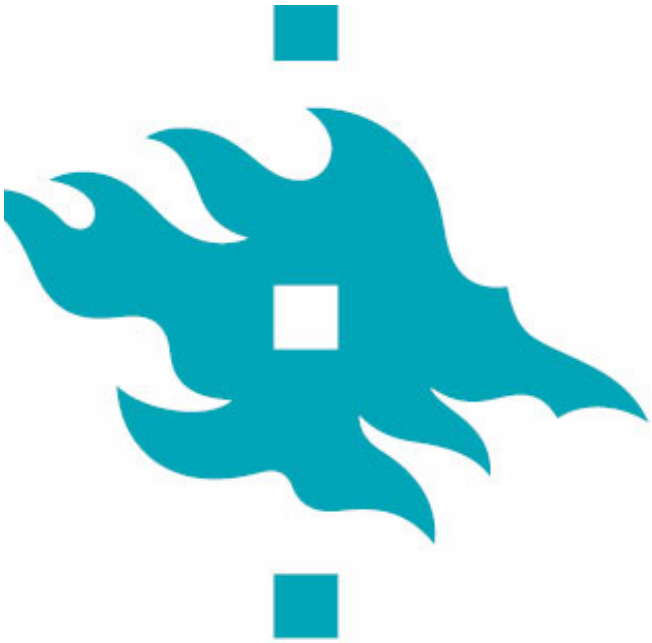

UNIVERSITY OF HELSINKI
FACULTY OF MEDICINE

ELECTROCARDIOGRAPHIC PARAMETERS OF VENTRICULAR REPOLARIZATION – MODIFIERS AND THE PROGNOSTIC VALUE

KIMMO PORTHAN

Division of Cardiology,
Department of Medicine,
Helsinki University Central Hospital,
University of Helsinki,
Helsinki, Finland
2011

ELECTROCARDIOGRAPHIC PARAMETERS OF VENTRICULAR REPOLARIZATION – MODIFIERS AND THE PROGNOSTIC VALUE

Kimmo Porthan

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in lecture room 3 of Biomedicum Helsinki, on May 13th 2011, at 12 noon.

Supervisors Docent Lasse Oikarinen
Division of Cardiology
Department of Medicine
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

Docent Matti Viitasalo
Division of Cardiology
Department of Medicine
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

Reviewers Docent Antti Hedman
Heart Center
Kuopio University Hospital
University of Eastern Finland
Kuopio, Finland

Docent Kari Ylitalo
Division of Cardiology
Department of Internal Medicine
Institute of Clinical Medicine
University of Oulu
Oulu, Finland

Opponent Professor Heikki Huikuri
Department of Internal Medicine
Institute of Clinical Medicine
University of Oulu
Oulu, Finland

ISBN 978-952-92-8747-5 (Paperback)
ISBN 978-952-10-6902-4 (PDF)
http://ethesis.helsinki.fi/

Unigrafia
Helsinki 2011

CONTENTS Page

LIST OF ABBREVIATIONS

DNA	deoxyribonucleic acid
ECG	electrocardiogram, electrocardiographic, electrocardiography
$I_{Ca,L}$	L-type calcium current
I_{Kr}	rapidly activating delayed rectifier potassium current
I_{Ks}	slowly activating delayed rectifier potassium current
KCNE1	potassium voltage-gated channel, Isk-related family, member 1 gene
KCNE2	potassium voltage-gated channel, Isk-related family, member 2 gene
KCNH2	potassium voltage-gated channel, subfamily H (eag-related), member 2 gene
KCNJ2	potassium inwardly-rectifying channel, subfamily J, member 2 gene
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1 gene
LQTS	long QT syndrome
LV	left ventricular
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
NOS1AP	nitric oxide synthase 1 adaptor protein gene
PCA	principal component analysis
QTc interval	QT interval with Bazett's formula adjustment for heart rate
QT_{end} interval	QT interval to the T-wave end
QTNc interval	QT interval with the nomogram method adjustment for heart rate
QT_{peak} interval	QT interval to the T-wave peak
RAAS	renin-angiotensin-aldosterone system
SCN5A	sodium voltage-gated channel, type V, alpha subunit gene
SD	standard deviation
SNP	single nucleotide polymorphism
TCRT	total cosine R-to-T
TdP	torsade de pointes
TMD	T-wave morphology dispersion
TPE	T-wave peak to T-wave end
TWR	T-wave residuum

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are reproduced with the kind permissions from their copyright holders. These studies are referred to in the text by their Roman numerals.

I Porthan K, Virolainen J, Hiltunen TP, Viitasalo M, Väänänen H, Dabek J, Hannila-Handelberg T, Toivonen L, Nieminen MS, Kontula K, Oikarinen L. Relationship of electrocardiographic repolarization measures to echocardiographic left ventricular mass in men with hypertension. J Hypertens 2007;25:1951–7.

II Porthan K, Viitasalo M, Hiltunen TP, Väänänen H, Dabek J, Suonsyrjä T, Hannila-Handelberg T, Virolainen J, Nieminen MS, Toivonen L, Kontula K, Oikarinen L. Short-term electrophysiological effects of losartan, bisoprolol, amlodipine, and hydrochlorothiazide in hypertensive men. Ann Med 2009;41:29–37.

III Marjamaa A[*], Newton-Cheh C[*], Porthan K, Reunanen A, Lahermo P, Väänänen H, Jula A, Karanko H, Swan H, Toivonen L, Nieminen MS, Viitasalo M, Peltonen L, Oikarinen L, Palotie A, Kontula K, Salomaa V. Common candidate gene variants are associated with QT interval duration in the general population. J Intern Med 2009;265:448–58. [*]Equal contribution.

IV Porthan K, Marjamaa A, Viitasalo M, Väänänen H, Jula A, Toivonen L, Nieminen MS, Newton-Cheh C, Salomaa V, Kontula K, Oikarinen L. Relationship of common candidate gene variants to electrocardiographic T-wave peak to T-wave end interval and T-wave morphology parameters. Heart Rhythm 2010;7:898–903.

V Porthan K, Viitasalo M, Jula A, Reunanen A, Rapola J, Väänänen H, Nieminen MS, Toivonen L, Salomaa V, Oikarinen L. Predictive value of electrocardiographic QT interval and T-wave morphology parameters for all-cause and cardiovascular mortality in a general population sample. Heart Rhythm 2009;6:1202–8.

Original publication III has also been used in the thesis of Annukka Marjamaa.

ABSTRACT

Normal electric activity of the heart consists of repeated cardiomyocyte depolarizations and repolarizations. Repolarization of the ventricular action potential is dependent on both inward and outward ion fluxes across cell membranes. Repolarization abnormalities may predispose to ventricular arrhythmias, and several factors may modify repolarization-related arrhythmia vulnerability. Ventricular repolarization generates the T wave, that is measured in body surface electrocardiogram, and several electrocardiographic measures have been developed both for clinical and research purposes to detect repolarization abnormalities.

In the series of studies reported here, the aim was to investigate modifiers of ventricular repolarization with the focus on the relationship of the left ventricular mass, antihypertensive drugs, and common gene variants, to electrocardiographic repolarization parameters. The prognostic value of repolarization parameters was also assessed. The study subjects originated from a population of more than 200 middle-aged hypertensive men attending the GENRES hypertension study, and from an epidemiological survey, the Health 2000 Study, including more than 6000 participants. Ventricular repolarization was analysed from digital standard 12-lead resting electrocardiograms with two QT-interval based repolarization parameters (QT interval, T-wave peak to T-wave end interval) and with a set of four T-wave morphology parameters.

The results showed that in hypertensive men, a linear change in repolarization parameters is present even in the normal range of left ventricular mass, and that even mild left ventricular hypertrophy is associated with potentially adverse electrocardiographic repolarization changes. In addition, treatments with losartan, bisoprolol, amlodipine, and hydrochlorothiazide have divergent short-term effects on repolarization parameters in hypertensive men. Analyses of the general population sample showed that single nucleotide polymorphisms in *KCNH2*, *KCNE1*, and *NOS1AP* genes are associated with changes in QT-interval based repolarization parameters but not consistently with T-wave morphology parameters. T-wave morphology parameters, but not QT interval or T-wave peak to T-wave end interval, provided independent prognostic information on mortality. The prognostic value was specifically related to cardiovascular mortality.

The results indicate that, in hypertension, altered ventricular repolarization is already present in mild left ventricular mass increase, and that commonly used antihypertensive drugs may relatively rapidly and treatment-specifically modify electrocardiographic repolarization parameters. Common variants in cardiac ion channel genes and *NOS1AP* gene may also modify repolarization-related arrhythmia vulnerability. In the general population, T-wave morphology parameters may be useful in the risk assessment of cardiovascular mortality. The results of this work may help to better understand the modifying factors of ventricular repolarization and the prognostic value of electrocardiographic repolarization measures.

Keywords: antihypertensive drugs, electrocardiography, epidemiology, genetics, hypertension, hypertrophy, left ventricular, mortality, prognosis, QT interval, repolarization, T wave.

1 INTRODUCTION

Myocardial ventricular repolarization is determined by a balance between inward and outward ion currents across cardiomyocyte cell membranes, and disturbances in this process may precipitate ventricular arrhythmias. Arrhythmias caused by repolarization abnormalties may potentially be malignant and lead to sudden cardiac death, which is a global health problem (Huikuri et al. 2001, Zheng et al. 2001).

Congenital long QT syndrome (LQTS) is a well-characterized monogenic repolarization abnormality affecting ion channels, where the patient-attributable risk of arrhythmias may be relatively high. Due to its rareness, however, LQTS cannot account for the total burden of ventricular arrhythmias seen in the general population. In contrast, common variants of repolarization-associated genes, although typically not associated with high patient-attributable arrhythmia risk, may nonetheless be significant risk modifiers at the population level (Newton-Cheh et al. 2009, Pfeufer et al. 2009). The impact of genetic variants differs between populations with different genetic backgrounds, and the relationship of common genetic variants to repolarization measures has not been previously characterized in a large Finnish population-based cohort.

Hypertension, or elevated arterial blood pressure, which was prevalent in 26% of adults worldwide in 2000 (Kearney et al. 2005), is also a potential risk factor for repolarization abnormalities. Hypertension predisposes to left ventricular hypertrophy (LVH), which is associated with increased cardiovascular morbidity and mortality, including sudden cardiac death (Levy et al. 1990, Haider et al. 1998). Experimental evidence suggests that the unfavourable effect of LVH on sudden cardiac death may be mediated by arrhythmogenic repolarization abnormalities (Kowey et al. 1991, Yan et al. 2001), and clinical studies have shown that marked LVH is associated with adverse electrocardiographic (ECG) repolarization changes (Oikarinen et al. 2001a, 2002, 2004b). However, it has been unknown whether even mild hypertension-induced structural left ventricular (LV) remodelling, expected to be more common than marked LVH, is already associated with adverse ECG repolarization changes.

