Vectorcardiographic evaluation of ventricular repolarization in healthy individuals and LQTS mutation carriers

Farzad Vahedi, M.D.

Department of Molecular and Clinical Medicine
Institute of Medicine at Sahlgrenska Academy
University of Gothenburg
Sweden

University of Gothenburg

Gothenburg 2013

Vectorcardiographic evaluation of ventricular repolarization
in healthy individuals and LQTS mutation carriers

ISBN 978-91-628-8733-9
Printed in Gothenburg, Sweden by Kompendiet

To my family

CONTENTS

ABSTRACT

Ventricular arrhythmia is a well-known cause of syncopal attacks and sudden cardiac death in humans. Ventricular repolarization (VR) is the most unstable phase in cardiac electrical activity and its regulation depends on the coordinated activity in multiple ion channels. Several physiological factors such as heart rate (HR), autonomic nervous system (ANS) activity, gender and age influence the function of these ion channels. Meanwhile there are some genetic disorders e.g. the long QT syndrome (LQTS), which by changing (increase or decrease) the function of the ion channels can cause an imbalance in ion currents and subsequently increase the propensity for ventricular arrhythmias. Evaluation of VR in response to both physiological and pathophysiological factors is of great importance for understanding the mechanism of ventricular arrhythmias. Vectorcardiography (VCG) as a noninvasive tool in evaluation of global VR has proven superior to scalar ECG and, therefore, been used in this project.

Aims

To apply VCG as a noninvasive tool in VR analysis in order to:

1. Evaluate the influences of important physiological determinants for VR such as increase in HR, pharmacological modulations of ANS activity, and gender in healthy individuals.

2. Study the phenotypes in carriers of two common LQT1 mutations (R518X and Y111C in the *KCNQ1* gene) with biophysically different properties and also

compare the LQT1 mutation carriers with age and sex matched healthy control subjects as well as with a group of LQT2 mutation carriers.

3. Evaluate VR instability by calculating the beat to beat variability of VR measures in groups of LQTS mutation carriers and compare them with age and sex matched healthy control subjects.

Results and Conclusions

1. In healthy individuals the pure HR increase by atrial pacing decreased the heterogeneity of action potential (AP) morphology and VR, while similar increase in HR by β-adrenoceptor stimulation or vagal withdrawal had different effects on VR and resulted in changes that could increase arrhythmia risk.

2. At supine rest there was a modest but significant difference in QTcB but otherwise no difference in VR measures between carriers of two LQT1 mutations with in vitro different biophysical effects on potassium channel function.

3. There were no signs of increased VR dispersion in LQT1 and LQT2 mutation carriers at rest. In fact, there was an inverse relation between QTcB and measures of global heterogeneity of AP morphology and VR in both controls and LQT mutation carriers.

4. Although LQTS mutation carriers did not show any sign of increased heterogeneity of VR at rest compared to healthy controls, they had higher instability of VR duration and heterogeneity at beat to beat analysis. A greater instability of most aspects of VR already at rest thus seems to be a salient feature in both LQT1 and LQT2, which might pave the way for afterdepolarizations and ventricular arrhythmias.

SAMMANFATTNING

Ventrikulär arytmi är en välkänd orsak till synkopeattacker och plötslig hjärtdöd. Kamrarnas elektriska återhämtningsfas (ventrikulär repolarisation, VR) är den mest labila fasen i hjärtats elektriska aktivitet och är beroende av den samordnade aktiviteten i flera jonkanaler. Många fysiologiska faktorer såsom hjärtfrekvens, autonoma nervsystemets aktivitet, kön och ålder styr funktionen hos dessa jonkanaler. Samtidigt finns det vissa genetiska sjukdomar t.ex. långt QT syndrom (LQTS), som genom att ändra funktionen av jonkanaler kan orsaka obalans i jonströmmar och öka benägenheten för ventrikulär arytmi. Utvärdering av VR och dess svar på både fysiologiska och patofysiologiska faktorer och förändringar är av stor betydelse för att förstå mekanismen för ventrikulära arytmier. Vektorkardiografi (VKG) är ett icke-invasivt verktyg som har visats vara bättre än yt-EKG i utvärderingen av global VR och därför använts i detta projekt.

Syften

Att tillämpa VKG som ett icke-invasivt verktyg för VR-analys för att:

1. Utvärdera effekten av viktiga fysiologiska faktorer för VR som ökad hjärtfrekvens, farmakologiska modulationer av autonoma nervsystemet och kön hos friska individer.

2. Studera elektrofysiologiska fenotyper hos bärare av två vanliga LQT1 mutationer (R518X och Y111C i *KCNQ1* genen) med olika biofysiska egenskaper och också jämföra LQT1 mutationsbärare med ålders- och könsmatchade friska kontrollpersoner samt en grupp av LQT2 mutationsbärare .

3. Utvärdera VR instabilitet genom att beräkna slag till slag variationen av VR parametrar i grupper av LQTS mutationsbärare och jämföra dem med ålders- och könsmatchade friska kontrollpersoner.

Slutsatser

1. Hos friska individer, minskade heterogeniteten av aktionspotentialens (AP) morfologi och VR under ren hjärtfrekvensökning med förmaksstimulering, medan en liknande hjärtfrekvensökning genom β-adrenoceptor stimulering eller vagal blockad hade annorlunda effekter på VR och orsakade förändringar som kan öka arytmirisken.

2. I vila fanns en liten men signifikant skillnad i det hjärtfrekvenskorrigerade QT intervallet (QTcB) men annars ingen skillnad i VR parametrar mellan bärare av två LQT1 mutationer med in vitro olika biofysiska effekter på kalium kanal funktionen.

3. Det fanns inga tecken på ökad heterogenitet av VR i LQT1 och LQT2 mutationsbärare i vila. I själva verket var det ett omvänt förhållande mellan QTcB och mått på den globala heterogeniteten av AP morfologi och VR i både kontroller och LQT mutationsbärare.

4. Även om LQTS mutationsbärare inte visade några tecken på ökad VR heterogenitet i vila jämfört med friska kontroller, så hade de högre instabilitet i duration och heterogenitet av VR vid slag till slag analys. En större instabilitet i de flesta aspekterna av VR redan i vila verkar alltså vara en karakteristisk egenskap i både LQT1 och LQT2, vilket kan orsaka ventrikulära arytmier.

LIST OF PAPERS

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

I. Vahedi F, Haney MF, Jensen SM, Näslund U, Bergfeldt L: **Effect of heart rate on ventricular repolarization in healthy individuals applying vectorcardiographic T vector and T vector loop analysis.** Ann Noninvasive Electrocardiol. 2011; 16:287-294.

II. Vahedi F, Odenstedt J, Hartford M, Gilljam T, Bergfeldt L: **Vectorcardiography analysis of the repolarization response to pharmacologically induced autonomic nervous system modulation in healthy subjects.** J Appl Physiol 2012; 113:368-76.

III. Diamant UB*, Vahedi F*, Winbo A, Rydberg A, Stattin EL, Jensen SM, Bergfeldt L: **Electrophysiological Phenotype in the LQTS Mutations Y111C and R518X in the KCNQ1 Gene.** In manuscript

* Both authors contributed equally to this paper.

IV. Vahedi F, Diamant UB, Lundahl G, Bergqvist G, Gransberg L, Jensen SM, Bergfeldt L: **Instability of repolarization in LQTS mutation carriers compared to healthy control subjects assessed by vectorcardiography**. Heart Rhythm 2013; 10:1169–1175.

ABBREVIATIONS AND DEFINITIONS

ACA:	Aborted cardiac arrest
ANS:	Autonomic nervous system
AP:	Action potential
Atr:	Atropine
β-AR:	Beta-adrenoceptor
CaMKII:	Ca^{2+}/calmodulin-dependent kinase II
cAMP:	Cyclic adenosine monophosphate
DADs:	Delayed afterdepolarizations
EADs:	Early afterdepolarizations
ECG:	Electrocardiography
HR:	Heart rate
I_{CaL}:	L-type Ca^{2+} current
ICD:	Implantable cardioverter defibrillator
I_{Kr}:	Rapid component of delayed rectifiers
I_{Ks}:	Slow component of delayed rectifiers

I_{Na}:	Na^+ current
Iso:	Isoprenaline
I_{to}:	Transient outward K^+ current
LQTS:	Long QT syndrome
NCX:	Na^+/Ca^{2+} exchanger
PKA:	Protein kinase A
$QRS_{amplitude}$:	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 in X, Y and Z leads
QRS-T angle:	The angle between the maximum QRS and T vectors
QRS-T area angle:	The angle between the QRS_{area} vector and the T_{area} vector
QTc:	Heart rate corrected QT interval
QTcB:	Heart rate corrected QT interval according to Bazett
QTcF:	Heart rate corrected QT interval according to Fridericia
QTVI:	QT variability index
RyR2:	Ryanodine receptor type 2
SCD:	Sudden cardiac death

SD:	Standard deviation
STV:	Short-term variability
$T_{amplitude}$:	The 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 in X, Y and Z leads, which describes global dispersion of ventricular repolarization
T_{avplan}:	The mean distance between the periphery of the T loop and the preferential plane
$T_{azimuth}$:	The angle of the maximum T vector in the transverse plane (0^o left, $+90^o$ front, -90^o back and 180^o right)
TdP:	Torsades de Pointes polymorphous ventricular tachycardia
$T_{eigenvalue}$:	The squared quotient between the two largest perpendicular axes (eigenvalues) of the T loop in the preferential plane $(d1/d2)^2$ (where $d1 \geq d2$); the roundness of the T loop
$T_{elevation}$:	The angle of the maximum T vector in the cranio-caudal direction by us defined from 0^o (caudal direction) to 180^o (cranial direction)
T_{p-e}:	T_{peak} to T_{end}, the last part of the QT interval and final repolarization

Var:	Variance, e.g. in QT_{Var}
VCG:	Vectorcardiography
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 vectorial sum of the QRS_{area} vector and T_{area} vector, taking into account the angle between them; it describes the heterogeneity of action potential morphology throughout the ventricles
VR:	Ventricular repolarization
WPW:	Wolff-Parkinson-White
3-D:	3- dimensional

BACKGROUND

History of the electrical signal recording

The first documentation of a cardiac action potential (AP) was made by *Rudolph von Koelliker* and *Heinrich Müller* in 1856. Two decades later *Augustus Desiré Waller* recorded the first human electrocardiogram (ECG) and in 1887, he was able to obtain the first human electrocardiogram from the body surface.[1]

Willem Einthoven further investigated cardiac electrical activity and could identify four distinct waves on the ECG.[2]

The concept of "the area of QRST" was first introduced by *Wilson FN et al.* in 1934.[3] Vectorcardiography (VCG), based on Wilson's concepts, was presented three years later and then developed by *Ernest Frank* in the early 1950s for use in routine analysis of ECG tracings.[4]

The ventricular AP and ECG waves

The ventricular AP consists of four distinct phases which are the result of the coordinated activity of multiple ion channels; Figure 1. Voltage heterogeneity throughout the ventricles during the AP is the origin of QRS and T waves on surface ECG. Depolarization of myocardial cells during phase 0 of the AP is caused by a large inward current through voltage-gated Na^+ channels. In this phase the difference in depolarization instants between myocardial cells in various parts of the ventricles results in voltage heterogeneity creating the QRS wave. It is followed by

a transient repolarization (phase 1), reflecting Na^+ channel inactivation and the activation of voltage-gated outward K^+ currents. In normal circumstances during phase 2 or the plateau phase of the AP most of the myocardial cells have the same potential causing no voltage heterogeneity which results in an isoelectric line on ECG known as the ST segment. This phase is the result of a delicate balance between the inward (Na^+ and Ca^{2+}) and the outward (K^+) currents and is essential for Ca^{2+}-activated regulation of the release and recycling of Ca^{2+} by the sarcoplasmic reticulum, crucial for the electro-mechanical coupling. As the L-type Ca^{2+} channels undergo voltage-dependent inactivation, the outward K^+ current results in complete repolarization of myocardial cells during phase 3 of the AP. In ventricular myocytes the resting membrane potential remains almost constant throughout the diastolic interval (phase 4).

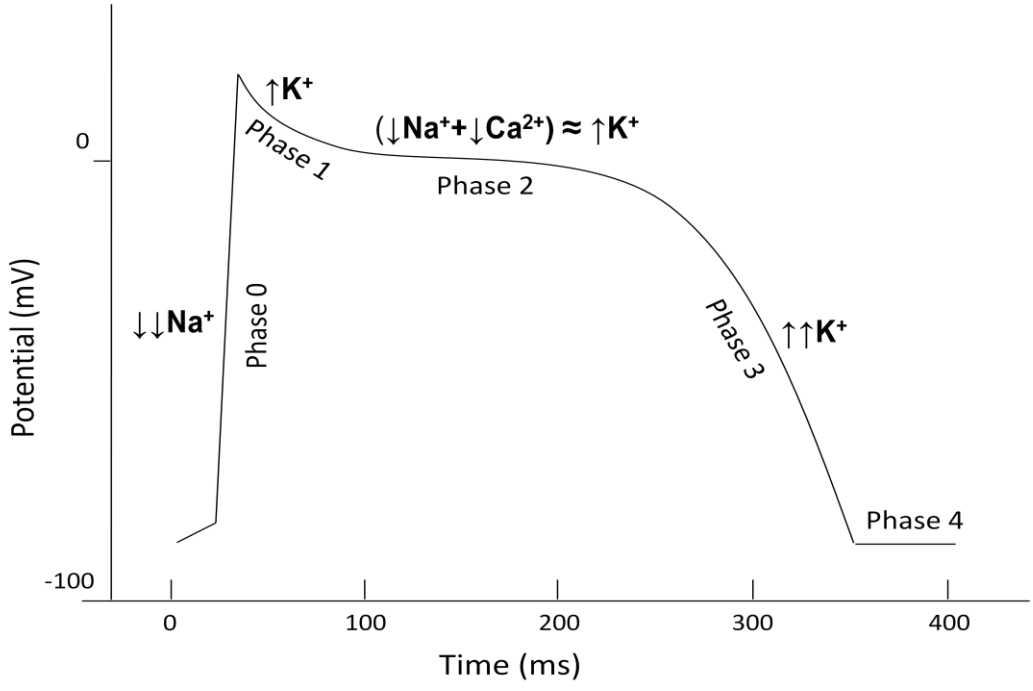

Figure 1. Main inward (↓) and outward (↑) ion currents during the ventricular AP.

