# Repolarization studies in the long QT syndrome

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To my family

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## ABSTRACT

**Background:** Sudden cardiac death in the young is predominantly caused by inherited cardiac conditions. Long QT syndrome (LQTS) is one of the most common of these disorders. Since risk stratification relies largely upon the heart rate (HR) corrected QT interval (QTc), it is crucial to identify an appropriate method for QT correction. Furthermore, cardiac events in LQTS type 1 (LQT1) occur commonly at HR increase. Repolarization duration and dispersion was therefore studied at HR increase.

**Aims:** The objectives were to describe the electrocardiographic and vectorcardiographic phenotype in LQTS patients and to compare repolarization response including dispersion to HR increase between LQT1 and healthy controls.

**Methods:** Paper I compared four different methods for HR correction of the QT interval in a group of LQTS patients using linear regression. In a subgroup, comparisons were made before and after the initiation of betablockers. In paper II and IV we used an intravenous bolus injection of atropine to increase HR in LQT1 patients and healthy controls. Vectorcardiography (VCG) was continuously recorded and VCG parameters were compared. Paper III compared the VCG reaction to increased HR induced by an exercise stress test in LQT1 patients and healthy controls.

**Results:** Bazett´s method yielded the only correction resulting in a QTc without relation to HR, irrespective of initiation of betablockers. Although a similar HR response to atropine, the QT adaptation was faster in LQT1 than in healthy controls. As a response to exercise, the QTcB and its components, the HR corrected QTpeak and Tpeak-end intervals, but not global dispersion parameters, separated LQT1 patients from controls.

Following a rapid HR increase induced by atropine, the majority in both groups showed a biphasic response for global measures of VR dispersion, including an overshoot; in LQT1 the overshoot was more pronounced.

**Conclusions:** Although questioned**,** Bazett´s method remains preferable for QT correction in LQT1 and 2. Faster QT adaptation following a rapid HR increase in LQT1 patients indicates a disturbed QT hysteresis. Timing of repolarization duration but not global dispersion parameters distinguished LQT1 patients from controls after exercise. The biphasic response in VR dispersion was exaggerated in LQT1 patients which could play a role in arrhythmogenesis, but further studies are warranted.

**Keywords**: long QT syndrome, vectorcardiography, repolarization, hysteresis, QT adaptation, ventricular repolarization

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# SAMMANFATTNING PÅ SVENSKA

**Bakgrund:** Hjärtstopp och plötslig hjärtdöd hos unga orsakas ofta av ärftliga hjärtsjukdomar där långt QT-syndrom (LQTS) är en av de vanligaste med en prevalens på ca 1:2000. LQTS orsakas av en mutation som drabbar hjärtmuskelcellernas jonkanaler vilket ofta leder till en förlängning av QT-intervallet på EKG och kan ge upphov till livshotande hjärtrytmrubbningar. Vid LQTS typ 1 (LQT1) uppstår arytmierna ofta i samband med hjärtfrekvensökning, såsom vid fysisk aktivitet.

Diagnosen bekräftas vanligtvis med genetisk utredning vilket också möjliggör testning av förstagradssläktingar. Allt fler asymtomatiska LQTS-patienter identifieras på detta sätt. De har en låg risk men ändå väsentligt högre än släktingar utan den aktuella mutationen.

**Syfte:** Syftet var att beskriva den elektrokardiografiska och vektorkardiografiska fenotypen hos LQTS-patienter och jämföra med friska, samt att med två olika pulshöjande provokationstester karakterisera repolarisationsstörningarna.

**Metod:** För delarbete I användes EKG-data från ca 200 LQTS-patienter från Göteborg och Umeå och de fyra vanligaste metoderna för hjärtfrekvenskorrigering av QT-tid jämfördes med statistiska metoder. I delarbetena II-IV genomgick patienter med LQT1 och friska kontroller olika pulshöjande interventioner (atropin-injektion respektive arbetsprov) och EKG och vektor-kardiografi (VKG) jämfördes mellan grupperna

**Resultat och slutsatser:** I delarbete I kunde vi visa att Bazett´s metod för frekvenskorrigering var den enda av de fyra metoderna som upphävde sambandet mellan hjärtfrekvens och QT hos LQTS-patienter, oavsett ålder eller behandling med betablockad. I delarbete II och IV sågs en accelererad QT-anpassning respektive en mer överdriven bifasisk adaptationsreaktion av dispersionsparametrar hos LQTS-patienter jämfört med friska trots samma grad av hjärtfrekvensstegring. I delarbete III visade det sig som väntat att QTcB ökade signifikant mer efter arbete hos LQT1. Däremot kunde vi inte se någon ökad global dispersion. LQT1 karakteriseras av en tidsmässigt störd anpassning vid hjärtfrekvensökning vilket kan öka risken för rytmrubbningar.

# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Dahlberg P, Diamant U-B, Gilljam T, Rydberg A, Bergfeldt L. **QT correction using Bazett´s formula remains preferable in long QT syndrome type 1 and 2.** *Ann Noninvasive Electrocardiol. 2021 Jan;26(1):e12804.*

II. Dahlberg P*, Axelsson KJ*, Jensen SM, Lundahl G, Vahedi F, Gransberg L, Bergfeldt L. **Accelerated QT adaptation following atropine-induced heart rate increase in LQT1 patients versus healthy controls: A sign of disturbed hysteresis.** *Physiol Rep. 2022 Nov;10(21):e15487.*

III. Dahlberg P, Axelsson KJ, Rydberg A, Lundahl G, Gransberg L, Bergfeldt L. **Spatio-temporal repolarization dispersion before and after exercise in patients with long QT syndrome type 1 vs controls.** *Accepted. AJP - Heart and Circulatory Physiology. 2023*

IV. Dahlberg P, Axelsson KJ, Jensen SM, Lundahl G, Gransberg L, Bergfeldt L. **Greater overshoot in the adaptation of repolarization dispersion following atropine-induced heart rate increase in LQT1 patients and healthy controls.** *In manuscript*

*Both authors contributed equally

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| AP | Action potential |
| APD | Action potential duration |
| BB | Beta blockers (β-blockers) |
| cB | HR corrected according to Bazett (as in QTcB) |
| DAD | Delayed afterdepolarizations |
| EAD | Early afterdepolarizations |
| ECG | Electrocardiogram |
| FGS | First-degree relative |
| HR | Heart rate |
| ICD | Implantable cardioverter defibrillator |
| $I_{Kr}$ | Rapid delayed rectifier/inward rectifier potassium channel |
| $I_{Ks}$ | Slow delayed rectifier |
| LCSD | Left cardiac sympathetic denervation |
| $L_{QT}S$ | Long QT syndrome |
| QRS | QRS interval, from start of Q until end of T wave: Ventricular depolarization on ECG |
| QT | QT interval, the interval from QRS start to T wave end: Ventricular de- and repolarization on ECG |
| QTc | QT interval corrected for heart rate |
| QTcB | QT interval corrected for HR (Bazett) |

| | |
|---|---|
| QTcFram | QT interval corrected for HR (Framingham) |
| QTcF | QT interval corrected for HR (Fridericia) |
| QTcH | QT interval corrected for HR (Hodges) |
| QTpeak | The interval from the onset of QRS to the peak of the T wave |
| SCD | Sudden cardiac death |
| Tamplitude | The amplitude of the maximum T vector in space (inscribed in the T-vector loop) |
| Tarea | The spatial area under the T wave curve in X, Y and Z leads |
| Tpeak-end | The interval from the peak to the end of the T wave |
| TdP | Torsades de Pointes (polymorphic ventricular tachycardia) |
| VCG | Vectorcardiography |
| VG | Ventricular gradient, the vectorial sum of the QRS area vector and the Tarea vector |
| VR | Ventricular repolarization |

# QT CORRECTION FORMULAE

| | |
|---|---|
| Bazett formula | $QTcB=QT/RR^{1/2}$ |
| Framingham | $QTcF=QT/RR^{1/3}$ |
| Fridericia | QTcFram=QT+0.154*(1-RR) |
| Hodges | QTcH=QT+1.75*(HR-60) |

# 1 INTRODUCTION

## 1.1 SUDDEN CARDIAC DEATH

Cardiac arrest or sudden cardiac death (SCD) in a seemingly healthy young individual is a rare but devastating event. The occurrence of SCD frequently draws substantial attention, particularly if it occurs in an athletic context with an audience. SCD is often defined as a sudden unexpected death from cardiac causes (1). The term can be applied in cases involving a previously known potentially fatal cardiac condition, when an autopsy has identified a cardiac anomaly as the cause of death or when an arrhythmic event is suspected due to the absence of pathological findings in an autopsy. The annual number of SCDs globally is around 4 million cases per year and the incidence is highest in association with older ages (2). The incidence of SCD among younger individuals is estimated to be 0.46 to 3.7 per 100 000 person-years (1, 3-5) and the estimated number in Sweden among those between 15 and 35 years of age is approximately 0.9 per 100.000 person-years (6). This translates to around 50 SCD cases per year among those between 15 and 35 years of age in Sweden (7). At older ages coronary artery disease is the dominant cause of SCD, but among younger patients inherited arrhythmic disorders, such as cardiomyopathies (hypertrophic cardiomyopathy, arrhythmogenic cardiomyopathy, dilatated cardiomyopathy) or channelopathies (such as long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, Brugada syndrome) are more common (8-10). Figure 1 illustrates the cause of death from SCD among men between 15 and 35 years of age in Sweden from 2000 to 2010.

Survival rates after sudden cardiac arrest are low, under 10% globally (11) although improvements have been observed due to increased awareness and enhanced strategies, for instance improved access to defibrillators (12). Since SCD generally occurs unexpectedly, preparation is often unfeasible. SCD frequently occurs out of hospital.

Identifying an underlying heart disease such as LQTS can help prevent cardiac events/SCD.

An autopsy may indicate the cause of death, such as cardiomyopathies or aortic disease. However, in some cases, the autopsy uncovers no signs of structural abnormalities, and hence, no explanation for the cause of death is found. These cases are believed to be caused by arrhythmia and are often called unexplained SCD or sudden arrhythmic death syndrome (SADS). In several nation-wide studies of the incidence of SCD, SADS has been demonstrated to be the leading explanation SCDs among in younger individuals (6, 13, 14).

In post-mortem evaluations, it is immensely important to determine whether there was a cardiac cause of death. Identifying an underlying heart disease, such as LQTS can be helpful for properly advising the surviving family and to prevent cardiac events and SCD among first-degree relatives (15, 16). In many countries a blood sample or other tissue is frozen and saved to enable DNA extraction for future genetic testing if appropriate.