Drug treatments for hypertension are associated with LVH regression, beneficial ECG repolarization changes, and improved prognosis (Rials et al. 1998, Rials et al. 2001, Oikarinen et al. 2003, Okin et al. 2004a). The favourable repolarization changes seen after long-term treatments with antihypertensive drugs may be a consequence of reverse structural remodelling. However, antihypertensive agents from different drug classes may have differing effects on angiotensin II and aldosterone (Burnier and Brunner 1992, Siscovick et al. 1994, Hoes et al. 1995, Delpón et al. 2005, Fischer et al. 2007), which are important blood pressure regulators with direct proarrhythmic potential (Delpón et al. 2005, Domenighetti et al. 2007, Fischer et al. 2007). In addition, angiotensin II receptor blockers and ß-blockers may have direct antiarrhythmic properties (Delpón et al. 2005, Zicha et al. 2006). Therefore, commonly used antihypertensive drugs may also have potentially clinically significant short-term repolarization effects, although this has not been assessed in previous studies.

The ECG T wave is generated by myocardial voltage gradients during ventricular repolarization (Yan and Antzelevitch 1998, Yan et al. 2003). The time from QRS onset to T-wave end, the QT interval, is used to assess ventricular repolarization, but this measure may detect changes only in repolarization duration. In contrast, novel T-wave morphology parameters, such as principal component analysis (PCA) ratio, T-wave

morphology dispersion (TMD), total cosine R-to-T (TCRT), and T-wave residuum (TWR) measure both temporal and spatial repolarization changes (Acar et al. 1999, Malik et al. 2000, Okin et al. 2005). Additionally, the T-wave peak to T-wave end (TPE) interval measures terminal repolarization, and has been linked experimentally to arrhythmogenic repolarization dispersion (Zabel et al. 1995, Yan and Antzelevitch 1998, Opthof et al. 2007). However, only a few previous studies have examined the predictive value of these novel repolarization measures in the general population.

In the present series of studies, ventricular repolarization was assessed from digital standard 12-lead resting ECGs with a comprehensive set of repolarization parameters including QT interval, TPE interval, and four T-wave morphology parameters. The study population was derived from a hypertension study, well suited to the assessment of the association of LV mass (LVM) and antihypertensive drugs, to ECG repolarization. Furthermore, a large Finnish population-based survey provided an optimal range of study subjects for assessing the relationship between selected common gene variants and ECG repolarization parameters, as well as for analysing the predictive values of these ECG repolarization measures.

2 REVIEW OF THE LITERATURE

2.1 Ventricular repolarization in the myocardium

Membrane currents and the cardiac action potential. Mechanical contraction of the myocyte is dependent on electric activity of the cell membrane. In the resting state, cell interior voltage is more negative than exterior, corresponding to a negative membrane potential of about –90 mV. This potential is generated by different ion concentrations between the two sides of the membrane and is ultimately maintained by ion pumps and transporters with the aid of energy-dependent mechanisms (Moczydlowski 2003b).

The action potential is an all-or-nothing event where the cell membrane potential rises (depolarization) and falls (repolarization) and is mediated by an integrated activity of ion channels embedded in the cell membrane. Ion channels allow ion flow across the cell membrane according to their electrochemical gradient, and most of the ion channels involved in the action potential are voltage-gated and highly selective to specific ions (Moczydlowski 2003a). Depolarizing ion currents make membrane potential more positive and trigger action potential if the threshold potential is reached. Repolarizing ion currents then make membrane potential gradually more negative, finally restoring it to its original resting value.

Action potential phases and major ion currents of the ventricular cardiomyocyte are shown in Figure 1 and Table 1. An external stimulus triggers the action potential, which starts with a rapid depolarization (phase 0) caused mainly by inward fast sodium current, followed by a downward deflection (phase 1) which is due to transient outward potassium current (Levy 2004). The cardiac action potential exhibits an extended "plateau" (phase 2), which is generated by an equilibrium between inward L-type calcium current ($I_{Ca,L}$) and outward delayed rectifier potassium currents (Levy 2004). The rapid repolarization (phase 3) is caused by delayed rectifier potassium currents, along with inactivation of $I_{Ca,L}$ (Levy 2004). The inward rectifier potassium current is active throughout phases 3 and 4, where it acts to restore the membrane resting potential in addition to other functions (Moczydlowski 2003a, Cerrone et al. 2006).

Cardiac ion channels. Ion channels are present in the cell membrane of all biological cells. There is a wide variety of ion channels, and their function is fundamental for several biological processes, such as nerve impulse, hormone secretion, and muscle contraction (Moczydlowski 2003b). In cardiomyocytes, ion channels mediate the action potential. In addition, they serve as a link between electric excitation and mechanical function via calcium-induced calcium release, where calcium influx into the myocyte through the $I_{Ca,L}$ channels triggers the release of calcium from the sarcoplasmic reticulum, the myocyte calcium store (Boulpaep 2003). The increased concentration of cytosolic free calcium, in turn, activates muscle contraction, forming a mechanism called excitation-contraction coupling (Apkon 2003).

Ion channels are constructed of proteins arranged around a cell membrane pore, typically containing four to six protein subunits or pseudosubunits arranged together like a rosette (Moczydlowski 2003b). Each subunit is encoded by a single gene and is made up of several transmembrane segments. In addition to the pore-forming subunits (α subunits), some ion channels have accessory subunits (named as β, γ, δ etc.), which act as channel gating regulators and linkage proteins (Roden et al. 2002). Ion channels are classified according to their structural and functional characteristics, such

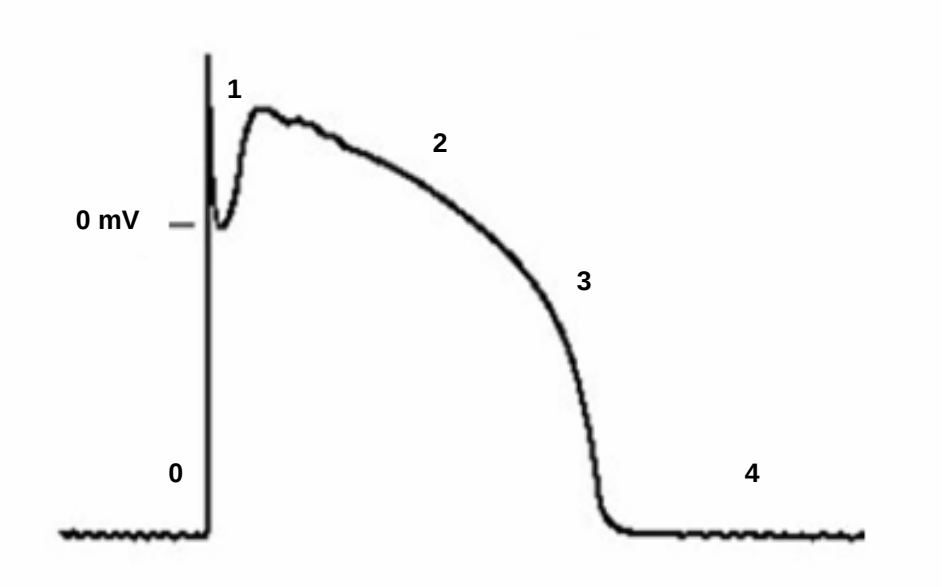

Figure 1 Action potential of the ventricular myocyte. Numbers 0 to 4 indicate action potential phases.

Table 1 Major ion currents* during the ventricular action potential.

Ion current	Ion	Action phase/role
I_{Na}	Na^+	0
$I_{Ca,L}$	Ca^{2+}	0–2
I_{to}	K^+	1
I_{Kr}	K^+	3
I_{Ks}	K^+	2, 3
$I_{K(ATP)}$	K^+	3, 4
I_{K1}	K^+	3, 4
$I_{Na/Ca}$	Na^+, Ca^{2+}	ion homeostasis
$I_{Na/K}$	Na^+, K^+	ion homeostasis
I_{pCa}	Ca^{2+}	ion homeostasis

*This list is not intended to be exhaustive. $I_{Ca,L}$=L-type calcium current, $I_{K(ATP)}$=ATP-sensitive potassium current, I_{K1}=inward rectifier potassium current, I_{Kr}=rapidly activating delayed rectifier potassium current, I_{Ks}=slowly activating delayed rectifier potassium current, I_{Na}=fast sodium current, $I_{Na/Ca}$=sodium-calcium exchanger current, $I_{Na/K}$=sodium-potassium pump current, I_{pCa}=plasma membrane ATPase calcium current, I_{to}=transient outward potassium current.

as classification by gating, referring to the mechanism that opens and closes the channel. The activation of voltage-gated channels is highly dependent on membrane potential. As such, in cardiomyocytes, the fast sodium current, $I_{Ca,L}$, transient outward potassium current, rapidly activating delayed rectifier potassium current (I_{Kr}), and the slowly activating delayed rectifier potassium current (I_{Ks}) are mediated through voltage-gated channels (Moczydlowski 2003a). Cardiac ion channel expression varies between species (Nerbonne 2004) and genders (Di Diego et al. 2002). In addition, different regions of the heart feature specific ion-current properties (Volders et al. 1999, Antzelevitch and Fish 2001, Szentadrassy et al. 2005), as described further in section 2.3.

2.2 Mechanisms of arrhythmias

Cardiac arrhythmias form a heterogeneous group of abnormalities in the electric activity of the heart. The basis for arrhythmias lies either in abnormal electric impulse initiation or conduction (Levy 2004). Abnormal electric impulse initiation forms the first category of arrhythmias, and involves pace-maker cells that are found in the sinoatrial node (normally the primary pace-maker), atrioventricular node, and His-Purkinje system. Pace-maker cells may experience diminished or loss of automaticity. In contrast, a positive shift in cardiomyocyte resting potential may accelerate activity in pace-maker cells (Lederer 2003). Spontaneous depolarizations may also occur in cells that normally do not have pace-maker activity, for example in ventricular myocytes. This may occur through early or late afterdepolarizations. Early afterdepolarizations appear in the late phase of the action potential, before full repolarization, and are caused by the activity of $I_{Ca,L}$ channels or sodium-calcium exchangers (Roden 2008). There is a direct positive correlation between action potential duration and susceptibility to early afterdepolarizations, which occur more often with lower rather than higher heart rates and in midmyocardial rather than endocardial or epicardial myocytes (Levy 2004). Early afterdepolarizations may also be caused by stretch-induced mechanisms (Eckardt et al. 2001). Early afterdepolarizations are the arrhythmia trigger mechanism for example for torsade de pointes (TdP) tachycardia in most subtypes of LQTS (Antzelevitch and Shimizu 2002, Hedley et al. 2009). In contrast, delayed afterdepolarizations appear near the very end of the action potential or after full repolarization, and occur more often with higher rather than lower heart rates. Delayed afterdepolarizations are associated with an increased intracellular concentration of calcium, which in turn may activate depolarizing ion channels, triggering an action potential (Levy 2004). Delayed afterdepolarizations are responsible for triggering arrhythmia, for example in catecholaminergic polymorphic ventricular tachycardia (Katra and Laurita 2005).

Abnormalities in electric conduction form the second main category of arrhythmias. Conduction tissue may become less excitable or inexcitable due to depolarization (e.g. in ischemia), or abnormal anatomy (e.g. accessory pathway) may be evident causing abnormal conduction. Reentry is a conduction disturbance whereby electric impulse travels recurrently in a circle within the heart instead of travelling through the heart and then stopping until the next impulse. Reentry has three requirements: a closed conduction loop, a region of unidirectional functional block within the loop, and a sufficiently slow conduction of action potentials around the loop (Lederer 2003). Reentry may be responsible for many arrhythmia types, such as atrial and ventricular tachycardia and fibrillation (Lederer 2003), and also includes the maintenance of TdP (El-Sherif et al. 1997).