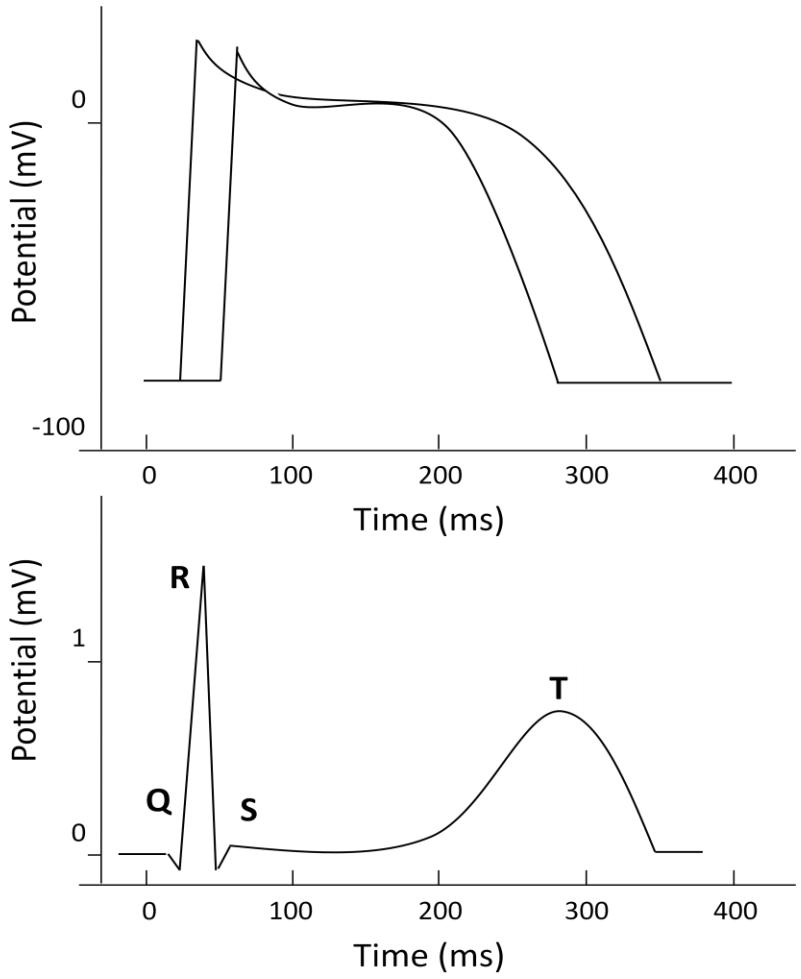

Figure 2. The QRS wave is caused by the heterogeneity of depolarization instants while the T wave is the result of superimposition of heterogeneity of depolarization instants and AP morphology throughout the ventricular myocardium.

The myocardial voltage heterogeneity in phase 3 of the AP gives rise to the T wave on the ECG which is the result of superimposition of the heterogeneity of depolarization instants and AP morphology throughout the whole ventricular myocardium; Figure 2.

Theoretically, if AP has the same instant and morphology in all the ventricular cells there will be no voltage heterogeneity during the depolarization and the

repolarization and the ECG will appear as an isoelectric line and there will be no ECG.

In ventricular myocytes in addition to the Na^+ (I_{Na}) and Ca^{2+} (I_{CaL}) currents, there are multiple types of K^+ currents i.e. transient outward currents (I_{to}) and both rapid and slow components of delayed rectifiers (I_{Kr} and I_{Ks}) as well as ion-exchangers. The AP morphology is highly influenced by the properties of the ion channels and any imbalance between these currents could affect AP duration, refractoriness of myocardial cells and propensity for cardiac arrhythmias.[5]

Beta-adrenergic receptor activation and ion channel regulation

There are four β-adrenoceptor (β-AR) subtypes, but there is little evidence for effects of β_3- and β_4-AR subtypes on cardiac electrophysiology.[6] The proportion of β_1- to β_2-AR in the human ventricle is approximately 3 to 1. As shown in Figure 3, by coupling to the adenylylcyclase stimulatory G protein they increase the level of cyclic adenosine monophosphate (cAMP) which activates protein kinase A (PKA). Substrates phosphorylated by PKA that may be relevant to cardiac arrhythmias include the L-type Ca^{2+} channel, the cardiac ryanodine receptor type 2 (RyR2), the α-subunit of the cardiac Na^+ channel, the cardiac isoform of the Na^+/Ca^{2+} exchanger (NCX), K^+ and Cl^- channels.[7-9]

VCG in VR evaluation

The voltage heterogeneity in the ventricular myocardium during the AP is of a 3-dimensional (3-D) character while the surface ECG in each lead is only a

2-dimensional projection of this phenomenon. Those regions of the myocardium which have the voltage heterogeneity parallel to each specific ECG lead have a more prominent effect on the amplitude of the ECG waves in that lead while the parts with perpendicular angle to any specific lead show little influence. Thus, different leads on the surface ECG make it possible to differentiate and assess regional changes in AP characteristics e. g. in acute myocardial infarction.

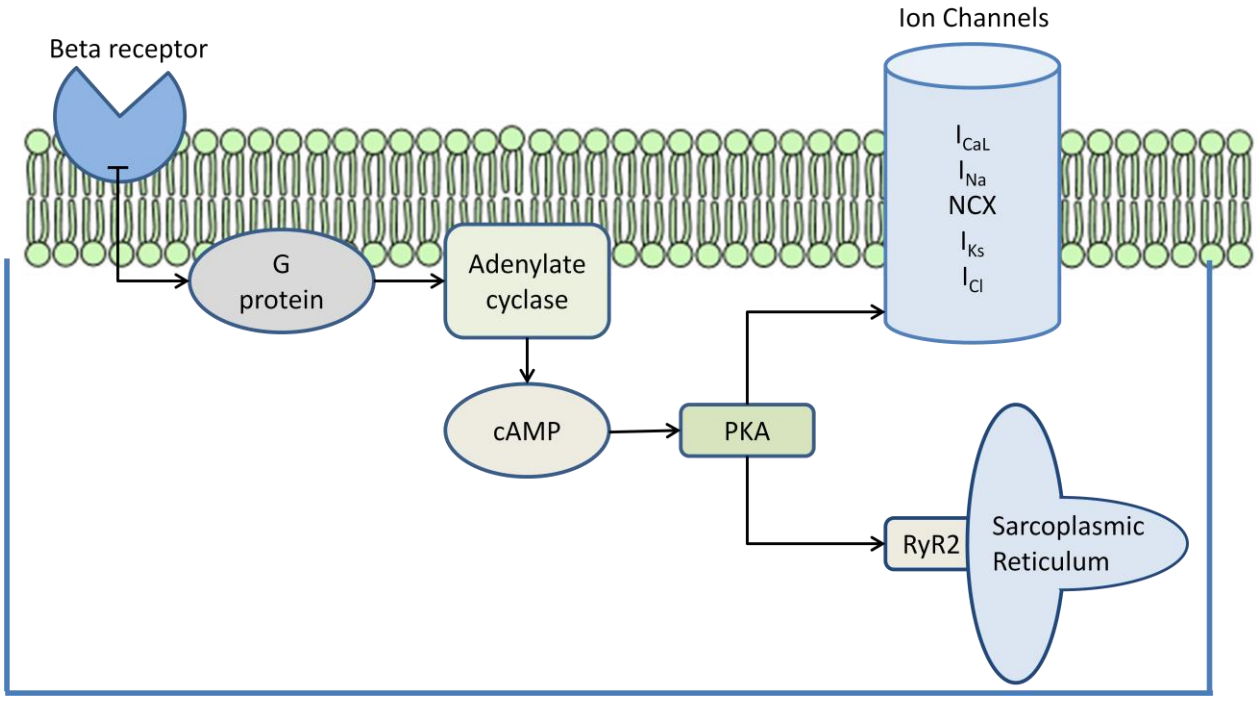

Figure 3. Schematic illustration of β-adrenoceptor regulation of ion channels through the adenylate cyclase and protein kinase A (PKA) pathway. cAMP: cyclic Adenosine Monophosphate; I_{CaL}: L-type Ca^{2+} current; I_{Cl}: Cl^{-} current; I_{Ks}: slow component of delayed rectifier; I_{Na}: Na^{+} current; NCX: Na^{+}/Ca^{2+} exchanger; RyR2: ryanodine receptor type 2.

In contrast, VCG based on the Frank orthogonal lead system, as shown in Figure 4, provides 3-D QRST derived "conventional" ECG measures e.g. the QRS, QT and $T_{peak\text{-}end}$ ($T_{p\text{-}e}$) intervals. Besides, it provides information about other global aspects of VR reflected by T vector and T vector loop morphology parameters such as the total heterogeneity of AP morphology (ventricular gradient, VG) and global dispersion of VR (T_{area}). It also outlines the spatial relationship between depolarization and repolarization forces.[10-16]

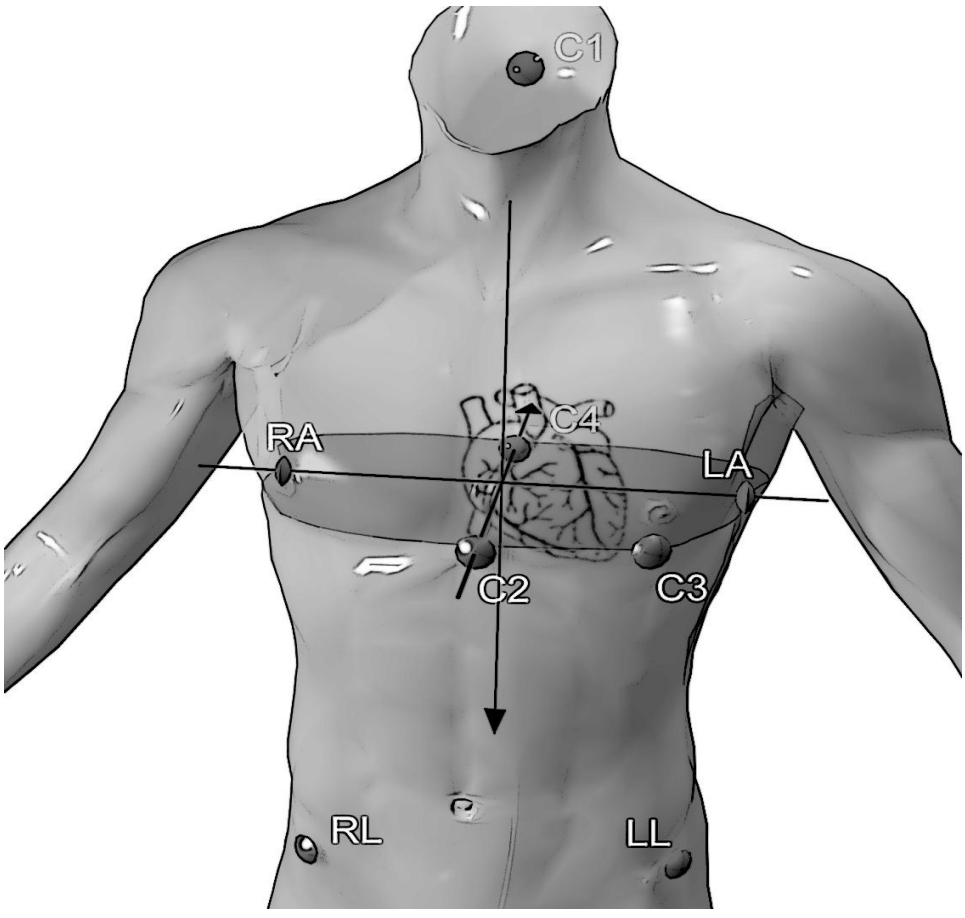

Figure 4. VCG recording according to the Frank orthogonal lead system is more anatomically representative than routine scalar ECG in evaluating the global ventricular electrical activity.

VCG is more anatomically representative than routine scalar ECG in evaluating the global VR and has proven superior to ECG for detection of VR changes of cardiac memory, as induced by ventricular pacing or appearing after ablation in the Wolff-Parkinson-White (WPW) syndrome.[15,16] VCG usage is especially relevant in evaluation of global VR in LQTS studies and also in measuring the beat to beat variability of different VR parameters. The exact duration of the QT interval in any selected ECG lead is not a consistent measure of the overall VR duration.[17] Each ECG lead represents a certain projection of the 3-D T loop, and can be affected by the orientation of the T loop in relation to the given ECG lead.[18] Chest movements due to e.g. respiration may change the orientation of the T loop and introduce variability into the serial QT measurements on the given ECG lead that has nothing to do with the variability of VR. In contrast, VCG recording creates a single spatial QRST complex which allows the measurement of QT interval unaffected by the T loop axis orientation. Furthermore, VCG based QTc measurement was found more accurate than both manual and automatic QTc analysis on scalar ECG in identifying LQTS.[19] Considering the above mentioned advantages of VCG in noninvasive evaluation of the global electrical activity of the heart we chose this method in our project to study both physiology and pathophysiology of global VR.

Although there are many factors that affect VR, the most important physiological are heart rate (HR), autonomic nervous system (ANS) activity, age and gender.[20,21] While the effect of HR and ANS on the AP and VR duration has been extensively studied and documented by many investigators[22-27] these effects on the VG and other VCG derived VR parameters are incompletely known. The purpose of the first two parts of our project was, therefore, to evaluate separately the influences of HR increase and ANS perturbations on VCG-derived VR measures. A secondary purpose was to compare the VR response in adult men and women.

The T loop and the corresponding T wave

The T loop emerges from inhomogeneous recovery throughout the ventricles or VR dispersion.[28,29] Such dispersion or heterogeneity is primarily due to differences in AP morphology throughout the ventricles superimposed on secondary factors such as differences in ventricular depolarization instants.[30]

The T vector reflects the voltage heterogeneity at any instant during VR. While $T_{amplitude}$ represents the maximum value of heterogeneity during VR, T_{area} or the area under the curve during the JT interval is an index of total heterogeneity of VR, as it represents the summed voltage gradients during the whole period when repolarization differences exist[31-33]; Figure 5.

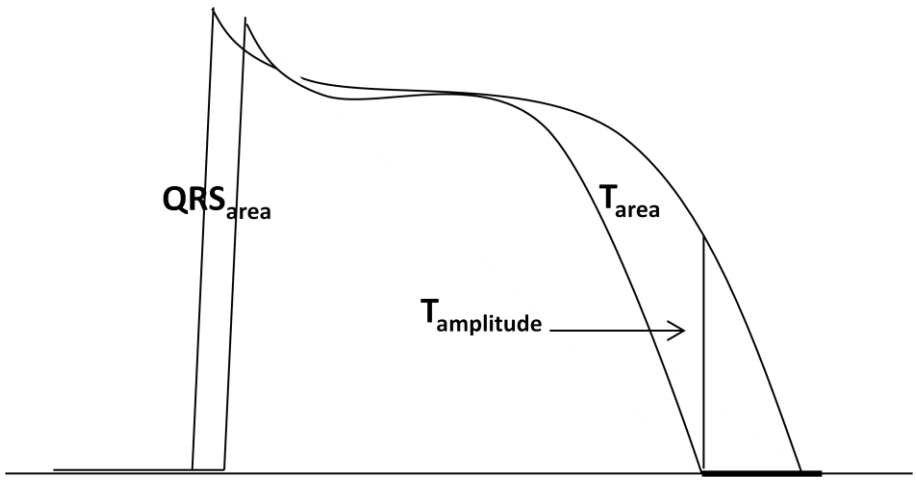

Figure 5. A simplified illustration of QRS_{area} and T_{area} based on voltage heterogeneity during the ventricular AP. $T_{amplitude}$ represents the maximum dimension of this heterogeneity during VR.

In ventricular wedge preparations, the differences in the time course of repolarization of the epicardium, endocardium, and midmyocardially located M cells were shown to result in transmural dispersion of repolarization. Full repolarization of the epicardium appeared to be coincident with the peak of the T wave and that of the subendocardially located M cells with the end of the T wave. Based on these findings the T_{p-e} was initially proposed as an index of transmural dispersion of VR duration.[34-36] However, *in vivo* studies have been unable to identify the delay in intramural repolarization consistent with distinct manifestation of M cells.[37,38] Besides, in animal studies the peak of the T wave was found clearly before full repolarization of the epicardium but coincided with earliest repolarization of the ventricles, whereas the end of the T wave coincided with the latest; Figure 6. T_{p-e} has, therefore, been suggested as an index of global dispersion of repolarization duration.[39-41]

VG

As mentioned above, dispersion of VR, reflected in the T wave, arises from superimposition of a primary factor i.e. the heterogeneity of the AP morphologies throughout the ventricles; and a secondary factor i.e. the heterogeneity of the ventricular depolarization instants as they result from the conducted impulse. The contribution of primary and secondary factors cannot be separated by T wave analysis alone. The power of the VG is its ability to assess heterogeneity of the ventricular AP morphology independent of secondary factors.