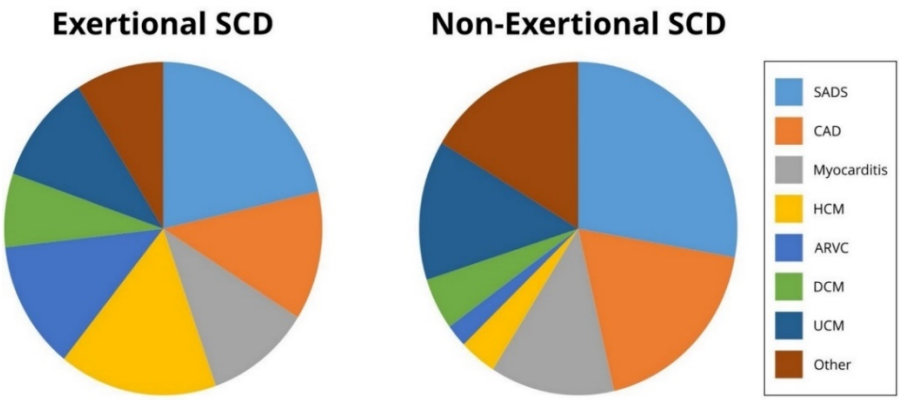


*Figure 1. Cause of death related to SCD in men aged 10-35 years in Sweden 2000-2010. SADS: sudden arrhythmic death syndrome, CAD: coronary artery disease, HCM: hypertrophic cardiomyopathy, ARVC: arrhythmogenic cardiomyopathy, DCM: dilatated cardiomyopathy. Reproduced from Wisten et al. Resuscitation Volume 144. November 2019, with permission from Elsevier*

## 1.2 CARDIOGENETICS

Cardiogenetics is a subspeciality in cardiology and clinical genetics that has developed within the last three decades. The discovery that variants in certain genes are causative of some, mostly rare heart diseases has contributed to our understanding of these diseases and to the possibility of pre-symptomatic screening for relatives at potential risk. In the case of more common

cardiovascular diseases, such as atrial fibrillation and ischemic heart disease, the potential benefits of genetic testing have not yet been demonstrated. When a potentially hereditary cardiac disease is discovered in an individual, referred to as the proband or index patient, genetic counselling is offered, and a pedigree is drawn. Figure 2 is an example of such a pedigree.

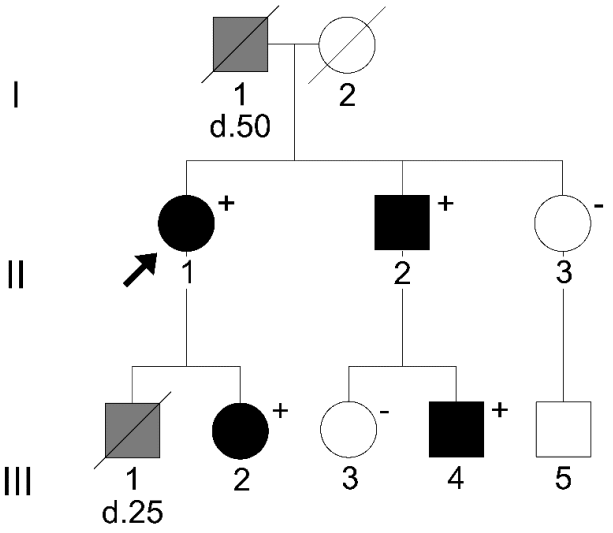


*Figure 2. Pedigree from a four-generation family. Circles denote females and squares denote males. The arrow denotes the proband or index patient. The individuals with a crossed-over symbol are dead. Filled symbols: affected individuals.*

It is beneficial if the cardiogenetic team is multidisciplinary, consisting of cardiologists, clinical geneticists and genetic counsellors (17). The counselling can include possible strategies to avoid unnecessary risks for mutation carriers, information about the genetic testing of children or in selected cases even information about preimplantation genetic testing. If possible and desired by the proband, sequencing of genes that are likely to be disease-causing, is performed. The pathogenicity of genetic variants is based on several criteria. It goes without saying that the phenotype must match the genetic variant found, but if the only phenotype is SCD, this is easier said than done. Other criteria include that the variant was previously described as being associated with the disease, that it aligns with the inheritance pattern, and that it is unusual in the normal population. It is also possible to use in silico approaches and in vitro

experiments to assess the functional consequences of a certain mutation. Genetic variants are classified into five classes, ranging from benign variants through variants of unknown significance (VUS) to pathogenic variants. Only pathogenic or likely pathogenic variants (class IV and V) can be used for predictive testing (18). If a pathogenic genetic variant is detected in the proband, first degree relatives can be offered cascade genetic testing for the same variant. Individuals who share the same variant can thereby be offered tailored follow-up and, in some cases prophylactic treatment, and individuals who do not share the variant can be discharged from the clinic.

Sometimes no genetic cause can be found in the proband despite an obvious hereditary pattern. In such cases, first-degree relatives are still offered cardiac examinations. If a VUS is detected it cannot be used for predictive testing, but re-evaluation can sometimes be feasible due to the potential addition of new information after a couple of years.

Most of these conditions are inherited in an autosomal monogenetic pattern, meaning that a first degree relative has a 50 % risk of sharing the genetic variant. However, many of these diseases have incomplete penetrance and a variable expression, meaning that not all mutation carriers develop the disease and the severity of disease may vary. As a consequence, a growing number of individuals have a known pathogenic genetic variant but have a yet unclear risk of developing the actual disease (17, 19). In cardiogenetics we meet patients mostly with hereditary cardiomyopathies, such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and arrhythmogenic cardiomyopathy (ARVC), hereditary thoracic aortic disease such as Marfan syndrome, Loeys-Dietz syndrome, vascular Ehlers-Danlos syndrome but also with primary arrhythmia syndromes such as long QT syndrome (LQTS), Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia (CPVT).

In some cases, a so-called molecular autopsy, genetic screening of an individual who has died suddenly from unexplained SCD can contribute to providing a diagnosis. However, this type of investigation is not always straight forward. Selecting a gene panel that is sufficiently large to contain possible pathogenic variants but still sufficiently narrow to not detect excessive “background noise” is challenging and should be done by a dedicated multidisciplinary team (15). If a pathogenic genetic variant is found, the

surviving family can be offered cascade genetic testing for the same variant. The affected individuals can be offered appropriate management with tailored follow-up. If no variant is found, the diagnosis remains unclear, but the family can be offered clinical screening which could include echocardiography, ECG, exercise stress test, and Holter monitoring (20).

The first cardiogenetic clinics in Europe were initiated in the 1990s and in our region, it was opened in 2013. Since then, nearly 2000 patients have undergone genetic testing in our center and nearly 100 have been tested using an LQTS panel.

# 1.3 LONG QT SYNDROME

Congenital long QT syndrome is the prototypical channelopathy, characterized by a prolonged QT interval in the ECG and an increased propensity of syncope, cardiac arrest and sudden cardiac death in individuals without structural heart disease.

## 1.3.1 HISTORY

The first report of LQTS, published in 1957, described a severely affected Norwegian family who lost three children to sudden cardiac death (21). Prior to their deaths, they had had a history of syncope and in addition, the children suffered from congenital deafness. One of them underwent an autopsy which revealed no signs of structural changes in the heart. ECGs were available for two of the children and indicated markedly prolonged QT intervals. The report contained detailed descriptions of a diagnostic work up of the oldest child, and of the experiments performed. Exercise (running on stairs) was demonstrated to prolong the QT interval, as well as the infusion of adrenaline. The inheritance pattern was described as autosomal recessive. A few years later Romano and Ward independently published descriptions of similar cases with syncope and prolonged QT, but with normal hearing and an autosomal dominant inheritance pattern (22, 23). Until the acronym LQTS was introduced in the 1970s encompassing both the autosomal recessive and the dominant variants, the disease was referred to as Jervell and Lange-Nielsen syndrome (JLNS, recessive) and Romano-Ward syndrome (dominant).

In the early 1990s the first evidence of LQTS being a genetic disorder was discovered. In 1991 a strong linkage between a DNA marker and LQTS was shown and published by Keating et al (24). In 1995 the first causative mutations for LQTS were found, specifically in genes encoding cardiac ion channels. The first were in the gene hERG (later KCNH2) encoding a cardiac potassium channel (25). In the same year, the discovery that mutations in the gene SCN5A causing LQTS type 3 (LQT3) was published (26) and the following year, mutations in the gene KCNQ1 were discovered as causative of LQTS type 1 (LQT1) (27). This facilitated identification of gene specific triggers (28) as well as treatments (29). In the late 1990s LQTS was also found to have a variable penetrance which explained normal QT intervals in some individuals despite pathogenic variants (30).

Initially, LQTS was believed to be exceptionally rare, but in 2009 a study concerning the prevalence was published based on genetic testing in neonates with prolonged QTc (exceeding 460ms) suggesting the prevalence to be around 1:2000 which remains valid (31).

### 1.3.2 DIAGNOSIS

Most patients with LQTS exhibit a prolonged QT interval making the diagnosis quite straight forward, especially in individuals experiencing cardiac events such as syncope (1, 32, 33). A diagnostic score for LQTS was first published in 1985 by Schwartz and updated in 1993 (34, 35). It has been further developed since then and in 2011, a prolonged QTc four minutes after exercise was added to the score (36, 37). Apart from the heart rate corrected QT interval, the diagnosis relies on clinical and family history (Figure 3). However, an increasing number of individuals harboring a pathogenic genetic variant, but lacking the typical QT prolongation, are being discovered. The prevalence of this condition, sometimes referred to as concealed LQTS, varies between 20 and 40% of the LQTS population (38, 39).

| Criteria | Points |
|---|---|
| Electrocardiographic findings[a] | |
| QTc[a] | |
| ≥480 ms | 3 |
| 460–479 ms | 2 |
| 450–459 (male) ms | 1 |
| QTc[b] 4th minute of recovery from exercise stress test ≥480 ms | 1 |
| Torsade de pointes[c] | 2 |
| T-wave alternans | 1 |
| Notched T-wave in three leads | 1 |
| Low heart rate for age[d] | 0.5 |
| Clinical history | |
| Syncope[c] | |
| With stress | 2 |
| Without stress | 1 |
| Congenital deafness | 0.5 |
| Family history | |
| Family members with definite LQTS[e] | 1 |
| Unexplained sudden cardiac death below age 30 among immediate family members[e] | 0.5 |

*Figure 3. Diagnostic criteria, the Schwartz score. LQTS score ≤1: low probability of LQTS. 1.5-3 points: intermediate probability of LQTS. ≥3.5 points: high probability. Reproduced from Europace. Wilde et al. EHRA/HRS/APHRS/LAHRS Expert consensus statement on the state of genetic testing for cardiac diseases. With permission from Oxford University Press*

In an expert consensus statement from 2011 the prevalence of a pathogenic genetic variant was also added as a means of diagnosing LQTS, although it is not part of the Schwartz score (40). According to recent guidelines, a diagnosis of LQTS can be made in the case of repeated QTc of 480ms or above, if the Schwartz score is more than 3, if a pathogenic variant is found, or in cases with arrhythmic syncope and a QTc of 460 ms or above (1, 20).

| Recommendations | Class[a] | Level[b] |
|---|---|---|
| **Diagnosis** | | |
| It is recommended that LQTS is diagnosed with either QTc ≥480 ms in repeated 12-lead ECGs with or without symptoms or LQTS diagnostic score >3. | I | C |
| In patients with clinically diagnosed LQTS, genetic testing and genetic counselling are recommended. | I | C |
| It is recommended that LQTS is diagnosed in the presence of a pathogenic mutation, irrespective of the QT duration. | I | C |
| The LQTS diagnosis should be considered in the presence of a QTc ≥460 ms and <480 ms in repeated 12-lead ECGs in patients with an arrhythmic syncope in the absence of secondary causes for QT prolongation.[952,962,963] | IIa | C |

*Figure 4. Diagnostic criteria for LQTS. Reprinted from the 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: Developed by the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC) Endorsed by the Association for European Paediatric and Congenital Cardiology (AEPC)*

## 1.3.3 LQTS GENETICS

As described in section 1.3.1, the era of LQTS genetics began in the 1990s. In addition to the first three described genes the number was gradually increasing until at least 17 genes were suggested as causative. In 2020 a large international, multicentered, evidence-based reappraisal of 17 LQTS genes was performed. The conclusion of this report was that at least half of the genes previously reported, were lacking sufficient evidence as being causative of LQTS. For typical LQTS, the "original" three had definitive evidence. Another four genes (CALM 1-3 and TRDN) were found to be associated with LQTS with atypical features. In addition, two genes associated with multiorgan syndromes including QT prolongation exist, but their role as causative genes in isolated LQTS is disputed (41).