Action potential prolongation and increased dispersion of repolarization are central to repolarization-related arrhythmias. In LQTS, action potential prolongation and the increased (transmural) dispersion of repolarization and refractoriness, which lead to early afterdepolarizations and reentry, form the arrhythmia mechanism (Hedley et al. 2009). However, repolarization may also be abnormal without action potential prolongation, such as is seen in short QT syndrome and Brugada syndrome, where the increased dispersion of repolarization and refractoriness underlie the arrhythmia vulnerability (Antzelevitch and Oliva 2006).

Ion channel dysfunction is fundamental for the genesis of several arrhythmias, many of which may be seen as an imbalance between depolarizing and repolarizing ion

currents. An ion channel may feature gain-of-function or loss-of-function due to a gene mutation. Accordingly, congenital mutations in genes affecting the structure and function of ion channels and transporters form a heterogeneous group of inherited arrhythmia syndromes including, among others, LQTS, short QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, and the congenital forms of atrial fibrillation, sick sinus syndrome, and conduction defects (Lehnart et al. 2007, Ackerman and Mohler 2010). Ion channel function may also be abnormal due to acquired causes, such as drugs, electrolyte imbalances, or bradycardia (Antzelevitch and Shimizu 2002). Furthermore, a chronic structural heart disease or abnormality, such as hypertophy, may lead to changes both in ion channel gene expresssion and function as well as alterations in conduction, intercellular coupling, extracellular matrix, and dispersion of refractoriness. These changes are termed “electric remodelling” and together contribute to the arrhythmia mechanism and increased arrhythmia vulnerability (Hill 2003).

Altogether, arrhythmia vulnerability is a dynamic process and is dependent on the sum of several congenital and acquired factors. Recognition of multiple factors affecting the duration of ventricular repolarization and thus the propensity for arrhythmias led to the concept of repolarization reserve (Roden 1998). This is a multiple-hit theory, in keeping with the idea that the normal heart has some tolerance or reserve against excessive prolongation of the QT interval, and an arrhythmia is triggered only if several risk factors are present in an individual simultaneously (Roden 1998).

2.3 Ventricular repolarization in the electrocardiogram: T-wave genesis

ECG, a recording of extracellular voltage gradients generated by the heart, is the most important tool for diagnosing disturbances in the electric activity of the heart, including arrhythmias and repolarization abnormalities. Ventricular depolarization produces the ECG QRS complex, and the T wave represents ventricular repolarization (Lederer 2003). In addition to the T wave, the ST segment and the J and U waves, not discussed further here, may be classified as components of ventricular repolarization (Yan et al. 2003).

From the vectorcardiographic point of view, ventricular repolarization may be seen as a three-dimensional loop, and the T waves in different ECG leads represent the projection of the repolarization loop to a specific lead (Kors et al. 2008). However, the cellular and ionic mechanisms responsible for the T wave were poorly understood throughout almost the entire 20th century. Nearly 20 years ago, a population of myocardial cells, M cells, with specific repolarization properties was discovered from the midmyocardium (Sicouri and Antzelevitch 1991). M cells have specific ion-current features. Compared to epicardial and endocardial myocytes, M cells feature smaller I_{Ks} as well as larger sodium-calcium exchanger current and larger late sodium current (Antzelevitch and Fish 2001). Epicardial and M cells feature prominent transient outward potassium current (Antzelevitch and Fish 2001), which also may be more dominant in right than left ventricle (Volders et al. 1999). In contrast, densities of I_{Kr} and inward rectifier potassium current are even in the three myocardial layers (Antzelevitch and Fish 2001). M cells have a tendency to prolong their action potential more than epicardial or endocardial cells in response to QT-interval prolonging drugs or heart-rate slowing (Sicouri and Antzelevitch 1991, Antzelevitch et al. 1999). Different

repolarization features of the three myocardial cell layers also seem to be linked to the arrhythmogenic mechanism in LQTS, short QT syndrome, and Brugada syndrome (Antzelevitch 2008, Hedley et al. 2009).

After the discovery of M cells, the T wave has been proposed to result mainly from voltage gradients on either side of the M cell region (Yan and Antzelevitch 1998, Antzelevitch 2007). These transmural gradients are produced by differences in the final repolarization of epicardial, endocardial, and midmyocardial myocytes (Yan and Antzelevitch 1998). Figure 2 shows the QRS complex and the positive T wave as well as contemporaneous action potentials from three different myocardial layers from a canine ventricular wedge preparation. The T wave starts to rise when the action potentials of different myocardial layers diverge in phase 2, reaches the peak coincident with the full repolarization of epicardium, and returns to the baseline when the M region has repolarized (Yan and Antzelevitch 1998). In smaller species, the entire endocardium has the properties of M cells and the T-wave end coincides with the end of endocardial repolarization (Gupta et al. 2008). However, the results from experimental studies using animals and cell/wedge preparation samples cannot be directy extrapolated to the whole heart in humans, and the role of transmural voltage gradients in the T-wave genesis has been questioned by some researchers (Conrath and Opthof 2006). Thus, despite the research advances during the past two decades, the debate on human T-wave genesis is ongoing.

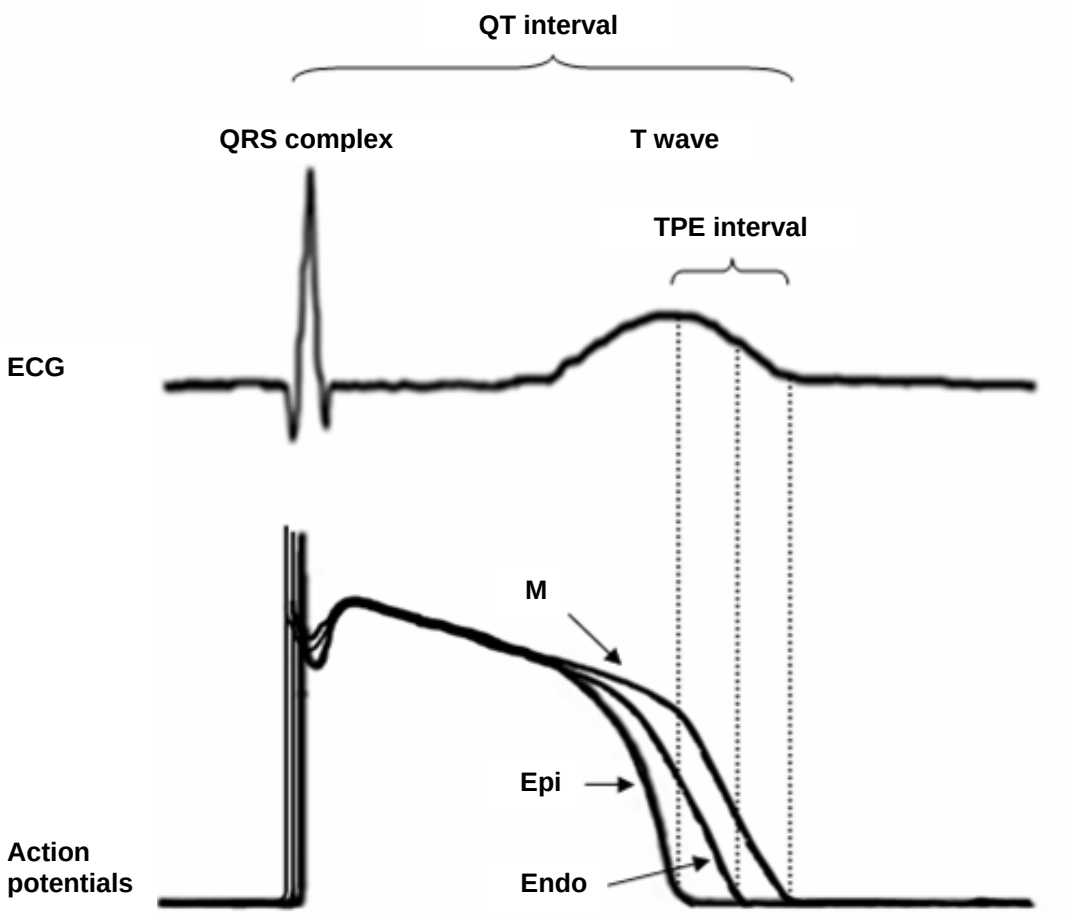

Figure 2 Simultaneous action potentials from epicardial (Epi), endocardial (Endo), and midmyocardial (M) regions of a canine ventricular wedge preparation, and the temporal relationship with surface electrocardiogram (ECG). TPE=T-wave peak to T-wave end.

2.4 Electrocardiographic repolarization parameters

As the evidence that repolarization abnormalities and arrhythmia vulnerability are linked together is convincing, efforts have been made to characterize repolarization changes for risk evaluation. Repolarization indexes have been studied, for example in resting and excercise ECGs as well as in standard 12-lead ECGs and body surface potential maps. Unfortunately, finding an informative marker of repolarization abnormalities has proven difficult. For clinical purposes, repolarization assessment from 12-lead resting ECG has been limited mainly to QT-interval measurement and detection of "non-specific" changes in the ST segment and/or T wave, which nonetheless provide prognostic information (Rose et al. 1978, Kreger et al. 1987, Schillaci et al. 2004). In the 1990s, the range of QT-interval durations, the QT dispersion, was studied vigorously, but appeared not to be a reliable measure (Malik and Batchvarov 2000, Rautaharju 2002). LV wedge preparation studies indicated that the TPE interval may be used as an index of repolarization dispersion (Yan and Antzelevitch 1998). More complex approaches, including mathematical data processing and analysis of T-wave morphology, have already been studied decades ago (Abildskov et al. 1977, Lux et al. 1981). In 1995, measures called T-wave amplitude (Dekker et al. 1995) and T-wave area (Zabel et al. 1995) were presented. In 1997, PCA was applied to 12-lead ECG yielding the PCA ratio (Priori et al. 1997). In addition to these measures, many potentially useful repolarization parameters assessing the two- or three-dimensional shape of the T-wave from standard 12-lead resting ECG have been introduced (Merri et al. 1989, Kors et al. 1998, Acar et al. 1999, di Bernardo and Murray 2000, Kardys et al. 2003, Kanters et al. 2004, Badilini et al. 2008, Graff et al. 2009).

2.4.1 QT-interval based repolarization parameters

QT interval. The time from the QRS onset to the T-wave end, the QT interval, encompasses ventricular depolarization and repolarization and is a measure of global repolarization duration (Figure 2). The QT interval depends on heart rate as well as on gender, age, plasma electrolyte concentrations, use of medications, congenital and acquired diseases (Pfeufer et al. 2005). Currently, approximately one third of the variation in the QT interval is considered to be heritable (Newton-Cheh et al. 2009). Measuring the QT interval from ECG is relatively fast and straightforward. In addition, the cellular basis of the QT interval is easily understandable. Consequently, both in clinic and research, the QT interval is widely used to detect short QT syndrome, LQTS, and disease- and drug-related repolarization effects. However, the QT interval also has several limitations: 1) QT interval is affected by changes in the duration of QRS complex, 2) precise determination of the T-wave end with acceptable reproducibility may be difficult when the T wave is flat, and especially when there is a fusion of T and U waves or when the T wave has a bizarre pattern, 3) there are several methods for heart-rate adjustment, and each method has weaknesses (Batchvarov et al. 2002b, Dogan et al. 2005), 4) changes in the T-wave shape cannot be detected by measuring QT interval, 5) there is significant overlap in the QT intervals of healthy subjects and those with abnormal repolarization (Vincent el al. 1992).