In the vectorial approach VG, known also as the QRST integral or $QRST_{area}$, is the spatial integral of the area formed by the moving heart vector during the QT interval

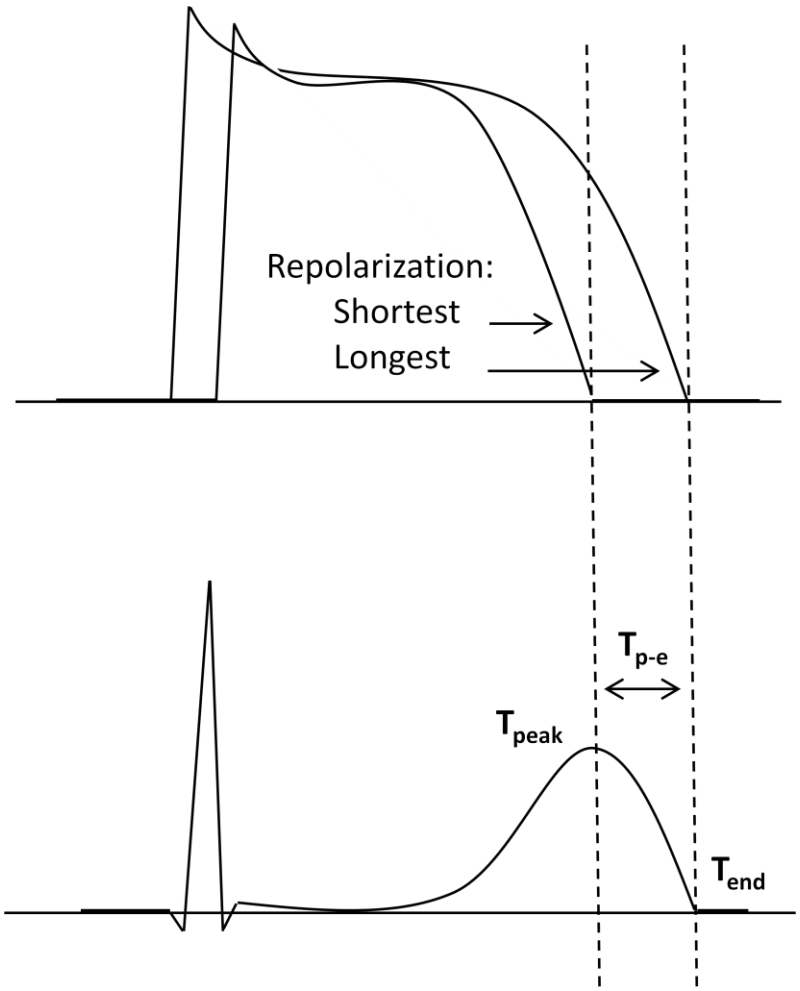

Figure 6. In animal studies the peak and the end of the T wave coincided with the end of the shortest and the longest repolarization, respectively. T_{p-e} has, therefore, been suggested as an index of global dispersion of repolarization duration. Note; there is an inverse relation between the onset of repolarisation and the end of repolarisation.

and depends solely on the heterogeneity of the AP morphology in the muscle fibers and not on the order in which the muscle cells are activated.[3,42-44]

The VG measure is equal to the vectorial sum of the QRS_{area} and the T_{area}; Figure 7. In humans, the QRS axis usually points slightly posterior, inferior, and to the left,

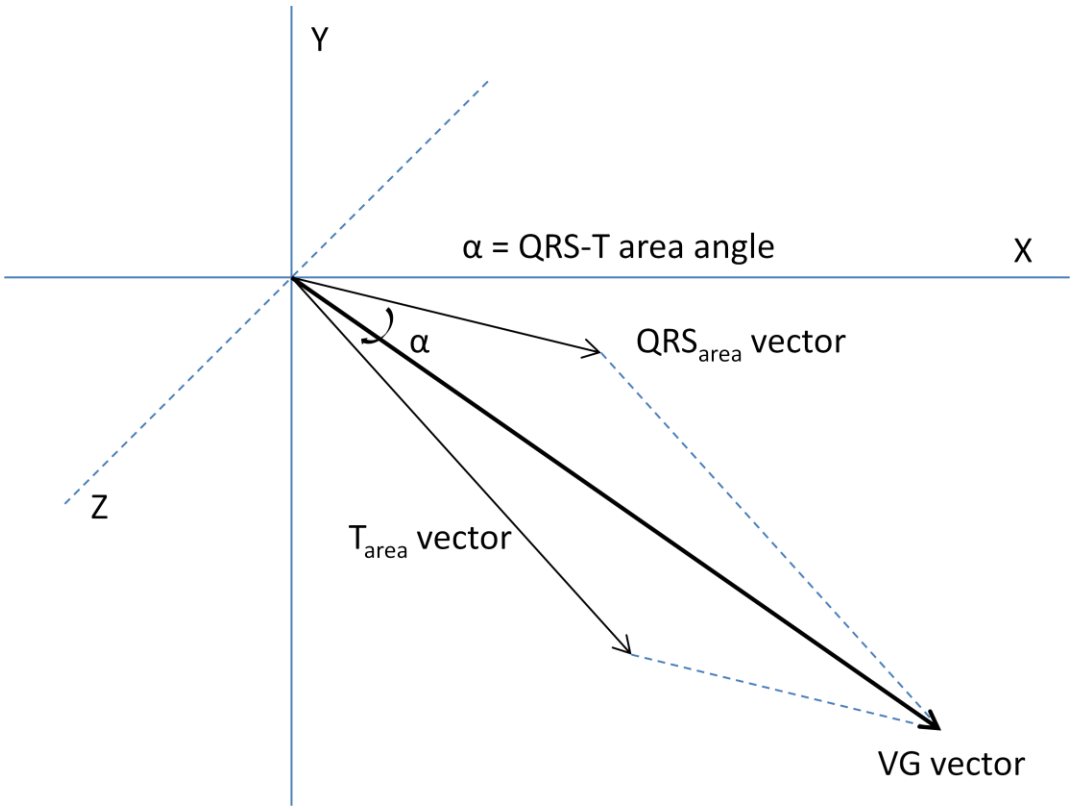

Figure 7. VG dimension is equal to the vectorial sum of the QRS_{area} and the T_{area}. The QRS-T area angle is the angle between the QRS_{area} vector and the T_{area} vector.

and the T axis usually points anterior, inferior and to the left. Consequently, the VG points usually anterior, inferior, and to the left, roughly along the long axis of the heart, in the direction of the apex.[45] Increased QRS_{area} and T_{area} are not necessarily associated with a large VG. Even in a normal heart, pure secondary changes (e.g. an altered intra-ventricular activation sequence due to a ventricular ectopic beat) yield a wide and bizarre QRS complex and T wave with large QRS and T integrals or areas. However, in this case the angle between the QRS and T area vectors i.e. QRS-T area angle will be large (discordance), and the VG may remain almost unchanged (no primary changes i.e. no changes in the heterogeneity of AP morphology). It has previously been shown that the spatial QRS-T angle based on ECG recordings has

some prognostic value.[46,47] The spatial QRS-T area angle may increase from secondary changes and combining it with the VG could still further increase this prognostic value, as this would add information about the absence or presence of primary changes. With pure secondary changes, only the spatial QRS-T area angle would be enlarged, while the VG remains unchanged. With additional primary changes (a less favorable condition), the VG would be enlarged as well.

Long QT syndrome

Congenital long QT syndrome (LQTS) is the prototypical monogenic inherited disorder of VR with variable penetrance and expressivity. LQTS mutation carriers have an increased propensity for Torsades de Pointes (TdP) polymorphous ventricular tachycardia, syncope and sudden cardiac death (SCD).[48] Mutations in up to 13 different genes have so far been identified as causally associated with different types of LQTS and several modifier genes have also been postulated.[49-51] The affected genes encode either directly for the proteins making up the channels that regulate different ion flow in and out of the cardiac myocyte or proteins that modify the function of these channels.[52] The two most common forms LQT1 and LQT2 arise from loss-of-function mutations in *KCNQ1* and *KCNH2* (also known as *HERG*), which encodes the slowly activating and the rapidly activating delayed rectifier K^+ channel, respectively.[53,54] Ion current perturbations caused by these mutant channels disrupt the delicate balance normally occurring during VR and could e.g. allow a predominant effect of adrenergic influence on the L-type Ca^{2+} channel and facilitate Ca^{2+} and Na^+ channel-mediated afterdepolarizations; Figure 8.

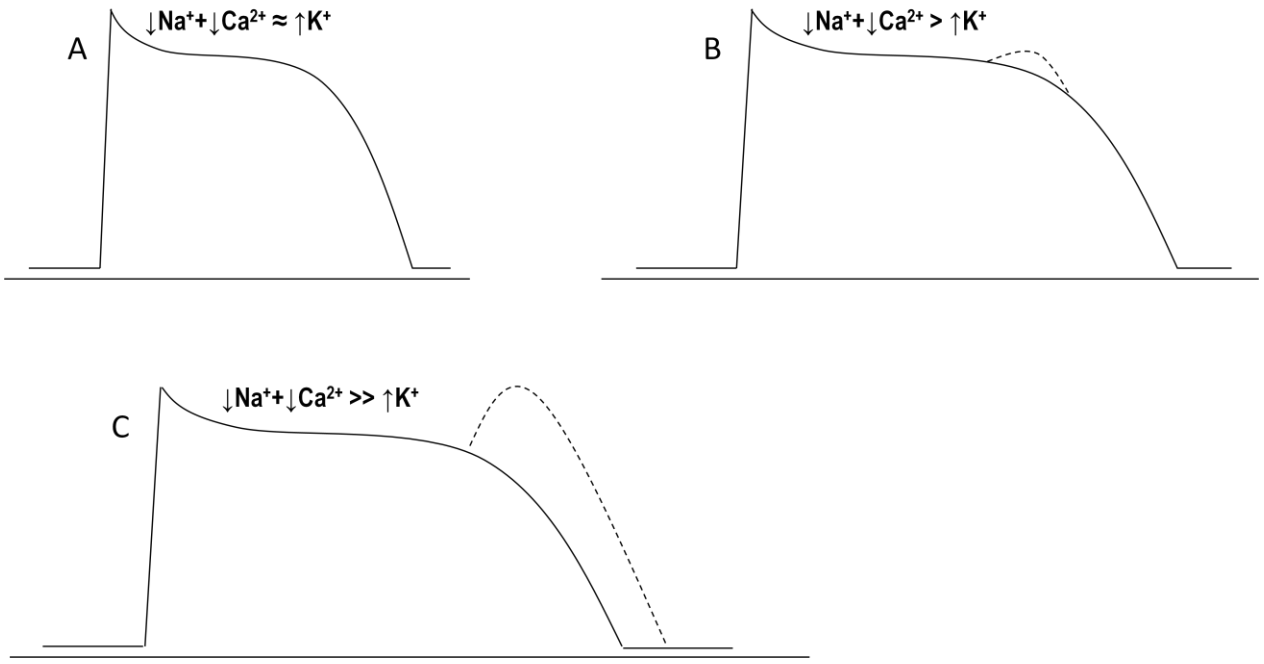

Figure 8. The normal AP is the result of a delicate balance between the inward and the outward ion currents (A). Prolongation of AP and the formation of afterdepolarization (B) and triggered activity (C) may be the result of the imbalance between these currents.

Transmembrane voltage oscillations during the last parts of the cardiac AP are referred to as afterdepolarizations.[55] Early afterdepolarizations (EADs) occur during phase 2 or 3 of AP while delayed afterdepolarizations (DADs) arise in phase 4. These oscillations can bring the membrane to its threshold potential and trigger a spontaneous AP or extrasystole and initiate TdP which can be responsible for syncope and SCD in e.g. LQTS[56]; Figure 9.

Reduced activity in I_{Kr} and I_{Ks} or increased activity in I_{CaL}, NCX or late I_{Na} can be the underlying mechanism for EADs. In contrast, DADs are caused by large increases in intracellular Ca^{2+} and consequently increased activity in NCX e.g. in digitalis toxicity and during myocardial reperfusion or during excessive β-AR

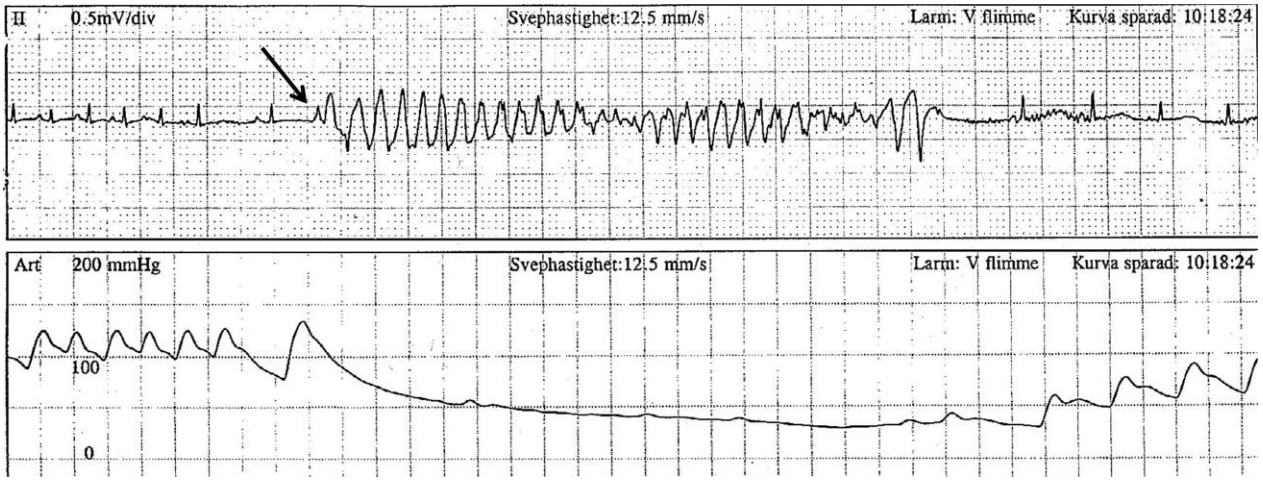

Figure 9. The upper panel shows an episode of TdP that presumably starts with a DAD triggered activity (arrow) and converts spontaneously to sinus rhythm. The lower panel is the simultaneous arterial blood pressure in the same patient; it drops to lower than 50 mmHg in less than three seconds following the start of tachycardia.

stimulation. DADs may also occur in hypertrophied and failing hearts as well as in Purkinje fibers surviving myocardial infarction.[57]

While EADs and DADs may underlie the premature beat that initiates TdP, in vitro studies on myocardial wedge preparations and also some in vivo animal models provide evidence in support of circus movement reentry as the mechanism responsible for the maintenance of the arrhythmia in LQTS.[58-62] The studies based on myocardial wedge preparations suggest the presence of electrical heterogeneity principally in the form of transmural dispersion of repolarization which gives rise to a vulnerable window for the development of reentry. However, the accuracy of this assumption has not been evaluated in a whole-heart setting in humans.

The presence of an LQTS mutation should be regarded as a risk factor that increases the probability of cardiac arrhythmias. But given the incomplete penetrance of the disease[63] it is impossible to define the individual risk of events in each subject. Even

within the same family, mutation carriers may show large variability of clinical manifestations ranging from presently asymptomatic to developing ventricular fibrillation.