In the EHRA/HRS consensus document from 2022, molecular genetic testing is recommended for definitive disease associated genes (KCNQ1, KCNH2,

and SCN5A) for index patients with a high probability of LQTS. In patients with a syndrome associated with LQTS, other genes can be added accordingly (42).

Genetic testing is recommended for children of affected individuals from birth onward, since treatment is recommended from an early age (42).

Genetic testing in LQTS provides the opportunity to offer genotype-specific management and treatment such as mexiletine for LQT3 (section on management).

**KCNQ1**: The most common LQTS-type, LQT1 is caused by mutations in the gene KCNQ1. KCNQ1 encodes the primary subunit of the voltage-gated potassium channel protein engaged in the repolarization phase of the action potential. In the heart, this channel protein mediates the slow delayed rectifying potassium current ($I_{Ks}$). Since this current ($I_{Ks)}$ is increased via sympathetic activation, it is important for QT adaptation during HR increase. A loss-of-function mutation here causes a reduced potassium current and thus, tachycardia does not induce the appropriate degree of QT shortening which can be arrhythmogenic (43). This potassium channel protein is also present in the neurons in the inner ear, which explains why bi-allelic KCNQ1 mutations are associated with congenital deafness.

**KCNH2**: The second most common LQTS gene is KCNH2 (LQT2) which encodes the α-subunit of the potassium channel that mediates the rapid delayed rectifier $I_K$ current ($I_{Kr}$). The prolongation of repolarization is induced through a reduced potassium current. KCNQ1 along with KCNH2, encode the rapid $I_{Kr}$ and the slow $I_{Ks}$ respectively, which are components of the delayed rectifier current and are both important for the phase 3, rapid repolarization, of the cardiac action potential (see section on cardiac action potential).

**SCN5A**: SCN5A encodes the α-subunit of the cardiac sodium channel. LQT3 is caused by variants in this gene. It is responsible for the depolarizing sodium inward current ($I_{Na}$). Mutations in this gene can cause many other conditions aside from LQTS (44). The LQTS phenotype is presumed to be caused by mutations that increase the delayed sodium inward current and thereby prolong the action potential duration.

#### 1.3.3.1 JERVELL AND LANGE-NIELSEN SYNDROME

The condition known as Jervell and Lange-Nielsen syndrome (JLNS) is an extremely rare form of LQTS, characterized by bilateral sensorineural hearing loss, prolonged QTc and a high risk of developing lethal ventricular arrhythmias. The inheritance pattern is autosomal recessive, meaning that homozygosity or compound heterozygosity is required for inheritance. Mutations in KCNQ1 or KCNE1 are most frequent (45).

#### 1.3.3.2 ACQUIRED LQTS

Acquired LQTS is a condition in which an external factor, such as a drug, induce a QTc of more than 500 ms or a QTc change of more than 60 ms. Genetic testing in these individuals can sometimes be indicated but the decision requires experience and individualized consideration (42).

### 1.3.4 PHENOTYPE

The clinical presentation of LQTS can vary, ranging from no symptoms at all to cardiac arrest. The clinical presentation can vary even within the same family. The ECG may appear completely normal or LQTS may be obvious with extremely prolonged QT intervals. Individuals with normal QTc are sometimes referred to as concealed LQTS. The factors that determine why some individuals seem to be protected from events can vary, but genetic modifiers have been suggested as an explanation. The exact mechanism behind these genes is not known.

#### 1.3.4.1 ECG ABNORMALITIES

A prolonged QT interval is the hallmark of LQTS. However, in up to 40% of cases, the QT interval may be normal (33). These individuals, with what is known as concealed LQTS, or normal-QT LQTS, have a substantially lower risk of cardiac events, but they still exhibit a 10-fold increase compared to their unaffected relatives. Figure 5 presents a Kaplan-Meier curve indicating the cumulative probabilities of severe cardiac events by genotype (positive or negative) and QTc. A QTc of 440ms or more was considered to be prolonged (38).

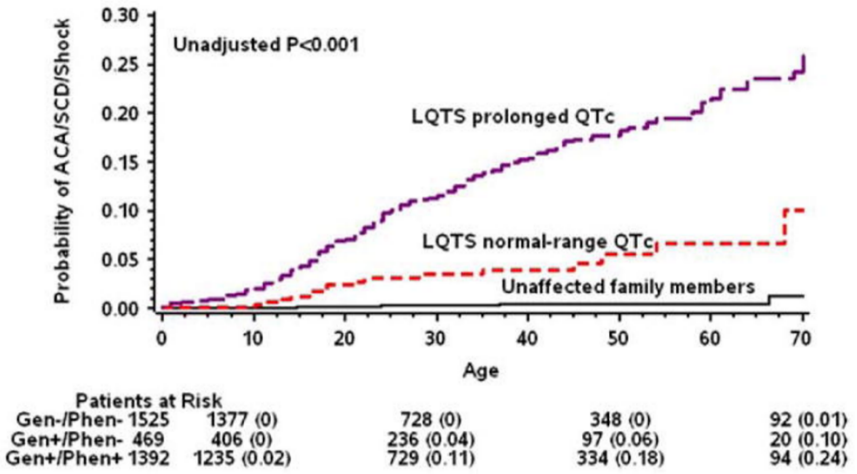


*Figure 5. Kaplan-Meier cumulative probabilities of aborted cardiac arrest (ACA) and sudden cardiac death (SCD) by genotype and corrected QT (QTc) subgroup. Reproduced with permission from Elsevier. JACC 2011. Goldenberg et al. Risk for Life-Threatening Cardiac events in Patients with Genotype-Confirmed LQTS and normal-range corrected QT intervals.*

On the other hand, the ECG phenotype T-wave alternans, which is a periodic beat-to-beat variation of the T wave amplitude or morphology, has been closely associated with a markedly increased risk of cardiac events (46). In LQT1 a typical broad T wave can be observed (47, 48). Exercise stress testing is frequently used as part of the diagnostic work up and for LQT1, a QT interval that fails to shorten is typically observed. Other ECG characteristics that can be found include flat, notched or negative T waves especially in LQT2 (49).

For LQT3 a long isoelectric ST segment is characteristic (48).

#### 1.3.4.2 ARRHYTHMIAS

Patients with LQT1 typically present with symptoms at an earlier age compared to LQT2 and 3. The prototypical arrhythmia in LQTS is the polymorphic ventricular tachycardia called torsade de pointes (TdP) which instantaneously causes syncope or cardiac arrest. As mentioned above, the arrhythmias typically occur as a response to increased HR, often related to physical exercise in the case of LQT1, in response to auditory stimuli or during the postpartum period in LQT2, and during states of increased vagal tonus in cases of LQT3 (50). In LQT2 the arrhythmias are frequently pause dependent (51). Arrhythmia can lead to syncope which typically has an abrupt onset and

a short duration, but it can also lead to ventricular fibrillation and cardiac arrest as discussed earlier.

## 1.3.5 RISK STRATIFICATION

Unlike hereditary cardiomyopathies, patients with hereditary channelopathies such as LQTS seldom present with symptoms prior to serious cardiac events (syncope or cardiac arrest). For this reason, the suggestion of lifestyle modification or the prescription of prophylactic treatment can be challenging, and adherence may be low due to a lack of understanding of risks. Nevertheless, risk stratification is important to offer the appropriate treatment for the right patients and to identify the group of patients with a high risk of cardiac events. The risk differs between genotypes as well as between individuals. The tools for risk stratification, based on the probability of a first cardiac event (before 40 years and before therapy), are somewhat blunt. Apart from genotype (LQT1-3), age, sex and QTc are considered. A QTc of >500ms indicates a higher risk than a shorter QTc duration (39). Figure 6 illustrates the scheme for risk stratification suggested by Priori et al.

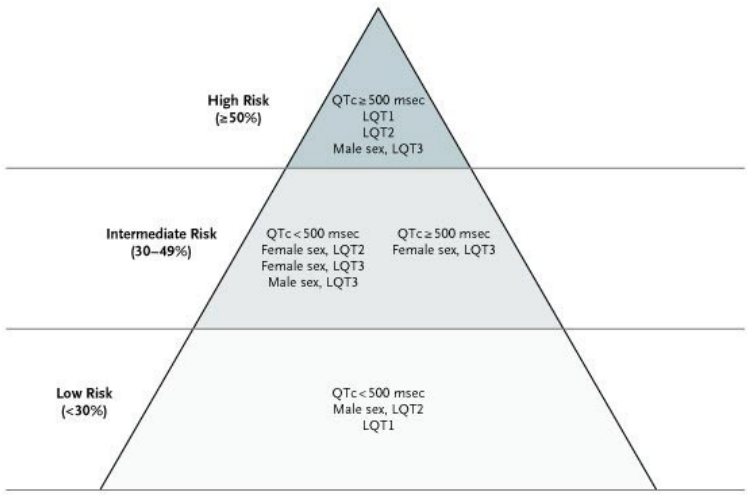


*Figure 6. A proposed scheme for risk stratification among patients with the long QT syndrome. Reproduced with permission from N Engl J Med. 2003 May 8;348(19):1866-74. Doi:10.1056 Copyright Massachusetts Medical Society*

A new risk prediction model for patients naïve to betablockers (1-2-3-LQTS-Risk) was suggested in 2018 and validated in 2022 in which only two parameters, namely genotype (LQT1-3) and duration of QTc interval, are taken into account (52, 53).

The genotype in LQTS is unequivocally linked to the risk of arrhythmia; specifically, LQT3 patients carry a higher risk of lethal events than LQT1 patients. But those who hope for a mutation-specific risk stratification have thus far been disappointed. The immense variety of mutations and the varying degree of penetrance do not enable precision medicine apart from genotype (LQT1-3) specific management. However, it has been demonstrated that LQT1 patients with a mutation located in the transmembrane part of the ion channel are at a higher risk of cardiac events compared to patients with mutations located at other parts of the ion channel (54, 55).

LQT2 patients with a mutation located in the pore region of the KCNH2 gene face a higher risk of cardiac events compared to non-pore region mutations (56, 57). Some suggest that risk stratification in LQT2 patients should also include the location of KCNH2 mutation (58) but it has not been added in guidelines.

It is not recommended to perform an invasive electrophysiologic study for risk stratification in LQTS (1).

## 1.3.6 MANAGEMENT

The management of LQTS consists of risk stratification, life-style modifications, pharmacological treatment and implantable cardiac defibrillator (ICD). The goal of management is to prevent syncope or ultimately SCD.

### 1.3.6.1 LIFESTYLE MODIFICATIONS

Several drugs have the ability to prolong the QT interval and patients with LQTS should be instructed to inform their physician about their condition prior to any new prescriptions. A meticulous individual decision must be made in each case and as a rule of thumb QT-prolonging drugs should be avoided. However, drugs with a QT prolonging potential can sometimes be “allowed” under certain circumstances, such as chemotherapy if no other option is available, and if certain combinations can be avoided. A list of drugs with the

potential to prolong the QT interval or the ability to induce TdP is available online (crediblemeds.org).

Patients should also be informed about the risks of electrolyte imbalances, particularly hypokalaemia, and should avoid or correct them if possible.

One goal should be to avoid gene-specific triggers as far as possible. This includes swimming in LQT1, unless supervised by others. Participation in competitive sports is debated (59-64).

