studied in genetically undefined LQTS populations in which diagnosis has been based on QT interval prolongation and/or symptoms (Lehmann et al. 1994, Malfatto et al. 1994). Malfatto et al. studied 53 LQTS patients and 53 controls. All LQTS patients had QTc > 440 ms whereas QTc of controls was < 440 ms. A biphasic of notched T -waves was found in 61 % of LQTS patients above the age of 15 and in 63 % under the age of 15 years. In controls, the corresponding figures were 23 % and 0 % (Malfatto et al. 1994). Lehmann et al. compared the prevalence of T -wave humps (double peaked T -wave, second peak termed as T2) in 254 family members of 13 clinically diagnosed LQTS families to that in over 2,900 healthy control subjects (Lehmann et al. 1994). Prevalence

was studied separately in LQTS family members according to the QTc classification (prolonged QTc >470, borderline ≥ 420 but ≤ 460 or normal ≤ 410 ms). T2 was found in 27 % of all blood relatives, 2 % of spouses and 1.5 % of healthy controls. Of those blood relatives with QTc ≥ 470 ms, T2 was present in 53 % but only in 16 % of those with QTc ≤ 460 ms. T2 was observed in any of the leads V4-6 or limb leads in 24 % of all blood relatives, and it was confined to leads V2 or V3 in only 2.9 % of cases. In control subjects, the corresponding figures were 0.9 % and 0.6 %. Thus, when the QT interval in a family member of LQTS patient is equivocal, the presence of T2 (even when relatively subtle) in limb leads or leads V4-6 seems to be highly suggestive of LQTS. However, since the true number of LQTS gene carriers in the group with normal or borderline QTc was unknown, it is not possible to estimate the sensitivity of T2 as a marker of LQTS in standard ECG -recordings.

In addition, complexity of repolarization has been measured from the 24-hour ambulatory ECG recordings. Principal component analysis can quantify T-wave abnormalities encountered in LQTS, but its performance in identifying LQTS gene carriers from healthy relatives has not been studied (Priori et al. 1997).

2.4. Duration and characteristics of action potentials

Transmembrane ion fluxes are responsible for voltage changes during electric activation and recovery of myocardial tissue. Cardiac action potential is the cellular counterpart of body surface ECG and it can be directly measured from myocardial surface. Duration of human cardiac action potential is dependent on heart rate; premature excitation shortens it and the longer the cycle length is, the longer the action potential duration (Franz et al. 1983). Monophasic action potential (MAP) duration at 90% repolarization reflects the QT interval of surface ECG (Franz et al. 1987). Its duration is inversely related to activation time, i.e. areas which activate later have shorter action potentials and vice versa (Cowan et al. 1988). The duration of action potential also shows regional heterogeneity both at endocardial and epicardial surface (Franz et al. 1987, Cowan et al. 1988). Of the transmural layers of myocardium, epicardial cells possess the shortest duration of action potential. The end of action potential of epicardial cells coincides with

the peak of T -wave, whereas the end of the T -wave is coincident with action potential duration of M cells. Duration of action potential in endocardial cells is intermediate to these two layers (Shimizu and Antzelevitch 1997). Rate dependency and shortening of cardiac action potential by increasing heart rates has also been demonstrated in clinically diagnosed LQTS patients in which MAP remains longer than in controls (Hirao et al. 1996). In addition, morphological alterations in LQTS patients (humps at phase 3 of repolarization) in MAP has been demonstrated (Bonatti et al. 1985).

2.5. Rate dependency of action potentials and QT interval duration

The relationship between action potential duration and heart rate has been studied both in cell models and in canine myocardial preparations mimicking LQTS subtypes. For *in vitro* cell models of LQT2 and LQT3, dofetilide and anthopleurin have been used to block rapid outward potassium current I_{Kr} and outward sodium current I_{Na}, respectively. These studies showed that increase in pacing rate shortened duration of action potential more in LQT2 model than in control cells and more in LQT3 model than in LQT2 model (Priori et al. 1996). Shimizu et al. created canine ventricular myocardium preparation models for LQTS using d-sotalol, which inhibits the I_{Kr} and ATX-II for inhibition of the fast sodium channel to mimic the LQT2 and LQT3 subtypes (Shimizu and Antzelevitch 1997). Steeper rate dependence of action potential duration was observed in LQT2 and LQT3 than in controls and furthermore, steeper rate dependence in LQT3 than in LQT2 model (Shimizu and Antzelevitch 1997).

Vincent et al. (Vincent 1986) demonstrated *in vivo* that the absolute QT interval duration in LQTS (later shown to represent LQT1) patients shortened as long as their heart rate increased. However, the maximal heart rate at the final stage of the exercise remained lower and concomitantly with that, the QT interval was longer than in controls.

A study in genetically defined LQTS patient cohorts has shown that QT interval rate dependence is steeper in LQT3 patients than in LQT2 patients or healthy population (Schwartz et al. 1995) but the number of studied patients was very small, only four patients with LQT2 type of LQTS.

In ambulatory ECG recordings, Neyroud et al demonstrated that the QT rate dependence was steeper in LQT1 patients than in controls (Neyroud et al. 1998). Classification of LQT1 patients and healthy age- and gender matched controls based on a Holter model constituting of different QT/RR interval slope parameters resulted in 96 % specificity and 88 % sensitivity, thereby misclassifying 14% of the study subjects. When QTc parameter was taken into account together with Holter parameters, only 4% of study subjects were misclassified (Neyroud et al. 1998). It is of note, however, that only LQT1 patients were studied and no healthy relatives were included in the control group.

2.6. T-wave alternans

Macroscopic alternation of T -wave configuration has been observed in molecularly undefined LQTS patients (Schwartz and Malliani 1975, Sharma et al. 1981). It is associated with the extent of spatial dispersion of repolarization (Chinushi et al. 1998). Patients with T -wave alternans have an increased risk of cardiac events as T -wave alternans may preceed the onset of torsades de pointes tachycardia (Schwartz and Malliani 1975). However, the risk is primarily related to the magnitude of QTc prolongation and T -wave alternans does not make an independent contribution to the risk of cardiac events (Zareba et al. 1994).

2.7. Heart rate and heart rate variability

Intrauterine and neonatal bradycardia is occasionally encountered in LQTS carriers (for review, see (Gorgels et al. 1998)). This is probably related to the most severe forms of LQTS like the homozygous carrier state. In newborns and children up to the age of 3 years with genetically undefined LQTS, the resting heart rate was found lower than in healthy controls (mean difference approximately 20 beats min^{-1}). However, in older children and adolescents, no difference in heart rate was observed (Vincent 1986). In heart rate variability parameters, no differences have been observed in LQT1 patients and age- and gender matched controls (Neyroud et al. 1998). So far, heart rate variability has not been studied in LQT2 and LQT3 patients.

2.8. Occurrence of arrhythmias as diagnostic feature of LQTS

Provocation of ventricular premature complexes on exercise stress testing has been considered a feature of LQTS, although not common, (Weintraub et al. 1990) and to be one of the factors associated with a high risk of syncope or sudden death (Moss et al. 1985). The incidence of ventricular premature complexes during exercise in LQTS patients has not been widely assessed since the number of reports in which exercise stress tests has been systematically performed is small. In pediatric populations, Garson et al. reported a 30 % incidence of ventricular arrhythmias in children during an exercise stress test (Garson et al. 1993). However, 12 % of these children had also another congenital heart disease (Garson et al. 1993). Since ventricular arrhythmias cannot be provoked in the majority of LQTS patients, reproducible appearance of such arrhythmias during exercise (test) should raise consideration of other differential diagnostic alternatives as well. In invasive electrophysiologic testing, programmed ventricular stimulation does not induce sustained ventricular arrhythmias (Bhandari et al. 1985)

2.9. Triggers of symptoms

The majority of symptomatic LQT1 patients (71 %) have experienced syncopal spell or cardiac arrest in conjunction with exercise, whereas the arrhythmias in 71 % of the cases have occurred during sleep in LQT3 type of LQTS. LQT2 has been reported to represent an intermediate form of these two including exercise as a triggering factor in only 16 % of cases (Schwartz et al. 1997). Swimming appears as a significant risk factor for LQT1 but not for LQT2 (Ackerman et al. 1999, Moss et al. 1999, Laitinen et al. 2000, Piippo et al. submitted). Symptoms occurring after auditory stimuli have been reported to happen frequently in LQT2 (60 % of 15 symptomatic LQT2 patients) but this is not a feature of LQT1 (0 out of 23 symptomatic LQT1 patients) (Wilde et al. 1999).

2.10. Response to catecholamines

Infusion of epinephrine prolonged QT interval and monophasic action potential duration at 90 % level of repolarization (MAP90) duration and increased MAP90 dispersion in

genetically unverified LQTS patients but not in healthy controls (Hirao et al. 1996). In molecularly defined LQT1 patients, epinephrine caused a marked increase in monophasic action potential duration and QT interval duration (mean difference with 0.1 mg/kg/min epinephrine infusion was 46 ms), but only a minor change (a 12 ms increase) was observed in QT interval duration and no changes in monophasic action potential duration of healthy controls (Shimizu et al. 1998). Dispersion of monophasic action potential duration is also increased by epinephrine in LQT1 patients (from 26±6 to 45±13 ms) but not in healthy subjects (from 26±6 to 24±6 ms) (Shimizu et al. 1998). The adverse effects of catecholamines on ventricular repolarization are thus shown in LQT1 subtype of LQTS but remain unexamined in other LQTS subtypes. Epinephrine has also been shown to induce torsades de pointes tachycardia in patients with congenital long QT syndrome, as reviewed by Jackman et al. (Jackman et al. 1988), although the underlying molecular defect has not been known and thus no conclusions about the vulnerability to arrhythmias in different LQTS subtypes can be based on these findings.

2.11. Proposed diagnostic criteria for long QT syndrome

The early clinical observations of normal QTc's and yet exercise related syncopal spells occurring in members of LQTS families led to use of a scheme of diagnostic criteria. First set of diagnostic criteria (**Table 1**) was proposed in 1985 (Schwartz 1985). The cut-off point for normal QTc proposed in that review has been commonly used in prognostic assessments (Moss et al. 1991) and in a number of other studies since then.

The apparent overlap of QT intervals in LQTS patients and healthy controls after the first linkage studies resulted in development of new criteria (**Table 2**). These took into account the extent of QT interval prolongation and tried to grade the likelihood of symptoms being related to LQTS aimed to enhance the recognition of affected patients (Schwartz et al. 1993). Proposed scoring system indicated the probability of LQTS in a given individual (Schwartz et al. 1993). Zero or one point indicates low probability, 2-3 points intermediate and 4 or more points high probablity of LQTS.

Since the publication of these criteria, they have been widely used as an inclusion criterion for definite LQTS carrier status in clinical studies where the underlying disorder have not been molecularly defined (Shimizu et al. 1995, Emori et al. 1997, Hofbeck et al. 1997, Krahn et al. 1997, Priori et al. 1997, Shah et al. 1997, Nakayma et al. 1998).

Table 1. *Diagnostic criteria of LQTS (1985)*

Major	Minor
Prolonged QT interval (QTc > 440 ms)	Congenital deafness
Stress-induced syncope	Episodes of T -wave alternans
Family members with LQTS	Low heart rate (in children)
	Abnormal ventricular repolarization

Diagnosis of LQTS can be made in the presence of 2 major criteria or 1 major and 2 minor criteria.

2.12. Prediction of symptoms

2.12.1. Duration of QT interval as a risk factor

In a study from 1980's, QTc > 460 ms did not appear as the strongest independent risk factor in the study by Moss et al. (Moss et al. 1985). Eight years later, the duration of QTc interval was prospectively shown to be a predictor of cardiac events in a larger patient cohort (Moss et al. 1991). In 1993, Garson et al. showed that in the non-compliant (untreated) LQTS children of which 61 % were symptomatic at the time of enrollment, the risk of sudden death increased linearly with the increase of QTc from values below 440 ms to longer QTc intervals (Garson et al. 1993). The aforementioned studies carried out once again in molecularly undefined cohorts have excluded LQTS patients with the shortest ("normal") QT intervals and possibly included non-carrier relatives with QTc > 440 but below 470 ms. These studies therefore cannot tell the true risk of LQTS patients with QTc intervals lower than the inclusion criteria used, and on the other hand, results in a diluting effect at QTc intervals of 440-470 ms as many non-carrier relatives are likely to be included in this group. The first study evaluating QTc as a risk factor in molecularly defined LQTS patients showed that each 10 ms increase in

Table 2. *LQTS diagnostic criteria 1993.*

	Points
ECG findings *	
A. QTc †	
≥ 480 ms	3
460-470 ms	2
450 ms (in males)	1
B. Torsade de pointes‡	2
C. T-wave alternans	1
D. Notched T -wave in three leads	1
E. Low heart rate for age§	0.5
Clinical history	
A. Syncope‡	
With stress	2
Without stress	1
B. Congenital deafness	0.5
Family history (either A or B)	
A. Family members with definite LQTS#	1
B. Unexplained sudden cardiac death below age 30 among immediate family members	0.5

*In the absence of QT interval prolonging drugs or other conditions.

†QTc calculated by Bazett formula (Bazett 1920).

‡Mutually exclusive.

§Resting heart rate below the second percentile for age (Davignon et al. 1979).

#Definite LQTS is defined by an LQTS score ≥ 4.

QTc increase the risk of cardiac events by 6 %, independent of genotype (Zareba et al. 1998). Of all patients with QTc < 440 ms, 5-6 % had been symptomatic. However, the number of mutation carriers with QTc < 440 ms was only 23 (Zareba et al. 1998).

2.12.2. QT dispersion and risk of arrhythmias

QT dispersion is a measure of inhomogeneity of repolarization in ventricular myocardium (Statters et al. 1994). It has been measured from surface ECG as the difference of the longest and shortest QT intervals in any of the leads. In experimental models on tissue preparations, dispersion of repolarization is defined as the difference in repolarization time between M cells and epicardial cells (Shimizu and Antzelevitch 1997, Yan and Antzelevitch 1998).

QT dispersion has been proposed to serve as an indicator of increased risk of arrhythmic events (Statters et al. 1994). Thus far, QT dispersion has been studied only in a very small number of clinically affected, untreated LQTS patients without genetic diagnosis. Untreated LQTS patients had relative QT dispersion ((the standard deviation of QT/mean QT) x 100) higher than any of the controls and beta-blocker non-responders higher than those who remained asymptomatic during the treatment (Priori et al. 1994). In addition, QT dispersion was found to be diminished after left cardiac sympathetic denervation, upon which patients remained asymptomatic. These data suggest that increased QT dispersion would indicate risk of arrhythmias in LQTS patients.

The possible quantitative differences in QT dispersion between different subtypes of LQTS are unknown. Similarly, the utility of QT dispersion in homogenous patient populations with different LQTS subtypes, as a risk marker of arrhythmias, have not yet been studied.

