Long QT Syndrome
- studies of diagnostic methods

Ulla-Britt Diamant
To the study participants and their relatives

The Indian heart, as seen by the Ojibwa Indians, is full of strength and beats powerfully in space.

The cover: Relief “I live by the clear space”

Artist Jörgge Sundqvist.
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Abstract

Background: The Long QT Syndrome (LQTS) is a hereditary heart disease with risk of malignant ventricular arrhythmia and sudden cardiac death. Despite our increased knowledge about genotype and phenotype correlation we still rely on the 12-lead ECG for assessment of the QT interval and the T-wave morphology for diagnosis and risk stratification. Intra- and -inter individual variability in manually QT measurement and, e.g., difficulties in defining the end of the T-wave may impair the diagnosis of LQTS. Increased heterogeneity in ventricular repolarization (VR) may be an important factor in the arrhythmogenicity in cases of LQTS. In a LQTS founder population the same mutation is carried by numerous individuals in many families which provide a unique opportunity to study diagnostic methods, risk assessment, VR and the correlation between genotype and phenotype.

Methods: Resting 12-lead ECG and vectorcardiogram (VCG) were recorded in 134 LQTS mutation carriers and 121 healthy controls, to investigate the capability and precision in measuring the QT interval. For assessment of the VR, VCG was compared in individuals with mutations in the KCNQ1 and KCNH2 gene. Genealogical and geographic studies were performed in 37 index cases and their relatives to determine if Swedish carriers of the Y111C mutation in the KCNQ1 gene constitute a founder population. To confirm kinship, haplotype analysis was performed in 26 of the 37 index cases. The age and prevalence of the Y111C mutation were calculated in families sharing a common haplotype.

Results: VCG by automatic measurement of the QT interval provided the best combination of sensitivity (90%) and specificity (89%) in the diagnosis of LQTS. VCG showed no consistent pattern of increased VR heterogeneity among KCNQ1 and KCNH2 mutation carriers. Living carriers of the Y111C mutation shared a common genetic (haplotype), genealogic and geographic origin. The age of the Y111C mutation was approximately 600 years. The prevalence of living carriers of the Y111C mutation in the mid-northern Sweden was estimated to 1:1,500-3,000.

Conclusion: We have shown that VCG provides a valuable contribution to the diagnosis and risk assessment of LQTS in adults and children. No consistent pattern of increased VR heterogeneity was found among the LQTS mutation carriers. The identified Swedish LQTS founder population will be a valuable source to future LQTS research and may contribute to increase our understanding of LQTS and the correlation of phenotype, genotype and modifying factors.
**Abbreviations**

- **AP** Action potential from ventricular myocytes
- **APD** Action potential duration
- **Bpm** Beats per minute
- **EAD** Early after depolarization
- **ECG** Electrocardiogram
- **LQTS** Long QT Syndrome
- **µV** Microvolt
- **µVs** Microvolt seconds
- **Ms** Millisecond
- **QRS amplitude** Amplitude of the maximum QRS vector in space
- **QRS area** The spatial area under the curve formed by the moving heart vector during the QJ interval
- **QRS-T angle** The angle between the maximum QRS and T vectors
- **T amplitude** Amplitude of the maximum T vector in space
- **T area** The spatial area under the curve formed by the moving heart vector during the JT interval
- **T avplan** The mean distance between the periphery of the T loop and both sides of the preferential plane
- **T azimuth** The angle of the maximum T vector in the transverse plane (0° left, +90° front, -90° back and 180° right)
- **TdP** Torsade de Pointes
- **Teigenvalue** The squared quotient between the two largest perpendicular axes (eigenvalues) of the T loop in the preferential plane \([(d_1/d_2)^2 \text{where } d_1 \geq d_2]\)
- **Televation** The angle of the maximum T vector in the cranio-caudal direction by us defined from 0° (caudal direction) to 180° (cranial direction)
- **Tp-e** T peak to T end, the last part of the QT interval and final repolarization
- **VCG** Vectorcardiogram
- **VG** Ventricular gradient or QRST area is the spatial area under the curve formed by the moving heart vector during the QT interval; also the sum of the QRS area vector and T area vector, taking into account the angle between
them; it describes the dispersion of action potential morphology throughout the ventricles.

VR  Ventricular repolarization

The intervals and waves in an ECG complex
Svensk sammanfattning

Bakgrund och syfte med avhandlingen: Långt QT syndrom (LQTS) är en ärftlig hjärtsjukdom med risk för plötslig död p.g.a. livshotande hjärtrytmrubbning (kammartakykardi). EKG är ett viktigt instrument vid diagnos och riskbedömning av LQTS. Avhandlingens syfte är att förbättra diagnos och riskbedömning av LQTS genom att undersöka olika EKG metoder, samt finna ut om svenska bärare av Y111C mutationen (sjukdomsanlag) i KCNQ1 genen har samma ursprung och utgör en founderpopulation. En founderpopulation utgör en genetisk homogen grupp och är en viktig källa för framtida forskning av bl.a. diagnostiska metoder.


Resultat: VKG erbjöd den bästa kombinationen av sensitivitet (0,90), specificitet (0,89) för diagnos av LQTS. Genealogiska studier visade att 26 bärare av Y111C tillhörde samman släktträd med en anfader/moder som levde för ca 400 år sedan. Alla nu levande bärare av Y111C visade sig ha ett gemensamt geografiskt och genetiskt ursprung.


Betydelse: För enskilda individer med risk för potentiellt livshotande hjärtrytmrubbningar är tidig diagnostik, riskvärdering och profylaktisk behandling av stor vikt. Vi har påvisat vektorkardiografins fördelar vilket skulle kunna ge metoden en ökad betydelse i kliniken. Dock krävs ytterligare studier för att befästa vektorkardiografins position vid diagnostik och riskvärdering vid LQTS. Att vi identifierat en founderpopulation öppnar unika möjligheter för internationell betydelsefull forskning inom ett snabbt expanderande område. På sikt kommer detta att kunna bidra till minskad dödlighet bland patientgrupper med LQTS.
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I  Two automatic QT algorithms compared with manual measurement in identification of long QT syndrome  
**Diamant UB**, Winbo A, Stattin EL, Rydberg A, Kesek M, Jensen SM  
*J Electrocardiol. 2010 Jan-Feb;43(1):25-30*  
*Reproduced with kind permission from Elsevier.*

II  Vectorcardiographic Recordings of the Q-T Interval in a Pediatric Long Q-T Syndrome Population  
**Diamant UB**, Jensen SM, Winbo A, Stattin EL, Rydberg A  
*Pediatr Cardiol. 2013 Feb;34(2):245-9*  
*Reproduced with kind permission from Springer Science and Business Media*

III  Electrophysiological Phenotype in the LQTS Mutations Y111C and R518X in the KCNQ1 Gene  
**Diamant UB***, Farzad Vahedi**, Winbo A, Rydberg A, Stattin EL, Jensen SM, Bergfeldt L  
*both authors contributed equally*  
Manuscript

IV  Origin of the Swedish long QT syndrome Y111C/KCNQ1 founder mutation  
Winbo A, **Diamant UB**, Rydberg A, Persson J, Jensen SM, Stattin EL  
*Heart Rhythm. 2011 Apr;8(4):541-7.*  
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Introduction

The Long QT Syndrome (LQTS) is a hereditary heart disease that has been further clarified in the era of molecular genetics. The electrocardiogram (ECG) is still one of the most important elements in the description of the clinical phenotype in LQTS, in spite of the increased knowledge about genotype and phenotype correlation. In the daily work of diagnosis and risk stratification, we still rely on the 12-lead ECG for the evaluation of the duration of the QT interval and the T-wave morphology. However, because of pitfalls in the manual measurement of the QT interval, the diagnosis of LQTS may be misclassified due to, e.g., difficulties in defining the end of the T-wave. There are also intra- and inter-individual differences in measurements of the QT interval (1), which further worsens the accuracy of manual measurements. The ECG is fundamental in determining the diagnosis and in the risk stratification of LQTS. Nonetheless, there is no consensus on how to measure the QT interval or how to correct the QT interval for heart rate.

The aim of this thesis was to improve the capability of ECG as a diagnostic and prognostic tool in LQTS. Paper I and II compare two automatic methods (12-lead ECG and Frank vectorcardiogram VCG) and manual measurement of the QT interval in healthy individuals and a population of LQTS subjects. In paper I we also state intra- and inter-variability of manual QT measurement for four observers. Promising results in the automatic measurement of the QT interval by VCG are included in paper I and II, which deals with the time-consuming and error-prone manual measurement of the QT interval. Increased ventricular repolarization (VR) heterogeneity may be an important factor in the arrhythmogenicity in LQTS. This has, to the best of our knowledge, not been evaluated in humans in any previous study. In paper III, VR was studied in two populations with two different LQTS mutations and a group of healthy individuals. Genetic homogeneous populations are of great value to develop and improve diagnostic methods and risk assessment tools. A so-called founder population - comprising individuals with a common geographic and genealogic origin and carrying a common mutation - is ideal for studies of correlation between genotype and phenotype including ventricular repolarization. During our work in the LQTS clinic we noticed a high number of families carrying the same mutation Y111C in the KCNQ1 gene. After systematic genealogic and genetic studies, presented in paper IV, we proved that these families formed a LQTS founder population.
Background

Historical background

The first ECG in a human was recorded by Augustus Waller in 1887 (2). Later William Einthoven invented the string galvanometer and named the wave deflections P, Q, R, S and T (3), instead of ABCD that had been used with the capillary electrometer. He avoided N and O, which were already in use with mathematic/geometric matters and started with P to label the first deflection. In 1912, he also defined the current standard ECG leads I, II and III also known as “Einthoven’s triangle” (4). Einthoven and colleagues described the first electrical heart vector and the angle that was between a horizontal reference line and the heart vector. It was time consuming to calculate the loop, since each lead (I, II and III) had to be aligned manually for the calculation of the loop in the frontal plane (5). The study of orthogonal leads became easier, and was further developed with the introduction of the cathode-ray oscillograf in the 1950’s. More than 30 corrected lead systems was developed but the Frank lead system became the most frequently used because it was a compromise between accuracy and practical manageability (6). With today’s computer technology, it is easy to record a VCG with presentation of the loops in real-time. During the 1930´s the augmented limb leads (aVR, aVF and aVL) and the precordial leads (V1-V6) came into use and in the 1950´s the use of the standard 12 lead ECG was widely spread. In 1930’s the American Heart Association and the Cardiac Society of Great Britain defined the standard positions and wiring of the 12-lead ECG, which today forms the basis of manual and automatic interpretation of ECG - one of the most important diagnostic tools for cardiac diseases (7, 8).