For patients with LQT2, sudden causes of arousal such as sudden noises emitted by alarm clocks or telephones during rest or sleep appear to be an important trigger and should be avoided if possible (28). Women with LQT2 have an increased risk of cardiac events during the post-partum period and should, if possible, avoid sleep deprivation during this period (65, 66).

Patients with LQT3 experience most events at rest (67) and for these patients lifestyle modification can be even more challenging.

#### 1.3.6.2 PHARMACOLOGICAL THERAPY

All symptomatic patients should be offered treatment and the medication is effective. Beta blocker (BB) treatment extensively reduces the risk of cardiac events, but it does not abolish it completely (68, 69). However, treatment in asymptomatic individuals is much less determined (33).

Considering that adrenergic triggers appear to be most important in LQT1 (33, 67), it is not surprising that BB treatment was already attempted in the 1960 and that the first report was published in 1970 (70). BB remain the cornerstone of the management of these patients due to the high response among LQT1, and they are surprisingly well tolerated without bradycardia (43). The choice of BB should preferably be one of the non-selective ones, nadolol or propranolol. For symptomatic patients, metoprolol or atenolol should be avoided (71, 72)

In a consensus document from 2013, treatment with BB was recommended for symptomatic LQTS patients who had experienced syncope of VT/VF as well as all LQTS patients with a QTc of 470ms or more. The document also stated that BB can be useful in patients with QTc of less than 470ms (73). European

guidelines from 2017 use the same limit of 470ms (20). Figure 7 illustrates the suggested limits for BB treatment and recommendations for ICD implantation.

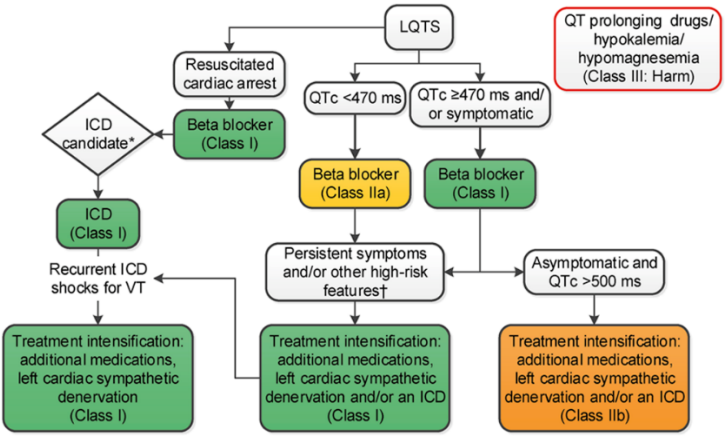


*Figure 7. Prevention of SCD in LQTS patients. Reproduced from Heart rhythm. Vol 15. Al-Khatib. 2017 AHA/ACC/HRS Guideline for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. With permission from Elsevier*

The contemporary ESC guidelines state that BBs are recommended in LQTS patients with a documented prolonged QTc, but no measures of the QT interval are specified, and BB should be considered (Class IIa) in patients with a normal QTc (1).

For patients with LQT3 BB can be less effective, and sodium channel current blockers (mexiletine) can be tried (29, 74). Mexiletine, a class Ib antiarrhythmic drug, was reported in 1996 to reduce the late $Na^+$ current (75). Mexiletine may shorten the QT interval in LQT3 patients. It has also been attempted in LQT2 patients and was also demonstrated to reduce the QT interval in this group (76).

#### 1.3.6.3 ICD

The implantation of a cardiac defibrillator (ICD) is an effective but invasive therapeutic option, that should be restricted to a minority of LQTS patients (77). For high-risk patients, the implantation of an ICD can be an option, with a class I recommendation for survivors of cardiac arrest (1, 5). It is also recommended for patients who are still symptomatic despite BB treatment, and it should be considered when BBs are not tolerated or contraindicated.

Implantation may be considered in patients with other high-risk features such as extreme QTc or certain hereditary factors (1, 52, 53). Pacemaker therapy can be considered in some LQT2 patients in whom arrhythmia is frequently pause-dependent (33).

#### 1.3.6.4 LCSD

Left cardiac sympathetic denervation (LCSD) is a method that is rarely used, at least in Sweden. It is recommended for patients who remain symptomatic despite treatment with appropriate pharmacological agents (1). It can be an alternative when ICD implantation is contraindicated for whatever reason. The outcomes are typically positive with a reduction of cardiac events. LCSD is now performed primarily through thoracoscopy (78, 79).

## 1.4 ELECTROCARDIOGRAPHIC RECORDINGS

#### 1.4.1.1 VENTRICULAR ACTION POTENTIAL

The ventricular action potential is composed of phases 0 through 4. These phases depend on the cardiac ion channels and their consecutive opening and closing. The cardiomyocytes are linked by intercalated discs, which enables the action potential to spread easily from one cell to another.

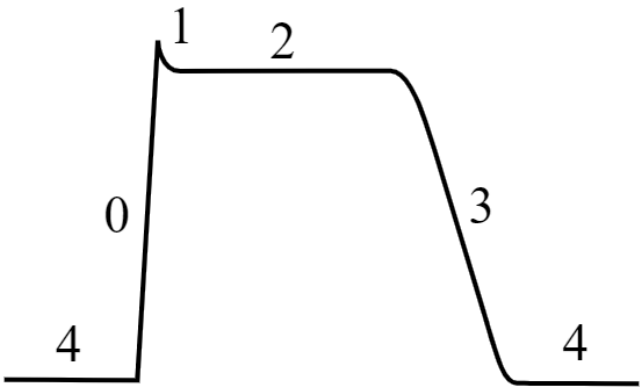


*Figure 8. Phases of cardiac action potential (zero to four). The depolarizing currents are the inward $Na^+$ current, causing the upstroke of the action potential, and the inward $Ca^{2+}$ current responsible for phase 2 (the plateau). The repolarizing currents are the rapid ($I_{Kr}$) and the slow ($I_{Ks}$) components of the delayed rectifier current.*

Figure 8 illustrates the phases of the cardiac action potential. Phase 0 means depolarization. The opening of voltage gated sodium channels causes an influx of $Na^{+,}$ which gives rise to the upstroke of the action potential. Phase 1 follows, indicating early repolarization, which is caused by the inactivation of $Na^{+}$ channels, reducing the influx of sodium into the cardiomyocyte, and the opening of potassium channels, thus leading to a rapid efflux of $K^{+}$. In phase 2, which is a plateau phase, the membrane potential remains constant due to a balance of currents in and out of the cell. Voltage gated $Ca^{2+}$ channels activate allowing $Ca^{2+}$ to move into the cell, and delayed rectifier potassium channels ($I_{Ks}$) open to allow $K^{+}$ to leave the cell. Phase 3 entails rapid repolarization and is caused by the closing of $Ca^{2+}$ channels. The $I_{Ks}$ remain open, and another type of potassium channels open, namely $I_{Kr}$, the rapid delayed rectifier $K^{+}$ current. Phase 4 entails resting membrane potential which results from a balance of different membrane pumps and the flux of ions that flowed in and out of the cell. This phase corresponds to diastole (80).

The QRS complex, as a reflection of ventricular activation or depolarization, is caused by the short upstroke of ventricular APD, propagated through the fast conduction system and lastly via conduction through the myocardium (81). There is a dispersion of activation moment throughout the heart and between different areas of the heart, and this heterogeneity is recognized as the QRS complex which normally lasts around 80ms. The electrical inhomogeneity in the ventricles during the entire repolarization phase is represented by the T wave. Since the depolarization is locally very fast, the subsequent repolarization of the first cells starts during the QRS complex. Repolarization is thus sometimes defined as the QT interval. The repolarization is substantially longer than depolarization, partly because there is no conduction system for repolarization (81, 82). Repolarization is more accurately described as a matter of synchronization.

The QT interval, which is the time from the start of ventricular depolarization until repolarization is finished, is approximately equal to the duration of mechanical systole. It is longer than AP of other tissue. The benefit of this is that it causes a long refractory period, preventing tetanus as in skeletal muscle. The refractory tissue also prevents circular activation (re-entry) (81).

#### 1.4.1.2 QT ADAPTATION

If increased cardiac output is required, such as when physical activity is initiated, an increase in HR and stroke volume is demanded (83). When HR increases, the duration of the ventricular action potential (APD) must shorten. To enable diastolic filling, it is primarily the systolic time that shortens. This adaptation of APD/QT to HR change is called electrical restitution (84). This can be observed as shorter QT intervals on the ECG when the RR intervals shorten. The QT interval depends on the preceding RR interval and the shorter the RR interval the shorter the QT. In fact, not only the one preceding RR interval determines the QT, but several RR intervals do, which contributes to the hysteresis function of the QT adaptation (85-89).

Hysteresis is a phenomenon in which something, in this case the change of the QT interval as a response to another factor, (here, to HR change), is lagging behind, creating a gradual change (90). This hysteresis function of the QT interval has been demonstrated to be a normal physiologic phenomenon in several studies (85, 86). The QT adaptation time is different if HR is increasing or decreasing, with a slower change following HR decrease (91). This means that QT intervals are longer at a given RR interval when HR is increasing as a response to exercise, and shorter when HR are decreasing after the exercise (90). The degree of hysteresis has been shown to be increased by ischaemia (92, 93). It has also been demonstrated that different autonomic effects influence hysteresis (90). The complete adaptation of the QT interval takes up to three minutes (86, 87, 89). We can investigate the QT adaptation to HR change using Holter ECG and by modification of HR as described in the Methods section, this may involve exercise tests, drugs and pacing (94).

The QT response to a sudden HR increase has been proven to have a biphasic pattern, in which an immediate response (IR) is followed by a gradual QT adaptation to a new steady-state value. This gradual adaptation can be approximated by a monoexponential function (89). QT adaptation to a new HR takes 2 to 3 minutes (86, 87, 89). The phenomenon of QT adaptation is explored in paper II.

Since QT varies with HR, we cannot compare QT values from time points with different heart rates unless we correct the QT interval in some manner. This was acknowledged over a hundred years ago (95, 96). Since then, many means of correcting the QT interval have been suggested (97-99). If a correction

formula works ideally, it eliminates the relationship between HR and QTc. Although several studies have been performed to compare the performance of the existing formulas, we could not identify any comparative studies involving a larger LQTS cohort.

The issue of these correction formulas is the subject of paper I.

#### 1.4.1.3 ELECTROCARDIOGRAM

The electrocardiogram (ECG) is a graph illustrating the voltage versus the time of the electrical activity of the heart recorded with electrodes on the body surface.

The first human electrocardiogram (ECG) was obtained in 1887 by Waller who recorded the electrical activity of the heart using saline. Einthoven continued the studies of action potentials from animal tissue and obtained ECGs with good quality, resembling our contemporary ECGs (100). At the beginning of the 20$^{th}$ century, he published several papers concerning the ECG techniques that attracted substantial interest and ECG machines were soon manufactured commercially (101-104). Contemporary ECGs are normally recorded digitally which enables computerized handling and storing. It can also be recorded for a longer time for instance in Holter technology. Modern ECGs can also be recorded using a watch or other handheld devices.

As mentioned earlier, the QRS complex corresponds to the depolarization phase, or the electrical activation of the ventricles. The T wave corresponds to the repolarization of the ventricles and the QT interval corresponds to the duration of the electrical systole.

When comparing QT intervals, QT is often corrected for HR (QTc) through mathematical formulas as described in the previous section.