Many studies have examined the relationship between QT-interval prolongation and adverse outcomes, such as increased risk of cardiovascular morbidity, mortality, ventricular arrhythmias, and sudden cardiac death. In LQTS, the duration of the QT

interval is a predictor of TdP events (Priori et al. 2003). In general, prolongation of the QT interval to a value of 500 ms or over is considered to confer an obvious TdP risk (Priori et al. 2003, Roden 2004, Sager 2008). In the general population, however, arrhythmia risk associated with a less pronounced QT-interval prolongation is less clear (Sager 2008). According to previous epidemiological studies, it has been somewhat controversial regarding whether QT-interval prolongation is a risk factor in the general population, with some of the studies reporting an elevated risk (Okin et al. 2000a, Robbins et al. 2003, Dekker et al. 2004, Straus et al. 2006); while other studies report risk increase only in subgroups, such as in subjects with cardiovascular diseases or with the most extreme category of QT-interval prolongation (Algra et al. 1991, Schouten et al. 1991, Dekker et al. 1994, Karjalainen et al. 1997, Elming et al. 1998, de Bruyne et al. 1999), or no risk increase at all (Goldberg et al. 1991). It is possible that methodological issues, such as differences in QT-interval measurement techniques and/or heart rate-adjustment, may have affected the results in some of the studies (Montanez et al. 2004).

T-wave peak to T-wave end (TPE) interval. The TPE interval is measured as the interval between T-wave peak and T-wave end (Figure 2). The TPE interval corresponds with the interval between the earliest and latest repolarization in the ventricle wall. TPE-interval prolongation is thus expected to represent a higher amount of transmural dispersion of repolarization or a wider "vulnerable window" during which parts of the ventricle have already repolarized while other parts have not, creating a milieu favoring reentry. The theory of transmural dispersion of repolarization has been well supported by experimental studies using ventricular wedge preparations (Antzelevitch et al. 1999, Emori and Antzelevitch 2001). However, some simulation studies (Van Huysduynen et al. 2005, Kors et al. 2008), as well as animal studies using intact hearts (Xia et al. 2005, Opthof et al. 2007), have suggested that the TPE interval may actually reflect global, not transmural, dispersion of repolarization *in vivo*. Smetana et al. (2003) studied the relationship between the TPE interval and heart rate in 24-hour ambulatory ECGs and concluded that the TPE interval correlates markedly with heart rate. In contrast, the TPE interval was practically independent of heart rate when analysed from resting ECGs (Haarmark et al. 2010) or daytime ambulatory ECGs (Andersen et al. 2008). It has also been suggested to use the TPE/QT interval ratio instead of the TPE interval to eliminate the possible confounding effect of heart-rate changes and inter-individual differences of the QT interval (Gupta et al. 2008).

Several clinical studies have assessed the value of the TPE interval as a surrogate marker of repolarization abnormalities, using either resting or ambulatory ECGs in relatively small patient groups, and none have been previously reported in large population samples. These studies were performed on patients with resistant hypertension (Salles et al. 2008), congenital LQTS (Lubinski et al. 1998, Tanabe et al. 2001, Viitasalo et al. 2002, Takenaka et al. 2003, Jeyaraj et al. 2008, Kanters et al. 2008), drug-related LQTS (Yamaguchi et al. 2003, Couderc et al. 2009), short QT syndrome (Anttonen et al. 2008), Brugada syndrome (Castro Hevia et al. 2006), catecholaminergic polymorphic ventricular tachycardia (Viitasalo et al. 2008), arrhythmogenic right ventricular cardiomyopathy (Haapalahti et al. 2008), hypertrophic cardiomyopathy (Shimizu et al. 2002), acquired bradycardia (Topilski et al. 2007), high risk patients with organic heart disease (Watanabe et al. 2004), patients receiving implantable cardioverter-defibrillator and cardiac resynchronization therapy (Lellouche et al. 2007), and in myocardial infarction patients (Lubinski et al. 2000, Oikarinen et al. 2001b, Haarmark et al. 2009). Altogether, findings from most of these clinical studies

have been promising and suggest that, in agreement with experimental studies, the TPE interval may serve as a marker of clinical arrhythmia vulnerability. However, the clinical use of the TPE interval requires further validation, and the methodological limitation of accurate determination of measurement points, such as the T-wave end, must also be acknowledged.

2.4.2 T-wave morphology parameters

Principal component analysis (PCA) ratio. The mathematical technique called PCA finds from multidimensional data the main ("principal") components which best explain the variance of the data (Paerson 1901). The technique uses the eight independent ECG leads (I, II, V1–6). In electrocardiology, PCA was originally used in body surface potential maps, with the aim of characterizing potentials from multiple leads with a few principal patterns (Lux et al. 1981, De Ambroggi et al. 1986). In PCA of the T wave, the main orthogonal components of the spatial T wave are quantified (Lux et al. 1981, De Ambroggi et al. 1986). Priori et al. (1997) adapted PCA to 12-lead ECG and hypothesized that the ratio of the second to the first component (PCA ratio; expressed as a percentage) may serve as a measure of T-wave complexity because the first component contains most of the repolarization energy in normal repolarization, whereas repolarization inhomogeneities increase the second and the following components. Morphologically, the PCA ratio may be visualized as the ratio of the short axis to the long axis of the spatial T-wave loop (Figure 3). Thus, the PCA ratio is a measure of the relative roundness or "fatness" of the three-dimensional T-wave loop, with increasing values referring to higher amounts of repolarization complexity and increases in the roundness of the T-wave loop (Okin et al. 2004b). The repeatability of the PCA ratio is considered reliable in serial ECG recordings (Extramiana et al. 2007).

According to previous studies, the PCA ratio may be used to distinguish patients with repolarization abnormalities. Compared to healthy controls, higher PCA values were found in LQTS (Priori et al. 1997), hypertrophic cardiomyopathy (Yi et al. 1998), and coronary heart disease (Rautaharju 2002). Importantly, the PCA ratio independently predicted cardiovascular mortality in the general population (Okin et al. 2002, Okin et al. 2005) and in patients with diabetes mellitus (Okin et al. 2004b). In a large sample of postmenopausal women, the PCA ratio independently predicted coronary heart disease events and congestive heart failure events, but was not a predictor of mortality (Rautaharju et al. 2006a,b).

T-wave morphology dispersion (TMD). TMD estimates the variation in T-wave morphology between different ECG leads (Acar et al. 1999). First, the three-dimensional T-wave loop is determined from the singular value decomposition of the eight independent ECG leads (Wall et al. 2003). Second, the two-dimensional T-wave loop is determined from the singular value decomposition of the three-dimensional T-wave loop. Third, reconstruction vectors of the eight independent ECG leads onto the two-dimensional T-wave loop are calculated. TMD is measured as the average angle (unit in degrees) between all T-wave reconstruction vector pairs excluding lead V1 (Acar et al. 1999) (Figure 4). Similar T-wave morphology in different ECG leads results in a small TMD value, and repolarization abnormalities increase the value.

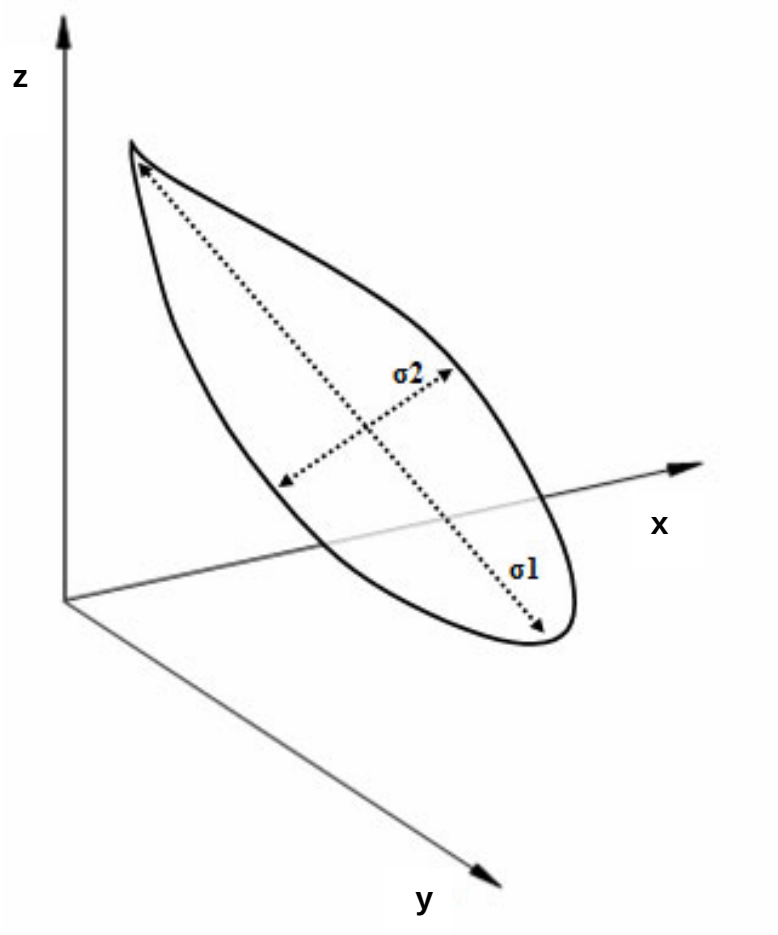

Figure 3 The principal component analysis ratio represents the ratio of the short axis (σ2) to the long axis (σ1) of the spatial T-wave loop.

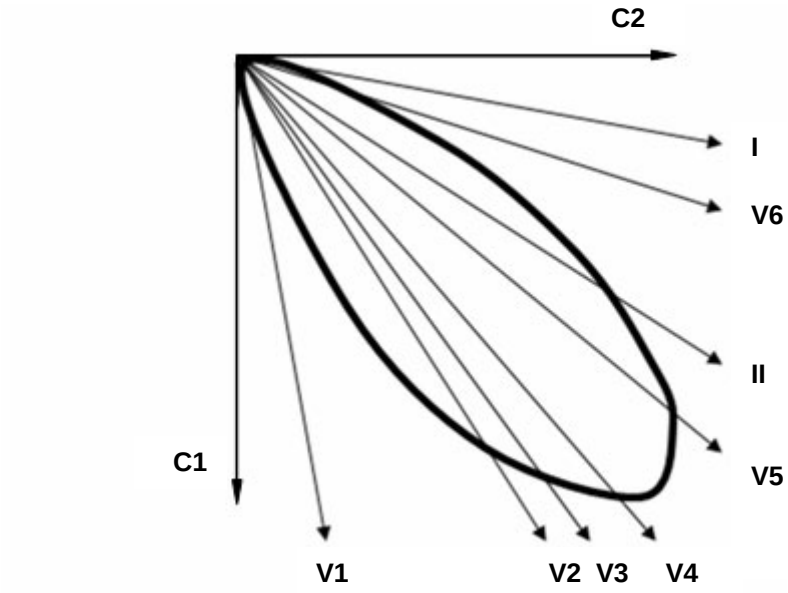

Figure 4 The two-dimensional T-wave loop and reconstruction vectors of the eight independent electrocardiogram leads onto the loop. The projection plane of the two-dimensional T-wave loop is determined by the two biggest components of the singular value decomposition (C1, C2) performed for the three-dimensional T-wave loop. T-wave morphology dispersion is measured as the average angle between all T-wave reconstruction vector pairs excluding V1, which usually differs from the other leads.