Congenital LQTS is a cardiac arrhythmogenic disorder associated with prolongation of the QT interval on the ECG, syncope and sudden cardiac death in young individuals with no structural heart disease. It was first described by Jervell and Lange-Nielsen 1957 in a family with congenital deafness, prolonged QT interval, syncope and sudden death transmitted in an autosomal recessive pattern (9). Romano reported in 1963 (10) and Ward in 1964 (11) a similar syndrome but without deafness and transmitted in an autosomal dominant way. In 1991 came the first report that presented LQTS as a genetic disorder, a finding that was achieved after gene linkage studies. The study was performed in a pedigree with both healthy and LQTS affected relatives, and uncovered a strong linkage between LQTS and a DNA marker at the Harvey ras-1 locus on chromosome 11 (12). In 1995, the connection between gene mutations and cardiac potassium and sodium channels was
discovered – since then LQTS has been considered as a cardiac ion channelopathy (13, 14). The early use of comprehensive national population records in Sweden provides good opportunities to track the ancestry of living persons back in time - this registration is a golden source for mapping of monogenetic diseases (15). Following a church law of 1686, the priests were enjoined since the late 17th and early 18th century to register in church archives every dweller in their parish. As a result of this we now, in Sweden, possess registers of all individual habitants of the Swedish parishes in the catechetical examination records. These were replaced in 1895-1900 by the “congregation book” which, in its turn, was abolished in 1991. The church registered all migration, births, deaths and marriages, and from 1860 every tenth year census were held and archived in the Swedish Central Bureau of Statistics. Tracking the geographical origin of the population carrying the Y111C mutation in the KCNQ1 gene brought us to the area along Ångerman River valley and its tributaries. As the other historical provinces of Northern Sweden, the landscape Ångermanland was first populated in the coastal areas and the river valleys (16). Occasional Swedish settlements were founded during the middle ages in the area where our founder couple settled in the mid-17th century. During the 16th, century there was also some colonization in the area by settlers coming from Finland. The settlers were principally farmers, but they also provided for themselves by hunting and fishing. There was a Sami indigenous population in the area that was predominantly nomads, living by keeping reindeers, hunting and fishing. The state encouraged the colonization of the interior of northern Sweden through the so called “Lappmarksplakatet” of 1673, and this was the starting point for the era of colonization of the inlands of northern Sweden that lasted for over 200 years. No great influx from other parts of the country occurred during the first half of the 18th century. In 1749, the Crown supplemented the bill with the Lapplands regulations settling a distribution of trades between the colonizers and the Sami natives. Settlers should foremost pursue farming, wherewith the trades of hunting and fishing would essentially be protected for the Sami. The main object of the regulations was to stimulate colonization and the settlers were granted 15-25 years free of taxation. The regulations didn’t accomplish any significant results towards an increase in settlings until a few decades later. During the 19th century, there was an increase of immigration from other parts of the country, and the population of the inland of northern Sweden grew. In the latter part of the 19th century there was a surge in the commercial interests in forestry affording new sources of income. The first half of the 20th century saw a certain increase of movement from the river valleys to the coastal cities, but not until after the 1950’s has there been a net decrease of population in the studied area.
LQTS a genetic cardiac ion channelopathy - Genotype

LQTS is the most well-known monogenetic heart disease and several hundreds of mutations in 13 genes have been described (Table 1). Most of the mutations can be found in 3 genes, the $KCNQ1$ (40-55%), $KCNH2$ (30-45%) and $SCN5A$ gene (5-10%) (14, 17, 18). LQTS is mostly a heterogenetic disease, meaning that each family carries a mutation unique for their family.

Table 1. LQTS genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Frequency</th>
<th>Locus</th>
<th>Functional effect Phenotype (in vitro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$KCNQ1$ (LQT1)</td>
<td>RWS, JLNS</td>
<td>40-55</td>
<td>11p15.5</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$KCNH2$ (LQT2)</td>
<td>RWS</td>
<td>30-45</td>
<td>7q35-36</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$SCN5A$ (LQT3)</td>
<td>RWS</td>
<td>5-10</td>
<td>3p21-p24</td>
<td>Gain-of-function</td>
</tr>
<tr>
<td>$ANKB$ (LQT4)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>4q25-q27</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$KCNE1$ (LQT5)</td>
<td>RWS, JLNS</td>
<td>&lt;1</td>
<td>21q22.1</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$KCNE2$ (LQT6)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>21q22.1</td>
<td>Loss-of-function</td>
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<tr>
<td>$KCNJ2$ (LQT7)</td>
<td>AS</td>
<td>&lt;1</td>
<td>17q23</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$CACNA1C$ (LQT8)</td>
<td>TS</td>
<td>&lt;1</td>
<td>12p13.3</td>
<td>Gain-of-function</td>
</tr>
<tr>
<td>$CAV3$ (LQT9)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>3p25</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$SVN4B$ (LQT10)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>11q23.3</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$AKAP9$ (LQT11)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>7q21-q22</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$SNTA1$ (LQT12)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>20q11.2</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>$KCNJ5$ (LQT13)</td>
<td>RWS</td>
<td>&lt;1</td>
<td>11q24</td>
<td>Loss-of-function</td>
</tr>
</tbody>
</table>

**Functional effects**

Gene mutations cause changes in the proteins of the cardiac ion channels. This creates a disturbance in the functional effect of the ion current through the cell membrane (19) (Figure 1). The mutations can be divided according to their phenotypical effects into different categories. A loss-of-function mutation is a mutation that does not create any functional gene product. A dominant loss-off-function mutation creates a defect gene product that disturbs the normal functional effect of the wild-type gene. A gain-of-function mutation results in a gene product that has a new feature, most of these mutations are dominant. A gain-of-function mutation can be due to a new amino acid sequence which alters protein function. Moreover, it can be a mutation in the regulatory regions, which expresses as a new tissue or in a new stage where it has not previously been active (20).

The KCNQ1 gene (LQT1) encodes the α-subunit of the K+ channel that generates the slow potassium current (IKs) (Figure 1) (21). During sympathetic activation with increased heart rate the repolarization duration is hastened by an increase of the IKs (19). When a KCNQ1 mutation causes defective IKs the repolarization duration does not shorten appropriately to sympathetic stimulation. This can be seen in an ECG as a prolonged QT interval that does not adapt properly to increased heart rate.

The KCNH2 gene (LQT2) encodes the α-subunit of the K+ channel and thereby for the rapid potassium current (IKr). A mutation in this region causes an effect similar to a mutation in the KCNQ1 gene with reduction of outwardly IKr and thus a prolongation of the duration of the repolarization. Both mutations in the KCNQ1 and KCNH2 gene reduces components of the outwardly potassium current (IK) through the cardiac ion channels.

The third common gene causing LQTS is the SCN5A gene (LQT3) that encodes the α-subunit of the cardiac sodium channel conducting the inward sodium current under the depolarization. The result of a mutation in the SCN5A gene produces an increase in the delayed Na+ inward current and the consequence is a prolongation of the action potential (AP) of the cardiac myocytes. The result of all of these mutations in the different genes thus creates an arrhythmogenic cardiac condition.
Figure 1: Illustration of the effect of altered ion-channel currents on the ventricular action potential (AP) duration in LQTS. The direction of ion currents: inward = below the line; outward = above the line. Hatched rectangles = time location of the effect of mutations in LQT1, LQT2 and LQT3 on sodium and potassium ion-channel currents. A prolonged AP is seen when (horizontal arrow) there is an inappropriate gain of function (GOF) in late sodium current (I_{Na}) or loss of function (LOF) in slowly (I_{Kr}) or rapidly (I_{Ks}) acting repolarization potassium currents. Reprinted from (21). Reproduced with kind permission from Elsevier.

Heredity in LQTS

Two hereditary variants of LQTS are known, the most common being Romano-Ward syndrome (RWS). If one parent carries a mutation the risk is 50 % for each child to develop RWS – this hereditary pattern is known as autosomal dominant. The other variant is Jervell and Lange-Nielsen syndrome (JLNS) which is transmitted in an autosomal recessive way. When both parents carry a mutation, the risk of each child inheriting the mutation is 50% (RWS) and 25% to inherit both mutations and get the disorder JLNS.
syndrome. JLNS is a serious disease and is in addition to cardiac arrhythmias associated with congenital deafness (22).

**Prevalence of LQTS**

The prevalence of LQTS has gradually increased due to the growing use of molecular genetic diagnostics which has consequently increased the number of diagnosed asymptomatic carriers (23). The estimated prevalence has increased from 1:10 000 in 2000 (24), to 1:5 000 in 2008 (25) and 1:2000 in 2009 (26). In paper IV, we present prevalence data from the area of mid-northern Sweden (counties of Jämtland, Västernorrland and Västerbotten) of the first Swedish LQTS founder mutation Y111C in the KCNQ1 gene.

**Genetic testing for LQTS**

Genetic testing for the LQTS has entered routine clinical practice and is a valuable instrument in the diagnosis of LQTS. An individual (index case) with the clinical diagnosis LQTS can be offered genetic testing to identify the mutation causing LQTS. Genetic testing identifies mutations in up to 75-80% of clinical affected individuals (27). When a mutation is identified in a family, this gives the first-degree relatives the opportunity to undergo genotyping thus providing important information in guiding the management of individuals.

**Founder mutations and founder populations**

A founder mutation can be described as a mutation that appears in a limited gene pool in a population living in the same geographic area and thus being enriched (15). Environmental factors, socio-ethnical constructions and characteristics of the mutation itself affect the expression of the disease. LQTS founder mutations have been found in South Africa (28) and Finland (29). A population growth during the 17th century and onward made the population in northern Sweden a breeding ground for the growth of monogenetic diseases (15). The river valleys of northern Sweden running from northwest to northeast has separated the populations living within the river valleys from other populations. This has contributed to sub-isolates of people living in the same geographic area within the river valleys. Moreover, Sweden has a unique opportunity of genealogic studies via the comprehensive population records which allows genealogical mapping of monogenetic diseases in the population. A LQTS founder mutation with large numbers of individuals provides an opportunity to study the genotype-phenotype correlations and impact of modifying factors on the phenotype.
Clinical presentation of LQTS - Phenotype

The LQTS phenotype is diverse and may include syncope, sudden cardiac death and an ECG with or without QT interval prolongation. Specific factors have been showed to trigger syncopal episodes, where LQT1 patients are more likely to develop cardiac events during exercise and swimming (30). Emotions and sudden noise give rise to cardiac events in individuals affected with mutations in the KCNH2 gene (LQT2), and LQT3 carriers can experience events on awakening and during sleep. The family history of syncope and sudden death among first-degree and second-degree relatives is important in the initial evaluation of a patient with suspected LQTS. Notably, about 30-50 % of the carriers of a LQTS mutation are asymptomatic throughout life.

Arrhythmias in the LQTS

The ventricular arrhythmia associated with the cardiac events in LQTS is Torsade de Pointes (TdP) (Figure 2). TdP is a polymorphic ventricular tachycardia characterized by a gradual change in the amplitude and twisting of the QRS complexes around the isoelectric line. The onset of the TdP is often preceded by a short-long-short sequence in R-R intervals, with the last sequence interrupting the T-wave (R on T phenomenon). In most of the cases the TdP is self-terminating and causes palpitations and/or syncope, but in the worst case the TdP degenerates into ventricular fibrillation (VF) and causes cardiac arrest or sudden cardiac death (31).

Figure 2. ECG from a loop-recorder implanted in an 18 year old female with unknown syncope episodes, showing the typical pattern seen in Torsade de Pointes.

Experimental studies have shown that the abnormal prolongation of the AP creates triggered activity, e.g., early after depolarizations (EAD), and the abnormal dispersion of repolarization is the substrate that initiates and
perpetuates TdP (32-34). Why TdP stops or why it continues is not known. A study in a LQTS population of 50 patients including 151 episodes of TdP, showed that the majority of these episodes (56 %) and QT-related extra systoles (70%) originated from the outflow tract (35). The outflow tract was defined as the area inferior to the pulmonary and aortic valve and other essentially contiguous structures, e.g., the right and left ventricular outflow tracts, and areas superior to the mitral and tricuspid annulus.