#### 1.4.1.4 VECTORCARDIOGRAPHY

Although ECG is normally easily acquired and provides substantial information it is only a two-dimensional projection of the three-dimensional voltage heterogeneity of the ventricular myocardium. The de- and repolarization forces create an electrical field that extends to the body surface. Vectorcardiography was introduced as it measures both the magnitude and vectorial direction of the cardiac electrical forces (105). It was developed in

the 1930s (106) and further developed in the 1950s (107, 108). In 1956 Frank developed a model for VCG with an orthogonal X, Y, and Z lead system and with positioning that corrected for differences in the torso (109). The Frank system uses seven or eight electrodes, five in the transverse plane around the chest, one on the neck and one on the left foot. The foot electrode can also be replaced by electrodes on each hip. (Figure 9)

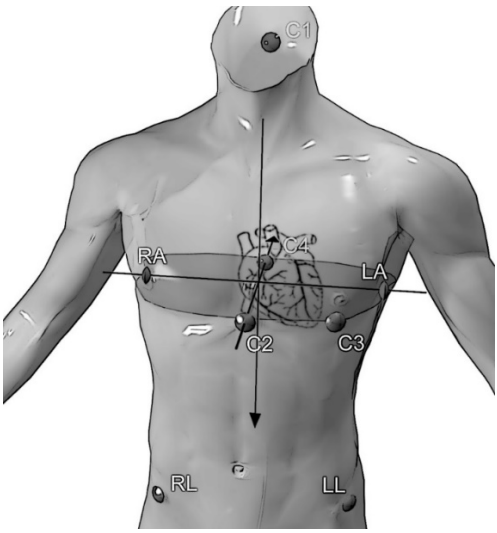


*Figure 9. Placement of VCG leads according to Frank.*

This system provides a three-dimensional representation of the electrical activity of the heart, the spatial VCG, which is constituted by continuous loops in three dimensions. The phases of the cardiac cycle are described by three consecutive loops, namely the P-loop (atrial depolarization), the QRS-loop (ventricular depolarization) and the T-loop (ventricular repolarization) (Figure 10). The VCG is often displayed as projections on three orthogonal planes, $QRST_X$, $QRST_Y$, and $QRST_Z$. The sum of these QRST complexes constitutes the global QRST complex.

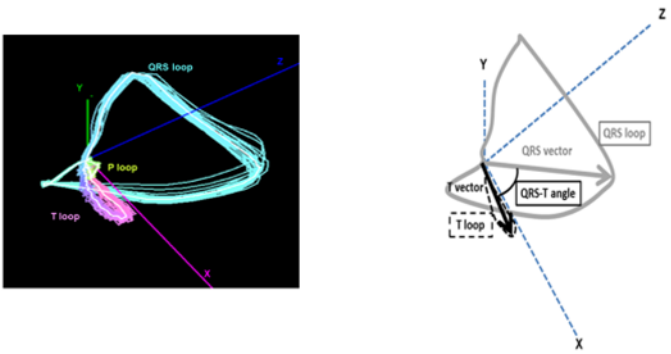


*Figure 10. P loop, QRS loop and T loop in space. To the left is a screenshot from an authentic recording and to the right is a cartoon based on the recording*

Aside from conventional ECG measures of ventricular de- and repolarization (VR) duration, QRS and QT intervals, VCG also provides other measures that give information regarding global VR dispersion. The fact that it is global means that regional differences can be detected, but not precisely located in the ventricles unlike on a regular 12-lead ECG in which, for instance, the location of a myocardial infarction can be determined from which leads are affected. One advantage of using VCG to determine for instance the QT interval is that unlike a regular ECG, in which the end of the T wave can be difficult to determine, the T loop has a distinct stop (110). The use of VCG can be relevant in studies of LQTS for evaluating global VR and it has also been used to measure the beat-to-beat variability of VR parameters (111). The VCG parameters used in each paper in this thesis are delineated in the "Methods" section.

VR duration can be measured using QT, QTpeak and Tpeak-end intervals. The QT interval is measured from the onset of the QRS complex to the end of the T wave, and the QTpeak interval is measured from the QRS onset to the maximum T amplitude. Tpeak-Tend is measured from the peak of the T wave (or from the nadir of a negative T wave) to the T end at the intersection between

the tangent at the steepest point of the downward slope of the T wave and the isoelectric line (112).

Different VCG measures exist to assess VR dispersion. In this thesis we used three: Tamplitude (μV), Tarea (μVs) and the ventricular gradient (VG, μVs).

The T loop can be used to calculate the Tamplitude which is the maximum T vector inscribed in the T vector loop. The Tarea is calculated as the root mean square of the Tarea in the three orthogonal leads (X, Y, Z). The VG means the vectorial sum of the QRS-area and T-area vectors. Figure 11 offers an illustration of VCG parameters based on voltage heterogeneity during ventricular action potential.

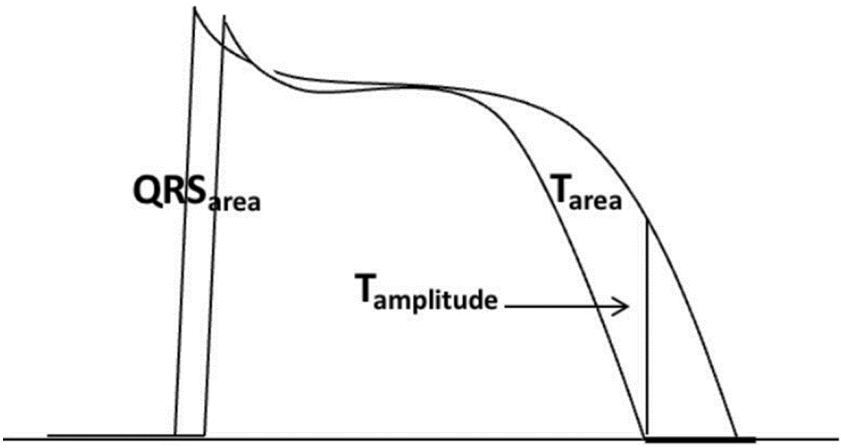


*Figure 11. Illustration of QRS area, Tarea and Tamplitude. (Reproduced with permission from the author, from an original figure in Vahedi F: Vectorcardiographic evaluation of ventricular repolarization in healthy individuals an LQTS mutation carriers. PhD thesis, Sahlgrenska Academy, University of Gothenburg. ISBN 978-91-628-8733-9)*

The use of VCG was previously more widespread but nowadays its use is primarily reduced to research. There are various techniques for deriving VCG from standard ECG (113). However, for the papers included in this thesis only VCG according to Frank has been used.

Figure 12 presents a vectorcardiographic recording, using Frank´s orthogonal X, Y and Z lead system with different colors for different leads. The vertical lines mark the annotation points. The annotations for T peak and T end are marked with arrows. The white complex (Mag) is the global QRST complex, which is the sum of the complexes in the orthogonal leads (X, Y, Z).

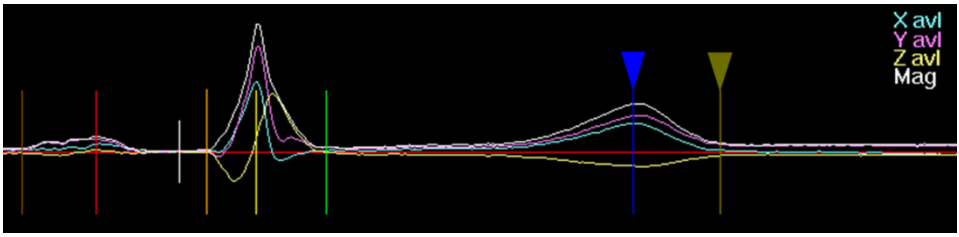


*Figure 12. Screenshot of vectorcardiographic recording using Frank´s orthogonal X, Y and Z lead system.*

#### 1.4.1.5 DISPERSION

The repolarization time of the heart differs regionally, partly due to the different distribution and function of the ion channels (80). Dispersion in repolarization, or heterogeneity, changes with HR. If HR changes suddenly, the repolarization time is not similar in all parts of the heart. If there are large differences in repolarization time between closely located areas, the propensity is higher for ventricular arrhythmia. This heterogeneity, whether it is exaggerated or not, is often believed to be important for arrhythmic events in structural heart diseases, such as ischemia. This remains to be shown for channelopathies, such as LQTS.

For ventricular arrhythmia to occur, a trigger is necessary, such as a ventricular extra systole. Additionally, if there is a substrate, it can be sustained. Such substrates can be structural heart disease (cardiomyopathies or scars after myocardial infarction) or electrical disorders, such as inherited channelopathies.

Animal experiments have found that when pacing heart tissue at a steady state the time to repolarization was similar on different sites, but a premature extra

systole could induce differences in repolarization time. In another experiment in which Kuo et al. induced increased dispersion by creating adjacent areas with different temperatures, ventricular arrhythmia was easily induced when the dispersion reached a certain value (114, 115).

A trigger is necessary to initiate arrhythmia, and this can be a ventricular extra systole. Early or delayed (EAD or DAD) afterdepolarizations can initiate arrhythmias if the membrane potential is depolarized to a certain threshold (116). The prolonged repolarization (QT) in LQTS patients predisposes for afterdepolarization.

# 2 AIMS

The overall aim of this thesis was to expand our knowledge regarding LQTS. As discussed in the Introduction section, there is a risk of ventricular arrhythmia in LQTS patients even with normal QTc. Since up to 40 % of all LQTS patients have a normal QTc, additional features to distinguish patients who are at risk from low-risk patients would be desirable. If risk stratification was refined, some patients could be safely left without treatment.

We aimed to compare the electrocardiographic and vectorcardiographic reactions to HR increase in LQTS patients and healthy controls and to characterize the repolarization disorders (Papers II-IV).

**Paper I:** The purpose of this study was to explore the performance of four common formulae for HR correction of the QT interval in LQTS patients. Since QTc is crucial for diagnosis and risk stratification in LQTS, it is of great importance to use the proper method.

**Paper II:** The aim of this paper was to study the adaptation of the QT interval to an increase in HR induced by a bolus dose of atropine in LQT1 patients compared to a group of healthy controls.

**Paper III:** The purpose of this study was to investigate whether VR dispersion differed between LQT1 patients and a group of healthy controls in response to an exercise test.

**Paper IV:** The aim was to analyze the adaptation of global measures of dispersion in response to an increase in HR induced by a bolus dose of atropine in LQT1 patients and a group of healthy controls.

# 3 PATIENTS AND METHODS

This is a summary of the more detailed descriptions contained in each paper.

## 3.1 STUDY SUBJECTS

**Paper I:** We enrolled 200 patients with a genetically confirmed diagnosis of LQT1 or LQT2 for this study and aimed to include patients of all ages. A cohort from Umeå University Hospital constituted approximately half of the study group. The other half was recruited from Sahlgrenska University Hospital in Gothenburg. The medical records were reviewed and patients with a genetically confirmed diagnosis of LQT1 or LQT2 were identified. Patients with at least one appropriate ECG were included in the study.

**Paper II and IV:** We recruited LQT1 patients (18-50 years old) from the outpatient cardiogenetic clinics at Sahlgrenska University Hospital and Umeå University Hospital. Apart from genetically confirmed LQT1, the patients were otherwise healthy and had not experienced previous cardiac events. Data acquired from a group of healthy controls (20-36 years old) participating in an earlier study were used as a comparison (117).

**Paper III:** Patients with LQTS (ages 6-68 years) were recruited at the Department of Cardiology at Umeå University Hospital and were invited to perform a semi-supine exercise test. A group of healthy volunteers (ages 6-72 years) were invited to perform a similar test for comparison. The individuals also participated in other substudies (118, 119). For this paper data from patients with LQT1 were selected.