2.13. Pharmacological interventions and risk of arrhythmias

Thus far, beta-antiadrenergic drugs have been the drugs of choice in reducing the risk of arrhythmias in LQTS, especially propranolol. In LQT1, propranolol reverses the QT and action potential duration prolonging effects of epinephrine (Shimizu et al. 1998), and treatment with beta-blockers in molecularly undefined LQTS patients is associated with a lower relative risk of syncope or cardiac death (Moss et al. 1985) but does not completely abolish cardiac events in symptomatic LQTS patients (Moss et al. 2000).

Preliminary results suggest, however, that beta-blockers are effective in preventing cardiac events in LQT1 patients (Vincent et al. 1996).

Studies evaluating the effects of other drugs in LQTS are scanty and only experimental, mostly *in vitro*. These studies utilize cell models, in which single channels have been pharmacologically blocked. Examples of these are chromanol 293B which has been used to block the slow outward potassium current I_{Ks} as a surrogate to LQT1 (Shimizu and Antzelevitch 1998), dofetilide or *d*-sotalol to block I_{Kr} (HERG), and anthopleurin or ATX-II to block I_{Na}.

In vivo, mexiletine has been shown to shorten QT interval in a small number of patients (Schwartz et al. 1995). This QT shortening effect was reported specifically in LQT3 but not in LQT2 patients. In that study, however, there was a large variation of QTc intervals in LQT2 patients and only paired t-test was used for statistical testing in a small number of patients. Therefore, the positive effects of mexiletine should be studied in larger LQT2 cohorts because the in vitro studies have shown positive effects of mexiletine on HERG channel function (Shimizu and Antzelevitch 1997) and it may thus be beneficial in decreasing the risk of arrhythmias in LQT2 as well as in LQT3 patients. In 1993, an evaluation of treatment and prognosis of 287 children showed that in the small number of patients (n=12) treated with mexiletine, the incidence of symptoms was not higher than in patients with propranolol (Garson et al. 1993).

I_{Kr}, which is impaired in LQT2 type of LQTS, is activated by an increase in serum potassium. This phenomenon, which would seem to run counter to simple electrochemical gradient considerations, is believed to result form effects of extracellular potassium on HERG inactivation kinetics (Yang et al. 1997). Indeed, a reduction of QT interval duration is obtained by acute administration of potassium in LQT2 patients (Compton et al. 1996) and also in LQT1 patients (Swan et al. 1999). Theoretically, high-potassium diet or drugs elevating the serum potassium level could thus be beneficial for LQT1 and LQT2 patients.

Nicorandil, a potassium channel opener, has been shown to reverse the effects of epinephrine on action potential and QT interval duration and dispersion of monophasic action potential duration in LQT1 patients (Shimizu et al. 1998). Propranolol, however, resulted in more marked improvement in these parameters in the presence of epinephrine (Shimizu et al. 1998).

2.14. Differential diagnosis of LQTS and other congenital arrhythmogenic cardiac disorders

Hypertrophic cardiomyopathy, dilated cardiomyopathy, familial idiopathic ventricular fibrillation, arrhythmogenic right ventricular dysplasia and familial polymorphic ventricular tachycardia are frequently associated with life-threatening ventricular arrhythmias. Patients with hypertrophic cardiomyopathy and dilated cardiomyopathy also often have moderate QT interval prolongation (Martin et al. 1994) which may mislead to diagnosis of LQTS. Opposite to these conditions, QT interval is normal in familial idiopathic ventricular fibrillation (Brugada syndrome) (Brugada and Brugada 1992) and rarely prolonged in patients with right ventricular dysplasia (Martin et al. 1994). QT interval prolongation may also be encountered in polymorphic ventricular tachycardia (for review, see Leenhardt et al. 1995)

2.14.1. Hypertrophic cardiomyopathy

The presence of ventricular hypertrophy in a normotensive individual without other congenital heart diseases or acquired valvular abnormalities is most probably due to a mutation in one of the genes encoding sarcomeric proteins. Histologically, the disorder is characterized by myocardial disarray and hypertrophy of myocytes which may as well be present as only local macroscopic hypertrophy. Electrocardiographic manifestations include QRS axis deviation, high voltage and/or inverted T -waves. Clinical criteria proposed for hypertrophic cardiomyopathy are presented in **Table 3** (McKenna et al. 1997). These criteria are to be used in the relatives (potential carriers) of a patient with known hypertrophic cardiomyopathy.

2.14.2. Dilated cardiomyopathy

The hallmarks of dilated cardiomyopathy include end-diastolic dimension greater than expected for age and body surface area as well as reduced left ventricular shortening

Table 3. *Proposed diagnostic criteria for hypertrophic cardiomyopathy in adult members of affected families* (McKenna et al. 1997)

Major criteria	Minor criteria
Echocardiography	
Left ventricular wall thickness ≥ 13 mm in the anterior septum or posterior wall, or ≥ 15 mm in the posterior septum or free wall	Left ventricular wall thickness of 12 mm in the anterior septum or posterior wall, or 14 mm in the posterior septum or free wall
Severe systolic anterior motion (septal-leaflet contact) of anterior mitral valve	Moderate systolic anterior motion (no leaflet-septal contact)
	Redundant mitral valve leaflets
Electrocardiography	
Left ventricular hypertrophy + repolarization changes	Complete bundle branch block or (minor) intraventricular conduction defect
T -wave inversion in leads I and aVL (≥3 mm) (with QRS-T -wave axis difference ≥ 30°), V3-6 (≥3 mm) or II and III and aVF (≥5 mm)	Minor repolarization changes
	Deeps S V2 (≥ 25 mm)
Abnormal Q (≥ 40 ms or 25 % R wave) in at least 2 leads from II, III, aVF (in abscence of left anterior hemiblock), V1-4; or I, aVL, V5-6	
	Unexplained chest pain, dyspnoea or syncope

fraction or ejection fraction, features that are being used for phenotypic classification (Olson et al. 1998). In more than 20 % of cases, dilated cardiomyopathy is familial

(Goerss et al. 1995). For the time being, the underlying gene abnormalities identified are sarcomere protein actin encoding gene ACTC (Olson et al. 1998), dystrophin protein linking myofibrils to an extracellular matrix (Franz et al. 1995, Muntoni et al. 1997) and lamin A/C gene (Fatkin et al. 1999). Prolonged QT interval in some dilated cardiomyopathy patients suggests ion channel dysfunction resulting in repolarization abnormalities and increased risk of arrhythmias (Martin et al. 1994). These may be secondary to structural or metabolic changes in failing heart, in general. Experimental evidence for prolongation of action potential prolongation has been gained from single myocytes obtained from failing hearts, and these are due to alterations in at least potassium currents (Tomaselli et al. 1994).

2.14.3. Familial idiopathic ventricular fibrillation

Although same SCN5A ion channel gene causes both LQT3 and familial idiopathic ventricular fibrillation, the latter is brought about mutations which result in loss of function, opposite to those in LQT3 which result in gain of function (Chen et al. 1998). The clinical manifestations are sudden death, ventricular tachycardia and ventricular fibrillation. Typically, arrhythmias occur during sleep or at rest (Alings and Wilde 1999). The diagnostic hallmarks of familial idiopathic ventricular fibrillation are ST -segment elevation in the right precordial leads and complete or incomplete right bundle branch block (Brugada and Brugada 1992, Gussak et al. 1999) which can be unmasked by administration of strong sodium channel blockers (class I C antiarrthytmic drugs) (Antzelevitch et al. 1996). In electrophysiologic study, HV interval is usually prolonged and ventricular fibrillation is inducible (for review, see Alings and Wilde 1999). QT interval is usually normal and arrhythmias during exercise stress test occur very infrequently whereas late potentials are common in signal-averaged ECG (Alings and Wilde 1999).

2.14.4. Arrhythmogenic right ventricular dysplasia

The diagnostic criteria for arrhythmogenic ventricular dysplasia were published in 1994 (see **Table 4**) and the spectrum of the clinical findings assessed in a multi-center study in 1997 (Corrado et al. 1997). The criteria are based on the presence of structural alterations: replacement of cardiomyocytes by fibrofatty infiltration leading to dyskinesia and aneurysms of the ventricle. Both of them are encountered primarily in the right ventricular myocardium. Electrocardiograpic consequences include right-sided T -wave abnormalities and late potentials in signal-averaged ECG and the disease manifests as ventricular tachycardia of left bundle branch block configuration (often associated with exercise), syncope or sudden death. Thus far, the genetic origin of arrhythmogenic right ventricular dysplasia has been linked to 4 chromosomal loci (Rampazzo et al. 1994, Rampazzo et al. 1995, Severini et al. 1996, Rampazzo et al. 1997).

2.14.5. Familial polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia was first described in 1975 (Reid et al. 1975). In this syndrome, any form of increased adrenergic stimulation (exercise, frightening, infusion of isoproterenol) can induce polymorphic ventricular premature beats followed by seemingly bidirectional and finally, polymorphic tachycardia. The morphology of ventricular complexes suggest that the arrhythmias arise from anywhere in the myocardium (Leenhardt et al. 1995). The genetic nature of the disorder is demonstrated in those 21 children studied by Leenhardt et al., (Leenhardt et al. 1995) among whom a familial history of syncope or sudden death was present in 30 % of cases. Some reports indicate that prolonged QT intervals are encountered in patients with polymorphic ventricular tachycardia (Shaw 1981, Rutter and Southhall 1985). In addition, presence of a prominent U wave has been reported as reviewed by Leenhardt et al. (Leenhardt et al. 1995). In their own study, only subjects with QT interval with < 440 ms were included (Leenhardt et al. 1995). A noticeable feature in their patients was the sinus bradycardia; mean resting heart rate being only 60 beats per minute in children of 10 years of age on average.

Table 4. *Criteria for diagnosis of right ventricular dysplasia* (McKenna et al. 1994).

Major	Minor
Global and/or regional dysfunction and structural alterations	
Sever dilatation and reduction of right ventricular ejection fraction with no (or only mild) left ventricular impairment	Mild global right ventricular dilatation and/or ejection fraction reduction with normal ventricle
Localized right ventricular aneurysms (akinetic or dyskinetic areas with diastolic bulging)	Mild segmental dilatation of the right ventricle
Severe segmental dilatation of the right ventricle	Regional right ventricular hypokinesia
Tissue characterization of walls	
Fibrofatty replacement of myocardium on endomyocardial biopsy	
Repolarization abnormalities	
	Inverted T -waves in right precordial leads (V2 and V3) (people aged more than 12 years; in absence of RBBB)
Depolarization/conduction abnormalities	
Epsilon waves or localized prolongation (>110 ms) of the QRS complex in right precordial leads (V1-V3)	Late potentials in signal averaged ECG
Arrhythmias	
	LBBB type ventricular tachycardia (sustained and non-sustained, recognizable on Holter, exercise stress testing)
	Frequent ventricular extrasystoles (more than 1000/24h) (Holter)
Family history	
Familial disease confirmed at necropsy or surgery	Familial history of premature sudden death (<35 years) due to suspected right ventricular dysplasia
	Familial history (clinical diagnosis based on present criteria)

Diagnosis of arrhythmogenic right ventricular dysplasia can be based on the presence of two major criteria or one major + two minor criteria or four minor criteria.

3. AIMS OF THE STUDY

When starting the present study, the molecular background and heterogeneity of the long QT syndrome was still unknown. This series of studies has been performed to improve diagnostic accuracy of long QT syndrome and identifying patients at highest risk of cardiac events. Revelation of the heterogenetic molecular etiology have expanded these studies towards the search of differential diagnostic features to recognize the type of ion channel disorder when molecular level diagnosis is not available.

The aims of this study were to evaluate the ability of electrocardiographic measures and characteristics to identify the LQTS patients from the healthy relatives and from patients carrying a congenital arrhythmogenic disorder closely resembling LQTS. The focus is on the commonly reported diagnostic features of LQTS, namely QT interval prolongation and its dynamic changes concurrently with heart rate changes, T -wave abnormalities, and finally, exercise-provoked arrhythmias.

First, QT interval behavior during and after exercise were assessed in non-homogenous pediatric LQTS patient group and their healthy relatives to determine, whether QT interval behavior during heart rate changes differ between healthy controls and LQTS patients in general.

Second, the diagnostic performance of QT interval duration and dispersion were evaluated in highly homogenous LQTS population to exclude the bias which might arise from genetic heterogeneity.

Third, the diagnostic performance of potentially diagnostic electrocardiographic features of LQTS were evaluated in the most common forms of LQTS, that is in genetically identified LQT1 and LQT2 patient populations and compared to healthy relatives.

Finally, the diagnostic features of another inherited arrhythmogenic disorder resembling LQTS by arrhythmia triggers and fatality were studied to make a distinction between it and the LQTS.

4. PATIENTS AND METHODS

4.1. Patients

Index patients who underwent diagnostic evaluation of congenital long QT syndrome were either referred from other domestic health care institutions or identified at the Department of Medicine or the Children's Hospital of Helsinki University Hospital. They fulfilled at least two of the following three criteria:

- presence of syncopal spells and/or documented torsades des pointes tachycardia,
- prolonged QT interval,
- family history of long QT syndrome or sudden death

A detailed, extended family history was obtained from all index patients to identify the potential carriers of the arrhythmogenic disorder and the clinical course of the disease in each pedigree.

A summary of study patients and controls is presented in **Table 5.** The studies were approved by the Ethical Review Committee of the department.

Table 5. *Number of study patients in studies I-III*

	Study I	Study II	Study III
Clinical LQTS	19		
Clinical controls	19		
LQT1			
pore region mutation *		30	23
C-terminal mutation *			22
LQT2*			20
Molecularly defined controls *		44	33

* identification of the mutation or, in controls, exclusion of the mutation found in the family from each individual's DNA sample.

Study I

Nineteen children, age 13±3 years, with congenital LQTS underwent an exercise test. The criterion for the diagnosis of LQTS was unexplained syncope or a positive family history of LQTS and QT_c > 440 ms (Bazett's formula) in standard 12 lead ECG. Patients were not molecularly defined. The QT_c of the patients at rest was 494±44 ms. Fifteen patients had a positive family history. Ten children had had syncopal spells. Four had lost their consciousness during exercise, 5 awake without exercise and one asleep with convulsions. In two patients, syncopal spells could not be related to sleep, awakening or any stressful situation. Seven out of nine asymptomatic children had at least one family member with symptoms (syncope or sudden death) which had occurred during exercise. All symptomatic patients were using beta blocking medication during the time of examination. No other medications were in use. Nineteen clinically and electrocardiographically healthy relatives of the patients, age 12±4 years, were studied as control group. None of them had any symptoms or medications and the QT_c in standard 12 -lead ECG at rest did not exceed 440 ms. None of the children had bundle branch block in baseline ECG.