Other ECG changes associated with LQTS

A prolonged QT interval has been shown to be associated with a high-grade AV-block especially in children (36). T-wave alternans is a macroscopic every-other-beat variation in T-waves and is a sign of a major electrical instability, and identifies particular patients with high risk of malignant arrhythmias (37) (Figure 3). In infants, subtle notches in the T-wave can be normal. However, in adults these notches are not normal, and subtle notched T-waves in lead II or V4-V6 should give suspicion of LQTS (38). It has been recognized that a typical T-wave morphology may be associated with specific genetic types. However, because there are overlaps in the T-wave morphology between the affected genes, the T-wave morphology is difficult to use in prediction of the affected gene in individuals with LQTS (39).

Figure 3. Twenty-four hour Holter recording from an 11 year old boy with Long QT syndrome showing T-wave macroscopic alternans.
The “Schwartz score”

The “Schwartz score” was presented in 1993 (updated 2011) and proposed as a quantitative approach in the diagnosis of LQTS (Table 2) (19). The scoring system evaluates not only the length on the QT interval but includes other ECG findings together with clinical history and family history.

**Table 2. LQTS Diagnostic Criteria Schwartz 2011**

<table>
<thead>
<tr>
<th>Electrocardiographic Findings*</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>A QTC **, ms</td>
<td></td>
</tr>
<tr>
<td>≥480</td>
<td>3</td>
</tr>
<tr>
<td>460-479</td>
<td>2</td>
</tr>
<tr>
<td>450-459 (men)</td>
<td>1</td>
</tr>
<tr>
<td>B QTC** 4th minute of recovery from exercise stress test ≥480</td>
<td>1</td>
</tr>
<tr>
<td>C Torsade-de-Pointes***</td>
<td>2</td>
</tr>
<tr>
<td>D T-wave alternans</td>
<td>1</td>
</tr>
<tr>
<td>E Notched T-wave in 3 leads</td>
<td>1</td>
</tr>
<tr>
<td>F Low heart rate for age ****</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical History</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A Syncope ***</td>
<td></td>
</tr>
<tr>
<td>With stress</td>
<td>2</td>
</tr>
<tr>
<td>Without stress</td>
<td>1</td>
</tr>
<tr>
<td>B Congenital deafness</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family History</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A Family member with definite LQTS *****</td>
<td>1</td>
</tr>
<tr>
<td>B Unexplained SCD &lt;30 year of age among immediate family members *****</td>
<td>0.5</td>
</tr>
</tbody>
</table>

LQTS long QT syndrome; *absence of medications or disorders known to affect these electrocardiographic features; **QTC calculated by Bazett formula QTC=QT/√RR (RR in seconds); ***mutually exclusive; ****resting heart rate below the second percentile for age; *****the same family member cannot be counted in A and B.

Score: ≤1 point: low probability of LQTS; 1.5-3 points: intermediate probability of LQTS; ≥3.5 points: high probability. Modified from (19).
Risk stratification in the LQTS

In the general LQTS population, and in particular in the LQT1 population there is a higher risk of cardiac events until puberty in males, whereas females has a higher risk during adulthood (40). Based on the probability of a first cardiac event (syncope, cardiac arrest or sudden death) before the age of 40, and without therapy it has been proposed a protocol for risk stratification among patients with LQTS according to genotype and sex. For instance, there is a high risk (≥ 50 %) if the QTc is ≥ 500 ms in a carrier of a mutation in the KCNQ1 or KCNH2 gene or in a male carrier with a mutation in the SCN5A gene (41). LQTS patients have variability in the QTc between follow-up ECGs. The maximum QTc interval contain prognostic information in addition to the baseline ECG, therefore it has been suggested in the risk stratification of LQTS to always record follow-up ECGs (42).

Prophylactic treatment of symptoms in Long QT syndrome

With prophylactic medication and minor lifestyle changes it is possible to effectively reduce mortality and morbidity in LQTS (43). It is thus important to identify even asymptomatic individuals and for this we need accurate diagnostic tools. β-adrenergic blocking agents are the first choice in prophylactic therapy of LQTS patients (43). This medication is most effective in individuals with mutations in the KCNQ1 gene, but somewhat less effective in KCNH2 gene (44). Many of the cardiac events during prophylactic treatment are thought to be caused by non-compliance or use of QT-prolonging drugs (45).

Implantable cardioverter defibrillator (ICD) is recommended in patients with cardiac arrest (43, 46, 47). Left cardiac sympathetic denervation (LCSD) may be used when prophylactic beta-blocking therapy has failed and/or were there has been “storms” of repeated defibrillation because of TdP in subjects with ICD (47).
Physiologic basis of the ventricular repolarization

Action potential of the ventricular myocytes in the normal heart

The cardiac action potential (AP) is divided in four different phases (48). The cardiac AP of the cardiac myocytes is illustrated in the upper part of Figure 1. **Phase 0:** the rapid depolarization in the ventricular tissue, caused by the rapid movement of Na⁺ (I_{Na}) through ion channels (when the membrane potential is approximal -65 mV) into the intracellular spaces of the myocytes. After just a few milliseconds the ion channels are inactivated and the channels are closed. **Phase 1:** a slight repolarization, the upstroke of phase 0 activates the channels which causes the transient efflux of K⁺ from the myocytes (“transient outward current” I_{to}). This brief repolarization phase is rapidly over and is seen as a notch in the AP. **Phase 2:** the plateau phase, this is the major determinant of the duration of the AP and is caused by the inwardly depolarization of calcium currents (I_{Ca}) and the outwardly repolarization of potassium currents (I_{K}). The ion channels (L-type calcium channels and potassium channels) have a slow activation rate and are opened in connection to phase 0, the balance of inward and outward currents between the competing ion channels determine the duration of the plateau phase. **Phase 3:** inactivation of the depolarizing current of calcium (I_{Ca}) is coupled to an increasing net outward potassium current (I_{K}) consisting of rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed potassium channels. **Phase 4:** during phase 4 the resting membrane potential is restored (-90 mV) and the baseline potential is maintained by the inward-rectifier potassium current (I_{Kr}).

There are slight differences between the AP in the myocardium and the AP in the Purkinje fibers in the ventricular conducting system. There is also a subtle difference between the myocardial layers APs where the mid-myocardium (M cells) having the longest action potential duration (APD) (49). The duration of ventricular repolarization is longer in the endocardium than the epicardium, and shorter in de basal regions compared with the apex (50). This heterogeneity of repolarization is seen in healthy adult hearts.

The interval between the beginning of Q and end of T in a surface ECG is an indirect measure of the duration of the ventricular action potentials (51) (Figure 4). Measured on a surface ECG, the QT interval consists of two parts, the QRS interval and the JT interval, which reflects the depolarization (in the His-Purkinje system and ventricles) and the duration of the repolarization, respectively. The depolarization is spread through the
Purkinje fibers from endocardium to epicardium and the repolarization is synchronized in opposite direction from epicardium to endocardium.

**Figure 4.** The QT interval corresponds to the interval between the Q wave and the end of the T-wave. When the QT interval is rate corrected (Bazett's formula) the preceding R-R interval (seconds) is used QTc=QT/(√RR).

**Prolonged action potential**

To maintain the correct balance between internal and external ion concentrations, it is essential that the ion currents can flow through the cell membrane of the myocytes. If there is an increase of the inward current or decrease of the outward current during the repolarization, a prolonged AP occurs (50). This will be reflected in the ECG and appears as a prolonged QT interval with alterations of the ST segment and T-wave morphologies. Abnormalities in the ST segment and T-wave of the QT interval can be classified as primary or secondary repolarization abnormalities (52). In absence of changes in the depolarization, the primary changes depend on changes in the shape and/or the duration of the repolarization phase 3 of the AP. Such changes may be caused by, e.g., ischemia, electrolyte abnormalities (Ca++ and K+), myocarditis, ion channelopathies (e.g., LQTS) but also abrupt changes in heart rate or body position. A change in the QRS that gives rise to abnormalities in the ST segment and T-wave is called secondary repolarization abnormalities. These may occur because of voltage gradients and become manifest due to changes in the depolarization that alter the repolarization. Secondary ST and T-wave abnormalities occur with bundle-branch blocks, ventricular pre-excitation and ectopic ventricular beats and paced ventricular complexes.

**Ventricular gradient**

Wilson *et al.* introduced the concept ventricular gradient (VG) that deals with primary versus secondary repolarization abnormalities (53, 54). The QRST area in a single ECG lead reflects the summarized local electrical activity in the myocardium, viewed from the angle of the particular ECG-lead. Commonly the orthogonal leads X, Y and Z in a VCG are used, since the
QRST-areas in the X, Y and Z-leads, together with the angle between the separate QRS and T areas, are used in the mathematical expression of the VG. An abnormal T-wave axis with a normal QRS axis indicates primary repolarization abnormalities. Secondary repolarization abnormalities, e.g., in left bundle-branch block the ST and T-wave vectors changes in the opposite direction of the mean QRS vector (52).

Heterogeneities of ventricular repolarization

The differences in the time-course of the repolarization between cells in different myocardial layers (endocardium, mid-myocardium and epicardium) contribute to the T-wave of the ECG (55). Voltage gradients, developing as a result of the different time course of repolarization of phase 2 and 3 in cells of the three myocardial layers, give rise to opposing voltage gradients on either side of the M region (mid-myocardium), which are in large part responsible for the inscription of the T-wave (56). In an upright T-wave, the earliest repolarization is seen in the epicardial cells and the latest is in the AP of the M cells. Full repolarization of the epicardial cells’ AP and the M cells’ AP, coincides with the peak of the T-wave and end of the T-wave, respectively. Therefore, the duration of the M cells’ AP, determines the QT interval, whereas, the duration of the epicardial AP determine the QTpeak interval.

Heterogeneities of VR have long been associated with arrhythmogenesis. Increase of the spatial heterogeneity (hereafter called dispersion) of repolarization has been identified as the substrate of arrhythmias both in LQTS and acquired LQTS (55). Triggered activity, which is both substrate and trigger for TdP in LQTS, may be induced by EADs which in turn may be induced by accentuated spatial dispersion secondary to an increase of the transmural, trans-septal or apico-basal dispersion of repolarization (55). Experimental models of LQT1, LQT2 and LQT3 have been developed from canine arterially perfused left ventricular wedge preparations (57, 58). Such studies suggests that the prolongation of the APD in the M cells leads to a prolongation of the QT interval, as well as an increased transmural dispersion of repolarization, both of which contributes to development of TdP (59-61).

The T_{peak}-T_{end} interval (T peak to T end, the last part of the QT interval and final repolarization) has been proposed as an index of transmural dispersion of repolarization (62). It has been shown that this interval is increased in patients with LQTS (63). T_{peak}-T_{end} have been suggested to have a potential value in predicting the risk of developing TdP (30, 64-66). However, further
studies are needed to evaluate these indices of electrical dispersion and the prognostic value in the assignment of arrhythmic risk.