## 3.2 VCG AND DATA COLLECTION

**Paper I:** For the Umeå cohort ECG data (automated HR and QT) from 12-lead ECGs were collected when the individuals participated in an earlier study (110). For the Gothenburg cohort, ECGs for eligible patients were retrieved from the digital ECG system. All ECGs were inspected to verify sinus rhythm, adequate quality and that the automatic HR and QT measurements were adequate. From these patients we used the earliest available ECG with sufficient quality. For 82 patients HR and QT intervals were determined

automatically and for 7 patients we measured the QT interval manually using the tangent method (120). In a subgroup of 44 patients ECGs were analyzed before and after the initiation of treatment with BB.

**For papers II-IV** Frank VCG was used as described in the Introduction section. We used a coronet II system (Ortivus, Danderyd, Sweden) with 8 electrodes and the signals were sampled at 500 Hz with an amplifier bandwidth of 0.03-170 Hz. The recording started after at least 5 minutes of rest before the exercise to allow stabilization and to minimize the effect of QT hysteresis on the baseline values. A global QRST complex was calculated by the system from the three orthogonal leads (X, Y, Z) and the annotation points were set automatically for onset and offset for the QRS complex and the T wave, and for the T wave also the peak. The VCG analysis was performed using a customized non-commercial software developed on a platform owned by Ortivus (Danderyd, Sweden). The VR duration was represented using QT, QTpeak and Tpeak-end intervals. The QT interval was measured from the onset of the QRS complex to the end of the T wave, the QTpeak interval from the QRS onset to the maximum T amplitude and the Tpeak-end from the peak or nadir of the T wave to the end of the T.

The T loop was used to calculate the Tamplitude which is the maximum T vector inscribed in the T vector loop. The Tarea, which is the root mean square of the T area in the three orthogonal leads (X, Y, Z) was calculated. The VG, meaning the vectorial sum of the QRS-area and T-area vectors was also calculated.

**Papers II and IV:** For both LQT1 patients and healthy controls Frank VCG was used with the study subjects in a supine position. VCG was recorded continuously before, during and after an intravenous bolus dose of atropine. HR and QT were analyzed as were the VCG parameters Tamplitude, Tarea and VG. Data points from each cardiac cycle for the adaptation of the selected parameters were fitted into exponential curves with the mean-square fit method. In this manner quantitative and reproducible measures could be identified and compared. In paper IV the curve fitting was more complex and is described in more detail in the manuscript. Figure 13 illustrates the adaptation of HR and QT following an atropine injection. Figure 14 illustrates a curve fit to the changes in Tamplitude following atropine injection in one patient, demonstrating the biphasic reaction pattern.

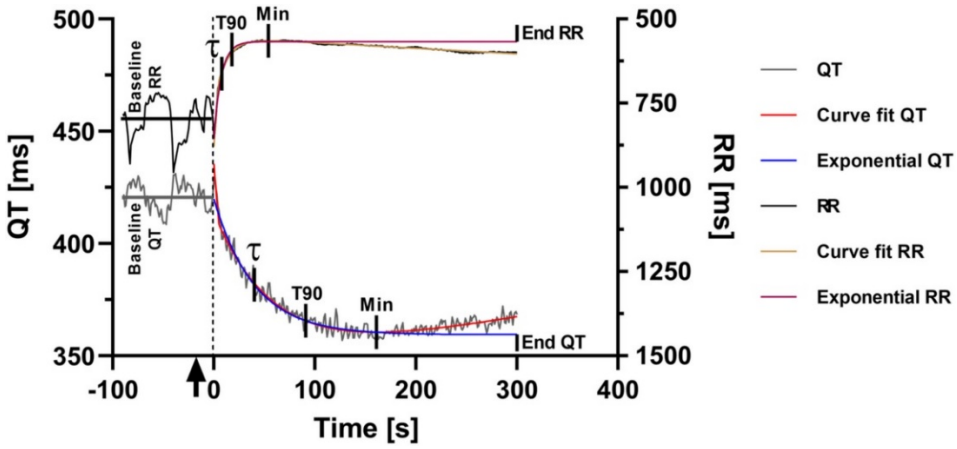


*Figure 13. Heart rate (RR interval) and QT adaptation following atropine. The atropine bolus is represented by the arrow. The start of the HR/RR response is represented by the time point 0. Reproduced under the terms of the Creative Commons Attribution License from Physiological Reports. Volume 10. 2022. Dahlberg et al. Accelerated QT adaptation following atropine-induced heart rate increase in LQT1 patients versus healthy controls: a sign of disturbed hysteresis.*

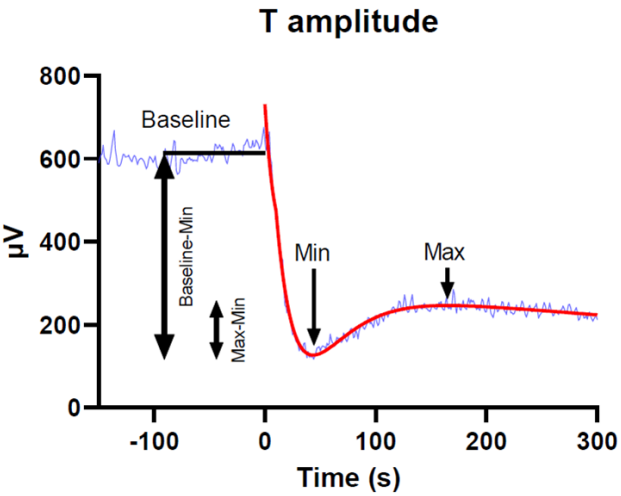


*Figure 14. A curve fit (red) to the changes in Tamplitude (blue) following atropine injection in one LQT1 patient, illustrating the biphasic reaction pattern. The time point 0 denotes the start of the VR dispersion adaptation to the atropine induced HR increase. Min represents the minimum value at the nadir of the initial dip. Max is the maximum value subsequent to Min. Max-Min is the overshoot of the reaction during the adaptation to the HR increase. The same principles were applied for Tarea and ventricular gradient.*

All curve fits were inspected for quality before analysis. For paper IV derivated curves of the dispersion measures were also constructed for all study subjects, to visualize the rate of change for each measure. In individuals with a clear biphasic rection pattern (the majority) analyses were made on the fitted curves for the three dispersion parameters, to identify the measure points as illustrated in Figure 14. These measures describe the amplitude of the reaction in relation to the baseline value as well as the time points for Min and Max. The overshoot of the reaction is defined as the difference between Min and Max, and the overshoot ratio is defined as the percentage of the overshoot of the total change in amplitude during the adaptation to HR increase.

**Paper III:** Frank VCG was recorded before, during and after an exercise stress test with the individual in a semi-supine position. The VCG parameters were calculated from 10s-averages of the QRST complex. One-minute-long sequences with high quality and minimal variability from two time points before the exercise and two time points after the exercise were identified. 10s-samples were selected from these sequences. As a "before" value the average from the two time points before the exercise was used. The time point of the maximum HR was used as the end of the exercise. As the "after" value we selected a sequence within 7±2 minutes after the exercise.

The VR duration was assessed as the duration of the entire QT interval as well as its components, namely QTpeak and Tpeak-end. These duration measures were corrected for HR using Bazett´s method (112) resulting in the parameters QTcB, QTpeakcB, and Tpeak-endcB. In addition to VR duration, VR dispersion was assessed as Tamplitude, Tarea and VG.

To assess the intensity of the exercise, we first calculated the individual HR reserve (HRR), as the difference between the estimated maximum HR (220 minus age) and the HR at baseline. The maximum HR during exercise was measured. Finally, the individual exercise intensity was calculated as the percentage of the HRR used during the exercise. For a study subject with an age of 40 years, and a resting HR of 60 bpm, the estimated maximum HR would be 180 bpm and HRR would be 120 bpm. If the study subject has a maximum HR of 135 during exercise, the exercise intensity is 62.5 %.

## 3.3 HEART RATE MODIFICATION

**Paper I:** We used data from routine ECGs, and no modification of heart rate was used in this study.

**Paper II and IV:** We increased HR by using an intravenous bolus injection of atropine (0.04mg/kg b.w.) over 30 seconds.

Atropine is an anticholinergic medication used for several purposes such as for decreasing saliva production, dilating the pupils and to treat bradycardia. Common side effects include dry mouth and blurred vision. Atropine is a competitive antagonist that blocks the muscarinic receptors. In high doses it causes a blockade of $M_2$ receptors on the SA node which induces a rapid HR increase (often exceeding 100 bpm, tachycardia).

Parasympathetic activity on the sinoatrial node slows the heart but ventricular contractility is only affected by the sympathetic tone (121).

**Paper III:** An exercise stress test was used as a physiological means of increasing HR. The exercise was performed using a bicycle ergometer with the study subjects in a semi-supine position. The exercise started at a low workload which was gradually raised until perceived fatigue. VCG was recorded continuously before, during and after the test.

## 3.4 STATISTICS

For descriptive purposes in all four papers, data were presented in terms of mean ± standard deviation (SD), numbers and percentages. In the case of the parameter age, the full range was presented to emphasize the broad age spectrum in the cohorts. In all four papers non-parametric statistical tests were used to obtain the most robust results possible. A p-value of $<0.05$ was considered significant.

In **paper I**, four formulae for HR correction of the QT were compared. Scatterplots were constructed for QTc/HR pairs for four correction formulae (Bazett´s formula, Framingham, Fridericia, and Hodges). The formulae are found in the Definitions section at the beginning of this thesis. The Spearman correlation coefficients were calculated using a linear correlation analysis. The

slopes and the correlation coefficients were compared. The same analyses were applied in a subgroup with ECGs available before and after the initiation of betablockers.

A concordance analysis was made using QTc based on Bazett´s formula as the reference to evaluate the agreement between formulae. The reference values were set to 480ms and 500ms respectively. These reference values were chosen because the first value is used in the Schwartz diagnostic criteria and the higher value indicates a high risk of events (39, 122).

In **paper II**, we used the Mann-Whitney U test to compare the differences in QT and QTpeak adaptation between LQT1 patients and healthy controls.

In **paper III**, the Mann-Whitney U test and Wilcoxon´s test were used to compare the groups. The comparisons were made between the groups, LQT1 and controls, and between the time-points, before and after the exercise test. A univariate and multivariate regression analysis was performed to evaluate the association between the LQT1 genotype and different variables and VCG parameters.

In **paper IV**, we used the Mann-Whitney U test to compare patients and controls.

## 3.5 ETHICS

All four projects were conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethical Review Board (*Regionala Etikprövningsnämnden*) in Gothenburg or Umeå (paper I: diary number Dnr 1021-15 and T253-18, paper II and IV: Dnr 138-07 and 1021-15, paper III: Dnr 2011-339-31M). For papers II-IV written informed consent was obtained from all study subjects.

# 4 RESULTS

**Paper I:** The age span ranged from 0.1 to 77.5 years and the median age was 31.5 years. 123 (62%) were female and 52 (26%) were children. When the slopes and the correlation coefficients of the scatterplots were compared, only Bazett´s method produced a QTc without a significant correlation with HR. The initiation of BB did not alter the result. The result was also similar when the subgroup of children was analyzed separately. Having identified Bazett´s method as the preferable correction formula, a QTcB of ≥480ms was used as a reference for a concordance analysis. The Cohen´s kappa values for QTcF, QTcFram and QTcH were 0.629, 0.588 and 0.487, respectively. When a QTcB of ≥500 ms was used as a reference the corresponding kappa values were 0.647, 0.612 and 0.469 respectively. The agreement can be categorized in 5 levels, 0.41-0.60 can be considered to indicate "moderate" agreement and 0.61-0.80 can be regarded as "good" (123).