Study II

The study group consisted of members of a family in which 30 individuals were identified to be carriers of D317N mutation of KVLQT1 gene causing LQTS type 1 (LQT1 patients). Twelve of the patients in LQT1 group had experienced an exercise-related syncope. The youngest symptomatic patient was 3 years old at the time of the first syncope. Eight patients had experienced syncope at the age of 5 to 25 years and 3 patients between 30 to 37 years. In addition to them, one boy drowned at the age of 10. Although no genetic analysis was performed, he was included in the study group since his QTc was 450 ms, he had been treated for LQTS because of multiple exercise-related syncopal attacks before his death and his mother is a carrier of the D317N mutation.

As a control group, 44 non-carriers of the mutation from the same pedigree were studied. One diabetic subject was excluded from the study because diabetes mellitus may

affect the repolarization time. Seven LQT1 patients (5 symptomatic, 2 asymptomatic) and one control subject were receiving beta-adrenergic blocking agents during the time of ECG registration. In two cases (both LQT1 patients) beta-adrenergic antagonists had been discontinued for at least five half-lives before ECG was obtained. No other medications known to affect the repolarization were in use.

Study III

The first patient group consisted of 23 subjects with the mutation D317N in the pore domain of the KvLQT1 gene (Saarinen et al. 1998) (LQT1 pore region group). Seven of these patients were symptomatic. All symptoms were associated with physical exercise. The second patient group included 22 subjects with the G589D mutation close to the C-terminus of the KvLQT1 gene (Saarinen et al. 1998) (LQT1 C-terminus group). Four of the patients in the LQT1 C-terminus group had experienced a syncopal spell; three of these were exercise-related. Thus, a syncopal spell was associated with exercise in 10 out of 11 cases in LQT1 patients. The third patient group consisted of 20 patients from 10 families with a variety of HERG channel mutations (R176W, L552S, Y569H, G584S, G601S, 453delC and 1631delAG) (LQT2 group) (Laitinen et al. 2000). Each of these mutations resulted in translation frameshift or a substitution of a conserved amino acid and was found to be present in affected family members but absent in controls. In every family, at least one patient had experienced a syncope and had QTc $\geq$ 480 ms. Altogether 8 out of the 9 symptomatic LQT2 patients had their symptoms at rest or during night. Thirty-three healthy relatives were included as a control group. No beta-blocking or other medications known to affect the repolarization were used by patients or control subjects during the study.

In the Results chapter of this book, the total number of subjects has been expanded in the comparison of QTc intervals of carriers of D317N (n=25) and G589D (n=253) mutations of KvLQT1, as well as del453C (n=13) and L552S (n=30) mutations of HERG gene and non-carrier relatives (n=464) (**figure 1**). In addition, in **figures 2** and **3**, QT intervals of altogether 160 male and 188 female carriers of G589D mutation of KvLQT1 gene and

those of 166 male and 202 female non-carrier relatives have been plotted against heart rate.

Study IV

Two families with a total of 51 living members were evaluated because of family members with only marginally prolonged QT interval but reproducible arousal of ventricular arrhythmias during exercise. Six members had died suddenly at adolescence or early adulthood, and five patients had been evaluated because of an exercise-related syncope. A total of 43 members of these two families underwent cardiological examination, including electrocardiographic tests and cardiac ultrasonography. Fourteen were considered affected due to the appearance of exercise-induced polymorphic ventricular premature complexes, and nine of them were subjected to further evaluation including cardiac catheterization and angiography, electrophysiologic testing and endomyocardial biopsy. All patients were otherwise healthy and had no medications. QTc's were compared to age- and gender-matched healthy controls. Patients with frequent ventricular premature complexes (>10 during any minute) or ventricular tachycardia during exercise stress test were considered affected. Individuals < 18 years of age with normal exercise stress test findings were classified as unknown. Of the deceased family members, those whose unexplainable sudden death had occurred under the age of 30 years were also considered affected.

4.2. Genetic studies

For all genetic analyses, genomic DNA was isolated from venous EDTA-anticoagulated blood samples using standard methods. An informed consent was obtained from patients and their healthy relatives. Control DNA samples were obtained from 100 apparently healthy adult individuals.

Study II: Forty-four unrelated probands were screened for mutations of the cardiac potassium channel gene KVLQT1 using single-strand conformational polymorphism (SSCP) and subsequent DNA sequencing. As a result, a mutation D317N (former

D188N) was identified in one large pedigree. Relatives in this pedigree were studied by digesting PCR products of genomic DNA (Saarinen et al. 1998).

Study III: Two Finnish unrelated JLN patients were screened for mutations of the minK and KVLQT1 genes by direct sequencing of PCR products of genomic DNA. A G589D mutation was detected in two unrelated probands with JLN. Thereafter, a total of 120 unrelated Finnish probands with clinically diagnosed LQTS were screened for this mutation and altogether 32 (27 %) of the probands were found to be heterozygous for the G589D mutation.

Exons of HERG gene were sequenced after their amplification by PCR and each of the mutations R176W, L552S, Y569H, G584S, G601S, 453delC and 1631delAG were identified in one of the probands of 89 unrelated pedigrees. Altogether 87 carriers of these mutations were identified in the pedigrees.
In addition, 100 DNA samples from control subjects were screened for each mutation.

Study IV: Nine affected and 16 unaffected individuals from the family 1, and four affected and six unaffected individuals from the family 2 were included in the genetic linkage study. Highly polymorphic microsatellite markers were used in genotyping.

Linkage analyses were performed with MLINK and ILINK options of FASTLINK v. 3.0P package (Cottingham Jr et al. 1993, Schaffer et al. 1994) and GENEHUNTER (Kruglyak et al. 1996). A penetrance of 0.9 for the disease and an affected allele frequency of 0.0002 were assumed. Multipoint lod scores were calculated in affected only mode in pedigree 1 while in two-point calculations the whole pedigree was included.

4.3. Resting 12-lead ECG

A twelve-lead ECG was recorded at rest (50 mm/s, 0.1 mV/mm). QT interval was measured from lead II and adjusted for heart rate (QTc) according to the Bazett's formula. Total QT interval was measured from the beginning of Q wave to the end of T -

wave manually by tangent method and early QT interval (QTm) to the peak of T -wave. Late QT interval was the interval from the peak of T -wave to the end of T -wave. A tangent method was chosen to enable a uniform way of measuring QT interval even at higher heart rates when the end of T -wave merges into P wave. It should be noted however, that this method may result in somewhat shorter QT intervals than when measured to the point where the terminal limb of T -wave is joined to the TP baseline. In cases of notched or double-peaked T-waves, the first peak of T -wave was used for measurements. If the interval between the peaks was longer than 150 ms, the second was considered as a U-wave and was not included in the measurements (Chou 1979). All ECG measurements were carried out blinded to the genotype.

4.4. Assessment of QT interval normality

QT interval was adjusted for subject's heart rate using Bazett's formula $QT_c = QT/RR^{1/2}$ (sec) (Bazett 1920). In order to compare the sensitivity and specificity of different methods to adjust for heart rate in separating LQT1 patients from their healthy relatives, corrected QT intervals (QT_{fc}) adjusted for heart rate by the Fridericia cubic root formula ($QT_{fc} = QT/RR^{1/3}$) (Fridericia 1920) was also calculated. In addition to these adjustment methods, actual QT values were also compared at resting heart rates to upper normal limits for both genders. As the upper reference limit, we used the mean plus 1.96 standard deviation (SD) measured in a large survey of 10717 middle-aged Finnish subjects (Karjalainen et al. 1997). Measurements from lead II were used for comparisons between these normal values and different heart rate corrected QT intervals.

4.5. Rate adaptation of the QT interval

To analyze the rate adaptation of the repolarization intervals, we studied the behavior of total (study I and III), early and late (study I) QT by plotting the measurements against the heart rate and calculating the slopes by least squares linear regression analysis in each individual. QT intervals were measured at specified heart rates from 80 to 150 (study I) and from 100 to 130 (study III) by steps of 10 beats whenever available. At least 3 measurements in each phase were required for each patient or control person in order to

create a slope for the individual. These slopes of the QT to heart rate relationship were used to assess the differences in repolarization phenomenon between the groups and to evaluate the intraindividual changes between physical effort and recovery. Lead V3, which often has the largest T-wave amplitudes, was used for measurements (Cowan et al. 1988). In studies I and III, total QT intervals were measured. In addition, also early QT intervals were measured in study I. The late QT was the subtraction of early QT from total QT. The registered measurement was a mean of at least 4 consecutive QRST complexes. In cases of notched or double-peaked T-waves, the first peak of T -wave was used for measurements. No macroscopically evident T -wave alternans which would have interfered with QT interval measurements, was observed.

4.6. QT dispersion

QT dispersion (QTD) was defined as the difference between the maximal and minimal QT interval in any of the leads measured. Leads with T -waves of less than 1 mm amplitude were rejected. At least 9 out of 12 leads were available for measurements in each subject. Relative QT dispersion (rQTD) was calculated as follows: (the SD of QT/mean QT) x 100. Relative QTm dispersion (QTmD) was defined as (the SD of QTm/mean QTm) x 100.

4.7. Application of proposed clinical criteria for LQTS

The criteria take into account the ECG findings, clinical history and family history (Schwartz et al. 1993). For details please see **table 2**.

4.8. Exercise stress test

Exercise stress test was performed with a bicycle ergometer (Marquette Case 006, Marquette Electronics, Milwaukee, Wisconsin) with continuous recording of leads II, aVF, $V_{1\text{-}3,5}$. Initial load 30 W was increased by 15 W per minute until exhaustion. When patients could not continue with the exercise any more, they immediately laid down and continuous ECG was recorded at supine position for 7 minutes. QT interval was

measured from lead V_3. Maximum heart rate and achieved load (studies I, III and IV) and heart rate at which ventricular bigeminy first appeared were recorded (study IV only). QRS morphology was used to determine the origin of ventricular arrhythmias according to criteria obtained from ventricular tachycardia mapping in study IV. Expected maximum heart rate (studies I and III) was calculated as follows: expected maximum heart rate = 205 - (0.5 x age in years) beats per minute which follows the guidelines reviewed by Hammond and Froelicher (Hammond and Froelicher 1985).

4.9. Ambulatory ECG recording

The ambulatory electrocardiograms were recorded on tape with commercial recorders (Marquette Electronics, Milwaukee, Wisconsin) on an out-patient basis. Bipolar leads resembling standard electrocardiography leads V1 and V5 were used. The recorded electrocardiograms were analyzed using a Marquette Series 8000 Holter analysis system. Mean heart rate, number of ventricular premature complexes and ventricular tachycardias ($\geq$3 consecutive ventricular complexes) were calculated.

4.10. Differential diagnostic studies in study IV

4.10.1. Signal-averaged ECG

Signal-averaged ECG was recorded (Marquette Electronics, Milwaukee, WI) using Frank XYZ leads and a band pass filtering of 40-250 Hz in study IV. Simpson's criteria were applied for late potentials (Simson 1981).

4.10.2. Provocative testing for familial idiopathic ventricular fibrillation

Acute effects of intravenous flecainide (2 mg/kg), a class IC sodium channel blocking agent were tested in four patients (study IV) to unmask the potential ECG abnormalities encountered in familial idiopathic ventricular fibrillation (Antzelevitch et al. 1996). A 12 - lead ECG was obtained prior to, during and 15, 30, 60 and 120 minutes after the administration of the flecainide.

4.10.3. Cardiac ultrasonography

Cardiac ultrasonography with a 2.5 MHz transducer was performed using parasternal long- and short-axis and apical 4-chamber views. Left ventricular dimensions and wall thickness were measured from the M-mode recordings. Doppler echocardiography was used to exclude any valvular stenosis or regurgitation.

4.10.4. Programmed ventricular stimulation

Programmed ventricular stimulation was performed from right ventricular apex and outflow tract using 8-beat drive trains at cycle lengths of 600 and 400 ms with single and double extrastimuli with and without infusion of epinephrine (50 ug/kg/min).

4.10.5. Right ventriculography

Right ventriculography in 30^{o} right and 60^{o} left anterior oblique views and coronary arteriography were carried out in 11 adult affected subjects with polymorphic ventricular extrasystoles in study IV. Right ventricular wall abnormalities were assessed according to Daliento (Daliento et al. 1990), and end-diastolic and end-systolic volumes were calculated according to Ferlinz (Ferlinz et al. 1975). Cineangiograms of 10 patients with paroxysmal supraventricular tachycardia served as normal controls in a blinded analysis by two cardiologists independently.

4.10.6. Magnetic resonance imaging

Six patients in study IV underwent magnetic resonance imaging (MRI). The MRI heart studies were performed using a 1.5 Tesla superconducting Siemens unit (Magnetom Vision), a phased-array body coil and electrocardiogram triggering. T1-weighted spin echo axial and sagittal images were used to analyze the right ventricular anterior wall and a gradient-echo axial and sagittal cine sequence was used to evaluate the possible right ventricular dyskinesia. A breath-hold technique was combined to improve the image quality.

4.10.7. Right ventricular endomyocardial biopsy

Right ventricular endomyocardial biopsy specimens were obtained from 12 patients in study IV. Biopsies from patients less than 6 weeks after heart transplantation and no signs of rejection or infection were used for reference. Formalin-fixed, paraffin-embedded tissue samples were stained with hematoxylin-eosin, van Gieson and Masson's trichrome. The extent of myocardial fibrosis (van Gieson and Masson staining), lipid degeneration, nuclear changes and interstitial inflammation were scored blindly by two pathologists. Inflammation was confirmed by immunohistochemistry using a leukocyte common antigen monoclonal antibody (DAKO, Denmark). Scores from 0 to 3, (0 indicating no and 3 advanced changes) were used.

Summary of the methods used in the substudies is presented in **table 6**.

Table 6. *Summary of methods used in studies I-IV.*

	Study I	Study II	Study III	Study IV
12-lead ECG	X	X	X	X
QT dispersion		X		
Exercise ECG	X		X	X
Rate adaptation of QT	X		X	
Signal-averaged ECG				X
Ambulatory ECG recording				X
Molecular genetic diagnosis		X	X	X
Cardiac ultrasonography	X	X	X	X
Other cardiac imaging and histology				X
Programmed ventricular stimulation				X

5. RESULTS

5.1. QT interval duration

Although mean QTc intervals are significantly longer in patients carrying mutations of KvLQT1 (D317N; n=25, G589D; n=253) or HERG mutations (del453C; n=13, L552S; n=30) than in healthy population, QTc intervals show overlap with healthy relatives (mean QTc 412±24 ms, n=464), **Figure 1**. Healthy men have shorter QTc intervals than women (407±24 vs. 418±24 ms).

The sensitivity and specificity of two different QT adjustment formulas (Bazett 1920, Fridericia 1920) to correctly diagnose the carriers of one of these mutations, the KVLQT1 D317N was evaluated in study II. QT intervals adjusted for heart rate according to Bazett's square root formula or Fridericia's cubic root formula exceeding 440 ms were considered abnormal. Diagnostic sensitivity and specificity based on Bazett's formula were 90% and 88%, respectively, while the assessment by heart rate, adjusted QT intervals according to the Fridericia cubic root formula yielded sensitivity of 80% and specificity of 100% (**Table 7**).