**Manual methods to define the end of the T-wave**

It has been shown in several studies that the manual measurement of the QT interval is bound to have errors because of the difficulties to define the end of the T-wave. This leads to both intra observer variability and variability between different observers.

The difficulty to identify the end of the T-wave may be amplified by a fusion of the U-wave and the T-wave, by a T-wave that coincide with the following P-wave (high heart rates), by a biphasic T-wave, and by low amplitudes of the T-wave. There is also a problem to choose in which lead the QT interval should be determined since no consensus has emerged among different researchers. In paper I and II we used the method to define the end of the T-wave that was described by Goldenberg (67) (Figure 4). This method was proposed to be used in the day-to-day practice in the diagnosis of LQTS and other repolarization disorders. The longest QT value from the mean of 3-5 ECG complexes in lead II and V5 or V6 should be used. As with other similar methods, this may lead to elements of subjectivity, in particular when biphasic T-waves and U-waves interrupt the return of the T-wave to the baseline.

Another way to measure the QT interval, is by using the intersection of a tangent to the steepest slope (“peak-slope”) of the last limb of the T-wave and the baseline in lead II or V5 (Figure 5). The proponents of the tangent method believe that it gives a greater consistency in the measurement of the QT interval (68). When comparing the tangent method with the “threshold method” (T-wave offset when the T-wave reaches the isoelectric baseline) in healthy individuals with a normal T-waves, the tangent method gives shorter QT interval by up to 10 ms (69). The tangent method provides longer QT intervals than the threshold method when used in subjects with changed T-wave morphology, flat T-waves and U-waves as seen in drug induced QT prolongation (70).
Figure 4. A) Normal T-wave: T end is when the descending limb returns to TP baseline. B) Separated T and U wave: T end is when the descending limb of the T-wave returns to TP baseline before the onset of the U wave. C) Biphasic T-wave: T1 and T2 with similar amplitude, T end is when T2 returns to TP baseline. D) When a second low-amplitude interrupts the end of the larger T-wave: T end can both be at the nadir (1) of the two waves and at the final return to TP baseline (2) U wave? TP baseline corresponds to the baseline between the end of the T-wave and the start of next P wave. Modified from (67).

Figure 5. Tangent method: With a tangent drawn to the steepest slope in the end of the T-wave in lead II or V5. The intersection between baseline and the tangent is the end of the T-wave. The R to R interval is measured from the preceding R-R interval.

“Normal” QT interval

Is the QTc normal? This is the question that we face every time we measure and evaluate the QT interval. In two large population-based studies, including 12,500 and 40,000 healthy individuals, the following normal limits of QTc were suggested according to the 97.5th percentiles for the lower
and upper limits in healthy subjects: Adult males 350-450 milliseconds (ms) and, for adult females 360-460 ms (71, 72). In the guidelines from AHA/ACC/HRS QTc values ≥ 450 ms for men and ≥ 460 for women have been stated as prolonged QT (52). For the diagnosis of LQTS, Sami Viskin has proposed an upper limit for prolonged QTc ≥ 470 ms for males and QTc ≥ 480 ms for females, even if the individual is asymptomatic and have a negative family history (23). It is unusual that the LQTS diagnosis is based only on a prolonged QTc - usually more evidence is required (73). The most accepted QTc limits among both adults and children have been suggested by Goldenberg et al. (67, 74) (Table 3). Goldenberg’s study further revealed a difference in QTc depending on age and sex among adults. The QTc difference depending on sex seems to disappear with old age (75).

Table 3. Bazett corrected QTc values for diagnosing QT prolongation

<table>
<thead>
<tr>
<th></th>
<th>Children 1-15 y (ms)</th>
<th>Adult male (ms)</th>
<th>Adult female (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal QTc</td>
<td>&lt;440</td>
<td>&lt;430</td>
<td>&lt;450</td>
</tr>
<tr>
<td>Borderline QTc</td>
<td>440-460</td>
<td>430-450</td>
<td>450-470</td>
</tr>
<tr>
<td>Prolonged QTc</td>
<td>&gt;460</td>
<td>&gt;450</td>
<td>&gt;470</td>
</tr>
</tbody>
</table>

Modified from (67).

Other sources used to define limits for the QT interval are studies of populations with genetically confirmed carriers and non-carriers of LQTS mutations (76). Such studies have documented carriers with normal QTc as well as non-carriers with prolonged QTc. It has been found that the diagnostic criteria QTc ≥ 450 ms fails to identify 10% of the carriers and incorrectly diagnose 10% of the non-carriers. It is known that the mutation carriers with normal QTc account for about 36% of the LQT1 carriers, 19% of the LQT2 carriers and 10% of the LQT3 carriers (41).

There is a pronounced diurnal variation of the QT interval in the normally innervated heart. This follows changes in neurally mediated autonomic tone, mainly parasympathetic, during sleep and on the circadian variation in circulating catecholamines (77). It has been demonstrated in healthy individuals that autonomic conditions, independent of the heart rate, directly affects the ventricular myocardium and causes changes in the QT interval, and this can complicate the clinical assessment (78).
The QT interval corrected for heart rate

In healthy individuals, the QT interval shortens with increasing R-R interval. Therefore, it has become standard practice to use a correction formula, such as the Bazett formula (79), to normalize the QT interval to a heart rate of 60 beats per minute (bpm), yielding the rate-corrected QT or QTc = QT/(√RR), where RR is the preceding R-R interval in seconds. When using the Bazett formula for heart rates below 60 bpm the QTc is somewhat overcorrected resulting in too short QTc intervals (false-negative values). The opposite takes place when correcting heart rates above 90 bpm, where Bazett's formula gives too long QT intervals (false-positive values) (67). In borderline cases, it would be advisable to repeat the measurements during heart rate > 60 bpm or < 90 bpm. The Fridericia formula (80) QTc=3√R-R has mostly been used in children because it reflects a more accurate QTc in higher heart rates, but it has the same limitations at slow heart rates as Bazett's formula (67). Numerous other formulas have been suggested that may give a more uniform correction over a wide range of heart rates (48). However, the Bazett and Fridericia formulas are the most frequently used because they provide nearly equivalent results for the diagnosis of QT prolongation in subjects with normal heart rate, i.e., between 60-90.

The relationship between the QT interval and the heart rate has a substantial inter-subject variability and a strong intra-subject stability (81). It is also known that the QT interval duration does not instantly adapt to heart rate changes, since there is a lag time before the QT is stabilized phenomenon known as QT-RR hysteresis (82, 83). The lag time has been shown to be individual and is approximately 2 minutes (84), but may be prolonged in cardiac disease and may be altered by autonomic perturbations (85).

The corrected QT interval and bundle branch block

In the left and right bundle branch-block (BBB) the excitation time is prolonged and QT interval prolongation is induced. The easiest correction would be to subtract 70 ms from the calculated QTc in case of left bundle-branch block (LBBB), or to subtract 40 ms in right bundle-branch block (RBBB) (86). To detect prolonged repolarization in BBB, the JT interval or Bazett’s QTc-QRS has been proposed (87). A study evaluating the previous proposal was performed in 11,739 individuals with normal conduction and 1,251 individuals with BBB (88), but still there was a residual correlation between the heart rate and the QT interval (r=0.54) in BBB, whereas the residual correlation was r=0.32 in the group with normal conduction. When comparing six different formulas for QT or JT correction in BBB in two different groups at different heart rates, the Hodges formula [QTcH = QT +
1.75 (heart rate – 60)] was found to produce the smallest heart rate dependency (89).

**Other clinical conditions associated with prolonged QT interval**

There are other conditions than LQTS that are associated with a prolongation of the QT interval and may cause TdP. Drugs commonly cause the “acquired long QT syndrome”, e.g., certain antibiotics, some antidepressants, antihistamines, diuretics, anti-arrhythmic drugs, cholesterol-lowering drugs, diabetes medications and some antifungal and antipsychotic (www.azcert.org). Also cardiac disorders such as chronic heart failure, cardiomyopathies and bradycardia due to sinus dysfunction or as mentioned, conduction block has been shown to prolong the QT interval. It is not uncommon to find a prolonged QT interval in, e.g., people suffering of anorexia-nervosa (90, 91), or subarachnoidal hemorrhage. Electrolyte imbalance especially hypokalemia, hypo magnesaemia and hypocalcaemia, is also a common cause of prolonged QT interval as well as intracoronary contrast injection and resuscitation (32).

**Electrocardiographic recordings**

The electromotive forces of the heart can be recorded by two different systems, the scalar and the vectorial system. The scalar system measures the difference in the APs of the heart between two leads, a negative and a positive on the body surface, or a positive on the body surface and a constructed zero potential. The vectorial system presents the projection of the electromotive forces of the heart viewed in three mutually perpendicular directions. In addition to the differences in potential and speed, the vectorcardiogram adds the direction of the electromotive forces.

**Vectorcardiography**

The clinical usefulness of vectorcardiography (VCG) is well documented. It has advantages over the 12-lead ECG, in particular in describing the increased dispersion in ventricular repolarization (VR) and the association to T loop morphology (92-95). With a distinct stop, the end of the T-wave loop is easy to detect with VCG compared with ECG. VCG also gives information about the spatial orientation of the repolarization and also a more
anatomically reliable T vector loop, as compared to a 12-lead ECG. VCG describes the variation in electrical activity in the heart as a single dipole. In each moment of a heartbeat, a spatial vector with an arrowhead is depicted representing the orientation and the magnitude (length of the vector) of the dipole. When each instantaneous single vector is plotted consecutively in a heartbeat, continuous vector loops are formed in space for each heartbeat. If the configuration of all three loops in all three planes are analyzed simultaneously, it is possible to calculate the vector magnitude by using the direction and magnitude of the spatial vector (Pythagorean formula); vector magnitude = √X²+Y²+Z² (Figure 6).

![Figure 6](image)

**Figure 6.** The vector magnitude constructed from Frank leads X, Y and Z. The interval between the letters Q and F correspond to the QT interval. Reprinted from (96). Reproduced with permission from Elsevier.
From the three orthogonal leads X, Y and Z, the spatial VCG can be recorded and projected. Ernest Frank developed a system to compensate for the nonspherical human torso and the eccentric origin of the heart’s electrical activity. The three Frank orthogonal leads X, Y and Z are calculated from the electrodes using the equation shown in \textbf{(Figure 7)} (6, 97, 98).

\begin{equation}
X = (0.610 \times A) + (0.171 \times C) - (0.781 \times I)
\end{equation}

\begin{equation}
Y = (0.655 \times F) + (0.345 \times M) - (1.000 \times H)
\end{equation}

\begin{equation}
Z = (0.133 \times A) + (0.736 \times M) - (0.264 \times I) - (0.374 \times E) - (0.231 \times C).
\end{equation}

\textbf{Figure 7.} Frank lead system, with the 3 orthogonal leads X, Y, and Z recorded by 8 electrodes. Reprinted from (96). \textit{Reproduced with permission from Elsevier.}
If two of the three leads X, Y and Z are depicted in a coordinate system a separate loop can be constructed for each ECG component P, QRS and T-loops and presented in three planes (Figure 8).