In previous studies, TMD was higher in patients with marked LVH (Acar et al. 1999, Oikarinen et al. 2002), and in a patient group with various heart diseases (Ono et al. 2005), when compared to healthy controls. In US male veterans with cardiovascular diseases (Zabel et al. 2002) as well as in a general population sample in US Indians (Okin et al. 2005), TMD predicted mortality in univariate analyses. In contrast, in studies analysing myocardial infarction patients (Zabel et al. 2000), and systolic heart failure patients (Huang et al. 2009), TMD was not a mortality predictor.

Total cosine R-to-T (TCRT). TCRT estimates the spatial deviation between depolarization and repolarization phases (Acar et al. 1999). TCRT corresponds conceptually to the measure called QRS-T angle (Kardys et al. 2003). In part, TCRT resembles methodologically an older repolarization measure called ventricular gradient (Batchvarov et al. 2002a). When TCRT is quantified, vectors from three-dimensional depolarization and repolarization loops are used after singular value decomposition of the eight independent ECG leads (Wall et al. 2003). TCRT is then calculated as the cosine of the angle between depolarization vector and repolarization vector (Acar et al. 1999) (Figure 5). TCRT is unitless and it varies between –1 and 1, corresponding to an angle of 180º and 0º, respectively. Thus, a high TCRT value refers to a small angle between R- and T-wave loop vectors, as is seen when depolarization and repolarization phases are normal.

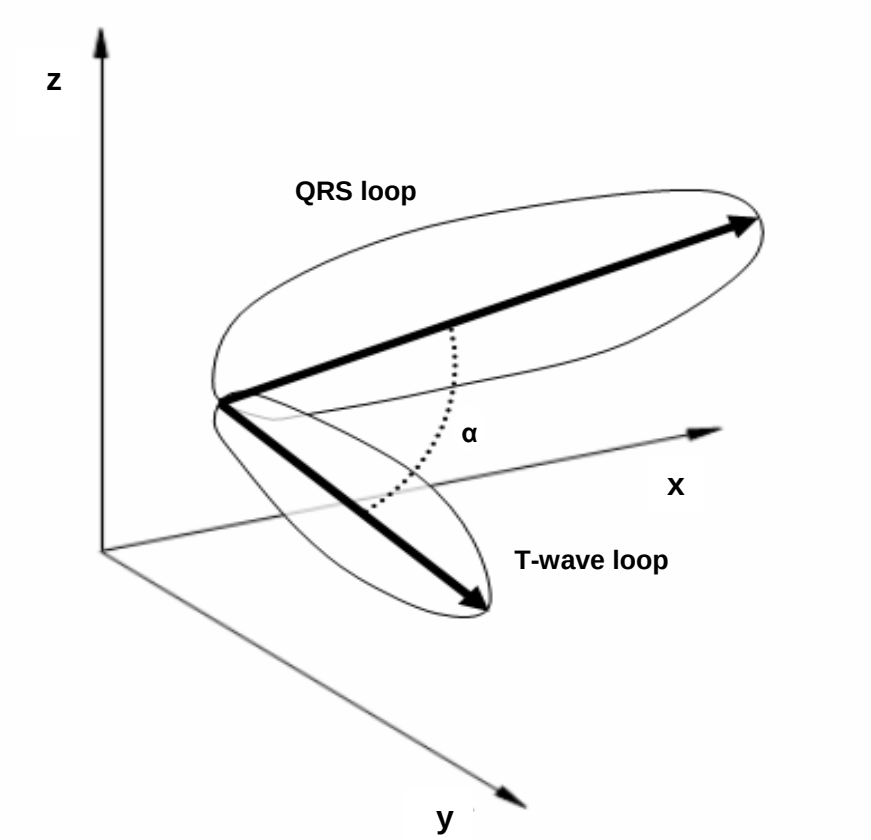

Figure 5 The three-dimensional QRS loop and T-wave loop and the respective depolarization and repolarization vectors. Total cosine R-to-T reflects the angle (α) between the depolarization and repolarization wavefronts.

In previous studies, TCRT has correlated with LVM and differed between healthy controls and patients with various heart diseases (Acar et al. 1999, Oikarinen et al. 2002, Ono et al. 2005, Anttonen et al. 2009). TCRT was a univariate predictor of mortality in males with cardiovascular diseases (Zabel et al. 2002) as well as in the general population (Okin et al. 2005). The QRS-T angle was an independent predictor of cardiac mortality in a Dutch general population study with elderly subjects (Kardys et al. 2003). In postmenopausal women, the QRS-T angle was one of the dominating predictors among several ECG parameters and showed an independent role as a predictor of coronary heart disease events, heart failure events, and both all-cause and coronary heart disease mortality (Rautaharju et al. 2006a,b). In systolic heart failure, TCRT was able to stratify the risk of cardiovascular mortality (Huang et al. 2009). What is particular for TCRT, is that several clinical studies have reported its independent prognostic value for mortality in myocardial infarction (Zabel et al. 2000, Batchvarov et al. 2004, Malik et al. 2004). Some studies on myocardial infarction patients have linked low TCRT values specifically to cardiac (Batchvarov et al. 2004, Perkiömäki et al. 2006) or arrhythmic deaths (Batchvarov et al. 2004, Malik et al. 2004).

T-wave residuum (TWR). After singular value decomposition of the eight independent ECG leads (Wall et al. 2003), the first three components represent the three-dimensional or “dipolar” signal content. In contrast, the vector magnitude of the fourth to eighth components are expected to reflect “non-dipolar” residual components that are contained within the ECG but are not reflected in the three-dimensional ECG, thus serving as an estimate of (regional) repolarization heterogeneity (Malik et al. 2000). The sum of vector magnitude of the fourth to eighth eigenvalues of the T-wave signal gives the absolute TWR (in technical units), and the relation between absolute TWR and the sum of vector magnitude of the first to eighth eigenvalues gives the relative TWR (Malik et al. 2000, Kesek et al. 2004). Higher TWR values are expected to indicate higher degrees of ventricular repolarization heterogeneity. Studies in 24-hour ambulatory ECGs have suggested that TWR as well as TCRT are dependent on heart rate (Smetana et al. 2004). In resting ECGs, however, both TWR and TCRT seem to be only weakly influenced by heart rate (Extramiana et al. 2007). Some concerns have been raised about the possibility that the repeatability of TWR and TCRT in serial ECG recordings is not optimal (Extramiana et al. 2007).

Both the absolute and relative TWR have been identified as independent predictors of all-cause mortality in males with cardiovascular diseases (Zabel et al. 2002), and later, absolute TWR was shown to be an independent predictor of all-cause and cardiovascular mortality in the general population (Okin et al. 2005). These studies both used a set of T-wave morphology parameters and demonstrated that TWR contained the highest predictive value among the parameters tested. In postmenopausal women, increased absolute TWR was an independent predictor of all-cause mortality but, unlike QRS-T angle, did not predict an increase in coronary heart disease events, heart failure events, or coronary heart disease mortality (Rautaharju et al. 2006a,b). In haemodialysis patients with end-stage renal disease, relative TWR was an independent predictor of cardiovascular and arrhythmia-related mortality (Lin et al. 2007).

2.5 Modifiers of repolarization

Multiple factors have an impact on arrhythmia vulnerability. These factors may be classified into three categories: proarrhythmic substrate, triggers, and modifiers/modulators (Malik 2008). Vulnerable cardiac tissue, such as a heart with infarction scars, forms the arrhythmia basis (substrate). Immediate causes or mechanisms initiating arrhytmia, such as ischemia or a premature beat, are referred to as triggers. A factor that alters the electrophysiological properties of the heart, thus changing the relationship between the proarrhythmic substrate and trigger, is a modifier. Many of the modifiers are considered not to contain high proarrhythmic potential alone but, when appearing simultaneously, they may become significant. Established factors modifying repolarization (directly or indirectly) include gender, age, heart rate, electrolyte imbalances, structural heart diseases, diabetes mellitus, liver malfunction, body temperature, autonomic nervous system, drugs (especially those prolonging the QT interval), and variants of ion channel genes.

2.5.1 Left ventricular hypertrophy (LVH)

In physiological LVH, the diameter of the LV wall or chamber (or both) increases as a response to physical exercise or pregnancy. Causes of pathological hypertrophy include hypertension, valvular heart disease, ischemic heart disease, and (primary) hypertophic cardiomyopathy. In ventricular volume overload or increased afterload, LVH is a secondary adaptive process which enables the heart to maintain normal wall-tension and cardiac output. However, pathological hypertrophy is associated with unfavourable long-term consequences, such as diastolic dysfunction, impaired coronary blood flow, and heart failure (Levy et al. 1996, Erdogan et al. 2007, Paulus et al. 2007). Epidemiological studies have confirmed the association between LVH and adverse prognosis (Levy et al. 1990, Haider et al. 1998, Vakili et al. 2001, Bombelli et al. 2009), as well as the risk reduction in LVH regression (Verdecchia et al. 2003, Okin 2009). LVH also predisposes to sudden cardiac death (Haider et al. 1998, Turakhia et al. 2008), which is presumed to be caused at least in part by LVH-induced malignant ventricular arrhythmias. Hypertrophied myocardium may predispose to arrhythmias by various mechanisms. First, myocyte loss and the associated fibrotic replacement slow electric conduction and form a substrate for reentry (Choudhury et al. 2002). Second, interstitial fibrosis may lead to intercellular uncoupling, which is associated with increased dispersion of repolarization (Conrath et al. 2004). Third, the reduced coronary blood flow predisposes to ischemia. Fourth, cardiomyocytes in the hypertrophied heart display arrhythmogenic alterations in ion channel expression and function.

Data on hypertrophic electric remodelling in the human ventricle is limited. In various experimental models of cardiac hypertrophy the most consistent electrophysiological change has been the cardiomyocyte action potential prolongation (Ben-David et al. 1992, Rials et al. 1995, Hill 2003, Furukawa and Kurokawa 2006), predisposing to afterdepolarizations. Electric remodelling may be non-uniform, further increasing the repolarization heterogeneity between different regions of the heart and favouring reentry (Kowey et al. 1991, Rials et al. 1995, Furukawa and Kurokawa 2006). The mechanism of action potential prolongation has been linked to alterations in intracellular calcium homeostasis as well as to changes in both depolarizing and repolarizing ion currents, with the decrease in repolarizing potassium currents being the

most coherent finding (Hill 2003, Furukawa and Kurokawa 2006). Putative underlying changes in signalling pathways have also been described (Furukawa and Kurokawa 2006). Electric remodelling in hypertrophy and heart failure have common features but also dissimilarities (Hill 2003). Myocardial matrix metalloproteinase activation and remodelling in the cardiac transverse-tubule system may be important events during the unfavourable disease progression from hypertrophy to heart failure (King et al. 2003, Wei et al. 2010).