Classification according to the normal upper limit (mean ± 1.96 SD) of QT interval obtained from a large population study (Karjalainen et al. 1997) resulted in a sensitivity of 80 % and specificity of 100% (**Table 7**). The overlap of QT intervals is illustrated in male carriers of G589D mutation (n=160) of KvLQT1 gene and their non-carrier male relatives (n=166) in **figure 2.** The corresponding plot for females (n of carriers is188 and non-carriers 202) is presented in **figure 3**.

5.2. QT dispersion

Dispersion of QT in symptomatic patients with the mutation in the pore region of the KvlQT1 gene was increased (66±48 ms) compared to controls (45±19 ms, p=0.02)

(study II). QT dispersion and mean QT did not correlate significantly with each other in LQT1 patients (r=0.34, p=0.07) or controls (r=0.28, p=0.64)**.**

Table 7. *Application of various diagnostic alternatives in LQTS in regard to molecular classification of patients and their relatives.*

Diagnostic classification	Genetic status	
	D317N +	D317N -
Bazett (Bazett 1920)		
QT_c < 440 ms	3	38
QT_c > 440 ms	27	5
Fridericia (Fridericia 1920)		
QT_{fc} < 440 ms	6	43
QT_{fc} > 440 ms	24	0
Population study (Karjalainen et al. 1997)		
QT < upper normal limit	6	43
QT > upper normal limit	24	0
Proposed diagnostic criteria for the LQTS (Schwartz et al. 1993)		
Low probability	8	43
Intermediate probability	6	0
High probability	16	0

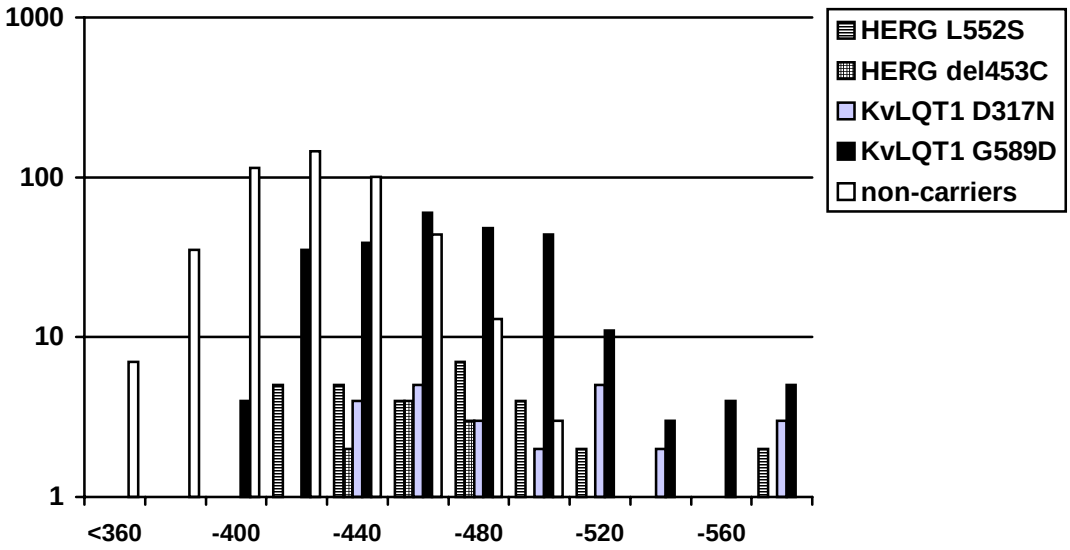

Figure 1. *Distribution of QTc intervals among LQT1 and LQT2 patients and non-carriers.*

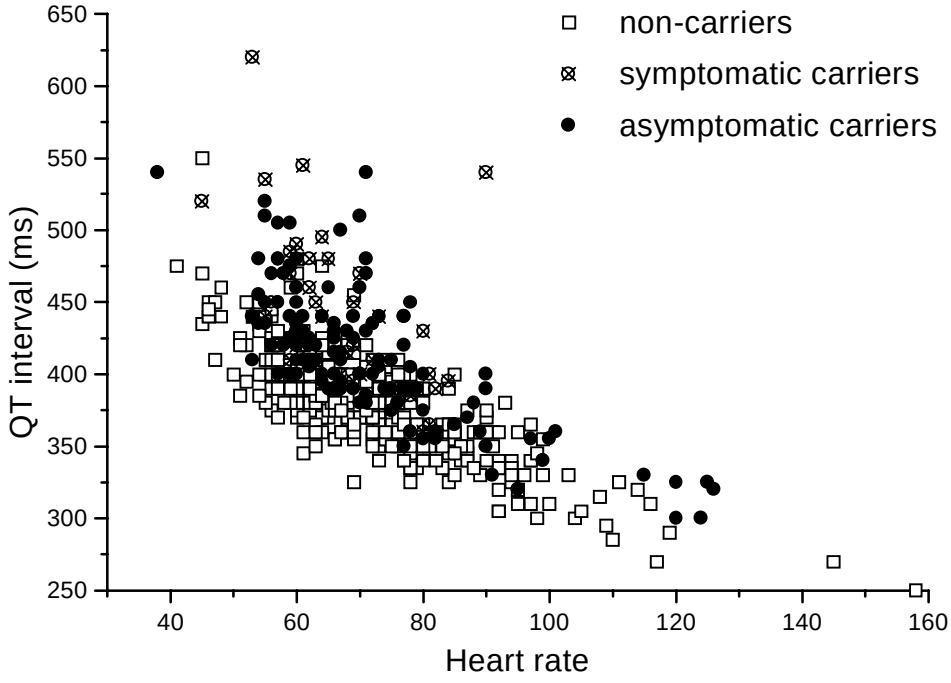

Figure 2. *Distribution of QT intervals according to heart rate in male carriers and non-carriers of KvLQT1 G589D genotype.*

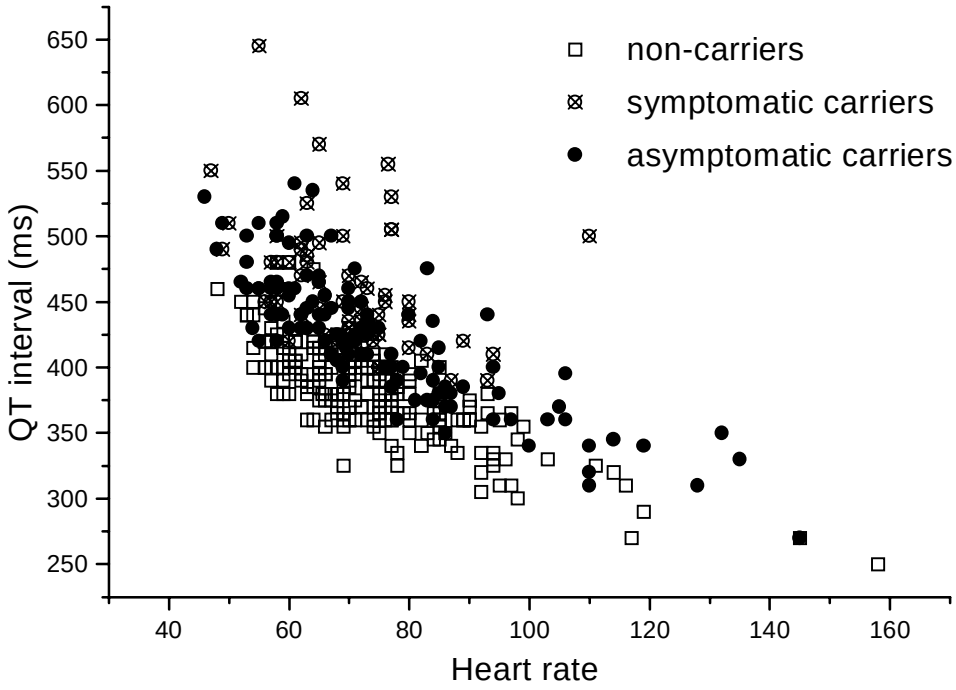

Figure 3. *Distribution of QT intervals according to heart rate in female carriers and non-carriers of KvLQT1 G589D genotype.*

5.3. T -wave morphology

A biphasic or double-peaked T-wave in any of the precordial leads was observed at rest in 10, during exercise in 8 and after exercise in 10 of 19 pediatric LQTS patients (study I). In the control group (n=19), double-peaked T-wave was exhibited at rest by 8, during exercise by one and during recovery phase by no one. In the LQTS group, three out of 11 cases showing prolongation of total QT from exercise to recovery did not exhibit double-peaked T -waves at rest, during or after exercise.

5.4. Resting heart rate

Resting heart rates in LQT1 or LQT2 patients were not different from that in healthy controls. LQT1 patients with a mutation in the pore region of the channel had similar

resting heart rates as their healthy relatives (68±12 bpm in both groups, study II) and similar to that of LQT2 patients (68±9 bpm, study III).

5.5. Maximal heart rate

In the heterogenous pediatric LQTS group (study I) without beta antiadrenergic medication, the maximal heart rate attained was lower than in the control group (172±20 vs. 195±11 bpm, p=0.0003).

Gene-specific analysis in adolescent and adult population showed differences between genes (LQT1 and LQT2 types of LQTS) as well as between different mutations (study III), **Table 8**.

Table 8. *Maximal actual and relative heart rates in LQT1, LQT2 and control subjects.*

	LQT1 pore region n=23	LQT1 C-terminal n=22	LQT2 n=20	Controls n=33	p-values
Maximal heart rate (beats/min)	140±13	161±7	187±14	181±13	*
Maximal heart rate of expected (%)	76±5	86±4	99±6	96±7	*

* <0.001 between all groups except LQT2 and controls.

No difference was found in the achieved heart rate between symptomatic and asymptomatic LQT1 patients (LQT1 pore region and C-terminus groups combined). The maximal load achieved was 236±46 W, 248±46, 187±48 W and 237±46 W for men and 135±40, 149±31, 161±17 and 152±32 W for women of each group, respectively, p=NS.

Impairment in heart rate was related to QT interval duration, examined as a correlation between achieved/expected heart rate -ratio and QT interval. **Figure 4** shows the relationship of QT interval duration at the heart rate 130 bpm during exercise and the achieved/expected heart rate ratio. The mean QT interval of controls at this heart rate is 282±12 ms.

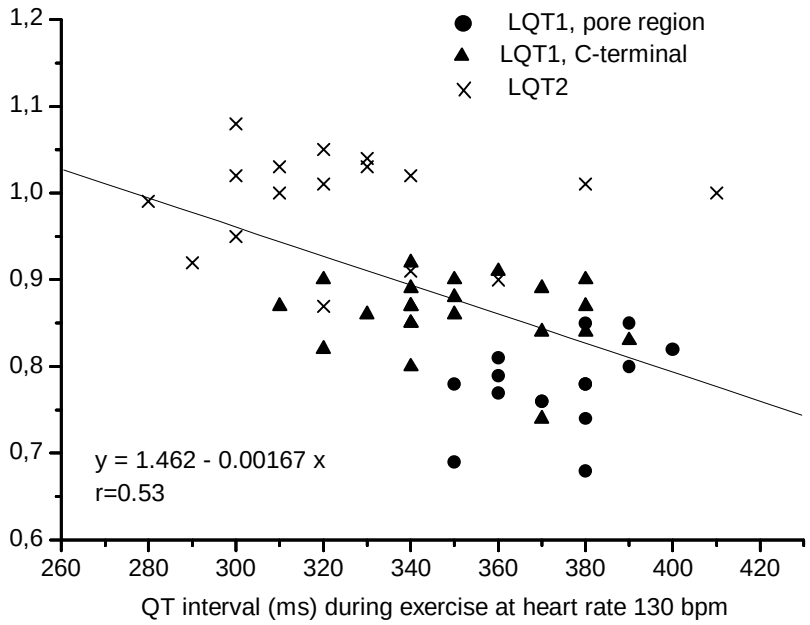

Figure 4. *Relationship between QT interval and the relative maximal heart rate achieved during exercise.*

Altogether, 56 % of the LQT1 patients and only 3 % of the controls failed to reach the provisional limit of 85 % of the expected maximal heart rate, whereas all the LQT2 patients exceeded this limit. The predictive value (R^2) of relative maximal heart rate was 28 % during exercise (r=0.53) and 30 % (r=0.55) during recovery at heart rate 130 beats per minute.

5.6. QT interval during and after exercise

QT intervals were significantly longer in both LQT1 groups and LQT2 group than in the control group at all heart rates during exercise and recovery (**Figure 5, Table 9)**. Comparison between mutation types showed that QT intervals among LQT1 patients were longer in patients with the mutation in the pore region than in those with the mutation in the C-terminal region of KvLQT1 gene throughout the exercise phase except at the lowest heart rate studied (100 beats min^{-1}). LQT2 patients had QT interval significantly longer than that of LQT1 C-terminus patients at the lowest heart rate, but

thereafter exhibited shortening to values less than in either of the LQT1 groups. During the recovery phase, LQT1 patient groups did not differ from each other, but both showed significantly longer QT intervals than LQT2 patients (**Figure 5**).

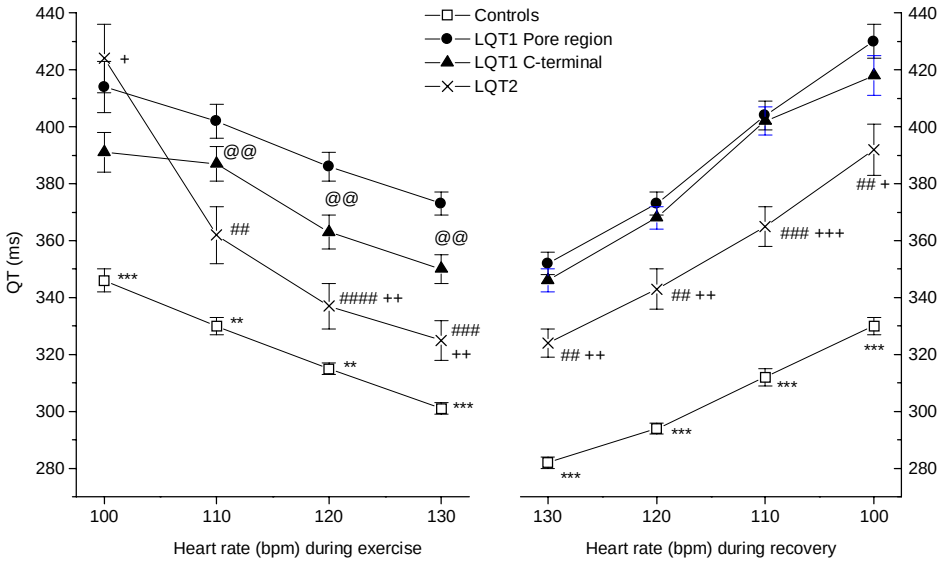

Figure 5. *Group mean QT interval ± SE at specified heart rates in two LQT1 patient groups, LQT2 patients and controls.*

In the control group, the QT interval was at all heart rates significantly shorter during recovery than during exercise (**Table 9**). In LQT1 patient groups, QT intervals were equal to or longer than during exercise at heart rates of 100 and 110 beats min^{-1} (**Table 9**). In LQT2 group, QT intervals at the lowest examined heart rate were shorter during recovery than during exercise. At higher heart rates, there was no difference between exercise and recovery phases in LQT2 patients.