X; Y Frontal plane

X; Z Transversal plane (or horizontal)

Y; Z Sagittal plane

Figure 8. QRS and T loops calculated from leads X, Y, and Z. The upper loops showing the QRS and T loop together, the lower only the T loop. Reprinted from (96). Reproduced with permission from Elsevier.
**VCG loop characteristics**

The depolarization and repolarization of the heart can be described by analyzing the direction, the morphology and the interrelationship between the characteristics of each loop.

The T vector orientation with its maximum vector in space under the repolarization is described in following parameters:

- **Tazimuth (°)** – is the angle in the transverse plane (X-Z). The angle is 0° when the vector is pointing to the left. Forward direction is defined as 0°-180° and backward direction as 0°-(-180°) (Figure 9).

- **Televation (°)** – is the angle between the vector and the Y axis. It is 0° when the vector is pointing downwards and 180° when the vector is pointing in the cranial direction (Figure 9).

![Figure 9](image)

**Figure 9.** Dotted line: T vector loop, dashed line arrow: maximum T vector in space. **A)** Tazimuth expresses the angle in the transverse plane (X,Z) vector pointing to the left 0°, forward direction 0° to 180°, backward direction 0° to -180°. **B)** Televation expresses the angle between the T vector and the Y-axis perpendicular to the transverse plane (0° = vector pointing downward, 180° = vector pointing in the cranial direction). Reprinted from (99). **Reproduced with permission from Elsevier.**
The QRS and T vector orientation and their interrelationship can be described by the orientation of the maximum vector in space.

- **QRS – T vector angle (°)** – Maximal angle between the QRS and T vector loop.

The morphology of the T vector loop can be described by $T_{avplan}$, $T_{eigenv}$ and $T_{area}$ (93);
- **$T_{avplan}$ (µV)** -is the mean distance between the sample values of the T loop and its preferential plane, a measure of the T loops bulginess. Increased dispersion of the repolarization is reflected as a high value of the $T_{avplan}$ (100) (Figure 10).

![Distance between T vector loop and preferential plane](image)

**Figure 10.** $T_{avplan}$ (µV) expresses the bulginess of the T loop in relation to a preferential plane; $T_{avplan}$ can also be defined as the mean distance of the T loop from the preferential plane. Reprinted from (101). *Reproduced with permission from Elsevier.*

- **$T_{eigenv}$** (unitless) expresses the form and symmetry of the T loop by the quotient between the two highest eigenvalues (≈ diameters; $d_1$ and $d_2$) of the matrix of inertia. $T_{eigenv} = d_1/d_2$ where $d_1 > d_2$, $T_{eigenv} = 1$ corresponds to a circular T loop (sign of increased dispersion of the repolarization) and a higher value correspond to a normal more elongated T loop. $T_{eigenv}$ can be described as the longest axis ($d_1$) of the T loop that can be rotated most easily and $d_2$ is the perpendicular axis to $d_1$ that allows the easiest rotation. There is a third perpendicular axis $d_3$, but it has a value close to 0 and is therefore negligible (Figure 11).
**Figure 11.** $T_{eigen}$ (unitless) expresses the form and symmetry of the T loop and is the quotient between the highest $d_1$ and $d_2$ (= diameters) of the T loop. Reprinted from (101). *Reproduced with permission from Elsevier.*

- **$T_{area}$** ($\mu$Vs) is the spatial area under the curve formed by the moving heart vector during the J-T<sub>end</sub> interval in X, Y and Z leads: $(T_{x^2} + T_{y^2} + T_{z^2})^{\frac{1}{2}}$.

- **QRS<sub>area</sub>** ($\mu$Vs) is the spatial area under the curve formed by the moving heart vector during the Q-J interval in X, Y and Z leads: $(QRS_{x^2} + QRS_{y^2} + QRS_{z^2})^{\frac{1}{2}}$.

**Other VCG parameters**

- **$T_{p-e}$** (ms) - $T_{peak}$ to $T_{end}$, the last part of the QT interval and final repolarization in the QRST complex.

- **Ventricular Gradient** ($VG\ \mu$Vs) describes the dispersion of action potential morphology throughout the ventricles, (Figure 12).
Figure 12. Ventricular Gradient (VG µVs). The spatial ventricular gradient (VG) sometimes referred to as the QRST area is the vectorial sum of the QRS area and T area vectors, taking into account the angle between them. VG = (QRS area² + T area² + 2*QRS area*T area*cosineα)¹/².

α is the angle between the QRS area vector and the T area vector (0° to 180°), QRS area and T area are the spatial areas between the baseline and the curve formed by the moving vector during QJ and JT intervals respectively. Reprinted from (102). Reproduced with permission from Elsevier.
Aims

The aim of this thesis was to improve the diagnostics and risk assessment in the LQTS by

I: Examining electrocardiographic methods for measurement of the QT interval

II: Determining the precision of QT measurements in a pediatric population

III: Comparing vectorcardiographic parameters between individuals with mutations in the KCNQ1 or KCNH2 gene.

IV: Investigating if Swedish carriers of the Y111C mutation compose a founder population which could be an important source for future studies.
Materials

Overview of the LQTS study population in paper I-IV

Subjects

The index cases and family members in paper I-III were recruited from the LQTS Family Clinic at the Centre for Cardiovascular Genetics at Umeå University Hospital. The healthy volunteers were recruited from hospital staff at Umeå University Hospital and their relatives. The healthy volunteers had to fulfill the following criteria: absence of known heart or lung disease; no medication affecting the cardiac repolarization; and no history of unexplained syncope or SCD in any relative younger than 40 years of age. Genetic testing was performed in index cases and family members, but not in the healthy volunteers.

The LQTS population (by genetic testing confirmed carrier of LQTS mutation) was matched for age and sex with a control population (healthy volunteers and healthy family members’, non-carriers of LQTS mutation) 1:1 (paper I-II).
Seventy-five LQT1 individuals (≥ 17 year of age) were matched (age and sex) 1:1; and 3 individuals were matched 1:2 with 78 healthy controls. Five LQT1 children (≤ 16 year of age) were matched 1:1; and 19 were matched 1:2 with 43 healthy children (paper III).

In paper IV, index cases and family members were included from the LQTS Family Clinic at the Centre for Cardiovascular Genetics at Umeå University Hospital and also through national referrals to the Department of Clinical Genetics at Umeå University Hospital.

Subjects in paper I, included from 2005 until 2008

*The LQTS population* consisted of 94 carriers: 84 LQT1 and 10 LQT2, 16 were index cases and 78 family members. There were 25 *adult males*: 12 carriers of Y111C, 5 R518X, 6 with other LQT1 mutations and 2 with LQT2 mutations. There were 45 *adult females*: 16 carriers of Y111C, 18 R518X, 6 with other LQT1 mutations and 5 with LQT2 mutations. There were 24 *children* of whom 10 were boys: 2 carrying Y111C, 6 R518X, one other LQT1 mutation and one LQT2 mutation; 14 were girls: 5 carrying Y111C, 7 R518X and 2 with LQT2 mutations.

*The control population* consisted of 28 family members that were genetically confirmed non-carriers and 66 healthy volunteers. There were 25 *adult males*: 11 non-carriers and 14 healthy volunteers. There were 45 *adult females*: 13 non-carriers and 32 healthy volunteers. There were 24 *children*: 10 boys of which 2 were non-carriers and 8 healthy volunteers; 14 were girls of which 2 were non-carriers and 12 were healthy volunteers.

Subjects paper in II, included from 2005 until 2009

*The pediatric LQTS population* consisted of 35 mutation carriers: 29 LQT1 carriers (13 Y111C, 14 R518X and 2 other LQT1 mutations) and 6 LQT2 carriers.

*The pediatric control population* consisted of 10 family members that were genetically confirmed non-carriers and 25 healthy volunteers.

Subjects in paper III, included from 2005 until 2011

*The LQTS population* consisted of 118 carriers: 99 LQT1 and 19 LQT2. There were 36 *adult males*: 21 carriers of Y111C, 7 R518X and 8 with LQT2 mutations. There were 58 *adult females*: 26 carriers of Y111C, 21 R518X and
11 with LQT2 mutations. There were 24 children: 10 carriers of Y111C and 14 carriers of R518X.

The control population consisted of 121 individuals. There were 30 adult males: 16 family members that were genetically confirmed non-carriers and 14 healthy volunteers. There were 48 adult females: 16 family members that were genetically confirmed non-carriers and 32 healthy volunteers. There were 43 children: 15 family members that were genetically confirmed non-carriers and 28 healthy volunteers.

Subjects in paper IV, included from 2005 until 2010

The LQTS population consisted of 37 index cases and 21 family members all carriers of the Y111C mutation in the KCNQ1 gene.

The control population consisted of 84 healthy military recruits, matched for origin (northern Sweden), corresponding to 168 control chromosomes.

Ethical considerations

All studies were conducted in accordance with the Helsinki Declaration for Ethical principles for Medical Research Involving Human Subjects (2000). All studies were approved by the Regional Ethical Review board at Umeå University (Umeå Sweden) Dnr: 05-127M.

All participants in the study gave written consent including the legal guardians of the participating children. Information concerning genetic testing and execution of haplotype analysis was provided to all.
Methods

Paper I-III: In all individuals a 12-lead ECG was recorded during rest in a supine position, and approximately 2 minutes later this was followed by the recording of a VCG. For VCG recordings, five electrodes were applied around the chest at the level where the 4th intercostal space meets the sternum, one in the neck, one on the left hip, and one zero point on the right hip (according the Frank system) (103, 104). All the recordings were performed by the same person.

12-lead ECG

*Automatic measuring and interpretation of the QT interval*

In paper I and II the 12-lead ECGs were recorded with a Mac 5000 version 008B using the equipments 12SL algorithm for automatic calculation and interpretation of the QT interval (GE Medical System, Information Technologies, Milwaukee, WI, USA) (105). The paper speed was 50 mm/s, the amplitude gain 10 mm/mV, and the sampling rate 500 samples per second. The signal was amplified in a band between 0.31 and 150 Hertz (Hz). QRS complexes of the same morphology were aligned in time during 10 seconds recording without noises and a representative complex was generated from the medians obtained at the successive sampling points. The end of the T-wave was defined on the vector magnitude, which was calculated from the sum of the absolute value of I, II and V1-V6 and its first-order difference. The T-wave end was defined as when a new point contributes to the T area and when that point in the accumulated T area is below a threshold value (1%). The QT interval was then calculated from the earliest Q wave in any lead to the T-wave end. According to the equipments 12SL algorithm the QT interval was categorized as normal, borderline, or prolonged according to the specific criteria of age, sex and heart rate (105) (Figure 13).
**Figure 13.** Presentation of values and interpretation in 12-lead ECG from the equipment Mac 5000 version 008B. Calculated parameters: *Heart rate 56 BPM, PQ-interval 130 ms, QRS-duration 94 ms, QT/QTc 524/505 ms and PR-T axis 56, 52 and 39.* Interpretation: *Slightly slow sinus rhythm with sinus arrhythmia, prolonged QT-interval, pathological ECG.*

*Manual measurement of the QT interval*

All 12-lead ECGs were coded and, no automatic values or interpretations were visible for the observers (paper I-II). Four experienced observers repeated manual measurement of the QT interval in 42 12-lead ECGs on two occasions with a one week interval between measurements (paper I). All observers had verbal and written instructions on how to perform the measurements (Appendices I): the T-wave end was defined according to the proposal by Goldenberg I et al. (67). The QT interval was corrected for heart rate according Bazett’s formula. Each observer made a statement based on cut-off QTc values according to age and gender for diagnosing LQTS (*Table 3*); normal, borderline (suspected LQTS) or prolonged QT interval (LQTS). The observer with the lowest intra-observer variability performed the measurements in the subsequent 146 ECG recordings in paper I and also the 70 ECGs in paper II.