A combination of LQTS-type, age, gender, symptoms and the heart rate corrected QT (QTc) is presently used clinically for risk stratification.[64] However, the clinical diagnosis of the syndrome is not always easy since some patients with positive genetic test may have a normal QTc. In addition, even if the magnitude of QTc prolongation has been recognized as a powerful predictor of risk, syncope and SCA occur in 5% of LQTS-family members despite a normal QTc interval.[65-68]

In the last few years other ECG variables than QTc have been evaluated and found useful in the diagnosis and risk stratification of patients with LQTS or other proarrhythmic conditions. For example, increased T-wave amplitude variability has been demonstrated in genotyped LQTS patients.[69] In addition, in both animal and human models, especially in drug-induced LQTS affecting I_{Kr} function, increased short-term variability of the QT interval (STV_{QT}) and AP instability have been shown to be more important in predicting proarrhythmia than QT prolongation *per se*.[70-75] Increased STV_{QT} has even been suggested as a useful noninvasive additive diagnostic marker in congenital LQTS.[76] Furthermore, the QT variability index (QTVI), based on the variance and mean of QT and RR intervals has been found valuable in predicting arrhythmia, SCD, and the total mortality in several patient populations.[77-83]

The purpose of the third and fourth parts of my PhD project was, therefore, to provide a more complete understanding of the link between genotype and phenotype in LQTS.

In collaboration with Umeå University we studied two distinct LQT1 mutations with in vitro, different biophysical properties. The study came as close to the human counterpart of knock-out mice as possible and offered special opportunities to correlate genotype and electrophysiological phenotype. We also compared them with healthy controls and a group of LQT2 mutation carriers. In addition, we applied beat to beat analysis to compare variability of different VCG parameters between LQTS and healthy controls.

There is no doubt that increased heterogeneity and instability of VR can set the stage for ventricular arrhythmias and by studying both physiological and pathophysiological aspect of VR in our project we hoped to take a step forward in developing a reproducible noninvasive method with sufficient predictive accuracy to better identify the high risk individuals.

AIMS

To apply VCG analysis for noninvasive evaluation of VR in humans in order to:

1. Study the influences of important physiological determinants for VR such as increase in HR by atrial pacing, pharmacological modulations of ANS, and gender (Paper I and II).

2. Assess the phenotypes in carriers of two common LQTS mutations (R518X and Y111C in the *KCNQ1* gene) with biophysically different properties and also compare the *KCNQ1* mutation carriers with age and sex matched healthy control subjects as well as a group of LQT2 mutation carriers (Paper III).

3. Evaluate VR instability by calculating the beat to beat variability of VR measures in groups of LQTS mutation carriers and compare them with age and sex matched healthy control subjects (Paper IV).

MATERIALS and METHODS

This chapter is a summary of methods used in our four papers. For more detailed descriptions please refer to each paper.

The study protocols in all of the four papers were performed following the principles of the Declaration of Helsinki and approved by the regional ethics committee.

As far as possible, in all parts of the project, care was taken to enroll similar proportions of male and female subjects.

Paper I

Nineteen otherwise healthy patients 20-80 years of age (8 men and 11 women), scheduled for ablation therapy for atrioventricular nodal reentrant tachycardia, concealed WPW or ectopic atrial tachycardia were included. All chronic medications were discontinued 4 days before catheterization and no medication was given before or during the procedure. After successful ablation pacing was started in the right atrium at a rate 5–10 bpm higher than the patient's resting HR for 6 minutes. Depending on the resting HR, the lowest pacing rate was 80 or 90 bpm. The pacing rate was then increased stepwise with 10 bpm for periods of 4 minutes and HR up to 120 bpm was used. VCG was recorded during each step and in order to allow sufficient time to reach rate adaptation and VR steady state only the last minute of all 4-minute recordings was analyzed. In order to evaluate the effect of a HR change over a range of at least 30 bpm, subjects were divided into two partly

overlapping groups with HR ranges of 80–110 (n = 8) or 90–120 (n = 9) bpm. In the correlation analysis, however, the differences in the parameter values from the lowest to the highest paced rates (range 10, 20, 30, or 40 bpm) were used for all individuals, that is, 19 paired values. Only when results were consistent over both HR ranges and in the correlation analysis, a judgment was made in relation to HR dependence or independence.

Paper II

Thirty-one healthy volunteers 20-45 years of age (16 men and 15 women), were included. Under continuous VCG recording pharmacological modulation of ANS activity was achieved by sympathetic (β-AR) stimulation with isoprenaline (Iso) infusion in three consecutive steps followed by a washout period and subsequent parasympathetic blockade (muscarinic receptors) with atropine (Atr) and sympathetic (β-AR) blockade with propranolol; Figure 10. Five minutes VCG recording at each steady state phase was marked for off-line assessment. In addition, during the first 4 minutes following Atr injection six periods of 10-s VCG recordings were analyzed (A1–A6); A1–A3 during the first minute of HR increase, followed by A4–A6 at constant HR with 1-min interval between each step.

Paper III

This study included 99 genetically confirmed carriers of a mutation in the *KCNQ1* gene (57 Y111C and 42 R518X carriers). In addition, 121 healthy subjects were age and sex matched to the gene carriers and served as controls. Furthermore, 19 adult

LQT2 mutation carriers were included for comparison. For sex and age distribution please see the results. VCG was recorded after at least 5 minutes rest in the supine position.

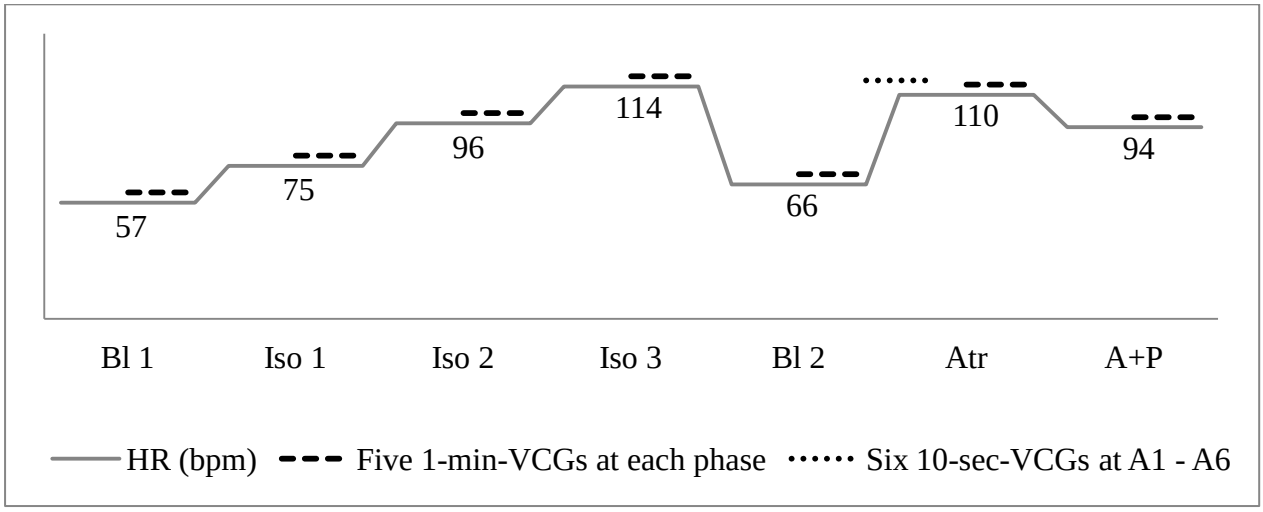

Figure 10. An overview of the protocol in Paper II, including the average heart rate (HR), at each steady state level and a specification of the periods for analysis of the vectorcardiography (VCG), which was recorded continuously. Bl: baseline; Iso: isoprenaline; Atr: atropine; bpm: beats/min; A+P: atropine + propranolol.

Paper IV

Forty-one individuals 18-65 years of age, with LQTS mutation (31 LQT1 and 10 LQT2; 15 men and 26 women) were recruited together with 41 healthy volunteers matched for age and sex. As in paper III VCG was recorded after at least 5 minutes rest in the supine position.

VCG

In Paper I a MIDA 1000 system and in the other three papers a CoroNet II system (Ortivus AB, Danderyd, Sweden) were used. These systems use eight electrodes positioned according to the Frank orthogonal lead system (X, Y, and Z). In paper I an averaged 3D QRST complex was constructed from all cardiac cycles recorded during a 15 seconds sampling period, and the mean of four such averages from the last 1-minute period was used. In paper II five 1-min averaged complexes were constructed during each 5-min steady state phase of the study and averaged. The time resolution was increased during the first 4 min following Atr injection as described above.

The last minute of the VCG recording was selected to construct a one 1-min averaged complex in paper III and also for beat to beat analysis in paper IV when every single cardiac cycle during the selected minute was compared with the previous cycle for calculating the beat to beat variability of different parameters. In the presence of occasional abnormalities such as premature atrial or ventricular contractions care was taken to select the last possible period of a one minute recording of undisturbed sinus rhythm.

In all of the four papers interval measures i.e. QRS, QT and T_{p-e} were calculated off-line on 3-D based QRST complexes as well as QRS and T vector and T vector loop parameters from QRS and T vector loops. The system automatically chooses annotation points (Q onset, J point, T_{peak}, and T_{end}) based on amplitude criteria. These annotation points were manually checked and adjusted when necessary, after which the accepted QRST complexes were tagged and automatically measured. In summary, ventricular gradient (VG), QRS_{area} and T_{area} (μVs), which are the spatial areas between the isoelectric baseline and the curve formed by the moving vector

during the QT, QJ and JT intervals, respectively, were computed from the QRST complex in the 3 orthogonal directions: $QRST_{area} = (QRSTx^2 + QRSTy^2 + QRSTz^2)^{1/2}$, $QRS_{area} = (QRSx^2 + QRSy^2 + QRSz^2)^{1/2}$ and $T_{area} = (Tx^2 + Ty^2 + Tz^2)^{1/2}$ (μVs). The QRS-T area angle was defined as the angle between the QRS_{area} vector and the T_{area} vector (0° to 180°), and was accounted for in the calculation of the VG. The amplitudes of the maximum QRS and T vectors (mV) in space were defined as well as their relation, i.e. the QRS-T angle (0° to 180º). As is shown in Figure 11 the spatial orientation of the maximum T vector was defined by its azimuth and elevation. In addition, the T vector loop morphology was characterized by two parameters: Teigenvalue (roundness; unitless) and Tavplan (bulginess; μV); Figure 12.

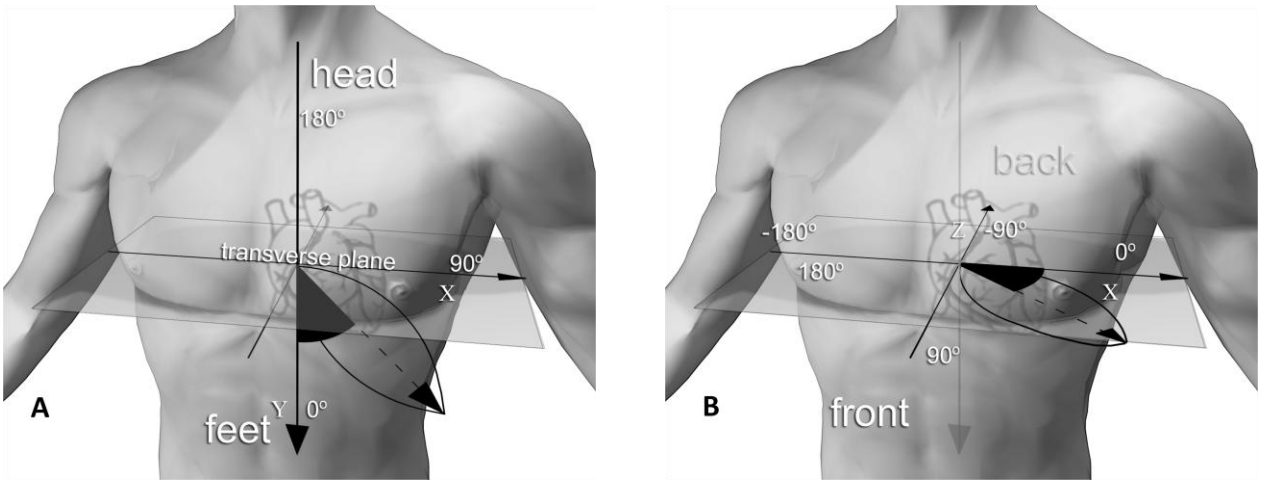

Figure 11. Vectorcardiographic parameters. **A:** Televation describes the angle between the maximum T vector and a cranio-caudal axis perpendicular to the transverse (horizontal) plane, which is depicted by the *rectangle* (also in panel B). At 0° the vector points downward (caudally), and at 180° it points upward (cranially). **B:** Tazimuth describes the angle between the maximum T vector projected on the transverse plane and the left extreme of the X-axis. At 0° the vector points to the left. Forward motions of the vector (left-front-right) are defined as 0° to 180°, and backward motions of the vector (left-back-right) are defined as 0° to -180°. Reproduced from Sahlén A et al.[13]

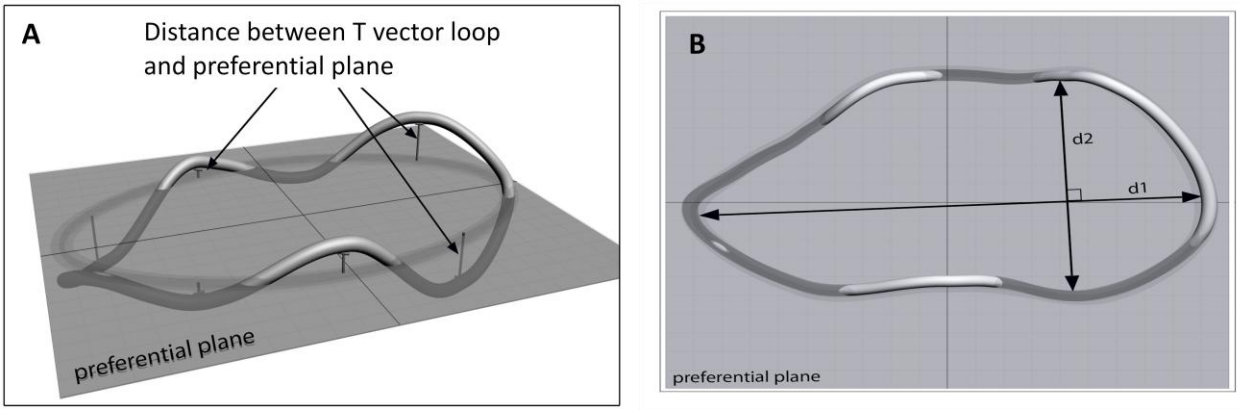

Figure 12. T-vector loop morphology. **A:** Tavplan = the mean distance between the T-vector loop periphery and its preferential plane, describes its distortion or bulginess (µV). **B:** Teigenvalue = the quotient of the 2 highest eigenvalues (d1 and d2; approximate diameters or axes) of the T loop, describes the morphology elliptical to circular (unitless). Reproduced from Wecke L et al.[15]

For evaluation of beat to beat variability of each parameter in paper IV, Poincaré plots were constructed by plotting each value (n) against its successor (n+1) from all cardiac cycles during one minute. Furthermore short-term variability (STV) for each parameter, describing the mean orthogonal distance to the line of identity on the Poincaré plot, was calculated

($STV_D = \Sigma\ |D_{n+1} - D_n| \ / \ [\text{number of cardiac cycles} \times \sqrt{2}]$, where D represents different parameters),

as well as

QT variability index ($QTVI = LOG\ [(QT_{Var}/QT_{Mean}^2) / (HR_{Var}/HR_{Mean}^2)]$.

Statistics

Mean (standard deviation [SD]) or median (inter-quartile range) was used for descriptive purposes.

In paper I Friedman's test was used for analysis of the overall effects and correlation analysis was used to test relations between HR increase and the parameter changes.

In paper II Friedman's test was used for analysis of the overall effects during the seven steady state phases, Bl1 - A+P, as well as during Bl2 - A3 and A3 - A6. Wilcoxon signed rank test was used for post hoc comparison of data from two specific phases. The Mann-Whitney U-test was used for testing for sex differences, both at baseline and with regard to the responses to interventions.

In paper III simple regression was used to compare LQT1 mutation carriers with controls. Taking into account age and gender between group comparisons were performed using Mann-Whitney and/or unpaired t-test.