During exercise, the QT intervals of the patients and the controls overlapped at all analyzed heart rates (**Figure 6**). During recovery at lowest measurable heart rate of 100 beats min^{-1} or 110 beats min^{-1}, LQT1 patients and controls showed no overlapping but 2 (10 %) LQT2 patients had QT values within the range of controls (**Figure 6, Table 10**).

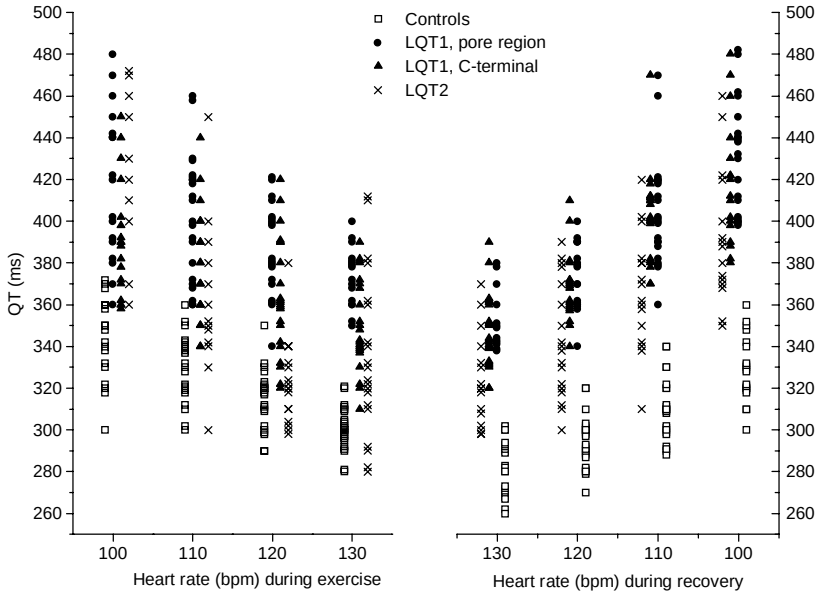

Figure 6. *QT intervals during exercise and recovery.*

Table 9. *QT interval during and after exercise.*

	Group	QT (ms), exercise	QT (ms), recovery	p-value *
100	LQT1 pore region	414±38	428±28	NS
	LQT1 C-terminus	386±21	417±31	<0.01
	LQT2	420±40	402±36	<0.05
	Controls	345±20	329±16	<0.01
110	LQT1 pore region	402±30	404±26	NS
	LQT1 C-terminus	380±27	401±23	<0.05
	LQT2	362±37	368±29	NS
	Controls	328±14	313±16	<0.001

* exercise vs. recovery

Table 10. *Application of various diagnostic alternatives in LQTS in regard to molecular classification of patients and controls.*

Diagnostic classification	Molecular classification		
	LQT1	LQT2	Controls
Standard ECG			
$QT_c \leq 470$ ms	18	7	33
$QT_c > 470$ ms	27	13	0
QT interval during recovery phase			
normal *	0	2	31
abnormal *	44	17	0
not available	1	1	2

* Measured QT interval during recovery phase at heart rate 100 bpm $\leq$ 360 ms or if not measurable, $\leq$ 350 ms at heart rate 110 bpm.

5.7. Rate adaptation of QT interval

LQT2 patients have steeper QT/heart rate dependence during exercise than healthy controls or LQT1 patients with a mutation in the C-terminal end of the gene (**Table 11,** study III). However, as **figure 7** illustrates, the wide overlap in individuals' slopes between the groups impedes the use of QT/HR -slopes to make a gene-specific diagnosis.

During recovery, both LQT1 and LQT2 patients have steeper QT/HR -slopes than controls (**Table 11**). A QT/HR -slope less negative or equal to -1.6 during *recovery* excluded all LQT1 patients and 90 % the LQT2 patients. However, 36 % of healthy controls have QT/HR -slopes steeper than that. In the heterogeneous pediatric study population, QT/heart rate dependence was also steeper during recovery than in healthy children (-2.50$\pm$0.82 vs. -1.79$\pm$0.47, p=0.003).

Table 11. *QT/heart rate slopes (ms/min^{-1}) during exercise and recovery*

	LQT1 patients		LQT2 patients	Controls
	Pore region	C-terminus		
QT/heart rate				
Exercise	-1.8±1.1	-1.6±0.7 *	-2.2±0.8 *†	-1.4±0.3 †
p -value ‡	<0.01	<0.001	NS	NS
Recovery	-2.6±1.3 #	-2.4±0.5 #	-2.2±0.6 #	-1.5±0.4

QT = QT interval, NS = not significant, * p<0.01 between LQT1 C-terminal group and LQT2 patients, † p<0.001 between LQT2 patients and controls, ‡ between exercise and recovery phases, # p<0.001 compared to controls.

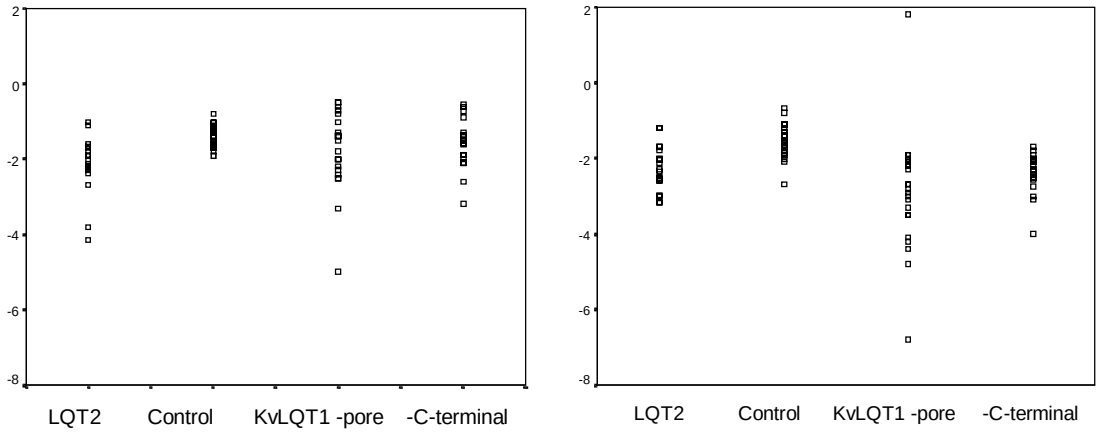

Figure 7. *QT/heart rate slopes during exercise* (left) *and recovery* (right).

It is the late QT/HR -slopes that behave differently in LQTS patients and controls (study I). The late QT/heart rate -slopes were steeper in the LQTS group than in controls during recovery (-1.27±0.74 and -0.46±0.29, respectively p<0.0001), whereas the early QT/heart rate -slopes did not differ between the groups or between phases (study I).

5.8. Occurrence of arrhythmias during exercise

The 45 LQT1 patients and 33 controls were devoid of arrhythmias during the exercise test but two out of the 20 LQT2 patients (10%) exhibited frequent ventricular premature complexes (>10 beats/minute) during exercise (study III). However, these arrhythmias were observed only at rest before, in the beginning and after the exercise; not at the higher heart rates during exercise.

5.9. Proposed diagnostic criteria for long QT syndrome

Application of the renewed diagnostic criteria for LQTS (Schwartz et al. 1993) to our LQT1 study population (study II) reached specificity of 100 %. However, the sensitivity of these criteria appeared low as 27 % of the D317N mutation carriers were classified into the category of low probability, 20 % into category of intermediate probability and only 53 % to the category of high probability of LQTS (**Table 7**).

5.10. Asymptomatic versus symptomatic mutation carriers

Both actual and rate-adjusted QT interval parameters (**Table 12**) as well as QTm values were similar in symptomatic and asymptomatic LQT1 patients with D317N mutation (study II).

However, analysis of larger patient population (n=249) with genetically uniform type of LQT1 (G589D mutation) shows that a higher proportion of mutation carriers are symptomatic the longer QT_c interval is (**Figure 8**) and that the duration of QTc interval is related to the risk of symptoms.

Although QT dispersion was increased in symptomatic LQT1 patients (n=12) compared to unaffected relatives (n=43) (66$\pm$48 vs. 37$\pm$15 ms, p=0.02), symptomatic patients LQT1 did not differ from asymptomatic (n=18) (45$\pm$19 ms). QT dispersion in the asymptomatic LQT1 patients did not differ significantly from that in the control individuals. Dispersion of early phase of repolarization (QTm) was increased in the group

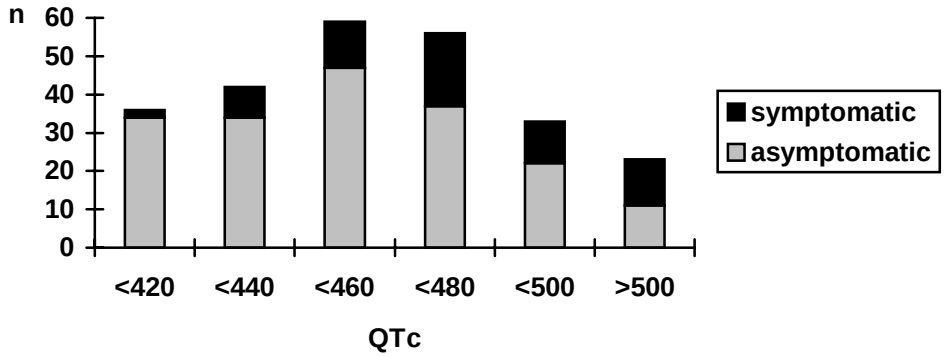

Figure 8. *Distribution of symptomatic and asymptomatic QTc carriers of G589D mutation of KvLQT1 gene.*

Table 12. *Total and early duration of repolarization and heart adjusted QT intervals in symptomatic and asymptomatic LQT1 patients and controls*

	Symptomatic LQT1 (n=12)	Asymptomatic LQT1 (n=18)	Controls (n=43)	p-value
Hr (beats/min)	70±9	65±13	69±12	NS
QT (lead II)	464±47	459±38	381±33	<0.001 *
QT_c (lead II)	496±38	475±36	404±25	<0.001 *
QT_{fc} (lead II)	485±38	469±31	396±22	<0.001 *
QTm (lead II)	391±44	380±33	310±32	<0.001 *

Hr = heart rate, QT = measured QT interval. $QT_c = QT/RR^{1/2}$, $QT_{fc} = QT/RR^{1/3}$, QTm = early duration of repolarization, * = symptomatic LQT1 patients vs. controls, and asymptomatic LQT1 patients vs. controls. No significant differences between symptomatic and asymptomatic patients.

of symptomatic LQT1 patients (57±32 ms) compared to controls (32±15 ms, p=0.006), whereas asymptomatic patients and control individuals did not differ significantly in this respect. Still, relative dispersion of early phase of repolarization (rQTm) did not differ significantly between any of the three groups. Overlapping of QT dispersion between groups was considerable (data not shown) and accordingly in the individual risk assessment the value of QT dispersion appears to be low.

5.11. Differential diagnostic studies in families with polymorphic ventricular tachycardia

Study IV shows that even carriers of familial polymorphic ventricular tachycardia have slightly prolonged QTc interval compared to healthy controls (430 ± 18 ms vs. 409 ± 19 ms, $p<0.01$). In none of the carriers, however, QTc was 470 ms or above and thus, none of them showed unequivocal QT interval prolongation.

Appearance of ventricular arrhythmias was observed in 14 out of 15 carriers of the 1q42-43 genotype. The arrhythmias consisted of frequent ventricular premature complexes in the beginning, followed by ventricular bigeminy and in most cases by salvoes of ventricular premature complexes. The arrhythmias appeared first at a sinus rate of 121±15 (range 104-153) bpm and disappeared immediately after cessation of work.

5.11.1. Findings of cardiac imaging studies

To exclude cardiomyopathies as the cause of the exercise-induced polymorphic ventricular tachycardia, imaging studies were performed to the affected family members. In echocardiography, the left ventricular end-diastolic and end-systolic indices were normal (**Table 13**). Interventricular septal and posterior wall thickness was ≤11 mm in all patients. Right ventricular cineangiography revealed no structural alterations or tricuspid valve insufficiency. One patient had a 70 % stenosis of the descending left anterior coronary artery. In MRI study, the structure of the myocardium was normal in all six studied cases. No findings suggestive to right ventricular cavity or outflow tract dilatation or adipose replacement of the myocardium were observed. Contraction of right ventricle was synergistic.

5.11.2. Other features of familial polymorphic ventricular tachycardia linked to chromosome 1q42 q43

Two affected subjects (14 %) had a late potential in signal-averaged ECG. In the electrophysiologic study, ventricular tachycardia was not inducible in any patient and AV- and HV -intervals were normal. Intravenous administration of flecainide in four

patients did not result in development of right bundle branch block or ST segment elevation.

Table 13. *Echocardiography and right ventricular cineangiography in familial polymorphic ventricular tachycardia*.

	Patients (N=9	Controls (N=9)
Echocardiographic left ventricular		
- end-diastolic diameter/BSA (mm/m^2)	28±3	29±4
- end-systolic diameter/BSA (mm/m^2)	18±3	19±3
-posterior wall thickness (mm)	9±2	9±2
Interventricular septal thickness (mm)	10±2	9±1
Cineangiographic right ventricular		
- end-diastolic volume/BSA (ml/m^2)	89±15	93±29
- end-systolic volume/BSA (ml/m^2)	36±8	38±9
- ejection fraction (%)	41±4	42±6

BSA = body surface area. None of the parameters showed statistically significant differences between groups.

Microscopic examination of the endomyocardial biopsies showed no significant differences in the extent of fibrosis, adiposity, nuclear changes and inflammation between patients and controls. Iron, Kongo and periodic acid-Schiff staining were negative in all samples excluding relevant storage diseases.

A significant positive LOD score (4.74 in the two families combined and over 3.0 in pedigree 1 alone) was obtained in linkage analysis. Based on the genotypes obtained in linkage analysis, only one carrier was devoid of arrhythmias during exercise stress test thus demonstrating high penetrance of the disease. This genotype was also associated with high mortality; the cumulative mortality by the age of 30 years was 31 % in the studied families.

6. DISCUSSION

6.1. QT interval and clinical criteria in the diagnosis of LQTS

Results of study II show that the QT interval measured on a resting ECG cannot reliably separate LQTS patients from the normal population, irrespective of method used to judge QT interval normality. Thus, a standard 12 -lead ECG is an insufficient method to diagnose all affected LQTS patients, even in a genetically homogenous population. As further demonstrated in study II, other clinical and ECG features applied as a proposed criteria score do not result in acceptable sensitivity in diagnosing the LQTS carriers with normal or borderline QT interval. It is also of note that those criteria have been applied in several studies in which they may have resulted in biased patient selection, since only the most obvious cases would have been included.