**VCG according Frank lead system**

*Automatic measuring of the QT interval*

The CoroNet II system (Ortivus AB, Danderyd, Sweden) was used to recording all VCGs in paper I, II and III. In paper I the older MIDA1000 was also used, but there are no relevant differences between the systems. The orthogonal leads X, Y and Z were recorded in 3-4 minutes, where the signals were sampled at 500 Hz (amplified band width 0.03-170 Hz). The recording was performed during period 60 seconds (s) for determination of the parameters describing the spatial QRS and T vector, T vector loop and QRST intervals.
The average vector magnitude was computed from the X, Y and Z leads (vector magnitude = \( \sqrt{X^2+Y^2+Z^2} \)), the detections points were located in relation to the Q-point which had the value 0 and the isoelectric line was located 30 ms before the start of Q. The detection point Q to F corresponded to the QT interval (Figure 6), and the mean heart rate for one minute was used in the automatic calculation of the QTc.

The CoroNet II algorithm defines the beginning of QRS and the end of the T-wave from the computed derivative of the vector magnitude. In order to find out the detections points for T end, the algorithm starts from the T peak and seeks forward until the magnitude decreases with 1/3 of the value of T peak. The algorithm seeks further forward to a predefined smaller value, and when the slope of the magnitude crosses that point the algorithm defines this as the T end. In a similar way the detection point of the QRS start is defined, from the highest derivative of the QRS complex and backwards until the magnitude has been decreased to 1/10 or a predefined value. When the detection point of T end is defined and a U wave is an early part of the T-wave, the U-wave was included in the QT interval. If the U-wave comes after the T-wave, it is not included in the QT interval.

**Off-line analysis of the VCG**

Paper III: All off-line analyses were performed by one observer. Detection points were automatically positioned and presented in the vector magnitude (Figure 6). The last minute of the recording was chosen for analysis; if there were extrasystolic beats the previous minute were chosen. The end of the T-wave was manually assessed by the tangent method (106). Roughly 90 % of the T-wave end detection points needed manual adjustment. After all recordings had been reviewed, the data was processed and the VCG parameters were determined.

**Genetic testing**

Genomic DNA was prepared from whole blood using the standard salting out method. Evaluation was performed in order to confirm the presence of mutation in \( KCNQ1 \) and \( KCNQ2 \) genes. Amplified Polymerase Chain Reaction (PCR) fragments were screened for sequence variants by denaturing high-performance liquid chromatography. In case of variant elution peaks, further analyses were performed with DNA sequencing (CEQ8000 sequencer Beckman Coulter, Fullerton, CA). When a mutation was identified in an index case, the family members subsequently underwent
direct analysis using MGB-probes by TaqMan 7000 (Applied Biosystem, Carlsbad, CA). Based on the molecular diagnosis each subject was classified as either genotype positive or genotype negative for LQTS (Paper I-IV).

**Genealogy, geography and haplotype analysis**

Paper IV investigated if the high number of index cases and family members carrying the Y111C mutation in the *KCNQ1* gene constitutes a population sharing a common haplotype, together with common geographic and genealogic origin. The age of the mutation and prevalence were also calculated.

**Genealogical and geographic analysis**

The genealogic searches were performed by tracing all maternal and paternal ancestors of 37 index cases, and then connecting as many index cases as possible in as few generations as possible with a common couple (founder couple). The maternal or paternal ancestors connecting to the index cases and the common founder couple were defined as obligate carriers of the family mutation. All obligate carriers including siblings and spouses were traced: sex, place of birth and death, were noted if possible. Spouses were traced to investigate the marriage pattern within or outside the river valley. The birth places were traced to establish a possible clustering of ancestors within a geographic area.

According to the population records initiated by the Crown in Sweden in the year of 1686, the priesthood was obliged to execute annual catechetical examinations in their local parishes and register the inhabitants by including births, deaths, marriage and migration. The documentation before 1686 was often based on e.g., estate inventory proceedings and court proceedings. These registries give us a unique opportunity to investigate our ancestors. Genealogical data were collected from church registries, parish books, and census archives at the Research Archive at Umeå University. Data were also collected from the Swedish archive information homepage (www.svar.ra.se) and researcher’s private genealogic database.

All genealogic data were entered and stored in DISGEN computer software (Lakewood, CO, USA). Open Source software (Inkscape vector graphic editor) and GIMP (GNU image manipulation program) were used to construct pedigrees and geographic maps.
Below is an example from a church registry of people who died in the parish, with names and causes of death documented. The individuals in the example registry have no connection with the LQTS families.

**Haplotype analysis**

To determine if the index cases and their family members were related and had the same genetically origin due to founder effect, we examined if they shared a common haplotype. The haplotype represents alleles inherited together in related individuals. The closer the microsatellite markers are to each other, the smaller is the probability of recombination.

We performed haplotype analysis in each index case whenever possible, including two generations from each family. These analyses were compared with 84 healthy controls of north Swedish origin.

The point mutation c.332A>G p.Y111C is located in the KCNQ1 gene on the short arm (p) of chromosome 11 at position 15, where the base A have been changed to G causing the amino acid to exchange from Tyrosin to Cystein.

We investigated 5-6 microsatellite markers upstream and 9 downstream from the point mutation. These microsatellites are located over a physical distance of approximately 8 x 10⁶ base pairs of the chromosome. To work out the pattern of shared alleles, we reconstructed the most likely ancestral haplotype. To compare the frequency of disease associated alleles in healthy non-carriers, analysis of the same microsatellite markers were performed in 168 control chromosomes in 84 healthy controls. We used the National Centre for Biotechnology Information Entrez Gene database, and the deCODE, Généthon and Marshfield genetic maps, to choose markers for the haplotype analysis. The markers with the highest heterogeneity according to the CEPH Genotype database were selected. For each separate marker forward and reverse primers flanking the microsatellite region were used (Sigma-Aldrich Inc.). Fragment analysis was performed according to manufactory instructions, and the PCR product was analyzed using an automated capillary electrophoresis–based DNA Sequencer (Wave® 3500 HT, Transgenomic Inc, Omaha, Nebraska). GE Healthcare (United
Kingdom) and Applied Biosystems (Foster City, California) supplied solutions and material for the PCR mix. GeneMapper software version 3.7 (Applied Biosystems Inc, Foster City, California) was used to analyze the microsatellite data.

*Mutation age and prevalence of the Y111C mutation in the KCNQ1 gene*

The prevalence and age of a mutation can be investigated by analyzing the haplotypes in mutation carriers from a common descent. To possibly reduce the uncertainty in these calculations two different computer programs were used. The extent of shared alleles in index cases, the frequency of disease associated alleles in healthy controls, and the recombination frequencies for the 15 microsatellite markers was computed in the ESTIAGE software (paper IV). The software estimated the age and determined the 95% confidence interval (CI). The mutation age was defined as the age of the most recent common ancestor of the index cases. Recombination frequencies were estimated using the distance from the Y111C mutation and the markers, and the standard correspondence 1 cM = 10⁶ base pairs.

The DMLE software (www.dmle.org) was used to estimate the prevalence of the mutation. Data were entered including the haplotypes of index cases and controls. The average population growth rate per generation was estimated based on regional demographic data from the Jämtland and Västernorrland Counties from the period 1570-1950 in Statistic Sweden (www.scb.se). The “true value” of the mutation age was assumed when the confidence interval (CI) of the DMLE and ESTIAGE software overlapped.

**Statistic methods**

Data were analyzed using SPSS version 16.0 and 18.0 (SPSS Inc. Chicago, IL, USA) in paper I-III. All values were calculated as mean, range, standard deviation (SD) and min-max. To assess and identify the efficacy in diagnosing LQTS for the different electrocardiographic methods (paper I and II), sensitivity, specificity and positive and negative predictive value (PPV and NPV, respectively) were computed.

In paper I-II, we compared the electrocardiographic methods by computing receiver operating curves (ROC), and the area under the curve (AUC) (SAS version 9.1.3; SAS Institute, Cary, NC). For nonparametric comparison of AUC, the SAS-macro %ROC version 1.6 was used.
Relative error (paper I) and κ coefficient were used to assess inter- and intra-individual observer variability and the agreement between the QT measurements. Relative error was calculated as; absolute error/mean error of the pair A – B/[(A + B)/2]). Absolute error was calculated as the error from two pairs (A, B) from 2 occasions of measuring as the absolute values of their difference A – B.

Molecular genetic diagnoses were used as gold standard when comparing the measured QTc between the different methods in paper I and II.

A value of the κ coefficient > 0.75 was considered as excellent agreement, and values < 0.40 was considered poor agreement. p ≤ 0.05 was considered statistically significant.

Regression analysis was used to investigate the influence of sex, age, KCNQ1 mutation on the VR (paper III). As we found a strong relationship between age and most VR parameters, we divided the LQTS population into two age groups: ≤ 16 y (children), and ≥ 17 y (adults), respectively.

We analyzed if healthy volunteers and non-carriers of the family mutation could be merged to one control group. Linear regression was used to compare controls and KCNQ1 mutation carriers.

**Limitations**

Paper I – III: DNA analysis was not performed in healthy volunteers in the control group. However, the risk for an individual to be carrier of LQTS in the healthy group is only 1/2000 (0.005%). The generalizability in paper I-III may be limited because the LQTS populations were composed preferably of KCNQ1 gene mutations and a low number of KCNH2 mutations, but no SCN5A gene mutations.

The electrocardiographic recordings were performed without consideration of the diurnal variation or day-to-day variation of the QT interval. More stringent registration times may possibly have given somewhat different results. Paper I, II and III: No consideration was taken to beta-blocking therapy in the analyses of the LQTS population. However, in study III we showed that in our LQTS population there was only a small change in the QT interval (16 ms) caused by beta blocking therapy and no changes in the QTc.
Paper IV: DNA was available for haplotype analysis in 26 out of 37 index cases, however, we found that index cases with or without genealogic connection to the common couple shared alleles to the same extent. This fact makes it likely that the analyzed index cases sample is representative. Twenty-six out of 37 index-cases were connected in a pedigree to a common couple. Some of the reasons why not all could be connected in the pedigree could be that a child had other parent/s then as stated in the church books, or because of destroyed church books, or that incorrect data were entered by the authorities (e.g., the vicar).
Summary of Results

Paper I and II

- We found a considerable intraindividual and interindivdual variability in the manual measurement of the QT interval in 12-lead ECG among 4 observers.
- The automatic interpretation of the QTc from 12-lead ECG showed the lowest sensitivity (0.40). It provided 38 out of 94 mutation carriers of LQTS with a correct diagnosis.
- Automatic QTc measurement using VCG showed the highest sensitivity (0.90) and a positive predictive value (PPV) 0.89 and an AUC 0.948. It provided 85 out of 94 mutation carriers of LQTS with a correct diagnosis based on an age and sex limited QTc value.
- Also in a pediatric population VCG had the highest accuracy for a correct diagnosis, 30 of 35 (86%) mutation carriers were correctly diagnosed as LQTS and 28 of 35 (80 %) non-carriers received the correct diagnosis.
- Automatic measuring with VCG, 12-lead ECG and manually measuring in 12-lead ECG showed roughly the same specificity 0.87-0.89. In the pediatric study specificity was nearly equal in the four methods.