In paper IV parameters were compared between the LQTS mutation carriers and controls using Mann-Whitney. In addition, the relation between the QT interval and the instability of VR measures was assessed by linear regression analysis.

RESULTS

Paper I:

The HR responses were similar in both subgroups (80-110 and 90-120 bpm, respectively) for almost all parameters. In summary (Table 1), in this group of generally healthy individuals changes in atrial paced HR from 80 to 120 bpm showed that the QRS and QT intervals together with VG, QRS_{area}, T_{area}, and $T_{amplitude}$ were markedly rate dependent and all decreased with increasing heart rate. In contrast, the $T_{p\text{-}e}$/QT ratio was rate independent and had a constant value of on average 0.20–0.21 irrespective of HR, as well as, the T-loop morphology parameter T_{avplan} and possibly $T_{eigenvalue}$. For other evaluated parameters, some rate-dependence might exist.

Table 1. The effects of step-wise heart rate increase from 80 to 110 bpm (n=8) on different VCG parameters presented as mean (SD). The correlation analysis was based on the changes from the lowest to the highest atrial paced rate in any individual (n=19). * $p \leq .05$, ** $p \leq .01$, *** $p < .001$, based on Friedman's test.

Heart rate (bpm)	**80 (n=8)**	**90 (n=8)**	**100 (n=8)**	**110 (n=8)**	**Correlation analysis (n=19) r**	**pvalue**
Interval based parameters						
QRS (ms)	91 (4)	89 (3)	89 (4)	86 (8)*	-0.51	0.025
QT (ms)	357 (8)	341 (5)	326 (0)	314 (2)***	-0.74	0.000
QTcB (ms)	412 (9)	417 (6)	422 (0)	426 (2)*	0.35	0.15
QTcF (ms)	393 (9)	390 (5)	387 (0)	384 (2)**	-0.13	0.60
$T_{p\text{-}e}$ (ms)	71 (13)	67 (12)	65 (5)	63 (5)*	-0.38	0.11
$T_{p\text{-}e}$/QT	0.20 (0.03)	0.20 (0.03)	0.20 (0.02)	0.20 (0.02)	0.023	0.92
Vector based parameters						
$QRS_{amplitude}$ (mV)	1.33 (0.41)	1.31 (0.43)	1.29 (0.47)	1.26 (0.46)	-0.59	0.008
$T_{amplitude}$ (mV)	0.40 (0.02)	0.37 (0.02)	0.32 (0.01)	0.28 (0)***	-0.64	0.003
$T_{azimuth}$ (º)	32 (30)	34 (32)	38 (31)	39 (33)***	0.28	0.25
$T_{elevation}$ (º)	62 (8)	63 (11)	64 (13)	66 (12)	0.07	0.79
QRS-T angle (º)	59 (25)	61 (26)	65 (24)	71 (23)*	0.43	0.068
QRS-T area angle (º)	74 (20)	75 (21)	79 (15)	85 (11)***	0.35	0.15
QRS_{area} (µVs)	33 (9)	33 (8)	31 (8)	30 (8)***	-0.53	0.02
T_{area} (µVs)	50 (12)	46 (10)	38 (6)	32 (5)***	-0.78	0.000
VG (µVs)	66 (14)	61 (13)	53 (13)	45 (14)***	-0.79	0.000
T loop morphology parameters						
T_{avplan} (µV)	0.58 (0.21)	0.59 (0.25)	0.62 (0.27)	0.60 (0.28)	0.31	0.19
$T_{eigenvalue}$	77 (63)	57 (87)	58 (155)	45 (74)	-0.06	0.79

Paper II:

B-AR stimulation (Iso infusion)

The achieved HRs at the three steady state Iso levels were: 75 (3), 96 (4) and 114 (8) bpm. Most parameters changed significantly (Table 2). In summary, although the absolute value of VR duration decreased (QT), its latter part (T_{p-e}) and consequently their ratio T_{p-e}/QT increased considerably, and so did QTc. QTcB exceeded 500 ms in 8 subjects and the T_{p-e}/QT ratio was >0.28 in 20 (see Discussion). Furthermore, because the QRS_{area} and T_{area} decreased and the angle between them (QRS-T area angle) widened, VG which is the vectorial sum of QRS_{area} and T_{area}, also decreased.

Muscarinic and β-AR blockade

The HR at Bl 2 was 66 (10) bpm and increased to 110 (9) bpm at steady state after atropine injection. As HR increased there was a paradoxical early prolongation of the QT interval at A1 compared to Bl 2 ($p < 0.001$), followed by rate adaptation and a decrease during the first minute; Figure 13. This paradoxical QT prolongation was fully reflected by the T_{p-e} increase which remained prolonged and resulted in the highest mean value of the T_{p-e}/QT ratio of 0.26 at A4. On the other hand QTcB reached its highest value at A3 and then decreased and stabilized within 2 minutes. In fact, QTcB increased to >500 ms in 14 subjects and the T_{p-e}/QT ratio was > 0.28 in 9 (6 had both). Furthermore, VG and T_{area} decreased as HR increased during A1 - A3 and remained unchanged during A4 - A6 with constant HR.

Table 2. Isoprenaline (Iso) effects on different parameters presented as mean (SD). The comparisons were made between baseline (Bl 1) and each dose level.
* $p \leq .05$, ** $p \leq .01$, *** $p < .001$.

	Bl 1	**Iso 1**	**Iso 2**	**Iso 3**
Heart rate (bpm)	57 (10)	75(3)***	96 (4)***	114 (8)***
MAP (mmHg)	73 (9)	75 (8)**	76 (10)**	71 (14)
Interval based parameters				
QRS (ms)	99 (11)	101 (11)***	100 (11)*	99 (11)
QT (ms)	399 (31)	397 (23)	372 (24)***	350 (25)***
QTcB (ms)	387 (21)	446 (25)***	469 (29)***	480 (26)***
QTcF (ms)	390 (20)	429 (24)***	434 (27)***	432 (25)***
$T_{p\text{-}e}$ (ms)	75 (8)	97 (19)***	110 (25)***	104 (23)***
$T_{p\text{-}e}$/QT	0.19 (0.02)	0.24 (0.04)***	0.29 (0.05)***	0.30 (0.05)***
Vector based parameters				
$QRS_{amplitude}$ (mV)	1.49 (0.40)	1.52 (0.41)	1.53 (0.38)	1.51 (0.39)
$T_{amplitude}$ (mV)	0.57 (0.18)	0.42 (0.16)***	0.48 (0.19)***	0.52 (0.18)
$T_{azimuth}$ (º)	21 (17)	20 (18)	36 (16)***	43 (14)***
$T_{elevation}$ (º)	57 (9)	60 (10)*	59 (11)	58 (10)
QRS-T angle (º)	30 (23)	38 (27)*	49 (30)***	55 (33)***
QRS-T area angle (º)	44 (25)	59 (27)***	73 (26)***	82 (28)***
QRS_{area} (µVs)	32 (11)	31 (11)*	30 (10)**	29 (11)**
T_{area} (µVs)	76 (24)	56 (21)***	59 (22)***	59 (21)***
VG (µVs)	100 (30)	74 (23)***	71 (22)***	67 (20)***
T loop morphology parameters				
T_{avplan} (µV)	0.58 (0.31)	0.67 (0.42)	0.76 (0.50)	0.80 (0.46)*
$T_{eigenvalue}$	176 (309)	103 (179)*	45 (51)*	31 (38)*

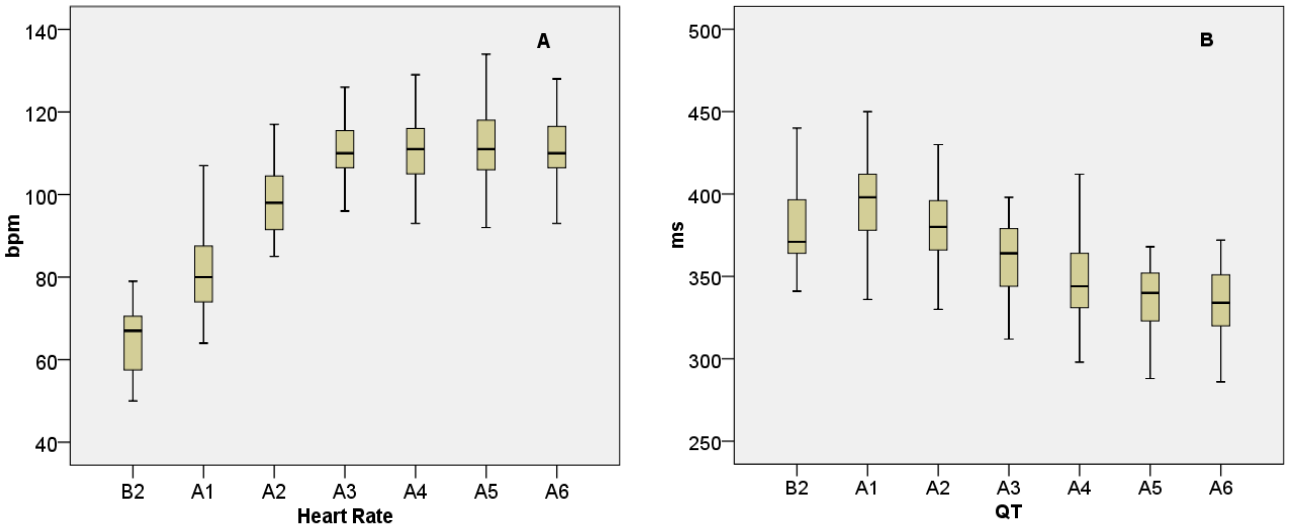

Figure 13. Box plots illustrating the changes in HR (A) and QT interval (B) from Bl2 following muscarinic receptor blockade with Atr at 6 consecutive steps: A1–A3 during the first minute and A4–A6 with 1-min interval.

In summary (Table 3), at steady state following atropine there were expected changes in "adrenergic" (sympathetic) direction, which were counteracted (as regards $QRS_{amplitude}$, QRS_{area}, QT, QTcB, T_{area}, $T_{amplitude}$, $T_{elevation}$, QRS-T area angle, VG, and $T_{eigenvalue}$) or fully reversed (as regards QRS and QTcF intervals) by propranolol, except for T_{avplan} which increased further.

Sex differences

At baseline, significant differences were observed for the QRS interval, $T_{amplitude}$, T_{area}, and VG; these parameters were significantly larger in men (QRS: 108 ± 7 vs. 90 ± 7 ms, $T_{amplitude}$: 0.69 ± 0.16 vs. 0.45 ± 0.08 mV, T_{area} 93 ± 22 vs. 58 ± 10 µVs; and VG 116 ± 32 vs. 82 ± 16 µVs; $P < 0.001$ for all comparisons). However, we did not observe any significant sex-related difference in the responses during the protocol.

Table 3. Effects of autonomic blockade on different measures presented as mean (SD). The comparisons were made between baseline (Bl 2) and atropine and atropine plus propranolol (A + P), respectively. * $p \leq .05$, ** $p \leq .01$, *** $p < .001$.

	Bl 2	**Atropine**	**A + P**
Heart rate (bpm)	66 (10)	110 (9)***	94 (9)***
MAP (mmHg)	75 (11)	84 (11)***	84 (11)***
Interval based parameters			
QRS (ms)	98 (11)	96 (10)***	98 (10)
QT (ms)	380 (29)	323 (20)***	334 (19)***
QTcB (ms)	394 (19)	437 (21)***	418 (18)***
QTcF (ms)	389 (19)	395 (20)**	388 (17)
$T_{p\text{-}e}$ (ms)	75 (9)	73 (10)	74 (8)
$T_{p\text{-}e}$/QT	0.20 (0.02)	0.23 (0.02)***	0.22 (0.02)***
Vector based parameters			
$QRS_{amplitude}$ (mV)	1.51 (0.39)	1.40 (0.36)***	1.46 (0.37)***
$T_{amplitude}$ (mV)	0.54 (0.17)	0.34 (0.15)***	0.43 (0.16)***
$T_{azimuth}$ (º)	24 (15)	27 (16)	25 (16)
$T_{elevation}$ (º)	58 (8)	64 (11)***	61 (10)***
QRS-T angle (º)	34 (24)	43 (27)***	41 (29)***
QRS-T area angle (º)	49 (25)	62 (27)***	54 (26)***
QRS_{area} (µVs)	33 (11)	28 (9)***	30 (10)***
T_{area} (µVs)	68 (23)	39 (18)***	49 (19)***
VG (µVs)	92 (27)	56 (19)***	70 (22)***
T loop morphology parameters			
T_{avplan} (µV)	0.59 (0.33)	0.69 (0.36)*	0.74 (0.37)**
$T_{eigenvalue}$	124 (183)	34 (54)***	57 (83)***

Paper III:

General analyses

The study population was divided into one group of adults ≥ 16 years of age: 47 Y111C carriers (27 women), 28 R518X carriers (21 women) and 78 age- and gender-matched controls (48 women); and one group of children 1-15 years of age: 10 Y111C carriers (6 girls), 14 R518X carriers (7 girls), and 43 controls (24 girls). In addition, 19 adult LQT2 mutation carriers were included (11 women).

Adult men and women

The two *KCNQ1* mutations differed significantly but modestly in QTcB. The difference was slightly larger in men than in women although women had longer QTcB intervals. In women there were also significant differences in the QRS duration and $T_{elevation}$, which both were larger in R518X carriers. Otherwise there was no significant difference in any other VR measures.

Comparing the 22 symptomatic LQT1 mutation carriers with the 53 mutations carriers without symptoms the only significant difference was a longer QT interval.

Comparison between controls and all LQT1 mutation carriers showed that QT, QTcB and $T_{p\text{-}e}$ intervals were significantly longer in LQT1. However, $T_{p\text{-}e}$/QT ratio was significantly larger in controls than in LQT1 carriers. Notably, there were no signs of increased heterogeneity of VR or of AP morphology in LQT1 subjects. In fact, $T_{amplitude}$ and VG (but not T_{area}) were both significantly larger in controls.

Comparing all LQT1 vs. all LQT2 mutation carriers revealed that QTcB, $T_{p\text{-}e}$, and

$T_{p\text{-}e}$/QT were significantly larger in LQT2 carriers but the VR dispersion (T_{area}, $T_{amplitude}$) was greater (within a pretty normal range) in LQT1 carriers.

Finally, there was an inverse relation between QTcB and VG both in LQT carriers and controls.

Children 1- 15 years of age

The QT and QTcB intervals were significantly longer in LQT1 mutation carriers while the $T_{p\text{-}e}$/QT ratio was larger in controls; otherwise there were no significant differences.

Paper IV:

Both LQTS and control groups were composed of 15 men and 26 women with the mean age (SD) 38 (13) in the LQTS and 37 (15) in the control group. As regards one-minute-averaged parameters, in line with our previous study (paper III), the QT interval was significantly longer in mutation carriers than in controls, however, there was no sign of increased heterogeneity of AP morphology (VG) or VR (T_{area}) in the LQTS carriers. In addition, LQT2 mutation carriers showed significantly longer $T_{p\text{-}e}$ and smaller $T_{amplitude}$ than LQT1 carriers and had a trend for longer QTcB and larger $T_{p\text{-}e}$/QT.

Beat to beat variability

As shown in Table 4 there were significant differences in beat to beat variability (instability) measured as STV for almost all parameters between controls and the LQTS group except for QRS, QRS_{area} and $QRS_{amplitude}$. In fact all VR parameters, except for $T_{eigenvalue}$, had higher STV (by approximately a factor of 2) in mutation carriers than in controls while STV_{RR} was higher in the control group ($p \leq .001$); Figure 14. Mutation carriers also had significantly higher QTVI than controls. In contrast, the comparison of beat to beat variability between LQT1 and LQT2 only showed a trend for higher STV_{QT} and QTVI in LQT2.