QT interval measurements carried out on specific heart rates showed, in study III, that although LQTS patients in general have longer QT intervals than the normal population, there is still considerable overlap. Opposite to QT intervals at rest and during exercise, QT intervals of LQTS patients and healthy controls during the recovery phase show significantly less overlap. Therefore, observing the QT interval at heart rates 110 or 100 bpm during the recovery phase following exercise is likely to contribute to clinical diagnosis in the most common types of LQTS, i.e. LQT1 and LQT2 variants.

6.2. Rate adaptation of QT intervals

The dynamic changes in QT/heart rate response are even capable to show differences between different mutations of same gene, i.e. mutations of pore and C-terminal region of KvLQT1. This was found in study III although these groups did not differ in respect to their baseline QT interval measurements.

The results of study III demonstrate that QT interval shortening in LQT2 patients is even more efficient than in healthy population. This finding is incongruous with preliminary

findings in molecularly defined LQTS patients (Schwartz et al. 1995). The small sample size of that study is probably the cause of this incongruence (Schwartz et al. 1995). The steeper QT/RR interval dependence during sleep in LQT1 type of LQTS patients, as demonstrated in 24-hour ambulatory ECG recordings by Neyroud et al. (Neyroud et al. 1998) may be due to lower heart rates as no significant difference to controls was observed at exercise in the studies I and III.

QT interval duration was significantly longer in patients with C-terminal mutation of KvLQT1 gene at slower heart rates during recovery than at similar heart rates during exercise. The repolarization disorder in terms of QT interval duration worsened in these patients during recovery and approximated that encountered in patients with mutations in the pore region. Thus the significance of even less severe mutations may be dependent on physiological conditions.

6.3. T -wave morphology

Results of study I show that a biphasic or double-peaked T-wave appeared to be a more specific marker of LQTS in the heterogenous pediatric LQTS population in ECG obtained after exercise than in standard resting ECG, when compared to normal controls (study I). However, QT interval prolongation from the exercise phase to recovery phase distinguished more LQTS carriers than morphological T -wave abnormalities. Nevertheless, based on previous findings in a mixed adult and pediatric population, T -wave humps or double-peaked T -waves in limb leads or leads V4-6 of a resting ECG appeared to be specific even if not sensitive findings for LQTS (Lehmann et al. 1994) and of additional diagnostic help. Of note, presence of T -wave *inversions* should raise the suspicion of hypertrophic cardiomyopathy or arrhythmogenic right ventricular dysplasia (in adults), depending on the location of the changes (see **Table 3** and **4**).

6.4. Heart rate

KvLQT1 mutations resulting in prolonged repolarization also impair the sinus rate response to exercise. Opposite to this, HERG mutations, even when causing markedly prolonged QT interval, do not restrict the maximal heart rate. Because the majority of

LQTS patients belong either to LQT1 or LQT2 subtypes, the inability to achieve the predicted age-adjusted maximal heart rate together with a significantly prolonged QT interval in resting ECG suggests the LQT1 rather than LQT2 subtype.

Abnormally low resting heart rate is not a diagnostic feature in adolescent or adult LQTS population in general. Only in a small number of male LQT3 carriers has resting heart rate been observed to be lower than in LQT1 or LQT2 carriers (Locati et al. 1998). In neonates and children with most severe repolarization abnormality, 2:1 atrioventricular block (Garson et al. 1993, Gorgels et al. 1998), or significant sinus rate decrease compared to healthy children of same age can occasionally be observed. Such a severe repolarization abnormality and atrioventricular block may result from homozygous carrier state of at least HERG mutation (Piippo et al. 1999) and probably also from KvLQT1 mutation. The atrioventricular block might be precipitated by the QT interval prolonging effect of pauses following a premature contraction. In LQT1, this may also be due to impaired QT and His-Purkinje refractory period shortening at high sinus rates. In addition, because the sinus rate is naturally highest in the youngest children, the direct negative effect of KvLQT1 gene on sinus node cells may also be easiest to note in the youngest children.

6.5. Risk of arrhythmic events

The duration of ventricular repolarization as measured by QT interval was similar in symptomatic and asymptomatic LQT1 patients with the same genotype in study II. Thus the carrier status of a specific mutation itself seems to be more of a determinant of cardiac events than QT interval duration, per se. However, the large variation in QTc intervals of carriers, as well as the higher proportion symptomatic patients among carriers with the longest QTc intervals, suggest that there are additional factors which also contribute to individual's risk of arrhythmias.

In LQT1 patients, the propensity to arrhythmias during and immediately after exercise may result from the relative QT interval prolongation concomitantly with increase of heart rate during exercise. In addition, the QT interval enlengthening after cessation of

exercise is most likely to play a role as well. Opposite to this, LQT2 patients, whose QT interval shortening is relatively more efficient compared to controls, rarely have symptoms during physical activities. This may imply that shortening the duration of repolarization by increasing the resting heart rate by pacemaker therapy may be beneficial particularly in LQT2 type of LQTS.

Dispersion of ventricular repolarization was increased in symptomatic LQTS patients. Increased dispersion of the QT interval in patients with LQTS has been reported in several earlier studies without performing molecular diagnosis (Day et al. 1990, Linker et al. 1992, Priori et al. 1994). However, conclusions on the relationship between degree of dispersion and risk of cardiac events have been inconsistent. The study by Linker et al. (Linker et al. 1992) showed no difference in the degree of dispersion between LQTS groups with frequent or infrequent symptoms, whereas Priori et al. found that LQTS patients who responded to beta-blockade had lower QT dispersion than non-responders while on beta-adrenergic antagonists therapy (Priori et al. 1994). In our molecularly defined cohort of LQTS patients, overlapping of QT dispersion between groups was considerable and accordingly, in the individual risk assessment, the value of QT dispersion appears to be low.

6.6. Occurrence of arrhythmias during exercise

Two out of 20 LQT2 patients exhibited ventricular premature beats and none of the 45 LQT1 patients had ventricular arrhythmias during exercise. On the other hand, the rate-dependent nature of augmentation of arrhythmias is typical for (catecholaminergic) familial polymorphic ventricular tachycardia. In the group of patients with familial polymorphic ventricular tachycardia the reproducible arousal of ventricular arrhythmias during exercise was similar to that in the series of Leenhardt et. al. (Leenhardt et al. 1995). In both studies, the average sinus rate at which the arrhythmias appeared during exercise was approximately 120 bpm. Relative physical inactivity during ambulatory ECG recording can fail to increase the heart rate enough to exceed the individual, arrhythmia-provoking limit. This will result in arrhythmia-free registration and false negative diagnosis.

6.7. Differential diagnosis

The spectrum of inherited arrhythmogenic cardiomyopathies and ion channel disorders is broad and contain subtle forms which are difficult to detect with conventional methods. Index case's false diagnosis will result in search of non-diagnostic and misleading clues like QT -interval or ventricular wall structure abnormalities. Besides in LQTS, long QT interval may be encountered in hypertrophic as well as in dilated cardiomyopathy (Martin et al. 1994). QT interval prolongation has not been described in conjunction of familial idiopathic ventricular fibrillation or arrhythmogenic right ventricular dysplasia except in rare cases. The value of cardiac imaging in the assessment of a patient with prolonged QT interval is therefore primarily in excluding hypertrophic or dilated cardiomyopathy, both of which may cause secondary QT interval prolongation.

In this study it was observed that arrhythmias are seldom induced during exercise test in the LQTS whereas the appearance of polymorphic ventricular premature complexes is the diagnostic feature in families with the exercise-induced polymorphic ventricular tachycardia. Although physical exercise is a common triggering factor for cardiac events in LQTS, the incidence of arrhythmic events is low compared to how often most people do some kind of physical exercise. In addition, other contributing factors may be needed for induction of arrhythmias besides the physical exercise, itself. Appearance of polymorphic ventricular tachycardia during exercise should raise the suspicion of the familial form of this arrhythmia.

6.7.1. Differentiating the familial polymorphic ventricular tachycardia from LQTS and other common inherited arrhythmogenic cardiac disorders

The clinical manifestation of familial polymorphic tachycardia resembles LQTS by the exercise-induced syncopal spell and sudden death occurring especially in young patients. QT intervals in the carriers of familial polymorphic tachycardia are slightly longer than normal but not unequivocally prolonged. In addition, exercise does not progressively increase the number of multifocal ventricular premature complexes in LQTS patients.

In contrast to arrhythmogenic right ventricular dysplasia, our patients of familial polymorphic ventricular tachycardia lacked the T-wave abnormalities (Corrado et al. 1997) and right ventricular wall abnormalities, and the polymorphic appearance of the ventricular tachycardia was clearly different from monomorphic tachycardia with left bundle branch block pattern encountered in arrythmogenic right ventricular dysplasia (Corrado et al. 1997). Left ventricular wall thickness and systolic function in all carries of familial polymorphic tachycardia also exclude hypertrophic and dilated cardiomyopathies.

6.7.2. Differentiating symptomatic LQTS patients from patients with syncopal spells from other, non-genetic causes

The risk of cardiac events in LQTS is highest in children and adolescents (Zareba et al. 1998). In these youngest age groups of syncope patients with apparently normal hearts, the most common differential diagnostic alternatives are vasovagal syncope and seizure in addition to those inherited arrhytmogenic disorders discussed in previous chapters. Prodromal weakness and nausea lasting tens of seconds or minutes is suggestive to vasovagal syncope whereas syncope starting with convulsions with or without preceding aura is likely to result from primary central nervous system disorder.

Therefore, detailed description of foregoing prodromal symptoms as well as eye-witness observations are important in differentiating the arrhythmia-induced syncope from the other relatively common causes. Careful family history may reveal sudden death at young age suggestive to inherited arrhythmogenic disorder, even to a specific type of LQTS, e.g. swimming or vigorous exercise to LQT1, auditory stimulus to LQT2 and sleep to LQT3.

6.8. Implications for future studies

The large variation of QT intervals found among carriers of even one specific mutation raises the question of modifying factors for phenotypic expression. Such determinants are many, including serum ion concentrations, sex hormones, other ion channel genes (either

mutated or allelic variants) as well as genes regulating autonomic nervous system signaling and secondary messengers in cells.

Even if mutations in the three most common LQTS genes in general result in approximately 6 per cent mortality (Zareba et al. 1998), the malignancy of the ion channel defect is likely to depend on the specific mutation; findings in study III showed that the magnitude of QT prolongation was different in various physiological states depending on the mutation. As some mutations are not found just in sporadic families but as founder mutations which are enriched in a population, larger cohorts with specific mutations will be available for subgroup analyses. These studies may provide data with significant differences in mortality depending on how the given ion channel is impaired.

The finding of gene- and mutation-specific differences in QT heart rate responses implies also that when antiarrhythmic drugs are being evaluated *in vivo*, not only should changes in QT interval duration at rest be assessed but also the changes in rate adaptation of QT interval. Thus, in the presence of a pharmacological agent, the impaired shortening of QT interval during exercise or excessive prolongation during recovery after exercise, might indicate a detrimental effect on ion channel function even if there was no change in baseline QTc. On the other hand, in LQTS patients, the opposite would most likely mean beneficial effect as in case of mexiletine in LQT3. Other currently used or potential antiarrhythmic agents in LQTS should be studied in like manner as well.

Totally new mechanisms for arrhythmias may be revealed in those inherited arrhythmogenic cardiac disorders which molecular mechanisms are not yet solved. An example of this is the familial polymorphic tachycardia linked to chromosome 1q42-43. It remains to be electrophysiologically further characterized in order to understand the arrhythmia mechanism. This could enhance both the therapy and the recognition of the underlying gene. Without doubt, the molecular genetic diagnosis will lead to more frequent identification of this disorder and enable early diagnosis and preventive measures in future.

7. CONCLUSIONS

Patient with anxiety or exercise-related syncopal spell or with family history of sudden death under such circumstances, should undergo cardiac evaluation which includes exercise stress test which improves the diagnostic accuracy of long QT syndrome, particularly when attention is paid to the recovery phase.

A normal QT interval on the resting ECG does not exclude the possibility of congenital long QT syndrome. Other resting electrocardiogram measures (T -wave abnormalities and QT dispersion) are capable of providing suggestive diagnosis of congenital long QT syndrome only in a limited proportion of LQTS gene carriers.

Although cardiac events are most common in patients with abnormal QTc interval (> 470 ms), it is essential that family members of LQTS patients with normal or only marginally prolonged QT intervals are evaluated to obtain or exclude the diagnosis of LQTS. This also enables the counseling of these individuals potentially at risk. If the molecular diagnosis is not available, the clinical diagnosis is often enhanced by observing the dynamic changes of QT interval *after* exercise

Normal or marginally prolonged QT interval together with exercise-induced ventricular arrhythmias in a structurally normal heart is suggestive of familial polymorphic ventricular tachycardia, the prognosis of which is poor. Although its symptoms are the same as in LQTS, the former can be differentiated from the latter by electrocardiographic measures. Systematic evaluation of family members to detect or exclude the exercise-induced arrhythmias is obligatory for correct diagnosis until molecular genetic diagnosis is available. The age-dependent manifestation of the disease necessitates repeated exercise stress tests for potential carriers of the disorder.

8. SUMMARY

Background

Congenital long QT syndrome is a spectrum of cardiac ion channel disorders resulting from mutations in several different genes. As a common feature, they share the prolonged ventricular repolarization which is associated with life-threatening ventricular arrhythmias. The conventional diagnosis is based on demonstration of a long QT interval which, however, is often absent thus resulting in false negative diagnosis. This series of studies was performed to improve diagnostic accuracy of long QT syndrome.

Patients and methods

A total of 114 long QT syndrome patients and 96 healthy controls, as well as 43 patients belonging to families with members exhibiting exercise-induced ventricular premature beats, were examined in this series of studies. Patients with molecularly defined diagnosis were used in three out of four studies. Family history, history of cardiac events and resting, exercise and ambulatory ECG recordings were obtained to determine the QT interval behavior as an indicator of propensity to ventricular arrhythmias. QT intervals were compared during physical exercise and subsequent recovery at specified heart rates. Cardiac imaging studies were used to exclude other disorders resulting in QT interval prolongation.

Results

Proposed diagnostic criteria for LQTS had diagnostic specificity of 100 % in a molecularly defined population of LQTS carriers and their healthy relatives, whereas the sensitivity was 73 %. During recovery from exercise, normal upper limits for unadjusted QT intervals exceeding 350 ms at heart rate 110 beats/min or 360 ms at heart rate 100 beats/min (lowest available) resulted in diagnostic sensitivity and specificity of 100 % for LQT1 patients and 89 % and 100 % for LQT2 patients. Mutations in KvLQT1 gene decreased the age-specific maximal heart rate in the study population from 96$\pm$7 % to 76$\pm$5 % (pore region mutation) or 86$\pm$4 % (C-terminal mutation) whereas mutations in

HERG gene had no effect on maximal heart rate. None of the LQT1 and only 10 % of LQT2 patients had ventricular premature complexes during exercise whereas they were encountered in 93 % of subjects with 1q42-q43 positive genotype.