**Paper II:** 21 patients and 31 controls were enrolled. For technical reasons not all recordings could be used but recordings from 18 LQT1 patients and 28 controls were included in this study. The LQT1 group was older, had higher blood pressure and a longer QTc compared to the control group. 6 patients (33%) were treated with BB. We investigated the adaptation of QT and QTpeak to a HR increase induced by an injection of atropine. The bolus injection induced an increased HR within 30s (T90 End on average 22-23s). The HR reaction in both groups was similar. Although the difference in QT and QTc (ΔQT and ΔQTc) was 48 and 32% greater respectively, we found that the QT adaptation was significantly faster in LQT1 patients compared to healthy controls. In the subgroup treated with BB the adaptation time was longer. The shorter adaptation time for LQT1 patients reflects the defective ion channel function. All study subjects experienced the expected side effects of the atropine, but we observed no complications.

**Paper III:** 43 LQTS patients and 60 healthy controls participated in the study. One patient and 9 controls were excluded due to missing or technically unsatisfactory registrations. To achieve comparable groups, the time point for registration after the exercise was decided to be within 7±2 minutes after max HR, which resulted in another 5 patients and 14 controls excluded as they did not have registrations available during this time period. The result was a total

of 37 patients and 37 controls. There was no significant difference in age, sex or in exercise intensity. The HR did not differ between the groups at baseline or at maximum, nor did the change in HR differ significantly between the groups. HR recovery after exercise was slower in LQT1 patients than in controls. The QT and QTcB were longer in LQT1 patients compared to controls. The QT interval decreased (ΔQT) in both groups after exercise but there was no significant difference between the groups. The LQT1 patients had a significantly larger prolongation of QTcB after exercise compared to controls. The lengthening of QTcB separated LQT1 patients from controls as expected, with a similar prolongation for both its components, Tpeak-endcB and QTpeakcB.

For the parameters reflecting global action potential duration, Tampl, Tarea and VG there were no significant differences between the groups.

**Paper IV:** We included recordings from 21 LQT1 patients and 31 healthy controls. The LQT1 patients were older and had higher blood pressure compared to controls. The HR reaction was similar in both groups with a prompt increase in response to the atropine injection. In the majority of subjects, we saw a biphasic adaptation pattern for the VCG parameters as illustrated earlier in Figure 14. The initial reaction in Tamplitude was significantly more pronounced for LQT1 patients compared to controls.

The rate of change in the dispersion parameters was more pronounced in patients compared to controls.

A subgroup analyses was conducted for the individuals with a clearly biphasic adaptation pattern. For Tarea and VG there were no significant differences in the overshoot reaction between LQT1 patients and controls. However, for Tamplitude the overshoot ratio was significantly larger for LQT1 patients, in other words, the reaction was exaggerated.

# 5 DISCUSSION

In this thesis we aimed to explore different aspects of repolarization in LQTS patients, with a focus on LQT1. We have examined the repolarization in relation to HR and the change following increased HR.

In the first paper we compared four of the most common formulae used for HR correction of the QT interval and demonstrated that Bazett´s method performed better for patients with LQT1-2. In paper II we investigated the QT adaptation as a response to an atropine injection. Although the HR reaction was similar for LQT1 patients and healthy controls, we found that the QT adaptation was faster in LQT1 patients compared to controls, indicating a disturbed hysteresis. In paper III we aimed to investigate whether altered global VR dispersion following an exercise stress test could distinguish LQT1 patients from healthy controls. We could find no such differences regarding global VR dispersion measures, but LQT1 patients exhibited a markedly prolonged QTc interval, as a whole and its components, the HR corrected QTpeak and Tpeak-end. In paper IV we investigated the adaptation of three global measures of VR dispersion as a response to an atropine injection. The adaptation pattern was bi-phasic with an overshoot in both LQT1 and controls. However, the LQT1 group displayed a more prominent overshoot but with significant overshoot ratio for Tamplitude.

## 5.1 HEART RATE INCREASE

As described in the Introduction section, already in the first publication regarding LQTS by Jervell and Lange-Nielsen heart rate modifications were used to examine the reaction of the QT interval (21). It has been known at least since then that a increase in HR often induces a QT prolongation in LQTS patients.

Resting HR has been shown to be an independent factor to predict cardiac events in LQT1 but not in LQT2 (124).

Over the years, several strategies to increase HR, physical exercise and pharmacological provocation, have been suggested to achieve sympathetic activation (or parasympathetic inactivation) and thereby amplifying phenotype

and unmasking individuals with “concealed LQTS” (mutation carriers with normal QTc). Postural and exercise provocations have been used in addition to pharmacological agents (36, 125, 126). Epinephrine was shown to increase the QT variability among LQTS patients (127). However, the latest guidelines advocate against routine epinephrine challenge in LQTS patients, presumably because reproducibility is low (1, 128). Isoprenaline infusion, which was used earlier by some centers in the diagnostic set up for LQTS patients, has been proven to prolong QTcB also in healthy controls, rendering it unsuitable to use for diagnosis of LQTS (117).

One challenge that afflicts exercise tests as a method to raise HR is that body movements induce disturbances on the ECG. This can possibly be overcome by using a supine or semi-supine exercise test. Nevertheless, in paper III we found that despite the semi-supine position, the registrations during the exercise test contained excessive noise to be able to be analyzed properly. However, the HR (or the RR intervals) could be accurately defined; thus, we were able to determine maximum HR during the exercise.

The use of a pharmacological agent enables better registrations since body movement can be minimized. This approach was selected for papers II and IV, with the use of an atropine bolus as a means of raising HR.

Atropine has been used by others as a means of blocking the parasympathetic activity and to allow sympathetic activity to raise the HR for study purpose (21, 117, 129). The dosage was selected to achieve a complete parasympathetic block (130). We opted to use atropine partly because it mimics the effects of an exercise. For healthy controls, Lecocq et al found that unlike the QT after isoproterenol, the decrease in QT observed during exercise was similar when atropine was used, suggesting that the QT regulation is mainly controlled by the parasympathetic system (131).

## 5.2 QT-ADAPTATION

As discussed in the Introduction section, the duration of QT is dependent upon HR. Failure to shorten the QTc when HR slows down after an exercise test is part of the diagnostic criteria for LQTS patients (36, 37). In paper II we showed that LQT1 patients had a faster QT adaptation to HR increase compared to healthy controls. Although the HR reaction was similar and the values of ΔQT

and ΔQTpeak were greater in LQT1, the adaptation time was shorter. The hysteresis function of the QT adaptation seems to be disturbed in LQT1 patients. The implication of the disturbed hysteresis function remains to be shown. However, the advantage of the hysteresis function of the QT adaptation is that it allows a more gradual adaptation of the time for coronary perfusion and ventricular function and also provides electrical stability (132). Since our study included only LQT1 patients without previous cardiac events, we can only speculate about whether symptomatic patients have an even more disturbed hysteresis.

The dynamic relation between HR and QT was studied by Halamek et al. on ambulatory Holter recordings. LQT1 patients were found to have an increased dependency of the QT interval to previous RR changes. LQT1 had a faster QT dynamic response to previous RR changes, which is consistent with our results. They showed this faster response to be present both in LQT1 with normal range QTc (<430ms) and with prolonged QTc. They suggest that measurement of this QT-RR dynamic coupling could be used in patients with suspected concealed LQT1 (88).

Mutations in the KCNQ1 gene, which is responsible for the genotype LQT1, induce a reduced current of the slow inward rectifying potassium channel ($I_{Ks}$). At rest, the contribution of this potassium channel to repolarization is small (133), but under conditions with increased HR, $I_{Ks}$ plays an important role for repolarization and consequently for the APD/QT shortening (133, 134). This explains why cardiac events in LQTS patients occur in situations with increasing HR and sympathetic stimulation (67).

We displayed an impaired hysteresis function for LQT1 in paper II which corroborates the notion that QT adaptation is intimately connected with the function of $I_{Ks}$.

## 5.3 QT CORRECTION IN LQTS

A discussion about which formula to use for the HR correction of the QT interval has been sustained for a long time (96, 97, 135-137). We identified Bazett´s method as the preferable method for QT correction for LQTS patients. We used standard ECGs from clinical everyday life. The purpose of using a correction formula is to enable the comparison of QT intervals between

individuals with different HR and within an individual between different situations with different heart rates. A correction formula is supposed to produce a QTc independent of HR. If HR is plotted against the QTc the slope factor should be zero (81). This method to compare formulae has been used by several others (136, 138). We also used a concordance analysis to compare the methods. As we demonstrated Bazett´s method to be the preferable one, how "bad/unfavourable" is it to use another formula? For both diagnostic and prognostic (threshold) values of QTc we found the best agreement to be between Bazett and Framingham and the worst to be between Bazett and Hodges in this cohort of LQTS patients. Interestingly, our hospital uses the Hodges formula, but there are easily accessible online calculators for those who wish to use another correction method.

Even though other formulae have been proven to be favorable in other groups, Bazett´s method has endured over 100 years. Its simplicity may be an explanation for that. The method has also been shown to be superior for neonates with their high HR, despite the fact that Bazett´s method is known to overcorrect the QT at HR over 90 bpm (139, 140). However, the debate is ongoing since the matter of QT prolongation and how to evaluate it in relation to pharmacology, appears to be infinitely interesting. A prolongation of QT leads to withdrawal of many new drugs that could otherwise have been useful (141).

In this thesis we have not compared measurements of the QT interval. But this is also of importance. The choice to use the tangent method or a threshold method for determining the end of the T wave, influences the measure of the QT interval. The tangent method provides a shorter QT (142). Part of this can be overcome by using VCG, as was demonstrated by Diamant et al. to be favorable for measuring QT in the LQTS group (110).

Practically all diagnostic and prognostic information about LQTS relies on QTcB (39, 143, 144). Apart from a study concerning a LQT3 family (135), we have not identified any previous reports about how different correction formulae perform in eliminating the inverse QT/HR relation in LQTS patients.

## 5.4 DISPERSION IN RESPONSE TO INCREASED HR

Ventricular arrhythmia can be triggered for instance by afterdepolarizations that prolonged VR (QT) predispose for, but it requires a substrate to be sustained (33). Such a substrate can be structural heart disease (cardiomyopathies or scars after myocardial infarction), or electrical disorders, such as inherited channelopathies.

Animal experiments have suggested that increased dispersion of VR can be a substrate for ventricular arrhythmia (114, 145-147). Some clinical studies have also suggested that altered or increased dispersion could be the arrhythmia substrate in LQTS (125, 148, 149)

VR dispersion is a normal physiologic phenomenon, and it is related with the time differences for repolarization in different parts of the ventricles as discussed in the Introduction section (1.4.1.5).

A biphasic adaptation of VR dispersion in response to HR increase is also a physiologic phenomenon. In an earlier study from our group, using atropine as a means of increasing HR, this biphasic response in VR dispersion was observed in healthy individuals (132). The same pattern was also seen following rapid atrial pacing (94, 150).

While papers I and II discuss with the duration of VR (QT) and its relation and adaptation to HR, the last two papers investigate VR dispersion in response to changes in HR. In paper III the VCG data are from recordings after an exercise stress test and are made from a time frame of 7±2 minutes after maximum HR. In paper IV the recordings were made during an atropine injection and hence during the actual rise in HR. These are important differences in time points of registrations. What is the initial effect and what effects last after a several minutes?

In a study by Vahedi et al., VR dispersion in terms of the VCG derived parameters Tarea, Tampl and VG were studied in a group of LQTS patients. LQT1 patients exhibited no signs of increased VR dispersion at rest compared to healthy controls (111). We continued this work to elucidate the arrhythmia

substrate in LQT1 by studying these dispersion measures in response to increased HR, via two different interventions.