Clinically, LVH is most often detected with echocardiography and/or ECG. Echocardiographically, LVH may be detected with relatively high accuracy, and is evaluated from wall-thicknesses and/or by indexing the approximated LVM to body size (Lang et al. 2005). Several ECG LVH criteria exist, and most of them are based on voltage measurements and feature high specificity and low sensitivity (Truong et al. 2010). Magnetic resonance imaging (Alfakih et al. 2004) and computer tomography (Truong et al. 2010) are more accurate in LVM evaluation, but impractical for solely this purpose. Previous clinical studies have shown that LVH is associated with ECG repolarization changes, such as QT-interval prolongation and changes in T-wave morphology (Yi et al. 1998, Oikarinen et al. 2001a, 2002, 2004b). However, although experimental evidence suggests that electric remodelling is an early process (Ben-David et al. 1992, Huang et al. 2000), little has been known about the potential clinically modifying effect of mild LVH or mild structural LV remodelling on ECG repolarization measures.

2.5.2 Drugs

The mechanism of “quinide syncope”, TdP tachycardia, was uncovered in the 1960s (Selzer and Wray 1964). Thereafter, it has become evident that both cardiac and non-cardiac drugs may directly modify cardiac repolarization, and the effect is typically observed as QT-interval prolongation with increased risk of TdP. Most drugs exert this effect by inhibiting the function of ion channels encoded by *KCNH2*, thus reducing I_{Kr} (Haverkamp et al. 2000). In the process of developing new drugs, routine *in vitro* and *in vivo* evaluation of potential drug-induced adverse repolarization changes is an important safety procedure (Haverkamp et al. 2000), and regulatory guidance notes currently dictate that the assessment of changes in ECG QT-interval duration and T-wave shape is mandatory (Darpo et al. 2006). Quantitative indexes of T-wave morphology have been suggested as potentially valuable tools for drug trials (Badilini et al. 2008, Graff et al. 2009), but the QT interval currently remains the standard for this purpose (Malik 2009). Despite the systematic evaluation process in drug development, the risk of drug-induced TdP may remain difficult to predict because the individual risk depends on several risk-modifying factors and, with the exception of most extreme values, the correlation between TdP risk and QT-interval prolongation is not strong (Roden 2008, Sager 2008). Different drugs may prolong the QT interval similarly, but may still modify afterdepolarization-susceptibility and repolarization dispersion differently (Sager 2008). The drug may also have an effect on several types of ion channels with a relatively low net TdP risk despite the marked QT-interval prolongation (e.g. amiodarone). In addition, minor drug-related changes in repolarization measures may be difficult to differentiate from their spontaneous, non-drug related variability. As a result, sometimes the adverse effect on repolarization becomes evident only after the new drug has been

released and widely used (e.g. cisapride, terfenadine). Comprehensive lists of QT-interval prolonging drugs are available in the Internet (e.g. www.azcert.org).

Hypertension has been recognised as the leading risk factor for mortality in the world, and nearly 30% of the adult population has been projected to have this condition (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) in 2025 (Kearney et al. 2005). In economically developed countries, approximately one-half to two-thirds of hypertensives are on antihypertensive medication (Kearney et al. 2004). Long-term antihypertensive medication is associated with LVH regression and beneficial repolarization changes (Malmqvist et al. 2002, Oikarinen et al. 2003, Okin et al. 2004a, Okin 2009). The renin-angiotensin-aldosterone system (RAAS) plays a key role in the generation of pathological LVH in hypertension (Mehta and Griendling 2007, Tomaschitz et al. 2010), and antihypertensive dugs that inhibit RAAS, the angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, may be more efficient in LVH regression compared to other drugs (Ruilope and Schmieder 2008). In addition, RAAS-inhibiting drugs may directly blunt the proarrhythmic actions of RAAS and modify cardiac repolarization by blood pressure-independent mechanisms (Caballero et al. 2000, Rials et al. 2001, Delpón et al. 2005). On the other hand, antihypertensive agents from other drug classes, such as ß-blockers and diuretics, may also have direct repolarization effects as they also can modulate RAAS and blood potassium level (Burnier and Brunner 1992, Blumenfeld et al. 1999). There are no clinical studies comparing the short-term repolarization effects of antihypertensive drugs. Nonetheless, short-term repolarization modifying effects might be significant at the population level because antihypertensive drugs are so widely used and a large number of hypertensive subjects may have LVH or structural remodelling, which may predispose them to repolarization-related arrythmias. The effect might be important particularly at the early phase of the treatment, before significant LV reverse remodelling has occurred.

2.5.3 Common gene variants

The majority of human genetic variation is expected to be in the form of substitutions at single base pairs, that is, single nucleotide polymorphisms (SNPs) (Kruglyak and Nickerson 2001). In this variation, a single deoxyribonucleic acid (DNA) nucleotide base (adenine, cytosine, guanine, thymine; called as alleles), differs between individuals or between paired chromosomes in an individual. Traditionally, the variation is classified as polymorphism if the minor (i.e. less frequent) allele frequency in the population is 1% or more (Kruglyak and Nickerson 2001). By 2007, more than 3 million SNPs had been documented (International HapMap Consortium 2007), and their linkage to various cardiac and non-cardiac diseases is under investigation. Nearly 10 million common SNPs are estimated to lie in the human genome (International HapMap Consortium 2007), but most of the SNPs may be innocent and without functional significance. SNPs may be located in the coding or non-coding sequences of a gene, or in the intergenic regions. Furthermore, a SNP may exhibit its effect on a gene by residing in the same (disease-causing) gene, but also from another locus, distinct from the disease gene (Scicluna et al. 2008).

Congenital LQTS is caused by a mutation in genes coding for cardiac ion channels or, more rarely, other specified cell proteins involved in cardiac repolarization (Hedley et al. 2009). Mutations causing LQTS often have a large effect on the QT interval in the

affected individual but, because these mutations are rare, they do not explain much of the QT-interval variation at the population level. In contrast, the impact of individual SNPs on cardiac repolarization may usually be low in a single individual, but because SNPs in repolarization-involved genes are more common, they may still have significant impact as repolarization modifiers at the population level.

Polymorphism in liver enzymes has been well-documented as a risk factor for QT-interval prolongation in the setting of a poor metabolizer and use of QT-interval prolonging drug (Lin and Lu 1998). During the last decade, several SNPs have also been found in genes coding for ion channels and their regulatory structures, and *in vitro* studies have demonstrated that SNPs may modify ion channel expression, function, and localization (Scicluna et al. 2008). Some of the variants are associated with potentially harmful, others potentially protective or unknown repolarization effects, and the effect may even vary whether the SNP is inherited *in cis* or *in trans* with the disease-causing allele (Hedley et al. 2009). Table 2 summarizes previous population studies on the relationship between the QT interval and SNPs in various LQTS genes. The clinical studies have typically used the QT interval as the repolarization measure and indicate a cumulative effect of various QT-interval altering SNPs (Pfeufer et al. 2009). Thus, the SNPs in LQTS genes play a role as disease modifiers, which in part explain the wide variability in the genotype-phenotype relationship in LQTS (Scicluna et al. 2008, Hedley et al. 2009).

Recently, several studies have also confirmed the association between the QT interval and polymorphism in the nitric oxide synthase 1 adaptor protein gene (*NOS1AP*), showing QT-interval prolongation with minor alleles (Arking et al. 2006, Aarnoudse et al. 2007, Post et al. 2007, Lehtinen et al. 2008, Tobin et al. 2008, Crotti et al. 2009, Newton-Cheh et al. 2009, Pfeufer et al. 2009, Raitakari et al. 2009, Tomás et al. 2010). *NOS1AP* may modify repolarization by altering the activities of $I_{Ca,L}$ and I_{Kr} (Chang et al. 2008). Of note, association between *NOS1AP* polymorphism and sudden cardiac death has been reported (Eijgelsheim et al. 2009, Kao et al. 2009). In genome-wide association studies, an association has been found between the QT interval and SNPs in genes which do not contain any established electrophysiological function (Newton-Cheh et al. 2009, Pfeufer et al. 2009). In addition, the currently known SNPs seem to explain only less than 10% of the variation in QT-interval duration (Newton-Cheh et al. 2009, Pfeufer et al. 2009), indicating the need for future research.

2.5.4 Gender

It has been well documented that cardiac repolarization exhibits gender differences. Previous clinical studies have demonstrated that women have a longer QT interval than men (Merri et al. 1989, Smetana et al. 2002, Wu et al. 2003). Furthermore, women prolong their QT interval more and have higher risk of TdP than men when using QT-interval prolonging drugs (Makkar et al. 1993, Benton et al. 2000). Gender may also affect the predictive value of QT-interval duration in the general population (Karjalainen et al. 1997, de Bruyne et al. 1999). A few clinical studies have also reported gender differences in numerical values of the TPE interval (Nakagawa et al. 2003), PCA ratio (Okin et al. 2000b, Okin et al. 2002), TMD (Ono et al. 2005), TCRT (Smetana et al. 2002), TWR (Smetana et al. 2002), and T-wave amplitude (Wu et al. 2003). Gender differences in the predictive value of T-wave morphology parameters have not been previously reported.

Table 2 A selection of population studies on the relationship between QT interval and SNPs.

Gene	SNP	Allele* and respective amino acid change	Minor allele effect† on QT interval	Reference
KCNQ1	rs757092	A→G	↑	Pfeufer et al. 2005, Gouas et al. 2007
	rs2074238	C→T	↓	Newton-Cheh et al. 2009
	rs12576239	C→T	↑	Newton-Cheh et al. 2009
	rs12296050	C→T	↑	Pfeufer et al. 2009
KCNH2	rs1805123	A→C, K897T	↑	Pietilä et al. 2002‡, Koskela et al. 2008‡
			↓	Bezzina et al. 2003, Gouas et al. 2005, Pfeufer et al. 2005, Newton-Cheh et al. 2007
			↔	Raitakari et al. 2009
	rs2968863§	G→A	↓	Pfeufer et al. 2009
	rs2968864§	A→G	↓	Newton-Cheh et al. 2009
	rs3807375	G→A	↑	Newton-Cheh et al. 2007
	rs3815459	G→A	↑	Pfeufer et al. 2005
	rs4725982§	C→T	↑	Newton-Cheh et al. 2009
SCN5A	rs1805124	A→G, H558R	↑	Aydin et al. 2005, Gouas et al. 2005
	rs11129795	G→A	↓	Pfeufer et al. 2009
	rs12053903	T→C	↓	Newton-Cheh et al. 2009
KCNE1	rs1805127	G→A, G38S	↑	Friedlander et al. 2005¶
			↔	Aydin et al. 2005, Akyol et al. 2007, Gouas et al. 2007
	rs1805128	G→A, D85N	↑	Gouas et al. 2005, Newton-Cheh et al. 2009
			↔	Aydin et al. 2005, Friedlander et al. 2005
KCNE2	rs2234916	A→G, T8A	↑	Aydin et al. 2005
			↔	Pfeufer et al. 2005
KCNJ2	rs17779747§	G→T	↓	Pfeufer et al. 2009

*Major→minor allele. †Prolonging effect=↑, shortening effect=↓, non-signicant effect=↔. ‡The effect was significant in women only and nonsignificant in men. §Located near the respective gene. ¶The effect was significant in men only and nonsignificant in women. SNP=single nucleotide polymorphism.