Table 4. Mean (SD) for STV of most parameters, as well as QTVI. P-values are based on comparisons of controls vs. all LQTS (3rd column) and LQT1 vs. LQT2 (5th column). * $p \leq .05$, ** $p \leq .01$, *** $p < .001$.

	Control	All LQTS	LQTS1	LQTS2
Interval based parameters				
STV_{RR} (ms)	38 (29)	22 (16)**	24 (17)	17 (12)
STV_{QRS} (ms)	1.4 (0.3)	1.6 (0.5)	1.6 (0.5)	1.6 (0.5)
STV_{QT} (ms)	2.0 (1.0)	4.8 (2.2)***	4.3 (2)	6.3 (2.2)*
Vector based parameters				
$STV_{QRSamplitude}$ (mV)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
$STV_{Tamplitude}$ (mV)	0.01 (0.01)	0.02 (0.01)***	0.02 (0.01)	0.02 (0.01)
$STV_{Tazimuth}$ (º)	1.8 (0.9)	4.2 (2.3)***	4.1 (2.4)	4.6 (2.1)
$STV_{Televation}$ (º)	1.7 (1.0)	4.4 (2.7)***	4 (2.3)	5.7 (3.6)
$STV_{QRS\text{-}T\ angle}$ (º)	2.5 (2.2)	4.2 (2.2)***	4.1 (2.6)	4.3 (1.7)
$STV_{QRS\text{-}T\ area\ angle}$ (º)	2.4 (1.3)	5.3 (2.9)***	5.3 (3)	5.3 (2.6)
$STV_{QRSarea}$ (µVs)	0.9 (0.4)	0.9 (0.3)	1 (0.3)	0.9 (0.4)
STV_{Tarea} (µVs)	2.8 (1.5)	5.4 (2.8)***	5.4 (2.9)	5.7 (2.8)
STV_{VG} (µVs)	3.3 (1.7)	6.1 (2.6)***	6.1 (2.7)	6.1 (2.3)
T loop morphology parameters and QTVI				
$STV_{Tavplan}$ (µV)	0.09 (0.05)	0.14 (0.08)***	0.14 (0.07)	0.15 (0.11)
$STV_{Teigenvalue}$	24 (55)	6 (9)**	6.5 (10)	5 (4)
QTVI	− 1.8 (0,4)	− 0.9 (0.7)***	− 1.1 (0.4)	− 0.3 (1)*

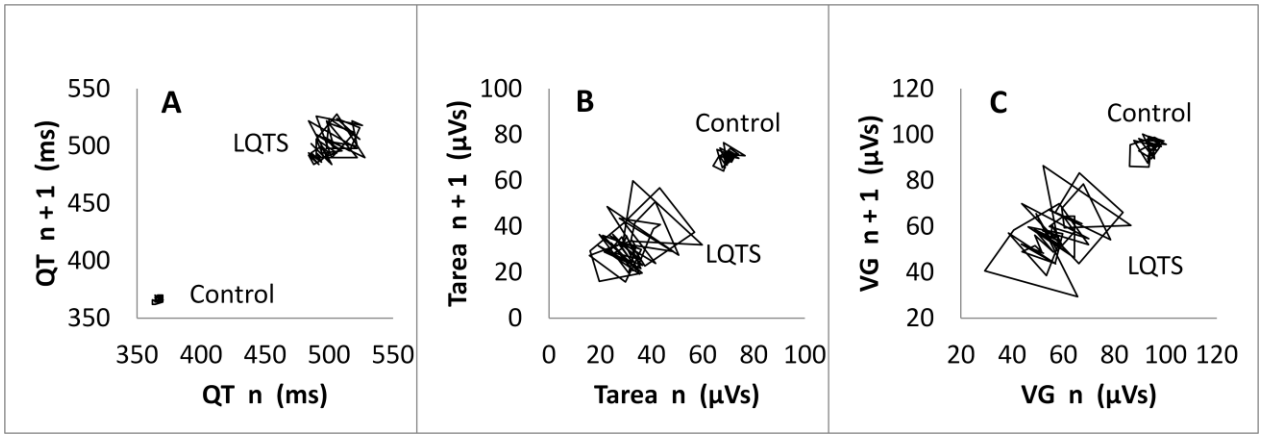

Figure 14. Poincare plots obtained from one subject in the LQTS group and one control subject illustrating increased instability of VR measures in the LQTS subject. Please note that the points are connected to show the sequential relation, while the calculations are based on the orthogonal distances from the identity line (not shown).

Furthermore there was a significant correlation ($p < .001$) between the QT interval and STV_{QT}, STV_{Tarea} and STV_{VG} in both controls and LQTS subjects; Figure 15.

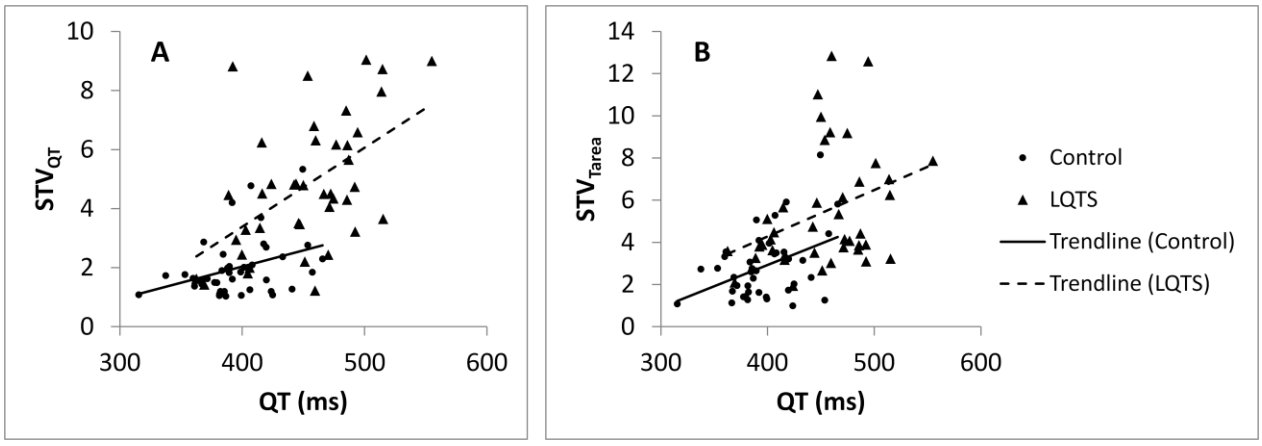

Figure 15. There was a significant correlation between the QT interval and STV_{QT} (panel A) and STV_{Tarea} (panel B) in both controls and LQTS subjects.

Influence of β-blocker therapy

Eighteen mutation carriers (44%; 11 LQT1 and 7 LQT2) were on β-blocker therapy. The comparison between them and those who were not on medication showed a trend ($0.01 < p < .05$) toward longer QTcB [470 (26) vs. 452 (25) ms], smaller T_{area} [58 (27) vs. 82 (34) μVs], and smaller $T_{amplitude}$ [0.37 (0.16) vs. 0.53 (0.20) mV] in the β-blocker group. We also found a trend toward lower STV_{Tarea}, STV_{VG}, $STV_{Tavplan}$ and $STV_{Tamplitude}$ in this group.

DISCUSSION

Our project was composed of two main parts, the first part (Paper I & II) dealt with the physiological aspects of VR where we tried to define both HR and ANS influences on VCG-derived VR measures in healthy men and women. We found that:

1. Changes in atrial paced HR from 80 to 120 bpm not only shortened VR duration but also decreased VCG-derived parameters such as VG, QRS_{area}, T_{area}, and $T_{amplitude}$. In contrast, T-loop morphology parameters and $T_{p\text{-}e}$/QT were not affected within this range of HRs.

2. By increasing HR, β-AR stimulation with incremental Iso infusion decreased HR-dependent parameters, reflecting the total heterogeneity of VR (T_{area}) and AP morphology (VG).

3. Both Iso infusion and Atr injection prolonged QTcB and increased the $T_{p\text{-}e}$/QT to levels observed in conditions with increased arrhythmia risk, such as congenital and acquired LQTS, thereby mimicking pathological conditions.

4. HR acceleration after muscarinic blockade with Atr was accompanied by a transient paradoxical QT prolongation and delayed HR adaptation of T_{area} and VG.

The second part of the project (Paper III & IV) concerns the pathophysiology of VR where two LQT1 mutations with in vitro different biophysical properties (Y111C and R518X)[52] were compared with each other, with age- and sex-matched controls, and with LQT2 mutation carriers (Paper III). In addition we compared beat to beat

variability of VCG derived VR measures between LQTS mutation carriers and healthy control subjects (Paper IV). The main findings were as follows:

1. Apart from a longer QTcB interval in Y111C mutation carriers, there were no significant differences between Y111C and R518X mutations at rest despite their different biophysical effects on ion channel function.

2. LQT2 carriers had longer QTcB and $T_{p\text{-}e}$ and a greater $T_{p\text{-}e}$/QT ratio suggesting higher arrhythmia risk at rest.

3. There was no consistent pattern of increased VR dispersion among LQT1 and LQT2 mutation carriers.

4. Mutation carriers showed significantly higher instability in most repolarization parameters than controls despite lower instantaneous HR variability (STV_{RR}).

5. Beta-blocker therapy seemed to have some effect on decreasing the instability of VR parameters in this group of LQTS mutation carriers.

AP duration

VR is responsible for the main part of AP duration (QT interval) and is well known to be HR or interval dependent, hence usually HR-corrected, most commonly according to Bazett as $QTcB = QT / RR^{1/2}$ (RR in seconds).

In contrast, based on measures from ECG-lead V6 in healthy individual, the $T_{p\text{-}e}$/QT ratio has a constant value in different HR but is significantly greater in patients at risk for arrhythmic events and a value more than 0.28 might indicate a higher

propensity for TdP in those with long and short QT syndrome, Brugada syndrome, and also in patients with acute myocardial infarction.[84]

Based on VCG measures (Paper I) we showed that in healthy individuals QTcB only changed minimally during atrial pacing (no case > 450 ms at HR 100-120 bpm) and the $T_{p\text{-}e}$/QT ratio had a constant value with a mean (SD) of 0.20-0.21 (0.03) irrespective of HR (no case > 0.26). In contrast, these parameters increased significantly in response to HR increase by Iso or Atr (Paper II). In fact, more than 50% of subjects had QTcB > 500 ms or $T_{p\text{-}e}$/QT to > 0.28 following Iso infusion or Atr injection, responses that are recognized as associated with increased propensity for malignant arrhythmias.[84] As mentioned above, animal studies have shown that T_{peak} and T_{end} almost coincide with the earliest and latest end of repolarization, respectively and $T_{p\text{-}e}$ has, therefore, been suggested as an index of global dispersion of VR duration.[39-41] Consequently, disproportional increase in $T_{p\text{-}e}$ may reflect a more heterogeneous repolarization which results in a higher dispersion in myocardial cell refractoriness. Increased heterogeneity of refractoriness between adjacent regions of myocardium can be responsible for wave-breaks in signal conduction which could result in reentrant arrhythmias.

The prolongation of $T_{p\text{-}e}$ following Iso infusion in our study could be interpreted as a sign of heterogeneous concentration of β-ARs and/or diverse response to Iso in different parts of the myocardium via other mechanisms, such as heterogeneous distribution of ion channels.

While β-AR activation of I_{Ks} results in shortening of VR, activation of I_{CaL} shifts the plateau of the AP to more positive values and prolongs VR.[85] Furthermore, some studies suggest that β-AR stimulation may even activate Ca^{2+}/calmodulin-dependent kinase II (CaMKII). This enzyme affects the function of both the membranous I_{CaL}

and the intracellular RyR2, phospholamban, as well as the sarcoplasmic reticulum Ca^{2+}-ATPase 2, which regulates the release and recycling of Ca^{2+} by the sarcoplasmic reticulum, crucial for the electro-mechanical coupling.[86,87] In addition, recent work has shown that CaMKII regulates Na^{+} channels, e.g., by enhancing late I_{Na}, which may prolong AP duration.[88] Our observations on QTcB and $T_{p\text{-}e}$/QT responses to Iso may be explained by either PKA-modulated I_{CaL} or CaMKII-mediated effects, or both.

A simple explanation for the role of QTcB prolongation and $T_{p\text{-}e}$/QT increase in arrhythmia formation and consequently the importance of studying these parameters is that during the phase 2 of the AP (plateau phase) different ion channels in the cell membrane have very low conductance, so relatively small changes in net current in this phase can not only result in a prolongation of AP but also in a significant change in membrane potential. As explained previously oscillations in membrane potential can cause EADs and triggered activities which in the presence of increased heterogeneity of refractoriness (increased $T_{p\text{-}e}$) may lead to wave-breaks and initiation of functional reentry resulting in the formation of TdP.

Paradoxical prolongation of the AP duration and the QT interval has previously been observed a few seconds following administration of adrenergic drugs in both guinea pig papillary muscle and dogs.[89,90] This initial prolongation has been suggested to be due to faster activation of I_{CaL} than of I_{Ks} by PKA.[91] This is (Paper II), to the best of our knowledge, the first time that paradoxical QT prolongation has been documented in humans following β-AR activation, here induced by muscarinic receptor blockade with Atr. The mechanism behind the effects of muscarinic receptor stimulation is less well understood compared with β-AR stimulation. It is, however, known that acetylcholine has an antagonistic effect on β-AR-stimulated I_{CaL} and Iso-induced activation of I_{Ks}.[85] In addition, muscarinic cholinergic agonists

inhibit release of norepinephrine from sympathetic nerve terminals.[92] Therefore, it is conceivable that, even following Atr injection and inhibition of muscarinic receptors, the faster activation of I_{CaL} should be the main underlying mechanism for the brief and transient paradoxical QT prolongation. In addition, our observation that, shortly after Atr injection, > 50% of the individuals had a prolonged QTcB > 500 ms, a $T_{p\text{-}e}/QT > 0.28$, or both, might indicate that vagal withdrawal can be as important for increasing the propensity for ventricular arrhythmias in patients with long QT syndrome as sympathetic stimulation in the presence of retained vagal activity.

The two LQT1 mutations compared in Paper III are located at different ends of the channel protein, Y111C in the N- and R518X in the C-terminal and they are also considered to have different effects on the I_{Ks} channel function.[93,94] R518X is a nonsense mutation and its functional effect is classified as haplo-insufficiency meaning ≤ 50 % reduction of channel function. Y111C is a point mutation with dominant negative effect, which means > 50 % reduction of channel function[95] and according to functional studies more than 75 % reduction in the in vitro setting.[93,94] A rather modest difference in QTcB was the only consistent difference (Y111C vs. R518X: 460± 26 vs. 446± 33ms; p=0.02). Any other existing difference between the two LQT1 mutations might not become apparent until the response to HR increase or catecholamines are assessed.

At least in LQT1, syncope or sudden death is triggered mostly by emotional or physical stress[96] and QT interval prolongation may be especially notable during or after exercise or epinephrine challenge.[97] Also in Y111C mutation carriers physical exercise and emotional stress were the most common triggers for syncopal episodes.[94] Our finding (Paper II) that even in healthy individuals a transient paradoxical QT prolongation results from β-adrenergic stimulation following

muscarinic blockade with Atr raises the question whether such responses are more prominent in LQTS mutation carriers and can be used to identify patients at risk for malignant arrhythmias. This issue obviously requires further studies.