Paper III

In adult men and women ≥17 years of age:

- A significant longer QT interval \((p=0.037)\) was found in the symptomatic group when comparing 23 symptomatic and 52 asymptomatic KCNQ1 mutation carriers.
- There was a significant difference \((p=0.02)\) in QTc between the populations carrying Y111C and R518X mutation in the \(KCNQ1\) gene, where the Y111C population had a longer QTc.
- There were a significant difference between the LQT1 population and the control population were the LQT1 population had longer QTc \((p=0.000)\) and longer \(T_{p-e}\) interval \((p=0.000)\).
- The QTc and \(T_{p-e}/QT\) was longer in LQT2 carriers and the \(T_{area}\) and \(T_{amplitude}\) were greater in LQT1 carriers, but all within a normal range.
In children 1-16 years of age:

- There were a significant difference between the LQT1 population and the control population, were the LQT1 population had longer QT interval \((p=0.009)\) and longer QTc \((p=0.000)\), while the \(T_{p-e}/QT\) ratio was larger in the control population \((p=0.000)\).

**Paper IV**

The Y111C population being a founder mutation is supported by:

- Genealogic studies connected 26 of 37 living carriers of the Y111c mutation in the \(KCNQ1\) gene through a line of obligate mutation carriers to a common founder couple born 1605 resp. 1614 (Figure 15).

- Geographic clustering back to a common geographic origin in the region of the Ångerman river valley and their tributaries were traced in ancestors to all 37 index cases (Figure 16).

- Haplotype analysis traced an original ancestral haploblock in the DNA in 26 index cases and 21 family members.

- The age of the mutation was calculated to 600 years (CI 450; 850) and 575 years (95% CI 400; 900) with the ESTAGE and DMLE software, respectively.

- A prevalence of 1:1,500 – 3,000 carriers was calculated for the Y111C mutation in the \(KCNQ1\) gene in the counties of Jämtland, Västernorrland and Västerbotten.
Figure 15 Twenty-six index cases carrying the Y111C mutation in the KCNQ1 gene connected through obligate carriers of the mutation to a common couple born in 1605 and 1614 spanning 13 generations. ◇=Index cases; H=Index cases with haplotype data. Reprinted from (107). Reproduced with permission from Elsevier.
Figure 16. Birthplaces of the 37 index cases and their ancestors, all carriers of the Y111C mutation in the KCNQ1 gene. Birthplaces in the 19th century are depicted in a map illustrating the geographic clustering of the mutation (grey shaded area). Depicted lines illustrate the rivers and tributaries that flow from northwest to southeast and the dots indicate the birthplace of the ancestors. The grey shaded geographic area of interest is magnified in the upper left corner. Reprinted from (107). Reproduced with permission from Elsevier.
Discussion

Manual measurements

Long QT is a widespread and potentially lethal disease because of the risk of the feared arrhythmia - known as TdP. The warning sign could be a noticed long QT interval, but it has been shown that many physicians, including many cardiologists, do not recognize a prolonged QT interval (108). This may be due to the difficulty in defining the end of the T-wave and/or a lack of experience in performing the measurements, which produces a significant intra- and inter-variability in the measurements of the QT interval. It is of utter importance to accurately measure and identify a long QT interval - both for the individual that is at risk of arrhythmia and for those without risk. In children, manual measurements of the QT interval are even more difficult due to high and variable heart rate (sinus arrhythmia).

In accordance with other studies (1, 108), we found (paper I) considerable problems with the manual method for measuring QT intervals. In a study by Postema et al., 151 students with no experience in manual measurement of the QT interval were taught to measure the QT interval by the tangent method (Figure 5). They measured and calculated QTc and interpreted four ECG (two normal and two LQTS) on two occasions and their results were compared with those of arrhythmia experts, cardiologists and non-cardiologists (109). The students achieved a correct classification rate of 71% and 77% on the first and second occasion, compared with 62% for the arrhythmia experts and less than 25% for the cardiologists and non-cardiologists. As a consequence the authors proposed that the tangent method should be used to achieve a better stratification for individuals that are at risk for sudden cardiac death (SCD).

The tangent method is reliable and easy to learn and has acceptable variability. However, the tangent method does not account for the entire QT interval and is problematic in T-waves with low amplitude (110), which may have implications for diagnosis and risk stratification. In paper I and II we used the method, as proposed by Goldenberg et al., since it includes the whole end of the T-wave and thereby the entire QT interval. The tangent method was used in the VCG off-line analysis (paper III), which makes study III comparable with other studies performed with the same VCG equipment (93, 102, 111-114).

In VCG recordings, the automatically measured QTc were compared with QTc manually measured, using the tangent method (unpublished results).
The study population consisted of both adults and children including 105 individuals with DNA verified LQTS and 118 healthy controls. In accordance with other studies we found that the mean QTc in all individuals was significant shorter, with the tangent method (427±35 ms) compared with the VCG method, (445 ±35 ms, p<0.0001) (Table 4) (69).

**Table 4.** QTc measured automatically with the VCG equipment and measured manually using the tangent method in the same recordings (unpublished results)

<table>
<thead>
<tr>
<th></th>
<th>All n=223</th>
<th>Control population n=118</th>
<th>LQTS population n=105</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCG QTc ms,</td>
<td>445±35</td>
<td>421±19</td>
<td>472±31</td>
</tr>
<tr>
<td>automatically</td>
<td>440:450</td>
<td>418:425</td>
<td>465; 476</td>
</tr>
<tr>
<td>VCG QTc ms,</td>
<td>427±35</td>
<td>404±21</td>
<td>454±29</td>
</tr>
<tr>
<td>manually tangent</td>
<td>423:432</td>
<td>400:407</td>
<td>447; 458</td>
</tr>
</tbody>
</table>

QTc: Heart rate corrected QT interval (Bazett’s formula); SD: Standard deviation; CI: Confidence interval; Control population: DNA confirmed non-carriers of a LQTS mutation and healthy volunteers; LQTS population: DNA confirmed carriers of a LQTS mutation; ms: milliseconds; VCG vectorcardiogram

**Automatic methods**

To overcome the intra– and inter-individual variability in manual measurements of the QT interval, a reliable automatic method would be valuable. But in paper I and II, we showed that automatic ECG interpretation may lead to an erroneous diagnosis. A study of 97 046 ECGs analyzed by the Marquette 12 SL ECG Analysis Program (GE Healthcare), a system that also was used in this thesis, showed a manifest underreporting of prolonged QT. Out of 16 235 (16.7%) ECGs with prolonged QTc only 7709 (47.5%) were stated as “prolonged QT” (115).

We showed in paper I a higher accuracy in automatic QTc measurement by VCG. The VCG recordings with electrodes applied according to the Frank lead system (Figure 7) and the algorithm that performed the automatic measurements of the QT interval provided the best combination of sensitivity and specificity (AUC 0.948).
The higher accuracy in automatic QTc measurement by VCG may be explained by that the vector magnitude is calculated from three orthogonal leads and that there are electrodes that cover the posterior parts of the heart (Z lead).

When investigating a pediatric population of LQTS mutation carriers and healthy controls we found that, based on QTc and a QTc limit of 440 ms, VCG identified 30/35 (86%) of the carriers. VCG incorrectly diagnosed five children as having LQTS. The automatic interpretation of the 12-lead ECG identified only 17/35 (49%) of the carriers. One reason to VCGs superiority and high precision in measurements of QT interval in a pediatric population could be that the VCG recording is longer compared to a standard ECG recording. The sampling time for the heart rate was 60 seconds for the VCG and 10 seconds for 12-lead ECG. This may have influenced the QTc, especially in a pediatric population with varying heart rate. When comparing the calculated mean heart rate between VCG and 12-lead ECG in 70 children, the mean heart rate was nearly the same. But, when analyzing each individual’s heart rate, calculated with both methods, we found that in 14 out of 70 children (20 %) the heart rate differed ≥10 bpm (max 22 bpm) between VCG and 12-lead ECG (unpublished data).

**QTc cut-off values**

There are carriers of LQTS mutations with normal QTc, and there are healthy individuals with prolonged QTc interval, as has been shown in earlier studies (73, 76). It is thus not possible to define a QTc value adjusted for age and sex that can sort out all carriers of a LQTS mutation from healthy individuals.

The distribution and different cut-off values of QTc were examined in 81 adult carriers (50 women) and 82 non-carriers (52 women) (unpublished results). Applying QTc cut-offs 430, 440 or 450 ms in the control population would state 24%, 12% and 2%, as having prolonged QT interval. If the same cut-off limits were used in the LQTS population, 96%, 90% and 80% would have prolonged QT interval. Table 5 show the percentages calculated for both populations. In Figure 14, individual QTc values are plotted against age for controls and carriers of a LQTS mutation, where lines mark the different investigated cut-off limits for prolonged QTc.

Over time there is a significant variability in the duration of the QTc. At baseline 25 % of 375 LQTS children had QTc ≥500 ms while 40 % during the
following 10 years at some point had QTc ≥500 ms (42). This highlights the importance of performing repeated ECG measurements of the QTc for the diagnosis of LQTS, and to investigate the clinical and family history before deciding to perform genetic testing (73).

Table 5. The effect of different QTc cutoff values on the statement - prolonged QT.

<table>
<thead>
<tr>
<th>QTc cut-off</th>
<th>Control Population</th>
<th>LQTS Population</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>All n=82</td>
<td>Female n=52</td>
</tr>
<tr>
<td>430 ms</td>
<td>20 (24%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>440 ms</td>
<td>10 (12%)</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>450 ms</td>
<td>2 (2%)</td>
<td>2 (4%)</td>
</tr>
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</table>

QTc: Corrected QT interval (Bazett formula); Control population: Healthy family members and healthy volunteers; LQTS population: Genetically confirmed carriers of a LQTS mutation.

Figure 14. QTc automatically measured with VCG against age for controls (left) and the LQTS study population (right). Lines show different QTc limits.
QTc cut-off levels for LQTS diagnosis were set to 430 ms, 440 and 450 ms for males, children and females respectively (paper I and II). These values correspond to the lower limit of borderline QTc, as proposed by Goldenberg et al. (67) (Table 3). These QTc limits should not be considered as absolute limits for a LQTS diagnosis, but as a wake up bell raising the question - could this be a LQTS patient?

**The “ideal” QT measurement**

The ideal ECG equipment would give us an accurate QT measurement without the manual procedure's problems. It has previously been shown that automated QT measurement provides considerable less variability (116). Modern ECG equipment provides digital on-line recordings with automatic measurements and interpretation of the ECG. It is however still important that the ECG is examined by an experienced person.