Gender difference in QT-interval duration appears after puberty when boys' QT interval shortens (Rautaharju et al. 1992), which suggests a gonadal-hormone mediated mechanism. Indeed, compared to controls, castrated men have prolonged repolarization and women with virilization have shortened repolarization (Bidoggia et al. 2000), which suggests that testosterone may have an important role in modulating (i.e. shortening) repolarization. Studies on women receiving hormone replacement therapy also indicate that estrogen may prolong the QT interval, and that progesterone may counteract this effect (Carnethon et al. 2003, Kadish et al. 2004, Gökçe et al. 2005). Although results vary according to the animal model, experimental studies have shown that the expression of several myocardial ion channels may vary between genders, and that

gonadal hormones may have effects on myocardial ion channels both via genomic and non-genomic pathways (Cheng 2006, Yang et al. 2010). Data on gender differences in myocardial ion channels in humans is limited. In failing human LV myocytes, women may have larger $I_{Ca,L}$ and smaller transient outward potassium current than men (Verkerk et al. 2005). Ultimately, gender differences in repolarization may in part be explained by gender differences in autonomic nervous system function (Nakagawa et al. 2009).

2.6 Summary

Function of the heart is dependent on electric activity of the cardiomyocytes whose action potentials are produced by a coordinated function of depolarizing and repolarizing ion currents through ion channels. Abnormal ventricular repolarization may predispose to arrhythmias, and repolarization-related arrhythmia vulnerability may be modified by several factors. Previously, little has been known about the ECG ventricular repolarization modifying effects of mild LVM increase and antihypertensive drugs in hypertension, as well as of SNPs in the Finnish general population.

In a standard 12-lead resting ECG, the duration of ventricular repolarization, the QT interval, has long been the most widely used repolarization parameter both clinically and in research. Efforts have been made to develop additional repolarization measures for risk stratification. Previous experimental and clinical studies suggest that the TPE interval may quantify arrhythmogenic repolarization dispersion. Furthermore, parameters evaluating temporospatial changes in the ventricular repolarization have been developed using computerized analysis of digital ECG. Previous clinical studies suggest that evaluation of T-wave morphology may differentiate between healthy individuals and those with repolarization disturbances, and that parameters such as PCA ratio, TMD, TCRT, and TWR contain independent prognostic information. Few previous studies have assessed the predictive value of T-wave morphology parameters, and none have reviewed the predictive value of the TPE interval, in the general population.

3 AIMS OF THE STUDY

The series of studies reported were undertaken to investigate modifiers of ECG repolarization parameters, and to assess the predictive value of these repolarization parameters for mortality. Specific aims were:

- To study whether mild LVM increase in hypertension is associated with ECG repolarization changes (study I).

- To investigate whether ventricular repolarization can be modified by the type of antihypertensive drug treatment (study II).

- To evaluate the relationship of common variants in *NOS1AP* and selected LQTS genes to ECG QT interval (study III), as well as to TPE interval and T-wave morphology parameters (study IV).

- To examine the predictive value of ECG QT interval and T-wave morphology parameters for all-cause and cardiovascular mortality in the general population (study V).

4 MATERIALS AND METHODS

4.1 Study subjects

In studies I and II, the study subjects originated from the GENRES study (a randomized, double-blind, cross-over, single-centre, placebo-controlled study on the molecular genetics of drug responsiveness in essential hypertension) (Hiltunen et al. 2007). The clinical part of the GENRES study was conducted at the Helsinki University Central Hospital in 1999–2004, and the study was approved by the Ethics Committee of Helsinki University Central Hospital and the National Agency for Medicines of Finland. The study subjects were white men aged 35–60 years with either prior antihypertensive drug treatment or diastolic blood pressure ≥95 mmHg on three separate measurements. All subjects were in sinus rhythm. Secondary hypertension, treatment with three or more antihypertensive drugs, drug-treated diabetes mellitus, congestive heart failure, and coronary heart disease were among the exclusion criteria. In study I, those GENRES study subjects were included who had both ECG and transthoracic echocardiogram recorded during the study run-in placebo period. Subjects with complete bundle branch block, ECG strain pattern of repolarization, or non-optimal ECG quality were excluded, and study I eventually included 220 subjects. In study II, those GENRES study subjects who had both ECG recorded and office blood pressure measured at the end of all four drug periods were considered eligible. Subjects with complete bundle branch block were excluded, and hence study II included 183 subjects.

In studies III–V, the study population was derived from an epidemiological survey, the Health 2000 Study, which was conducted in Finland in 2000–2001 with the permission of the Epidemiology Ethics Committee of the Helsinki and Uusimaa Hospital Region (Aromaa and Koskinen 2004). The Health 2000 Study population was a two-stage stratified cluster sample of 8028 Finnish adults aged ≥30 years. DNA samples were collected from 6334 subjects, and ECGs were available from 6292 subjects. In studies III–V, exclusion criteria were complete bundle branch block, QRS duration ≥120 ms (in study III only), Wolff-Parkinson-White pattern, paced rhythm, atrial fibrillation or flutter, poor ECG quality, confirmed genetic diagnosis of Finnish LQTS founder mutation (in studies III and IV only), and use of medication with possible effect on QT-interval duration. After exclusions, study III included 5043 subjects, study IV 5890 subjects, and study V 5917 subjects.

4.2 Clinical evaluation

In studies I and II, the subjects underwent a structured health interview, measurements (e.g. height, weight, ECG), and a physician's clinical examination at the screening visit of the GENRES study (Hiltunen et al. 2007). The study started with a four-week run-in placebo period, before which the subjects discontinued their possible previous antihypertensive medications. Transthoracic echocardiogram and ECG recording was performed at the end of the run-in placebo period. The subjects then received one of the four study drugs in a randomized order daily for four weeks, and placebo periods of four weeks were included between drug periods. The study drugs were, administered once daily doses of losartan 50 mg, bisoprolol 5 mg, amlodipine 5 mg, and

hydrochlorothiazide 25 mg. ECG was recorded, blood samples (serum potassium and fasting blood glucose were used in studies I and II) were taken, and office blood pressure and ambulatory blood pressure were measured at the same visit at the end of each placebo and drug period. Thus, for studies I and II, ECG and blood pressure data were gathered during eight different occasions (during treatment with four different antihypertensive drugs and during four placebo periods) from every study subject. The non-dominant arm was used to measure blood pressure. Office blood pressure measurements (Omron M4, Omron Healthcare, Tokyo, Japan) were performed after a 30-minute rest in sitting position three times with one minute intervals, and the mean of the last two measurements was used in the analyses (studies I and II). In study II, the average value of office blood pressure measurements from all four placebo periods was used as a reference to which measurements during each drug period were compared. The 24-hour ambulatory blood pressure, used in study I, was measured according to the guidelines of the European Society of Hypertension (O'Brien et al. 2003).

In studies III–V, clinical data were based on examinations performed at the Health 2000 Study baseline and included health questionnaires, measurements (e.g. height, weight, blood pressure, ECG), and a physician's clinical examination (Aromaa and Koskinen 2004). Disease definitions used in studies IV and V are listed in Appendix 1. Blood samples were taken after an overnight fast, and the total cholesterol/high density lipoprotein ratio was used in study V. Office blood pressure was measured from the right arm with a conventional mercury sphygmomanometer after a 10-minute rest in the sitting position twice with a two-minute interval. The mean of two blood pressure readings was used in studies III–V. In study V, both the mortality status and cause of death of the subjects were obtained from the Causes-of-Death Register maintained by Statistics Finland.

4.3 Electrocardiographic measurements

ECG recordings. In studies I–V, a digital standard resting 12-lead ECG was recorded with a Marquette MAC 5000 electrocardiograph (GE Marquette Medical Systems, Milwaukee, Wisconsin, USA). The 12 leads were recorded simultaneously. The digital median QRS-T complex automatically produced by QT Guard 1.3 software (GE Marquette Medical Systems) was used for measurements in each lead. In study II, the average values of ECG measurements from all four placebo periods were used as a reference to which measurements during each drug period were compared. ECGs used in studies III–V were classified according to guidelines from the Minnesota Code Manual (Prineas et al. 1982).

Adjustment for heart rate. In studies I–V, heart rate was automatically provided by QT Guard 1.3 software. In studies I and II, QT intervals were adjusted for heart rate with the nomogram method (yielding QTNc interval) (Karjalainen et al. 1994) with the correction numbers adopted from a study population closely resembling the population in studies I and II (Karjalainen et al. 1994). In studies III–V as well, QT intervals were adjusted with the nomogram method, but the rate-correction equations were determined in the study population which was used in these studies. The correction equations were determined separately for each 10 beats per minute heart rate range based on the previous description (Karjalainen et al. 1994). In studies IV and V, the equations were

calculated separately for both genders. To also compare our results to previous studies, Bazett's formula adjustment (QTc interval=QT interval/RR interval$^{0.5}$) was calculated (Bazett 1920) in studies III and V. TPE intervals were not rate-adjusted (Andersen et al. 2008).

QT intervals and TPE intervals. QT-interval measurements were performed based on a previously described and validated algorithm (Oikarinen et al. 1998) with a custom-made software. The software calculated the QT_{peak} (from QRS onset to T-wave peak), QT_{end} (from QRS onset to T-wave end), TPE interval, as well as QRS duration in each lead. A single observer reviewed measurements on-screen in a blinded fashion, and leads with low signal-to-noise ratio or flat T waves were excluded from QT- and TPE-interval analyses. In study I, QT and TPE intervals had to be measurable in ≥6 leads with ≥3 precordial leads for the ECG to be included in the study. For final analyses, the maximum QT_{peak} (studies I and II) and mean QT_{peak} (study II) interval of all leads, maximum QT_{end} (studies I, II, V) and mean QT_{end} (studies II and III) interval of all leads, maximum TPE interval of precordial leads (studies I, II, IV, V), and mean TPE interval of all leads (studies I and II) were used. In study IV, SNP effects on QT_{peak} and QT_{end} intervals were analysed from the precordial lead with the maximum TPE interval.

T-wave morphology parameters. All T-wave morphology parameters (studies I, II, IV, V) were calculated fully automatically by computer. QT Guard 1.3 software was used to measure the PCA ratio. Three additional T-wave morphology parameters (TMD, TCRT, TWR) were calculated by custom-made software, which uses a previously described algorithm (Acar et al. 1999, Oikarinen et al. 2002). The procedure involves singular value decomposition (Wall et al. 2003) of eight independent leads (I, II, V1–6) and reconstruction of an eight-lead orthogonal system. In such a system, the first three components correspond to the energy in the three-dimensional ECG vector (dipolar components), and the five remaining components correspond to the non-dipolar signal content of the original ECG (Acar et al. 1999, Malik et al. 2000) (see Appendix 2 for computation description). The absolute (not the relative) TWR was used as the measure of non-dipolar signal content (studies I, IV–V). T-morphology parameters showed non-significant or only weak correlations to heart rate (absolute *r* values from 0.005 to 0.08 in studies IV and V).