As mentioned previously, $T_{p\text{-}e}$/QT has been proposed as an index for arrhythmogenicity and a value of 0.28 has been suggested as a good cut-off point for propensity for TdP.[84,98] In Paper III LQT2 mutation carriers had significantly larger $T_{p\text{-}e}$/QT than LQT1 carriers (0.24 ± 0.07 vs. 0.19 ± 0.03, $p = 0.000$) and in fact more than 25% of them (5 of 19) had a $T_{p\text{-}e}$/QT larger than 0.28 while only one of the LQT1 mutation carriers reached this value. This finding might reflect that syncope or sudden death in LQT2 can occur even at rest.[96,99]

AP heterogeneity

VG reflects the heterogeneity of AP morphology.[44] As HR increases (Paper I), in the absence of conduction abnormalities, the heterogeneity in both ventricular depolarization instants (QRS_{area}) and AP morphology (VG) decreases which results in a less heterogeneous global VR. Figure 16 is a simplified illustration of how we interpret the effects of HR increase on the VG's components, QRS_{area} and T_{area} as well as on $T_{amplitude}$.

We showed that increasing HR from 80 to 120 bpm by atrial pacing decreased both VG and T_{area} in linear relation to HR changes (Paper I). Therefore the directional changes in these parameters as HR increased during Iso infusion (Paper II) were more or less expected, but their response to HR increase following β-AR stimulation was not linear, which may reflect differences in the rate dependency of β-adrenergic modulation of currents affecting repolarization.[100]

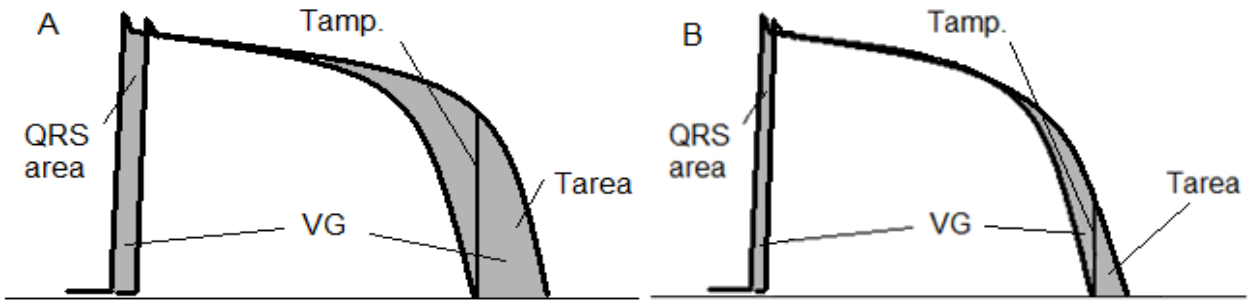

Figure 16. A simplified illustration of the effects of change from a lower (A) to a higher (B) heart rate on the ventricular gradient's components, QRS_{area} and T_{area}, and the relation to $T_{amplitude}$.

While the role of EADs and DADs in initiating TdP ventricular tachycardias is generally accepted, the role of VR dispersion is less clear. We did not observe any signs of increased dispersion of AP morphology or VR in LQT1 or LQT2 mutation carriers at rest (Paper III). Among both LQT1 and LQT2 carriers as well as among control subjects there was actually an inverse relation between QTcB and VG, T_{area} and $T_{amplitude}$. This is novel information suggesting that there is no permanent arrhythmia substrate due to increased dispersion of AP morphology and VR at rest in these mutation carriers. As a consequence, there emerges a need for a new explanation how the TdP becomes sustained.

AP Instability

In Paper IV we compared the QT variability in LQTS mutation carriers with controls using the two different parameters applied in previous studies, QTVI and STV_{QT}, and showed that both were significantly higher in LQTS by a factor ≥ 2. As regards STV_{QT} a similar difference has previously been shown by *Hinterseer M et*

al., who suggested that increased STV_{QT} was a useful noninvasive additive diagnostic marker in congenital LQTS.[76] STV_{QT} is an index of beat to beat variability of the QT interval, based on the mean orthogonal distance from the diagonal to the points of the Poincaré plot, Figure 14. In both animal and human models, especially in acquired LQTS, increased STV_{QT} was a better predictor of proarrhythmia than prolongation of QT.[70-75] By comparison, QTVI is based on the variance and mean of QT and RR intervals and is not affected by the order in which the intervals are recorded. QTVI was initially proposed by *Berger RD et al.* when they showed that dilated cardiomyopathy was associated with temporal VR lability.[77] Since the initial publication, increased QTVI has been shown to be associated with an increased risk for arrhythmias and SCD in several patient populations.[78-83] The novel aspect of our study is the use of VCG instead of surface ECG in our analysis. VCG based on the orthogonal Frank lead system is more anatomically representative than routine scalar ECG in evaluating the global VR which is especially relevant in LQTS studies and also when measuring the variability of different VR parameters (please see the introduction). To the best of our knowledge, except for the $STV_{QT,}$ this is the first time that increased STV of different VR parameters has been shown in LQTS mutation carriers. The correlation between the VR duration and instability as shown in Figure 16 is also of potential importance, as well as the observations during betablockade. However, the causal relationship and prognostic value of these findings warrants further evaluation.

Sex Differences

In our project we studied the sex differences in VCG-based VR parameters in healthy individuals at baseline and during ANS perturbations in Paper II and in LQTS mutation carriers compared to healthy subjects in Paper III.

While we did not observe any significant sex-related difference in the responses to ANS perturbations in healthy persons some VR parameters like $T_{amplitude}$, T_{area}, and VG were significantly larger in men at baseline. In addition, there were sex-related differences within the subgroups of LQTS mutation carriers emphasizing the importance of sex-related analyses in this context.

Clinical implications

We have found that increased HR through atrial pacing decreases both the duration and the heterogeneity of VR, in contrast, sympathetic stimulation by Iso or Atr increases two parameters indicating risk for arrhythmia formation i.e. QTcB and $T_{p\text{-}e}$/QT. Based on our findings and hypothetically a combination of beta-blocker therapy and overdrive pacing could be able to modulate the propensity for ventricular arrhythmias and can be used, for example, in patient with LQTS and also in emergency settings in patients suffering from electrical storm and/or incessant ventricular tachycardia specially those who already carry a pacemaker or an implantable cardioverter defibrillator (ICD). In fact, overdrive pacing has already been used in the immediate phase after atrioventricular junction (His' bundle) ablation to decrease the risk of ventricular arrhythmias and SCD.[101]

The observed paradoxical prolongation of QT interval following Atr injection in healthy subjects in Paper II is most probably even more prominent in LQTS mutation carriers which together with beat to beat variability of VR parameters could be used as a diagnostic tool and for risk assessment in LQTS.

Finally, by studying the variability of VR parameters in patient with documented ventricular arrhythmias, for example, in those with an ICD, or even by comparing the response of the variability of VR parameters to Atr injection in healthy subjects and LQTS mutation carriers we might be able to develop a noninvasive diagnostic tool for patients at higher risk for ventricular arrhythmias and SCD.

CONCLUSIONS

1. In healthy individuals the pure HR increase by atrial pacing decreased the heterogeneity of AP morphology and VR, while similar increase in HR by β-adrenoceptor stimulation or vagal withdrawal had different effects on VR and resulted in changes recognized in conditions with increased arrhythmia risk, such as congenital and acquired long QT syndromes. As regards VR, a substantial overlap between physiological and pathophysiological electrophysiology, therefore, seems to exist.

2. At supine rest there was a modest but significant difference in QTcB but otherwise no difference in VCG measures between carriers of two LQT1 mutations with in vitro different biophysical effects on the I_{Ks} channel function.

3. There were no signs of increased VR dispersion in LQT1 and LQT2 mutation carriers at rest. In fact, there was an inverse relation between QTcB and measures of global heterogeneity of AP morphology and VR in both controls and LQTS carriers.

4. Although LQTS mutation carriers did not show any sign of increased heterogeneity of VR at rest compared to healthy controls, they had higher instability of VR duration and heterogeneity at beat to beat analysis. A greater instability of most aspects of VR already at rest thus seems to be a salient feature in both LQT1 and LQT2, which might pave the way for afterdepolarizations and ventricular arrhythmias.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all colleagues and co-workers who have contributed to this thesis. In particular I would like to thank:

Lennart Bergfeldt, my research supervisor and electrophysiology mentor, for generously sharing your priceless knowledge and enthusiasm.

Thomas Gilljam, my co-supervisor and colleague, for all your help and support.

All my other **co-authors**, for your valuable contribution.

Per Hallgren, Mikael Holmberg and **Sigfus Gizurarson**, my former colleagues, for your invaluable clinical advice.

Lars Grip, **Göran Matejka** and **Bert Andersson**, my former bosses, for the grate educational opportunity.

All my **colleagues** and **co-workers** at the Department of Cardiology, Sahlgrenska University Hospital, for your support and friendship.

Arne Lie, the hospital director and **Olaf Rødevand**, the head of the Department of Cardiology, Feiringklinikken, Norway, for accepting me as a member of your team and for your transparent leadership.

All my **colleagues** and **co-workers** at Feiringklinikken, for making our clinic not only a very effective but also a quite joyful workplace.

My parents, brother and **sister,** for all the encouragement and your unconditional support.

My wife, **Jynfiaf**, for your patience and all the sacrifices you have made during these years, and for your utter love.

Alice and **Daniel**, for being my inspiration and joy of life.

REFERENCES

1. Waller AG: A demonstration on man of electromotive changes accompanying the heart's beat. J Physiol 1887; 8:229-234.

2. Lewalter T, Luderitz B: Historical milestones of electrical signal recording and analysis. In: Contemporary Cardiology: Cardiac Repolarization: Bridging Basic and Clinical Science. Edited by Gussak I, et al. Totowa, NJ: Humana, 2010.

3. Wilson FN, MacLeod AG, Barker PS, Johnston FD: The determination and significance of the areas of the ventricular deflections of the electrocardiogram. Am Heart J 1934; 10:46-61.

4. Frank E: An accurate, clinically practical system for spatial vectorcardiography. Circulation 1956; 13:737-749.

5. Nerbonne JM, Kass RS: Physiology and molecular biology of ion channels contributing to ventricular repolarization. In: Contemporary Cardiology: Cardiac Repolarization: Bridging Basic and Clinical Science. Edited by Gussak I, et al. Totowa, NJ: Humana, 2010.

6. Anderson KP: Sympathetic nervous system activity and ventricular tachyarrhythmias: recent advances. Ann Noninvasive Electrocardiol 2003; 8:75-89.

7. Walsh DA, Van Patten SM: Multiple pathway signal transduction by the cAMP-dependent protein kinase. FASEB J 1994; 8:1227-1236.

8. Kaumann AJ, Molenaar P: Modulation of human cardiac function through 4 beta-adrenoceptor populations. Naunyn Schmiedebergs Arch Pharmacol 1997; 355: 667-681.

9. Zygmunt AC: Intracellular calcium activates chloride current in canine ventricular myocytes. Am J Physiol 1994; 267:1984-1995.

10. Badilini F, Fayn J, Maison-Blanche P, Leenhardt A, Forlini MC, Denjoy I, Coumel P, Rubel P: Quantitative aspects of ventricular repolarization: relationship between three-dimensional T wave loop morphology and scalar dispersion. Ann Noninvasive Electrocardiol 1997; 2:146-157.

11. Nowinski K, Jensen S, Lundahl G, Bergfeldt L: Changes in ventricular repolarization during percutaneous transmural coronary angioplasty in human assessed by QT interval, QT dispersion and T vector loop morphology. J Intern Med 2000; 248:126-136.

12. Rubulis A, Jensen J, Lundahl G, Tapanainen J, Wecke L, Bergfeldt L: T vector and loop characteristics in coronary artery disease and during acute ischemia. Heart Rhythm 2004; 1:317-325.

13. Sahlén A, Rubulis A, Winter R, Jacobsen PH, Ståhlberg M, Tornvall P, Bergfeldt L, Braunschweig F: Cardiac fatigue in long-distance runners is associated with ventricular repolarization abnormalities. Heart Rhythm 2009; 6:512-519.

14. Wecke L, Gadler F, Linde C, Lundahl G, Rosen M, Bergfeldt L: Temporal Characteristics of cardiac memory in humans: vectorcardiographic quantification in a model of cardiac pacing. Heart Rhythm 2005; 2:28-34.

15. Wecke L, Rubulis A, Lundahl G, Rosen MR, Bergfeldt L: Right ventricular pacing-induced electrophysiological remodelling in the human heart and its relationship to cardiac memory. Heart Rhythm 2007; 4:1477-1486.

16. Wecke L, Poçi D, Schwieler J, Johansson B, Edvardsson N, Lundahl G, Bergfeldt L: Vectorcardiography shows cardiac memory and repolarization heterogeneity after ablation of accessory pathways not apparent on ECG. Int J Cardiol 2013; 5:152-157.

17. Malik M: Errors and misconceptions in ECG measurement used for the detection of drug induced QT interval prolongation. J Electrocardiol 2004; 37,Suppl:25-33.

18. Kors JA, van Herpen G, van Bemmel JH: QT dispersion as an attribute of T-loop morphology. Circulation 1999; 99:1458-1463.

19. Diamant UB, Winbo A, Stattin EL, Rydberg A, Kesek M, Jensen SM: Two automatic QT algorithms compared with manual measurement in identification of long QT syndrome. J Electrocardiol 2010; 43:25-30.

20. Larsen JA, Kadish AH: Effects of gender on cardiac arrhythmias. J Cardiovasc Electrophysiol 1998; 9:655-664.

21. Conrath CE, Opthof T: Ventricular repolarization: An overview of (patho)physiology, sympathetic effects and genetic aspects. Prog Biophys Mol Biol 2006; 92:269-307.

22. Attwell D, Cohen I, Eisner DA: The effects of heart rate on the action potential of guinea-pig and human ventricular muscle. J Physiol (Lond) 1981; 313:439-461.

23. Arnold L, Page J, Attwell D, Cannell M, Eisner DA: The dependence on heart rate of the human ventricular action potential duration. Cardiovasc Res 1982; 16:547-551.

24. Lecocq B, Lecocq V, Jaillon P: Physiologic relation between cardiac cycle and QT duration in healthy volunteers. Am J Cardiol 1989; 64:481-486.

25. Browne KF, Zipes DP, Heger JJ, Prystowsky EN: Influence of the autonomic nervous system on the Q-T interval in man. Am J Cardiol 1982; 50:1099-1103.

26. Magnano AR, Holleran S, Ramakrishnan R, Reiffel JA, Bloomfield DM: Autonomic nervous system influenced on QT internal in normal subjects. J Am Coll Cardiol 2002; 39:1820-1826.

27. Magnano AR, Talathoti N: Sympathomimetic infusion and cardiac repolarization. J Cardiovasc Electrophysiol 2006; 17:983-989.

28. Kootsey JM, Johnson EA: The origin of the T-wave. CRC Crit Rev Bioeng 1980; 4:233–270.

29. Franz MR, Bargheer K, Costard-Jackle A, Miller DC, Lichtlen PR: Human ventricular repolarization and T wave genesis. Prog Cardiovasc Dis 1991; 33:369-384.

30. Draisma HHM, Schalij MJ, Van Der Wall EE, Swenne CA: Elucidation of the spatial ventricular gradient and its link with dispersion of repolarization. Heart Rhythm 2006; 3:1092-1099.

31. Zabel M, Portnoy S, Franz MR: Electrocardiographic indexes of dispersion of ventricular repolarization: an isolated heart validation study. J Am Coll Cardiol 1995; 25:746-752.

32. Van Opstal JM, Verduyn SC, Winckels SK, Leerssen HM, Leunissen JD, Wellens HJ, Vos MA: The JT-area indicates dispersion of repolarization in dogs with atrioventricular block. J Interv Card Electrophysiol 2002; 6:113-120.

33. Van Huysduynen BH, Swenne CA, Draisma HHM, Antoni ML, Van de Vooren H, Van der Wall EE, Schalij MJ: Validation of ECG indices of ventricular repolarization heterogeneity: a computer simulation study. J Cardiovasc Electrophysiol 2005; 16:1097-1103.