Conclusions

The findings of this study indicate that QT intervals of long QT syndrome carriers show overlap with those of healthy population and cannot often be distinguished from them using resting ECG and clinical history only. Studies during exercise stress test show that different long QT syndrome genotypes have different influence on QT interval behavior in various physiological states. Even mutations of a given cardiac ion channel gene alter the dynamics of repolarization to different degree. Alterations in QT interval during standardized conditions like exercise stress test, enhance the diagnosis of long QT syndrome and separation between the two most common types of it, LQT1 and LQT2 type of long QT syndrome. In addition, exercise stress test is informative in the differential diagnosis of long QT syndrome from a familial arrhythmic disorder with symptoms similar to LQTS.

9. ACKNOWLEDGEMENTS

The subject of this study was suggested to me by Docent Lauri Toivonen, MD, Ph.D. and Docent Matti Viitasalo MD, Ph.D. To them I am grateful for all the guidance, support and sharing their knowledge of electrophysiology.

This study was performed at the Department of Internal Medicine at the Helsinki University. I want to express my gratitude to Professor M. Heikki Frick, former Head of the Division of Cardiology, Professor Markku S. Nieminen, present Head of the Division of Cardiology, Professor Kimmo Kontula, Professor Juhani Heikkilä and Professor Vesa Manninen for providing the use of the research facilities.

Furthermore, I wish to express my gratitude to Professor Michael H. Lehmann and Professor Heikki Huikuri for their careful review and constructive criticism of my manuscript of thesis.

In addition, without contribution of Professor Kimmo Kontula, Doctor Kirsi Piippo, Ph.D. and Doctor Kati Kainulainen, MD, Ph.D. in the molecular genetic studies, this study would not have been possible. The teamwork of ours has been enjoyable. I especially want to thank Doctor Kirsi Piippo, Ph.D. for her always so pleasurable cocoperation, patience and usefulness in the knowledge she has imparted to me.

My sincere thanks are due to my other co-authors Docent Päivi Heikkilä MD, Ph.D. Docent Pekka Keto, MD, Ph.D. Docent Timo Paavonen, MD, Ph.D. and Professor Juha Kere.

Also, to the staff of the Cardiovascular Laboratory and the Laboratory of Molecular Medicine, I express my gratitude for their devotion and flexibility. Nor could I forget the warmest thanks go to Mrs. Hanna Ranne, R.N. and Mrs. Päivi Kokko, R.N. for their excellent technical assistance.

I want to thank my colleagues at the Cardiovascular Laboratory for their optimistic and useful discussions. Especially I want to thank Docent Markku Mäkijärvi, MD, Ph.D. for guiding me to the world of cardiovascular research, and Doctor Lasse Oikarinen, MD, Ph. D. for the always kind and encouraging discussions.

I am grateful for all the colleagues who have been active in submitting patients and thus enabled the accumulation of pedigrees with inherited cardiac arrhythmias, especially for Docent Gottfried Härtel, MD, Ph.D. and Doctor Liisa Rovamo, MD, Ph.D. who had started the collection of the arrhythmia families. I also with to express special thanks to the entire staff of Children's Hospital of Helsinki University Central Hospital. Their help has been invaluable in carrying the studies on pediatric patients.

For the numerous happy moments during these years of research I want to thank Päivi, Kristiina, Sanna, Marjatta, Tuomas, Juha, Mika, David and all other friends of mine. Your company and the time we have spent together has been invaluable.

I wish to extend special thanks to all my patients and healthy volunteers for their time and assistance in this study. It is my desire that through these efforts, your lives have benefited in some way.

This study was supported by grants from the Finnish Foundation for Cardiovascular Reserach, the Paavo Nurmi Foundation and the Siegrid Juselius Foundation.

In closing, my deepest gratitude goes to my parents Aila and Juhani for their warm and loving support throughout the years of this study.

Helsinki, May 2000

Heikki Swan

10. REFERENCES

Abbott, G. W., F. Sesti, I. Splawski, M. E. Buck, M. H. Lehmann, K. W. Timothy, M. T. Keating and S. A. N. Goldstein. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97: 175-87, 1999.

Ackerman, M. J., D. J. Tester, C. J. Porter and W. D. Edwards. Molecular diagnosis of the inherited long-QT syndrome in a woman who died after near-drowning. N Engl J Med 341: 1121-5, 1999.

Alings, M. and A. Wilde. "Brugada" syndrome. Clinical data and suggested pathophysiological mechanism. Circulation 99: 666-73, 1999.

Antzelevitch, C., V. Nesterenko and G. X. Yan. Ionic processes underlying the action potential. Electrocardiology ’96: from the cell to the body surface. J. Liebman. World Scientific Publishing Co. Pte. Ltd, Singapore. 1996: 219-29.

Bazett, H. C. An analysis of the time-relations of electrocardiograms. Heart 7: 353-70, 1920.

Berthet, M., I. Denjoy, C. Donger, L. Demay, H. Hammoude, D. Klug, E. Schulze-Bahr, P. Richard, H. Funke, K. Schwartz, P. Coumel, B. Hainque and P. Guicheney. C-terminal HERG mutations. The role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. Circulation 99: 1464-70, 1999.

Bhandari, A. K., W. A. Shapiro, F. Morady, E. N. Shen, J. Mason and M. M. Scheinman. Electrophysiologic testing in patients with the long QT syndrome. Circulation 71: 63-71, 1985.

Bonatti, V., A. Rolli and G. Botti. Monophasic action potential studies in human subjects with prolonged ventricular repolarization and long QT syndromes. Eur Heart J 6 Suppl D: 131-43, 1985.

Brugada, P. and J. Brugada. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol 20: 1391-6, 1992.

Chen, Q. Y., G. E. Kirsch, D. M. Zhang, R. Brugada, J. Brugada, P. Brugada, D. Potenza, A. Moya, M. Borggrefe, G. Breithardt, R. Ortizlopez, Z. Wang, C. Antzelevitch, R. E. Obrien, R. E. Schulze-Bahr, M. T. Keating, J. A. Towbin and Q. Wang. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 392: 293-296, 1998.

Chinushi, M., M. Restivo, E. B. Caref and N. El-Sherif. Electrophysiological basis of arrhythmogenicity of QT/T alternans in the long-QT syndrome. Tridimensional analysis of kinetics of cardiac repolarization. Circ Res 83: 614-28, 1998.

Chou, T. C. Electrocardiography in Clinical Practice. Grune & Stratton, New York. 1979.

Compton, S. J., R. L. Lux, M. R. Ramsey, K. R. Strelich, M. C. Sanguinetti, L. S. Green, M. T. Keating and J. W. Mason. Genetically defined therapy of inherited long-QT syndrome. Circulation 94: 1018-22, 1996.

Corrado, D., C. Basso, G. Thiene, W. J. McKenna, M. J. Davies, F. Fontaliran, A. Nava, F. Silvestri, C. Blomstrom-Lundqvist, E. K. Wlodarska, G. Fontaine and F. Camerini. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. J Am Coll Cardiol 30: 1512-20, 1997.

Cottingham Jr, R. W., R. M. Iduri and A. A. Schaffer. Faster sequential genetic linkage computations. Am J Hum Gen 53: 252-63, 1993.

Cowan, J. C., C. J. Hilton, C. J. Griffiths, S. Tansuphaswadikul, J. P. Bourke, A. Murray and R. W. Campbell. Sequence of epicardial repolarization and configuration of the T wave. Br Heart J 60: 424-33, 1988.

Cowan, J. C., K. Yusoff, M. Moore, P. A. Amos, A. E. Gold, J. P. Bourke, S. Tansuphaswadikul and R. W. Campbell. Importance of lead selection in QT interval measurement. Am J Cardiol 61: 83-7, 1988.

Curran, M. E., I. Splawski, K. W. Timothy, G. M. Vincent, E. D. Green and M. T. Keating. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 80: 795-803, 1995.

Daliento, L., G. Rizzoli, G. Thiene, A. Nava, M. Rinuncini, R. Chioin and S. Dalla Volta. Diagnostic accuracy of right ventriculography in arrhythmogenic right ventricular cardiomyopathy. Am J Cardiol 66: 741-5, 1990.

Davignon, A., P. Rautaharju, E. Boiselle, F. Soumis, M. Megelas and A. Choquette. Normal ECG standards for infants and children. Ped Cardiol 1: 123-31, 1979.

Day, P. C., J. M. McComb and R. W. Campbell. QT dispersion: an indication of arrhytmia risk in patients with long QT intervals. Br Heart J 63: 342-4, 1990.

Donger, C., I. Denjoy, M. Berthet, N. Neyroud, C. Cruaud, M. Bennaceur, G. Chivoret, K. Schwartz, P. Coumel and P. Guicheney. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. Circulation 96: 2778-81, 1997.

Emori, T., T. Ohe, N. Aihara, T. Kurita, W. Shimizu, S. Kamakura and K. Shimomura. Effects of beta-blocker therapy on the dynamic QT/RR relation in patients with long QT syndrome during 24-hour holter ECG monitoring. A.N.E. 2: 40-6, 1997.

Fatkin, D., C. MacRae, T. Sasaki, M. R. Wolff, M. Porcu, M. Frennnex, J. Atherton, V. H. J. Jr., S. Spudich, U. De Girolami, J. G. Seidman, C. Seidman, F. Muntoni, G. Muehle, W. Johnson and B. McDonough. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. N Engl J Med 341: 1715-24, 1999.

Ferlinz, J., R. Gorlin, P. F. Cohn and M. V. Herman. Right ventricular performance in patients with coronary artery disease. Circulation 52: 608-15, 1975.

Franz, M. R., K. Bargheer, W. Rafflenbeul, A. Haverich and P. R. Lichtlen. Monophasic action potential mapping in human subjects with normal electrocardiograms: direct evidence for the genesis of the T wave. Circulation 75: 379-86, 1987.

Franz, M. R., J. Schaefer, M. Schöttler, A. Seed and M. I. M. Noble. Electrical and mechanical restitution of the human heart at different rates of stimulation. Circ Res 53: 815-22, 1983.

Franz, W. M., M. Cremer, R. Herrmann, E. Grunig, W. Fogel, T. Scheffold, H. H. Goebel, R. Kircheisen, W. Kubler, T. Voit and et al. X-linked dilated cardiomyopathy. Novel mutation of the dystrophin gene. Ann N Y Acad Sci 752: 470-91, 1995.

Fridericia, L. S. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. Acta Med Scand 53: 469-86, 1920.

Garson, A., Jr., M. d. Dick, A. Fournier, P. C. Gillette, R. Hamilton, J. D. Kugler, d. van Hare Gf, V. Vetter and G. W. d. Vick. The long QT syndrome in children. An international study of 287 patients. Circulation 87: 1866-72, 1993.

Goerss, J. B., V. V. Michels, J. Burnett, D. J. Driscoll, F. Miller, R. Rodenheffer, A. J. Tajik and D. Schaid. Frequency of familial dilated cardiomyopathy. Eur Heart J 16(Supp O): 2-4, 1995.

Gorgels, A. P., F. Al Fadley, L. Zaman, M. J. Kantoch and A. H. Z. The long QT syndrome with impaired atrioventricular conduction: a malignant variant in infants. J Cardiovasc Electrophysiol 9: 1225-32, 1998.

Gussak, I., C. Antzelevitch, P. Brehhegaard, J. Towbin and B. Chaitman. The Brugada syndrome: clinical, electrophysiological and genetic aspects. J Am Coll Cardiol 33: 5-15, 1999.

Hammond, H. K. and V. F. Froelicher. Normal and abnormal heart rate responses to exercise. Prog Cardiovasc Dis 27: 271-96, 1985.

Hirao, H., W. Shimizu, T. Kurita, K. Suyama, N. Aihara, S. Kamakura and K. Shimomura. Frequency-dependent electrophysiologic properties of ventricular repolarization in patients with congenital long QT syndrome. J Am Coll Cardiol 28: 1269-77, 1996.

Hofbeck, M., H. Ulmer, E. Sieber and H. Singer. Prenatal findings in patients with prolonged QT interval in the neonatal period. Heart 77: 198-204, 1997.

Jackman, W. M., K. J. Friday, J. L. Anderson, E. M. Aliot, M. Clark and R. Lazzara. The long QT syndromes: a critical review, new clinical observations and a unifying hypothesis. Prog Cardiovasc Dis 31: 115-72, 1988.

Jervell, A. and F. Lange-Nielsen. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J 54: 59-68, 1957.

Karjalainen, J., P. Ristola, A. Reunanen and M. Viitasalo. QT interval as a cardiac risk factor in a middle-aged population. Heart 77: 543-8, 1997.

Krahn, A. D., G. J. Klein and R. Yee. Hysteresis of the RT interval with exercise. A new marker for the long QT syndrome ? Circulation 96: 1551-6, 1997.

Kruglyak, L., M. J. Daly, M. P. Reeve-Daly and E. S. Lander. Parametric and non-parametric linkage analysis: A unified multipoint approach. Am J Hum Genet 58: 1347-63, 1996.

Laitinen, P., H. Fodstad, K. Piippo, H. Swan, L. Toivonen, M. Viitasalo, J. Kaprio and K. Kontula. Survey of the coding region of the HERG gene in long QT syndrome reveals six novel mutations and an amino acid polymorphism with possible phenotypic effects. Hum Mutat (online), mutation in brief, 334, 2000.

Leenhardt, A., V. Lucet, I. Denjoy, F. Grau, D. D. Ngoc and P. Coumel. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation 91: 1512-9, 1995.

Lehmann, M. H., F. Suzuki, B. S. Fromm, D. Frankovich, P. Elko, R. T. Steinman, J. Fresard, J. J. Baga and R. T. Taggart. T wave "humps" as a potential electrocardiographic marker of the long QT syndrome. J Am Coll Cardiol 24: 746-54, 1994.

Linker, N. J., P. Colonna, C. A. Kekwick, J. Till, A. J. Camm and D. E. Ward. Assessment of QT dispersion in symptomatic patients with congenital long QT syndromes. Am J Cardiol 69: 634-8, 1992.

Locati, E. H., W. Zareba, A. J. Moss, P. J. Schwartz, M. Vincent, M. H. Lehmann, J. A. Towbin, S. G. Priori, C. Napolitano, C. Robinson, M. Andrews, K. Timothy and W. J. Hall. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome. Circulation 97: 2237-44, 1998.

Malfatto, G., G. Beria, S. Sala, O. Bonazzi and P. J. Schwartz. Quantitative analysis of T wave abnormalities and their prognostic implications in the idiopathic long QT syndrome. J Am Coll Cardiol 23: 296-301, 1994.