A standardized algorithm and a uniform definition of recording circumstances would improve the diagnostic of diseases that alter the QT interval. It would also improve the possibilities to compare values from different studies. This is difficult today where several different methods are in use for QT measuring and often, also in high-impact LQTS studies, without accounting of how the QT intervals were measured (method, lead, etc.). ECG recordings are performed in various settings by staff with varying educations. To maintain the quality and the clinical value it is important to have well educated paramedical staff that correctly perform the ECG recordings, knowing the importance of: correctly placed electrodes, especially the precordial leads, in relation to body surface anatomical landmarks, connecting the correct electrode cable to the correct electrode, and assuring that there is minimal interference from skeletal muscles and artifacts from skin-electrodes or implanted or external electrical or electronic devices that otherwise hampers the use of the recordings.

**Electrophysiological phenotype**

The clinical risk stratification in LQTS is based on LQTS type, age, sex, symptoms, family history and QTc duration (41). Within a family and between families affected by the same LQTS type and even the same mutation QTc can vary considerably. We have shown in paper I and II that VCG accurately estimates QT interval as compared with 12-lead ECG (96,
VCG may be more sensitive to changes in VR and provide more information than just the duration of the QT interval (101). Therefore, VCG may be a valuable contribution in the assessment of increased VR dispersion.

The Y111C and R518X mutations in the KCNQ1 gene have different effects on the Iks ion channel function (118). R518X is a nonsense mutation causing a ≤50% reduction of the channel function (haplo-insufficiency). The Y111C mutation is a point mutation with a dominant negative effect with >50% reduction of channel function (>75% has been shown in vitro) (119).

The electrophysiological phenotypes (e.g., VG, Tarea, and Tazimut) of the two mutations were examined with VCG. The findings were compared with a healthy control population and a population of mutation carriers in the KCNH2 gene. Data analyses showed that age, sex and carriership of a LQTS mutation affected most VR parameters; consequently, the population was divided into adult men, adult women (≥17 years of age), and children 1-16 years of age. All recordings were performed during rest with the individuals in supine position, which might explain why we did not find signs of increased dispersion in the ventricular repolarization except for a longer QTc in the LQTS populations. We have earlier found that carriers of the Y111C mutation in Sweden has a low incidence of life threatening cardiac events despite the severe functional defects shown in in vitro studies (120).

Is this a sign of modifying factors? Could unknown mechanisms compensate the impaired channel function by physiological redundancy expressed as retained repolarization reserve?

A more prolonged QTc and a higher number of cardiac events have been found in LQTS populations with dominant negative effect compared with haplo-insufficiency (119). The clinical phenotype is linked to the mutation and its biophysical effects on the cell membrane. It has been shown that the localization of the mutation has importance, a first cardiac event were seen more often in missense mutations and mutations located in the transmembrane part of the protein rather than C-terminally located mutations (119).
**Ventricular repolarization**

In healthy individuals it has been shown that the VR duration shortens and the \( \text{QRS}_{\text{area}} \), \( \text{T}_{\text{area}} \) and \( \text{T}_{\text{amplitude}} \) decreases with increased heart rate (113). The conclusion was that an increased heart rate in healthy individuals decreases the ventricular depolarization instant, AP morphology and thereby the global VR. We found no significant difference in heart rate and no significant changes in VG, \( \text{T}_{\text{area}} \) and \( \text{QRS}_{\text{area}} \) in paper III.

Increased autonomous nervous activity can also be critical in LQTS if the repolarization duration does not shorten appropriately during sympathetic stimulation. Pharmacologic perturbations of autonomic nervous system (ANS) and the response of VR parameters recorded by VCG have been studied in healthy individuals (102). \( \beta \)-adrenergic stimulation after muscarinic blockade (atropine) gave a transient prolonged QT interval and delayed heart rate adaption of \( \text{T}_{\text{area}} \) and VG, thus mimicking pathological conditions with increased risk of arrhythmia such as LQTS. In Y111 carriers syncope was most commonly seen in association to physical activity or emotional stress (120), as also has been shown to be a trigger in other \( \text{KCNQ1} \) mutations.

**Risk markers**

LQTS is a genetic heterogenic inherited disease with variable penetrance (30-50% of the carriers never have symptoms) and expressivity. More knowledge of the association link between genotype and phenotype is needed. The degree of QT interval prolongation is a known risk marker for syncope and/or cardiac arrest in LQTS. Despite that, syncope and/or cardiac arrest happens in 5% of LQTS mutation carriers with a normal QT interval (121).

Other diagnostic parameters and risk markers than QTc have been evaluated in different LQTS populations. Increased short-term variability of the QT interval in drug-induced LQTS and AP instability were useful in the predicting arrhythmia (122, 123), and may be used as an additive non-invasive diagnostic marker in LQTS (124). We previously performed a beat to beat analysis of the QT interval and found that most VR parameters during resting conditions showed a significant larger instability (by a factor of 2) in carriers of a \( \text{KCNQ1} \) or \( \text{KCNH2} \) mutation compared with a control population (114). The beat to beat instability of the QT interval increased with increasing QT interval, also seen as increased VR dispersion measures -
$T_{area}$ (global dispersion) and VG (action potential morphology dispersion). This may be a marker of VR variability with valuable clinical implications, but this has to be confirmed in further studies with additional clinical (phenotype) and genetically (genotype) data (125). It would be valuable to perform such studies in populations with a high number of individuals with the same mutation.

**Founder population Y111C**

Founder populations are of special interest because they give, in a genetic homogenous population, a possibility to investigate the relation between genotype, phenotype and modifying factors.

With our genealogical, geographical, epidemiological, and haplotype data we have identified a founder population in the area around the Ångerman river valley consisting of carriers of an Y111c mutation in the $KCNQ1$ gene. The mutation was estimated to date back to the 15th century. The immigrants to the inland of the northern river valleys lived relatively isolated from other river valleys, the geographic isolation created condition for the occurrences of founding of genetic sub-isolates. We investigated the marriage-pattern of the obligate carriers in the pedigree and found that the spouses under the 17th and 18th centuries were born in the Ångerman river valley. Other LQTS founder mutations have been described, e.g., the A341V founder mutation in the $KCNQ1$ gene in South Africa (126), for which in vitro studies found it to be functional mild although the clinical phenotype is severe. These findings have given valuable contributions to the understanding of risk modifiers in LQTS.

When we started the LQTS Clinic at the Centre for Cardiovascular Genetics at Umeå University Hospital we found a high number of families (unaware of each other) carrying the Y111C mutation in the $KCNQ1$ gene. We have earlier described the mutation to have a mild clinical phenotype with a low 0.005% annual incident of life-threatening events (120). The mild phenotype enabled the enrichment in the isolated river valley and has contributed to the high number of today living carriers of the Y111C mutation (> 200 carriers 2013).

These findings do not correspond with the severe in vitro electrophysiological characteristic of the mutation which has >75% mutation-associated reduction of $Ks$ channel function (118, 127). Consequently, there must be other unknown factors affecting the clinical
phenotype. This large founder population is a valuable basis for future studies of phenotype and genotype correlations and modifying factors that affect the disease.

**Conclusion**

VCG was found to be an accurate method for the initial diagnosis of LQTS.

QT interval measurements performed with vectorcardiography have high precision in a pediatric population.

Ventricular repolarization analysis by resting VCG showed a significant difference in QTc between Y111C and R518X mutations in the KCNQ1 gene. VCG showed no increased ventricular repolarization dispersion in KCNQ1 and KCNH2 mutation carriers.

We have identified the first Swedish LQTS founder population carrier of the Y111C mutation in the KCNQ1 gene. This founder population will be an important source to future LQTS research in diagnostics methods and the correlation of phenotype, genotype and modifying factors.
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References


2. Waller A. A demonstration on man of electromotive changes accompanying the heart's beat. J Physiol. 1887;8:229-34.


59. Shimizu W, Antzelevitch C. Cellular basis for the ECG features of the LQT1 form of the long-QT syndrome: effects of beta-adrenergic agonists and antagonists and sodium channel blockers on transmural


77. Bexton RS, Vallin HO, Camm AJ. Diurnal variation of the QT interval--influence of the autonomic nervous system. Br Heart J. 1986


105. Healthcare GE. Marquette™ 12SL™ ECG Analysis Program Physician’s Guide. 1 February 2007;416791-004 Revision D.


115. Garg A, Lehmann MH. Prolonged QT interval diagnosis suppression by a widely used computerized ECG analysis system. Circulation


Appendices

I Instructions in manual measurement of the QT interval, translated.

II Long QT syndrome Questionnaire, translated.
Manual measurement of the QT interval

Measure 3 QT interval in lead II, V5 and V6, the interval should be specified in milliseconds (ms). Use a ruler or sender, whatever you normally use. Measure 3 RR-intervals in lead II, specify the interval in ms.

Mark clearly on the ECG the measuring points and write down the value under the ECG complex.

Each ECG has a code - 1, 2, 3 and so on - in the attached table. Enter your measurements from ECG coded 1 into code 1 in the table and so on. Leave the box or boxes empty if you have no value to enter.

At the end of each row in the table there are 3 options for the assessment of measured QTc. The 3 options are normal, borderline and prolonged QTc according to the table below. On each recorded ECG you can read age and sex on the individual. Cross mark the corresponding box for your assessment.

<table>
<thead>
<tr>
<th>Children 1-15 year (ms)</th>
<th>Adult male (ms)</th>
<th>Adult female (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;440</td>
<td>&lt;430</td>
</tr>
<tr>
<td>Borderline</td>
<td>440-460</td>
<td>430-450</td>
</tr>
<tr>
<td>Prolonged</td>
<td>&gt;460</td>
<td>&gt;450</td>
</tr>
</tbody>
</table>

It is well known that the T-wave end is difficult to assess because of low amplitudes, biphasic T-waves, discerning which is-U wave and which is T-wave. These are among the issues often mentioned that render the assessment problematic. You are one of four observers in a group where the measurements of the QT intervals are compared. Two observers are classified as habituated to measure the QT interval and two as less habit.

QT interval values, recorded and automatically measured by two different ECG equipments will be compared with each observer’s QT interval values. If you have any questions during the course of your work please contact any of the following. Thank you for participating in this work!

Ulla-Britt Diamant phone: 52729 or Steen Jensen phone: 51760.
II - Long QT syndrome questionnaire (translated)

Patient questionnaire

Patient information: name birth year, index relative with the disease

1. Have you had symptoms of the disease Long QT Syndrome that occurs in your family? (for example dizziness, loss of consciousness, convulsions) Yes/No

   If Yes, describe your symptoms.

   If No, go to question 5.

2. In which situations have you experienced the symptoms described?

3. How old were you at symptoms debut?

4. When did you experience your most recent symptom?

5. Have you had contact with the health care system in relation to syncope, loss of consciousness or convulsions? Yes/No

   If Yes, when, and at which hospital?

6. Do you have normal hearing? Yes/No

   If No, have you consulted an Auditory Clinic? At which hospital?

7. Do you have any additional diseases for which you attend controls and/or are receiving treatment for? Yes/No

   If Yes, what diseases and/treatments?

8. Have you been diagnosed with Long QT Syndrome?

   If Yes, when?

9. Have you been prescribed/recommended medical therapy? Yes/No

   If Yes, what medicine and in which dosage?

10. Are you currently taking the medication? Yes/No
If Yes, since when are you taking the medication? Are you taking the prescribed dosage?

If No, why are you not taking the medication?

11. When taking the medication, do you experience any adverse effects? Yes/No

If Yes, describe the adverse effects.

12. Is there anything you like to add with regards to the disease, symptoms, controls, medication or other aspects?