34. Antzelevitch C, Sicouri S, Litovsky SH, Lukas A, Krishnan SC, Di Diego JM, Gintant GA, Liu DW: Heterogeneity within the ventricular wall: Electrophysiology and pharmacology of epicardial, endocardial and M cells. Cir Res 1991; 69:1427-1449.

35. Antzelevitch C, Shimizu W, Yan GX, Sicouri S, Weissenburger J, Nesterenko VV, Burashnikov A, Di Diego J, Saffitz J, Thomas GP: The M cell: its contribution to the ECG and to normal and abnormal electrical function of the heart. J Cardiovasc Electrophysiol 1999; 10:1124-1152.

36. Yan GX, Martin J: Electrocardiographic T wave. A symbol of transmural dispersion of repolarization in the ventricles. J Cardiovasc Electrophysiol 2003; 14:639-640.

37. Anyukhovsky EP, Susunov EA, Gainullin RZ, Rosen MR: The controversial M cell. J Cardiovasc Electrophysiol 1999; 10:244-260.

38. Taggart P, Sutton P, Opthof T, Coronel R, Kallis P: Electrotonic cancellation of transmural electrical gradients in the left ventricle in man. Prog Biophys Mol Biol 2003; 82:243-254.

39. Xia Y, Liang Y, Kongstad O, Liao Q, Holm M, Olsson B, Yuan S: In vivo validation of the coincidence of the peak and end of the T wave with full repolarization of the epicardium and endocardium in swine. Heart Rhythm 2005; 2:162-169.

40. Xia Y, Liang Y, Kongstad O, Liao Q, Holm M, Olsson B, Yuan S: Tpeak-Tend interval as an index of global dispersion of ventricular repolarization: evaluations using monophasic action potential mapping of the epi- and endocardium in swine. J Interv Card Electrophysiol 2005; 14:79-87.

41. Opthof T, Coronel R, Wilms-Schopman FJG, Plotnikov AN, Shlapakova IN, Danilo P, Rosen MR, Janse MJ: Dispersion of repolarization in canine ventricle and the electrocardiographic T wave: Tp-e interval does not reflect transmural dispersion. Heart Rhythm 2007; 4:341-348.

42. Gardberg M, Rosen IL: Monophasic curve analysis and the ventricular gradient in the electrogram of strips of turtle ventricle. Circ Res 1959; 7:870-875.

43. Abildskov JA, Urie P, Lux R, Burgess MJ, Wyatt R: Body surface distribution of QRST area. Adv Cardiol 1978; 21:59-64.

44. Geselowitz DB: The ventricular gradient revisited: relation to the area under the action potential. IEEE Trans Biomed Eng 1983; 30:76-77.

45. Draper HW, Peffer CJ, Stallmann FW, Littmann D, Pipberger HV: The corrected orthogonal electrocardiogram and vectorcardiogram in 510 normal men (Frank lead system). Circulation 1964; 30:853-864.

46. Van Huysduynen BH, Swenne CA, Bax JJ, Bleeker GB, Draisma HH, Van Erven L, Molhoek SG, Van de Vooren H, Van der Wall EE, Schalij MJ: Dispersion of repolarization in cardiac resynchronization therapy. Heart Rhythm 2005; 2:1286-1293.

47. Kardys I, Kors JA, Van der Meer I, Hofman A, Van der Kuip DA, Witteman JC: Spatial QRS-T angle predicts cardiac death in a general population. Eur Heart J 2003; 24:1357-1364.

48. Moss AJ, Kass RS: Long QT syndrome: from channels to cardiac arrhythmias. J Clin Invest 2005; 115:2018-2024.

49. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, Keating MT: Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation 2000; 102:1178-1185.

50. Tester DJ, Will ML, Haglund CM, Ackerman MJ: Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. Heart Rhythm 2005; 2:507-517.

51. Schwartz PJ, Ackerman MJ: The long QT syndrome: a transatlantic clinical approach to diagnosis and therapy. Eur Heart J 2013 Mar 18. [Epub ahead of print]

52. Ackerman MJ: The long QT syndrome: ion channel diseases of the heart. Mayo Clin Proc 1998; 73:250-69.

53. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT: Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 1996; 12:17-23.

54. Roden DM: Clinical practice. Long-QT syndrome. N Engl J Med 2008; 358:169-176.

55. Cranefield PF: Action potentials, afterpotentials and arrhythmias. Circ Res 1977; 41:415-423.

56. Wit AL, Rosen MR: Afterdepolarizations and triggered activity: Distinction from automaticity as an arrhythmogenic mechanism. In: The Heart and Cardiovascular System. Edited by Fozzard HA, et al. New York: Raven Press, 1992.

57. Antzelevitch C, Burashnikov A, Di Diego JM: Cellular and ionic mechanism underlying arrhythmogenesis. In: Contemporary Cardiology: Cardiac Repolarization: Bridging Basic and Clinical Science. Edited by Gussak I, et al. Totowa, NJ: Humana, 2010.

58. Antzelevitch C, Sicouri S: Clinical relevance of cardiac arrhythmias generated by afterdepolarizations: The role of M cells in the generation of U waves, triggered activity and torsade de pointes. J Am Coll Cardiol 1994; 23:259-277.

59. El-Sherif N, Caref EB, Yin H, Restivo M: The electrophysiological mechanism of ventricular arrhythmias in the long QT syndrome: Tridimensional mapping of activation and recovery patterns. Circ Res 1996; 79:474-492.

60. Shimizu W, Antzelevitch C: Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade de pointes in LQT2 and LQT3 models of the long-QT syndrome. Circulation 1997; 96:2038-2047.

61. Shimizu W, McMahon B, Antzelevitch C: Sodium pentobarbital reduces transmural dispersion of repolarization and prevents torsade de pointes in models of acquired and congenital long QT syndromes. J Cardiovasc Electrophysiol 1999; 10:156-164.

62. El-Sherif N, Chinushi M, Caref EB, Restivo M: Electrophysiological mechanism of the characteristic electrocardiographic morphology of torsade de pointes tachyarrhythmias in the long-QT syndrome. Detailed analysis of ventricular tridimensional activation patterns. Circulation 1997; 96:4392-4399.

63. Priori SG, Napolitano C, Schwartz PJ: Low penetrance in the long-QT syndrome: clinical impact. Circulation 1999; 99:529-533.

64. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D: Risk stratification in the long-QT syndrome. N Engl J Med 2003; 348:1866-1874.

65. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, Hall WJ, Weitkamp L, Vincent GM, Garson A Jr, et al.: The long QT syndrome: prospective longitudinal study of 328 families. Circulation 1991; 84:1136-1144.

66. Shimizu W, Noda T, Takaki H, Kurita T, Nagaya N, Satomi K, Suyama K, Aihara N, Kamakura S, Sunagawa K, et al.: Epinephrine unmasks latent mutation carriers with LQT1 form of congenital long-QT syndrome. J Am Coll Cardiol 2003; 41:633-642.

67. Monnig G, Eckardt L, Wedekind H, Haverkamp W, Gerss J, Milberg P, Wasmer K, Kirchhof P, Assmann G, Breithardt G, Schulze- Bahr E: Electrocardiographic risk stratification in families with congenital long QT syndrome. Eur Heart J 2006; 27:2074-2080.

68. Hofman N, Wilde AA, Kaab S, van Langen IM, Tanck MW, Mannens MM, Hinterseer M, Beckmann BM, Tan HL: Diagnostic criteria for congenital long QT syndrome in the era of molecular genetics: do we need a scoring system? Eur Heart J 2007; 28:575-580.

69. Extramiana F, Tatar C, Maison-Blanche P, Denjoy I, Messali A, Dejode P, Iserin F, Leenhardt A: Beat-to-beat T-wave amplitude variability in the long QT syndrome. Europace 2010; 12:1302-1307.

70. Hondeghem LM, Carlsson L, Duker G: Instability and Triangulation of the Action Potential Predict Serious Proarrhythmia, but Action Potential Duration Prolongation Is Antiarrhythmic. Circulation 2001; 103:2004-2013.

71. Gbadebo TD, Trimble RW, Khoo MS, Temple J, Roden DM, Anderson ME: Calmodulin inhibitor W-7 unmasks a novel electrocardiographic parameter that predicts initiation of torsade de pointes. Circulation 2002; 105:770-774.

72. Kaab S, Hinterseer M, Nabauer M, Steinbeck G: Sotalol testing unmasks altered repolarization in patients with suspected acquired long-QT-syndrome - a case-control pilot study using i. v. sotalol. Eur Heart J 2003; 24:649-657.

73. Thomsen MB, Verduyn SC, Stengl M, Beekman JD, de Pater G, Van Opstal JM, Volders PG, Vos MA: Increased short-term variability of repolarization predicts d-sotalol-induced torsades de pointes in dogs. Circulation 2004; 110:2453-2459.

74. Hinterseer M, Thomsen MB, Beckmann B-M, Pfeufer A, Schimpf R, Wichmann H-E, Steinbeck G, Vos MA, Kaab S: Beat-to-beat variability of QT intervals is increased in patients with drug-induced long-QT syndrome: a case control pilot study. Eur Heart J 2008; 29:185-190.

75. Jacobson I, Carlsson L, Duker G: Beat-by-beat QT interval variability, but not QT prolongation per se, predicts drug-induced torsades de pointes in the anaesthetized methoxamine-sensitized rabbit. J Pharmacol Toxicol Methods 2011; 63:40-46.

76. Hinterseer M, Beckmann B-M, Thomsen MB, Pfeufer A, Dalla Pozza R, Loeff M, Netz H, Steinbeck G, Vos MA, Kaab S: Relation of Increased Short-Term Variability of QT Interval to Congenital Long-QT Syndrome. Am J Cardiol 2009; 103:1244-1248.

77. Berger RD, Kasper EK, Baughman KL, Marban E, Calkins H, Tomaselli GF: Beat-to-beat QT interval variability: novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation 1997; 96:1557-1565.

78. Atiga WL, Calkins H, Lawrence JH, Tomaselli GF, Smith JM, Berger RD: Beat-to-beat repolarization lability identifies patients at risk for sudden cardiac death. J Cardiovasc Electrophysiol 1998; 9:899-908.

79. Haigney MC, Zareba W, Gentlesk PJ, Goldstein RE, Illovsky M, McNitt S, Andrews ML, Moss AJ; Multicenter Automatic Defibrillator Implantation Trial II investigators: QT interval variability and spontaneous ventricular tachycardia or fibrillation in the Multicenter Automatic Defibrillator Implantation Trial (MADIT) II patients. J Am Coll Cardiol 2004; 44:1481-1487.

80. Piccirillo G, Magri D, Matera S, Pasquazzi E, Schifano E, Velitti S, Marigliano V, Quaglione R, Barillà F: QT variability strongly predicts sudden cardiac death in asymptomatic subjects with mild or moderate left ventricular systolic dysfunction: a prospective study. Eur Heart J 2007; 28:1344-1350.

81. Tereshchenko LG, Fetics BJ, Domitrovich PP, Lindsay BD, Berger RD: Prediction of ventricular tachyarrhythmias by intracardiac repolarization variability analysis. Circ Arrhythm Electrophysiol 2009; 2:276-284.

82. Dobson CP, La Rovere MT, Pinna GD, Bernardinangeli M, Veniani M, Midi P, Tavazzi L, Haigney M; GISSI-HF Investigators: QT variability index on 24-hour holter independently predicts mortality in patients with heart failure: analysis of GISSI-HF trial data. Heart Rhythm 2011; 8:1237-1242.

83. Oosterhoff P, Tereshchenko LG, van der Heyden MAG, Ghanem RN, Fetics BJ, Berger RD, Vos MA: Short-term variability of repolarization predicts ventricular tachycardia and sudden cardiac death in patients with structural heart disease: A comparison with QT variability index. Heart Rhythm 2011; 8:1584-1590.

84. Gupta P, Patel C, Patel H, Narayanaswamy S, Malhotra B, Green JT, Yan GX: T(p-e)/QT ratio as an index of arrhythmogenesis. J Electrocardiol 2008; 41:567-574.

85. Jalife J, Delmar M, Anumonwo J, Berenfeld O, Kalifa J: Ion channel regulation. In: Basic Cardiac Electrophysiology for the Clinician (2nd Ed.). Boston, MA: Blackwell, 2009; 72-91.

86. Couchonnal LF, Anderson ME. The role of calmodulin kinase II in myocardial physiology and disease. Physiology (Bethesda) 2008; 23:151-159.

87. Grimm M, Brown JH: Beta-adrenergic receptor signaling in the heart: role of CaMKII. J Mol Cell Cardiol 2010; 48:322-330.

88. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, Maier SK, Zhang T, Hasenfuss G, Brown JH, Bers DM, Maier LS: Ca^{2+}/calmodulin-dependent protein kinase II regulates cardiac Na^{+} channels. J Clin Invest 2006; 116:3127-3138.

89. Abildskov JA: Adrenergic effects on the QT interval of the electrocardiogram. Am Heart J 1976; 92:210-216.

90. Quadbeck J, Reiter M: Adrenoceptors in cardiac ventricular muscle and changes in duration of action potential caused by noradrenaline and isoprenaline. Naunyn Schmiedebergs Arch Pharmacol 1975; 288:403-414.

91. Kuzumoto M, Takeuchi A, Nakai H, Oka C, Noma A, Matsuoka S: Simulation analysis of intracellular Na^+ and Cl^- homeostasis during beta 1-adrenergic stimulation of cardiac myocyte. Prog Biophys Mol Biol 2008; 96:171-186.

92. Rardon DP, Bailey JC: Parasympathetic effects on electrophysiologic properties of cardiac ventricular tissue. J Am Coll Cardiol 1983; 2:1200-1209.

93. Dahimène S, Alcoléa S, Naud P, Jourdon P, Escande D, Brasseur R, Thomas A, Baró I, Mérot J: The N-terminal juxtamembranous domain of KCNQ1 is critical for channel surface expression: implications in the Romano-Ward LQT1 syndrome. Circ Res 2006; 99:1076-1083.

94. Winbo A, Diamant UB, Stattin EL, Jensen SM, Rydberg A: Low incidence of sudden cardiac death in a Swedish Y111C type 1 long-QT syndrome population. Circ Cardiovasc Genet 2009; 2:558-564.

95. Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, et al.: Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. Circulation 2007; 115:2481-2489.

96. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, et al.: Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation 2001; 103:89-95.

97. Vyas H, Ackerman MJ: Epinephrine QT stress testing in congenital long QT syndrome. J Electrocardiol 2006; 39(4 Suppl):107-113.

98. Yamaguchi M, Shimizu M, Ino H, Terai H, Uchiyama K, Oe K, Mabuchi T, Konno T, Kaneda T, Mabuchi H: T wave peak-to-end interval and QT dispersion in acquired long QT syndrome: a new index for arrhythmogenicity. Clin Sci (Lond) 2003; 105:671-676.

99. Brunner M, Peng X, Liu GX, Ren XQ, Ziv O, Choi BR, Mathur R, Hajjiri M, Odening KE, Steinberg E, et al.: Mechanisms of cardiac arrhythmias and sudden

death in transgenic rabbits with long QT syndrome. J Clin Invest 2008; 118:2246-2259.

100. Rocchetti M, Freli V, Perego V, Altomare C, Mostacciuolo G, Zaza A: Rate dependency of beta-adrenergic modulation of repolarizing currents in the guinea-pig ventricle. J Physiol 2006; 574:183-193.

101. Nowinski K, Gadler F, Jensen-Urstad M, Bergfeldt L: Transient proarrhythmic state following atrioventricular junction radiofrequency ablation: Pathophysiologic mechanisms and recommendations for management. Am J Med 2002; 113:596-602.