Martin, A. B., A. Garson, Jr. and J. C. Perry. Prolonged QT interval in hypertrophic and dilated cardiomyopathy in children. Am Heart J 127: 64-70, 1994.

McKenna, W. J., P. Spirito, M. Desnos, O. Dubourg and M. Komajda. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. Heart 77: 130-2, 1997.

McKenna, W. J., G. Thiene, A. Nava, F. Fontaliran, C. Blomstrom-Lundqvist, G. Fontaine, F. Camerini Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Br Heart J 71: 215-8, 1994.

Moss, A. J., J. L. Robinson, L. Gessman, R. Gillespie, W. Zareba, P. J. Schwartz, M. Vincent, J. Benhorin, E. L. Heilbron, J. A. Towbin, S. G. Priori, C. Napolitano, L. Zhang, A. Medina, M. L. Andrews and K. Timothy. Comparison of clinical and genetic variables of cardiac events associated with loud noise versus swimming among subjects with the long QT syndrome. Am J Cardiol 84: 876--9, 1999.

Moss, A. J., P. J. Schwartz, R. S. Crampton, E. Locati and E. Carleen. The long QT syndrome: a prospective international study. Circulation 71: 17-21, 1985.

Moss, A. J., P. J. Schwartz, R. S. Crampton, D. Tzivoni, E. H. Locati, J. MacCluer, W. J. Hall, L. Weitkamp, G. M. Vincent, A. Garson, Jr. and et al. The long QT syndrome. Prospective longitudinal study of 328 families. Circulation 84: 1136-44, 1991.

Moss, A. J., W. Zareba, J. Benhorin, E. H. Locati, W. J. Hall, J. L. Robinson, P. J. Schwartz, J. A. Towbin, G. M. Vincent, M. H. Lehmann and et al. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. Circulation 92: 2929-34, 1995.

Moss, A. J., W. Zareba, W. J. Hall, P. J. Schwartz, R. S. Crampton, J. Benhorin, G. M. Vincent, E. H. Locati, S. G. Priori, C. Napolitano, A. Medina, L. Zhang, J. L. Robinson, K. Timothy, J. A. Towbin and M. L. Andrews. Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. Circulation 101: 616-23, 2000.

Muntoni, F., A. Di Lenarda, M. Porcu, G. Sinagra, A. Mateddu, G. Marrosu, A. Ferlini, M. Cau, J. Milasin, M. A. Melis, M. G. Marrosu, C. Cianchetti, A. Sanna, A. Falaschi, F. Camerini, M. Giacca and L. Mestroni. Dystrophin gene abnormalities in two patients with idiopathic dilated cardiomyopathy. Heart 78: 608-12, 1997.

Nakayma, K., H. Yamanari, F. Otsuka, K. Fukushima, H. Saito, Y. Fujimoto and T. Emori. Dispersion of regional wall motion abnormality in patients with long QT syndrome. Heart 80: 245-50, 1998.

Neyroud, N., I. Denjoy, C. Donger, F. Gary, E. Villain, A. Leenhardt, K. Benali, K. Schwartz, P. Coumel and P. Guicheney. Heterozygous mutation in the pore region of potassium channel gene KvLQT1 causes an apparently normal phenotype in long QT syndrome. Eur J Hum Gen 6: 129-33, 1998.

Neyroud, N., P. Maison-Blanche, I. Denjoy, S. Chevret, C. Donger, E. Dausse, J. Fayn, F. Badilini, N. Menhabi, K. Schwartz, P. Guicheney and P. Coumel. Diagnostic performance of QT interval variables from 24-h electrocardiography in the long QT syndrome. Eur Heart J 19: 158-65, 1998.

Olson, T. M., V. V. Michels, S. N. Thibodeau, Y.-S. Tai and M. T. Keating. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. Science 280: 750-2, 1998.

Piippo, K., P. Laitinen, H. Swan, M. Viitasalo, M. Pasternack, L. Toivonen and K. Kontula. Homozygosity for a Finnish founder mutation of the HERG potassium channel gene causes a severe phenotype of long QT syndrome (LQTS) (abstract). Eur J Hum Gen 7(suppl1): 111, 1999.

Piippo, K., H. Swan, M. Pasternack, H. Chapman, K. Paavonen, M. Viitasalo, L. Toivonen and K. Kontula. A founder mutation of the potassium channel KvLQT1 in Long QT syndrome: implications for estimation of disease prevalence and molecular diagnostics. Submitted.

Priori, S. G., D. W. Montara, C. Napolitano, L. Diehl, V. Paganini, F. Cantù, G. Cantuù and P. J. Schwartz. Evalutation of the spatial aspects of T-wave complexity in the long-QT syndrome. Circulation 96: 3006-12, 1997.

Priori, S. G., C. Napolitano, F. Cantu, A. M. Brown and P. J. Schwartz. Differential response to Na+ channel blockade, beta-adrenergic stimulation, and rapid pacing in a cellular model mimicking the SCN5A and HERG defects present in the long-QT syndrome. Circ Res 78: 1009-15, 1996.

Priori, S. G., C. Napolitano, L. Diehl and P. J. Schwartz. Dispersion of the QT interval. A marker of therapeutic efficacy in the idiopathic long QT syndrome. Circulation 89: 1681-9, 1994.

Rampazzo, A., A. Nava, G. Buja, L. Daliento, G. Fasoli, R. Scognamilglio, D. Corrado and G. Thiene. The gene for arrhythmogenic right ventricular cardiomyopathy maps to chromosome 14q23-q24. Human Molecular Genetics 3: 959-62, 1994.

Rampazzo, A., A. Nava, P. Erne, M. Eberhard, E. Vian, P. Slomp, N. Tiso, G. Thiene and G. A. Danieli. A new locus for arrhythmogenic right ventricular cardiomyopathy (ARVD2) maps to chromosome 1q42-q43. Human Molecular Genetics 4: 2151-4, 1995.

Rampazzo, A., A. Nava, M. Miorin, P. Fonderico, B. Pope, N. Tiso, B. Livolsi, R. Zimbello, G. Thiene and G. A. Danieli. ARVD4, a new locus for arrhythmogenic right ventricular cardiomyopathy, maps to chromosome 2 long arm. Genomics 45: 259-63, 1997.

Reid, D. S., M. Tynan, L. Braidwood and G. R. Fitzgerald. Bidirectional tachycardia in a child. A study using His bundle electrography. Br Heart J 37: 339-44, 1975.

Romano, C., G. Gemme and R. Pongiglione. Aritmie cardiache rare dell'eta' pediatrica. II. Accessi sincopali per fibrillazione ventricolare parossistica. Clin Pediatr (Bologna) 45: 656-83, 1963.

Rutter, N. and D. P. Southhall. Cardiac arrhythmias misdiagnosed as epilepsy. Arch Dis Child 60: 54-70, 1985.

Saarinen, K., H. Swan, K. Kainulainen, L. Toivonen, M. Viitasalo and K. Kontula. Molecular genetics of the long QT syndrome: two novel mutations of the KVLQT1 gene and phenotypic expression of the mutant gene in a large kindred. Human Mutation 11: 158-65, 1998.

Saarinen, K., H. Swan, L. Toivonen, K. Viitasalo, M. Pasternack, E. Hirvelä, M. Saarma and K. Kontula. A founder mutation of the potassium channel KVLQT1 accounts for one third of cases with long QT syndrome in Finns (abstract). Circulation 98: I-456, 1998.

Schaffer, A. A., S. K. Gupta, K. Shiriram and R. W. Cottingham Jr. Avoiding recomputation in genetic linkage analysis. Hum Hered 4: 225-37, 1994.

Schwartz, P. J. Idiopathic long QT syndrome: progress and questions. Am Heart J 2: 399-411, 1985.

Schwartz, P. J. and A. Malliani. Electrical alternation of the T -wave: clinical and experimental evidence of its relationship with the sympathetic nervous system and with the long QT syndrome. Am Heart J 89: 45-50, 1975.

Schwartz, P. J., A. J. Moss, S. G. Priori, Q. Wang, M. H. Lehmann, K. Timothy, I. Denjoy, W. Haverkamp, P. Guicheney, V. Paganini, M. Scheinman and P. S. Karnes. Gene-specific influence on the triggers for cardic arrest in the long QT syndrome (abstract). Circulation 96: I-212, 1997.

Schwartz, P. J., A. J. Moss, G. M. Vincent and R. S. Crampton. Diagnostic criteria for the long QT syndrome. An update. Circulation 88: 782-4, 1993.

Schwartz, P. J., M. Periti and A. Malliani. Fundamentals of clinical cardiology: the long QT syndrome. Am Heart J 89: 378-90, 1975.

Schwartz, P. J., S. G. Priori, E. H. Locati, C. Napolitano, F. Cantu, J. A. Towbin, M. T. Keating, H. Hammoude, A. M. Brown, L. S. Chen and et al. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na+ channel blockade and to increases in heart rate. Implications for gene-specific therapy. Circulation 92: 3381-6, 1995.

Severini, G. M., M. Krajinovic, B. Pinamonti, G. Sinagra, P. Fioretti, M. C. Brunazzi, A. Falaschi, F. Camerini, M. Giacca and L. Mestroni. A new locus for arrhythmogenic right ventricular dysplasia on the long arm of chromosome 14. Genomics 31: 193-200, 1996.

Shah, M. J., T. S. Wieand, L. A. Rhodes, C. I. Berul and V. L. Vetter. QT and JT dispersion in children with long QT syndrome. J Cardiovasc Electrophysiol 8: 642-8, 1997.

Sharma, S., K. G. Nair and H. A. Gadekar. Romano-Ward prolonged QT syndrome with intermittent T wave alternans and atrioventricular block. Am Heart J 101: 500-1, 1981.

Shaw, T. R. D. Recurrent ventricular fibrillation associated with normal QT intervals. Q J Med 20: 451-62, 1981.

Shimizu, W. and C. Antzelevitch. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade de pointes in LQT2 and LQT3 models of the long-QT syndrome. Circulation 96: 2038-47, 1997.

Shimizu, W. and C. Antzelevitch. Cellular basis for the ECG features of the LQT1 form of the long QT syndrome. Effects of beta-adrenergic agonists and antagonists and sodium channel blockers on transmural dispersion of repolarization and torsade de pointes. Circulation 98: 2314-22, 1998.

Shimizu, W., T. Kurita, K. Matsuo, K. Suyama, N. Aihara, S. Kamakura, J. Towbin and K. Shimomura. Improvement of repolarization abnormalities by a K+ channel opener in the LQT1 form of congenital long-QT syndrome. Circulation 97: 1581-8, 1998.

Shimizu, W., T. Ohe, T. Kurita, M. Kawade, Y. Arakaki, N. Aihara, S. Kamakura, T. Kamiya and K. Shimomura. Effects of verapamil and propranolol on early afterdepolarizations and ventricular arrhythmias induced by epinephrine in congenital long QT syndrome. J Am Coll Cardiol 26: 1299-309, 1995.

Simson, M. B. Identification of patients with ventricular tachycardia after myocardial infarction from signals in the terminal QRS complex. Circulation 64: 235-42, 1981.

Splawski, I., M. Tristani-Firouzi, M. H. Lehmann, M. C. Sanguinetti and M. T. Keating. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. Nature Genet 17: 338-40, 1997.

Statters, D. J., M. Malik, D. E. Ward and A. J. Camm. QT dispersion: problems of methodology and clinical significance. J Cardiovasc Electrophysiol 5: 672-85, 1994.

Swan, H., K. Piippo, M. Viitasalo, K. Kontula and L. Toivonen. Accute effects of selective and non-selective beta-receptor antagonism and potassium on QT interval in LQT1 type of long QT syndrome (abstract). J Am Coll Cardiol 33 (Suppl A): 152A, 1999.

Tomaselli, G. F., D. J. Beuckelmann, H. G. Calkins, R. D. Berger, P. D. Kessler, J. H. Lawrence, D. Kass, A. M. Feldman and E. Marban. Sudden cardiac death in heart failure. The role of abrnormal repolarization. Circulation 90: 2534-9, 1994.

Wang, Q., M. E. Curran, I. Splawski, T. C. Burn, J. M. Millholland, T. J. VanRaay, J. Shen, K. W. Timothy, G. M. Vincent, T. de Jager, P. J. Schwartz, J. A. Toubin, A. J. Moss, D. L. Atkinson, G. M. Landes, T. D. Connors and M. T. Keating. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 12: 17-23, 1996.

Wang, Q., M. E. Curran, I. Splawski, T. C. Burn, J. M. Millholland, T. J. VanRaay, J. Shen, K. W. Timothy, G. M. Vincent, T. de Jager, P. J. Schwartz, J. A. Towbin, A. J. Moss, D. L. Atkinson, G. M. Landes, T. D. Connors and M. T. Keating. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 34: 9-16, 1996.

Ward, O. C. A new familial cardiac syndrome in children. J Ir Med Assoc 54: 103-6, 1964.

Weintraub, R. G., R. M. Gow and J. L. Wilkinson. The congenital long QT syndromes in childhood. J Am Coll Cardiol 16: 674-80, 1990.

Wilde, A. A. M., R. J. E. Jongbloed, P. A. Doevendans, D. R. Düren, R. N. W. Hauer, I. M. van Langen, J. P. van Tintelen, H. J. M. Smeets, H. Meyer and J. L. M. C. Geelen. Auditory stimuli as a trigger for arrhythmic events differentiate HERG-related (LQTS2) patients from KVLQT1-related patients (LQTS1). J Am Coll Cardiol 33: 227-32, 1999.

Vincent, G. M. The heart rate of Romano-Ward syndrome patients. Am Heart J 112: 61-4, 1986.

Vincent, G. M., J. Fox and K. W. Timothy. Beta-blockers markedly reduce risk and syncope in KVLQT1 long QT patients (abstract). Circulation 94: I-204, 1996.

Vincent, G. M., K. W. Timothy, M. Leppert and M. Keating. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. N Engl J Med 327: 846-52, 1992.

Yan, G.-X. and C. Antzelevitch. Cellular basis for the normal T wave and the electrocardiographic manifestatitions of the long-QTsyndrome. Circulation 1998: 1928-36, 1998.

Yang, T., D. J. Snyders and D. M. Roden. Rapid inactivation determines the rectification and $[K+]_o$ dependence of the rapid component of the delayed rectifier K+ current in cardiac cells. Circ Res 80: 782-9, 1997.

Zareba, W., A. J. Moss, S. le Cessie and W. J. Hall. T wave alternans in idiopathic long QT syndrome. J Am Coll Cardiol 23: 1541-6, 1994.

Zareba, W., A. J. Moss, P. J. Schwartz, G. M. Vincent, J. L. Robinson, S. G. Priori, J. Benhorin, E. H. Locati, J. A. Towbin, M. T. Keating, M. H. Lehmann and W. J. Hall. Influence of the genotype on the clinical course of the long QT syndrome. N Engl J Med 339: 960-5, 1998.