We hypothesized that if increased VR dispersion were an important substrate for arrhythmia in LQTS patients we would see differences in VR adaptation to increased HR between patients and controls.

In paper III we used an exercise stress test to increase HR. As described in paper III, signal disturbances from breathing and movement precluded the use of the registrations during the actual exercise, apart from HR which could be defined from the RR intervals. There was no significant difference in the baseline or maximal HR, nor in the exercise intensity between the groups. The HR recovery after exercise was slower in LQT1 patients, which was discussed in detail by Lundström et al (119). As expected, we found that QTcB, and its components QTpeak and Tpeak-end increased significantly more after exercise in LQT1 patients compared to controls. We found that the exercise induced prolongation of QTc lasted longer than 4 minutes after exercise, which is a time interval used in the Schwartz scoring system (37). This is consistent with a study by Aziz et al regarding a group of LQT1 children where the induced prolongation of QTc remained at least 9 minutes after exercise (151).

The difference in Tpeak-endcB (ΔTpeak-endcB) is an indication that exercise prolongs not only the whole VR, but also the interval between the earliest and latest parts of the ventricles to complete repolarization. This can be regarded as an example of increased temporal dispersion.

However, we could see no increase in the three global dispersion measures Tampl, Tarea and VG following exercise, for LQT1 patients or controls. The values of these global dispersion parameters were generally slightly larger in controls than in patients before exercise as well as after. There was, however, a larger proportion of men in the control group, which is known to affect these parameters. HR is also known to affect the parameters, and the values decreased after exercise as expected with higher “after”-values of HR (152, 153). However, there were no significant differences in the changes of the dispersion parameters, although HR was higher after exercise in patients.

Although arrhythmogenesis has been associated with increased dispersion of ventricular repolarization in different conditions including LQTS, we could

find no evidence of increased ventricular dispersion of action potential duration and morphology in LQT1 patients compared to controls, as reflected by Tampl, Tarea, and VG before or after exercise.

In an earlier study by Axelsson et al., the adaptation of Tampl, Tarea and VG to abrupt HR increase via atrial pacing, has been demonstrated to be a more dynamic process than the adaptation of the QT interval (94).

Paper IV describes the second part of the atropine stress test, in which a bolus injection of atropine was used to increase HR. The same three global measures of VR dispersion were studied as in paper III. The HR response was similar in both LQT1 and controls, as discussed earlier, and the majority in both groups exhibited a biphasic response of VR adaptation to HR increase. This biphasic response was more pronounced in LQT1, and the greatest difference was for Tampl with a significantly more pronounced initial biphasic reaction (overshoot ratio). A faster response is frequently associated with a tendency toward instability or overshoot. This larger overshoot therefore aligns with the faster QT adaptation that we showed in paper II. In an earlier study from our group, Vahedi et al demonstrated greater beat-to-beat variability in repolarization parameters in LQTS patients (111). LQT1 patients thus appear to display faster responses and tentatively greater instability in the adaptation of VR dispersion as a response to HR changes.

Due to the long half-life of atropine, our study cannot shed light on the adaptation of VR dispersion when HR returns to normal.

This thesis has primarily dealt with the electrical functions in relation to LQTS. And papers III and IV address the issue of electrophysiological dispersion and adaptation. But also mechanical dysfunction, at least subclinical, has been reported in LQTS (154). The proper adaptation of VR duration is important for the diastolic function of the heart, of time for emptying and filling of the ventricles and coronary perfusion. Several studies indicate impaired diastolic function, prolonged contraction duration and increased mechanical dispersion in LQTS (154-156). The concept of the electromechanical window (EMW) is defined as the time difference between the mechanical systole (time from onset of QRS complex to the second heart sound, i.e., the closing of the aortic valve) and the electrical systole, or the QT interval. EMW is typically positive in healthy individuals, meaning that the mechanical systole is longer than the QT

interval. In a study focusing on electromechanical coupling, ter Bekke et al showed a negative EMW in LQTS patients which was most pronounced in symptomatic patients (157). The result was confirmed in a later study by Charisopoulou et al, and they also added an exercise that showed more pronounced negativity at physical exercise and during recovery (118). These studies show a disturbed relation between cardiac electrical and mechanical function. Disturbed hysteresis in QT adaptation as in our study, might have consequences for mechanical function in line with the EMW studies, but also for coronary perfusion.

## 5.5 CONCEALED LQTS AND RISK STRATIFICATION

When LQTS was first described as an entity, all patients were believed to have a prolonged QT interval. The discovery that LQTS patients could also have normal QT intervals changed the arena and made the management more challenging. Over the years the proportion of LQTS patients with normal QT intervals have increased, due to a more proactive cardiogenetic approach with family screening programs. There is no consensus about the QTc limits for concealed LQTS, sometimes the limit of <430ms is used (88) and sometimes 460ms is used (158).

Up to 40 % of LQTS patients have a QTc with normal or at least non-diagnostic values and provocative tests have been attempted to unmask such patients with concealed LQTS (88, 159-161). As HR increase is known to prolong QTc in LQTS patients the tests are designed to achieve an increase in HR.

Despite the knowledge that cardiac events occur mainly in situations with increased HR, the only electrophysiologic parameter currently used for risk stratification, is QTc at rest. A prolonged QTc 4 minutes after exercise is used for diagnostics but not for prognostics. The QT adaptation during HR increase could mirror the phenotype in patients with a QTc of under 500ms. The atropine test may offer such possibilities but since only asymptomatic patients have been included in the study thus far, this remains to be shown.

Since many LQTS patients go through life without any symptoms, regardless of treatment with betablockers, additional means of separating high-risk

patients from those with very low risk would be desirable. There is still a clear gap of knowledge. I entered this research field to strive to uncover answers to this. Could we find ways to refine risk stratification?

Neves et al propose “intentional nontherapy” as an option, wherein the only preventive measures are the avoidance of QT-prolonging drugs, the correction of electrolyte imbalances and the reduction of fever (77). Earlier guidelines state that beta blocker treatment has a Class IIa recommendation (“should be considered”) in patients with a QTc of less than 470ms (20). The most recent guidelines state that beta-blockers should be considered in LQTS patients with a normal QTc (1, 20).

## 5.6 METHODOLOGICAL ASPECTS AND LIMITATIONS

There are some limitations to all the studies. As LQTS is a rare disease, the number of participants is limited.

In paper I the intention was to use automatic measurements of QT for all ECGs. However, since not all QT intervals were accurately measured; in fact, especially for cases with very long QT intervals, we were obliged to measure manually in some cases. The manual measurements can differ from the measurements made automatically, since the tangent method is often used. The tangent method is known to produce a QT interval that is shorter than when a threshold method is used. To minimize the influence of hysteresis, an ECG should be preceded by at least 3 minutes of rest. As the ECGs in this study were obtained as part of clinical routine, not every ECG was recorded in this manner.

In paper III the timepoints for measurements were selected when the quality was sufficient to make certain analyses for a whole minute. We ultimately opted to use only a 10-s average for our analyses. If this had been decided from the start, we could have chosen other timepoints for the analyses. The registrations after the exercise are from 7±2 minutes after the maximum HR. Since the value of QTc 4 minutes after exercise is well established in the modified Schwartz score, analyses of VCG parameters at this time-point would perhaps have provided the opportunity of interesting comparisons. However,

it has been shown that the HR induced prolongation of QTc in LQTS patients remains at least for 9 minutes (151).

In paper IV due to the supine position without any movements of the study subjects, the quality of the registrations was very good.

The use of VCG was more widespread earlier but nowadays its use is mainly reduced to research. There are techniques for deriving VCG from standard ECG (113) which can make the technique more widely available. However, for the papers included in this thesis only VCG according to Frank has been used.

## 5.7 ETHICAL COMMENTARY

All four projects were conducted in accordance with the Declaration of Helsinki and approved by the regional ethics committee as stated in the Methods section.

In paper I previously collected ECG data from LQTS patients were used.

Papers II-IV were based on results from human experiments. We aimed to include as many women as men.

In papers II and IV we used an intravenous injection of atropine to induce an increase in HR. The patients invited to participate in this study were selected from our cohort of asymptomatic LQTS patients. Although the LQT1 patients were considered to be low risk patients, there was at least a theoretical risk of arrhythmic events. The participants were thoroughly informed about expected side effects and the project took place in a hospital setting, with intravenous BB and equipment for resuscitation at hand and in the presence of at least one experienced cardiologist and nurses. No adverse arrhythmia effects were seen and apart from the expected side effects of atropine, tiredness, accommodation disturbances, and dry eyes and mouth, no other adverse effects were noted. The effects were observed in the LQT1 group as well as in the control group.

In paper III a group of LQT1 patients and a group of healthy controls performed an exercise test. Although physical exercise has a propensity to induce a prolongation of the QTc in LQT1 patients and is associated with an

increased risk of cardiac events, an exercise stress test is a method that is often used in diagnostic work-up in this patient group. The value of QTc 4 minutes after exercise is part of the Schwartz score (36, 37). The general experience is that adverse arrhythmic effects during exercise stress tests are exceptionally rare and during this project no such effects were seen.

# 6 CONCLUSIONS

We identified Bazett´s formula for HR correction of the QT interval as the only formula eliminating the relation between QT and RR in LQTS patients. Despite its widespread use for over 100 years, it had not been shown previously to be the preferable method for adult LQTS patients.

The impaired QT hysteresis function for LQT1 patients, mirrored as a shorter QT adaptation time in response to HR increase after atropine, emphasizes the importance of $I_{Ks}$ in APD/QT shortening. The atropine test could possibly serve as a tool for risk evaluation in the cohort of patients with concealed LQTS to identify true low-risk patients.

Physical exercise is known to prolong the QTc interval and to be linked to arrhythmias in LQT1 patients. As a response to an exercise stress test, the prolongation of the QTcB interval, an example of increased temporal electrophysiological dispersion, separated LQTS patients from healthy controls but not VCG parameters of global dispersion.

The initial biphasic reaction of VR dispersion following HR increase is a normal physiologic phenomenon, but it was more pronounced in LQT1, with an exaggerated overshoot. We suggest that this exaggerated reaction to HR increase in LQT1 patients might play a role in arrhythmogenesis.

# 7 FUTURE PERSPECTIVES

Research regarding congenital LQTS has been extensive during the years, as it is the prototypical channelopathy and the genetics are well known. Nevertheless, substantial research is still going on since there remain intriguing questions to be explored.

The question of precision medicine persists, although thus far, not much has been shown to support the possibility to predict clinical outcomes from a certain genetic variant. The introduction of polygenic risk scores might change the arena (42).

For patients with a severe phenotype, we can now offer medication as well as ICD which has led to reduced mortality. However, there are still small treatment-resistant groups such as CALM-mutation carriers or homozygotes with a need for more effective drugs.

There are a large number of individuals with a positive genotype-negative phenotype, sometimes referred to as concealed LQTS. This group is growing since cardiogenetic clinics are more common and work proactively. What makes some individuals prone to arrhythmia and some not, remains a matter of research and debate. It remains to be shown whether the atropine-test can have additional value in this group, but as we showed in paper II, patients with BB showed a more “normal” phenotype. Possibly, this test could be used to investigate the effects of BB on an individual level.

We have used VCG according to Frank. There are several transformation methods to achieve ECG derived VCG which could make the methods more spread.

Since LQTS is a rare disease, larger collaborations are necessary to gather a sufficient number of patients. Both national and international initiatives to gather such collaborations are needed.

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