Other ECG measurements. Custom-made software was also used to automatically measure R- and S-wave amplitudes (measured separately in each lead) as well as QRS duration (the same value used for all leads), and the results were reviewed on-screen in a blinded fashion by a single observer. With the 10 mm/mV calibration, Sokolow-Lyon voltage (SV1+RV5 or SV1+V6) >35 mm and Cornell voltage-duration product [(SV3+RaVL+8 in women)×QRS duration] ≥2440 mm×ms (Molloy et al. 1992) were used as ECG indexes of LVH. In studies III–V, ECG LVH was considered to be present if either of the criteria were fulfilled. In study I, the Cornell voltage-duration product was used as the sole criterion. In study II, the sum of QRS areas in all 12 ECG leads (QRS area sum) was calculated (Oikarinen et al. 2004a). The QRS area sum during placebo correlated with echocardiographically determined LVM index (LVMI) (r=0.41, P<0.001) and served as an estimate for LVM changes.

Reproducibility of ECG repolarization parameters. To test the intraobserver variability of ECG measurement techniques, repolarization measurements were performed twice from the same recordings in study I. The intraobserver coefficient of

variation was 1.5% for the maximum QT_{peak} interval of all leads, 1.3% for the maximum QT_{end} interval of all leads, 2.7% for the maximum TPE interval of precordial leads, 1.7% for the mean TPE interval of all leads, 0.0% for the PCA ratio, 1.6% for TMD, 0.0% for TCRT, and 2.5% for TWR.

4.4 Echocardiographic measurements

An experienced cardiologist performed echocardiograms during the run-in placebo period and stored two-dimensionally guided M-mode recordings on videotapes (studies I and II). Interventricular septal thickness, posterior wall thickness, and LV end-diastolic diameter were later measured on-screen by a single observer according to the recommendations of the American Society of Echocardiography (Sahn et al. 1978). Measurements were averaged from five cardiac cycles. Ejection fraction was calculated with the Teichholz method. LVM (in grams) was calculated by an anatomically validated formula $0.8\times[1.04\times((\text{interventricular septal thickness}+\text{LV end-diastolic diameter}+\text{posterior wall thickness})^3-\text{LV end-diastolic diameter}^3)]+0.6$ (Devereux et al. 1986), and LVMI by dividing LVM by body surface area (in square metres). The Mosteller formula $[(\text{height in centimetres}\times\text{weight in kilograms})/3600]^{0.5}$ was used to calculate body surface area (Mosteller 1987). LVMI >116 g/m^2 was used for LVH detection (Liu and Devereux 1998).

4.5 Genetic studies

In study III, the SNPs in *NOS1AP* and LQTS genes were selected based on their previous identification in the Finnish population and on the previous evidence of their functional role as a repolarization modifier (Fodstad et al. 2004, Arking et al. 2006, Aarnoudse et al. 2007). In study IV, seven SNPs were selected based on their shortening or prolonging effects on the QT interval in study III.

Peripheral blood sample lymphocytes were used for DNA analyses (studies III and IV). For *KCNH2* K897T, genotyping was performed using a Taqman Genotyping Assay (Applied Biosystems, Foster City, California, USA), which detects the alleles at the SNP site with fluorescence-labeled detectors. The fluorescence signals were measured with 7900HT Real Time PCR System (Applied Biosystems), and SDS2.2 software (Applied Biosystems) was used in analysing the data. For all other SNPs, genotyping was performed using Sequenom MALDI-TOF mass spectrometry (MassArray Compact Analyzer, Sequenom Inc., San Diego, California, USA), which utilizes primer extension reaction chemistry. In MALDI-TOF assays, the homogenous MassEXTEND method was used for *KCNH2* R1047L and the iPLEX chemistry for all other SNPs. Reactions for the MALDI-TOF assays were designed with Sequenom Assay Designer 3.1 software (Sequenom Inc.), and SpectroAnalyzer 3.4 software was used to assess the results.

4.6 Statistical analyses

Analyses were performed with SPSS 13.0 and 15.0 software (SPSS Inc., Chicago, Illinois, USA). Values are presented as mean±standard deviation (SD) for continuous variables and as number of subjects or percentages for categorical variables. Variable distributions were assessed with histograms and the Kolmogorov-Smirnov test, and the appropriate test was selected according to the distribution. In categorical variables, groups were compared with the Chi-square test. In continuous variables, the Mann-Whitney U test or independent samples *t* test (two-group comparison) or analysis of variance (multiple-group comparison) was used to compare groups. In repeated measures, either the repeated measures analysis of variance or Friedman test was used to compare groups. The Spearman test or Pearson's test was used to assess univariate correlations. Regression analyses were used to assess the relationship between independent and dependent variables. To normalize variable distributions in regression analyses, natural-logarithmic ($\log_e$) transformation or the Blom method (Blom 1958) was used. In study I, the relationship of repolarization parameters to five ordered LVMI categories was analysed with the Jonckheere-Terpstra test. In studies III and IV, the prevalence estimates were derived from the weighted study population, and the size effects of genetic variants to repolarization parameters were assessed with linear regression analyses including both additive and genotypic models. The additive model was a one degree of freedom model (the genotype was transformed to a variable which corresponded to the number of minor alleles) and the genotypic model was a two degrees of freedom model (the heterozygote and minor homozygote genotypes were converted to two dichotomous variables). All SNPs were tested for Hardy-Weinberg equilibrium with a Chi-square test. In study V, analyses were performed separately for men and women. In study V, the Kaplan-Meier curves and log-rank tests were used to assess the death rates in the groups dichotomized according to the median values of the repolarization parameters, and the Cox proportional hazards models were used to assess the relationship of repolarization measures and clinical variables to time to death. A two-tailed $P<0.05$ was considered statistically significant.

5 RESULTS

5.1 Relationship of repolarization parameters to left ventricular mass (study I)

For study I, 220 hypertensive men with a mean age of 51±6 years were subjects. No wall-motion abnormalities were seen with echocardiography, whilst mean LV ejection fraction was 62±7%, mean LVM 203±39 g, and mean LVMI 99±19 g/m^2. Only 39 subjects (18%) reported echocardiographic LVH, and only nine subjects (4%) demonstrated ECG LVH.

Table 3 shows repolarization parameters as well as clinical and echocardiographic variables relative to the echocardiographic LVH status. Of the clinical parameters, only 24-hour mean systolic blood pressure differed significantly between the groups. QT intervals, TPE intervals, PCA ratio, TMD, and TWR were higher in LVH versus non-LVH subjects, whereas TCRT was lower, as expected. To further study the relationship of repolarization parameters to LVM, five ordered LVMI categories were created and significant linear trends observed across the categories for all repolarization parameters (Figure 6). Five ordered LVMI categories in subjects without echocardiographic LVH were also created, and significant linear trends were observed for PCA ratio (P<0.001), TMD (P<0.001), TCRT (P=0.032), and TWR (P=0.025).

When ECG repolarization parameters and selected clinical and echocardiographic variables (age, body mass index, office and 24-hour mean systolic and diastolic blood pressure, blood glucose, serum potassium, interventricular septal thickness, LV end-diastolic diameter, LVMI) were correlated, the strongest correlation was observed between TMD and LVMI (r=0.39, P<0.001), and interventricular septal thickness and LVMI were the only variables that correlated statistically significantly with all ECG repolarization parameters (absolute r values from 0.15 to 0.29 for interventricular septal thickness and from 0.16 to 0.39 for LVMI). Multiple linear regression analyses including significant clinical variables and repolarization parameters showed that LVMI, though not an independent determinant of the QT_{peak} interval, was an independent determinant of all other repolarization parameters (P from 0.048 to <0.001).

5.2 Electrophysiological effects of losartan, bisoprolol, amlodipine, and hydrochlorothiazide (study II)

The mean age of the 183 hypertensive men in study II was 51±6 years. Table 4 shows the effects of antihypertensive treatment with losartan, bisoprolol, amlodipine, and hydrochlorothiazide on clinical and ECG parameters. Compared to placebo, both systolic and diastolic blood pressures were significantly lower during drug administration, but hydrochlorothiazide lowered blood pressure the least. Hydrochlorothiazide increased and losartan decreased the QRS area sum. Compared to serum potassium during the study run-in placebo period, serum potassium increased during bisoprolol and decreased during hydrochlorothiazide. Both losartan and bisoprolol shortened all studied QT intervals and the mean TPE interval, decreased TMD

Table 3 Characteristics of study subjects by LVH status in study I (LVH + if LVMI >116 g/m^2).

	LVH – (n=181)	LVH + (n=39)	*P*
Age (years)	50±7	52±5	0.084
Body mass index (kg/m^2)	26.9±2.8	26.8±2.8	0.795
Body surface area (m^2)	2.1±0.1	2.0±0.1	0.286
Office systolic blood pressure (mmHg)	150±16	155±16	0.106
Office diastolic blood pressure (mmHg)	100±9	100±9	0.706
24-hour mean systolic blood pressure (mmHg)	133±11	137±12	0.045
24-hour mean diastolic blood pressure (mmHg)	92±7	94±6	0.100
Serum potassium (mmol/L)	4.4±0.2	4.4±0.2	0.417
Ejection fraction (%)	62±7	60±8	0.077
Interventricular septal thickness (mm)	10.7±1.4	13.1±1.6	<0.001
Posterior wall thickness (mm)	10.4±1.3	12.3±1.4	<0.001
LV end-diastolic diameter (mm)	49±4	51±4	0.001
LVM (g)	190±29	261±26	<0.001
LVMI (g/m^2)	92±13	128±10	<0.001
Maximum QT_{end}Nc (ms)	400±21	410±23	0.007
Maximum QT_{peak}Nc (ms)	330±18	337±20	0.028
Maximum TPE V1–6 (ms)	82±12	89±13	<0.001
Mean TPE (ms)	72±7	76±6	0.003
PCA ratio (%)	12.9±4.9	16.3±6.1	0.002
TMD* (°)	11.1±6.2	16.6±12.3	<0.001
TCRT* (unitless)	0.45±0.45	0.22±0.54	0.006
TWR* (technical units)	12965±12292	16943±17852	0.035

Values are expressed as mean±standard deviation. The *t* test or *Mann-Whitney U test. LV=left ventricular, LVH=left ventricular hypertrophy, LVM=left ventricular mass, LVMI=left ventricular mass index, Nc=nomogram-corrected for heart rate, PCA=principal component analysis, QT_{end}=QT interval to the T-wave end, QT_{peak}=QT interval to the T-wave peak, TCRT=total cosine R-to-T, TMD=T-wave morphology dispersion, TPE=T-wave peak to T-wave end interval, TWR=T-wave residuum.

and increased TCRT. In addition, losartan shortened the precordial maximum TPE interval and decreased the PCA ratio. Hydrochlorothiazide prolonged both studied TPE intervals, but did not have an effect on the other repolarization parameters. Amlodipine had no significant ECG repolarization effects. Figure 7 shows the changes in ECG repolarization parameters, in percentages, during each drug treatment compared to placebo.

To determine the factors associated with changes in ECG repolarization parameters, they were correlated to the changes in systolic blood pressure, QRS area sum, and serum potassium level after the 4-week treatment. A decrease in systolic blood pressure was associated with shortening of QT intervals (*r* from 0.09 to 0.16, *P* from 0.011 to <0.001) and TPE intervals (*r* from 0.10 to 0.13, *P* from 0.005 to <0.001) as well as with an increase in TCRT (*r*=–0.09, *P*=0.015). A decrease in the QRS area sum was associated

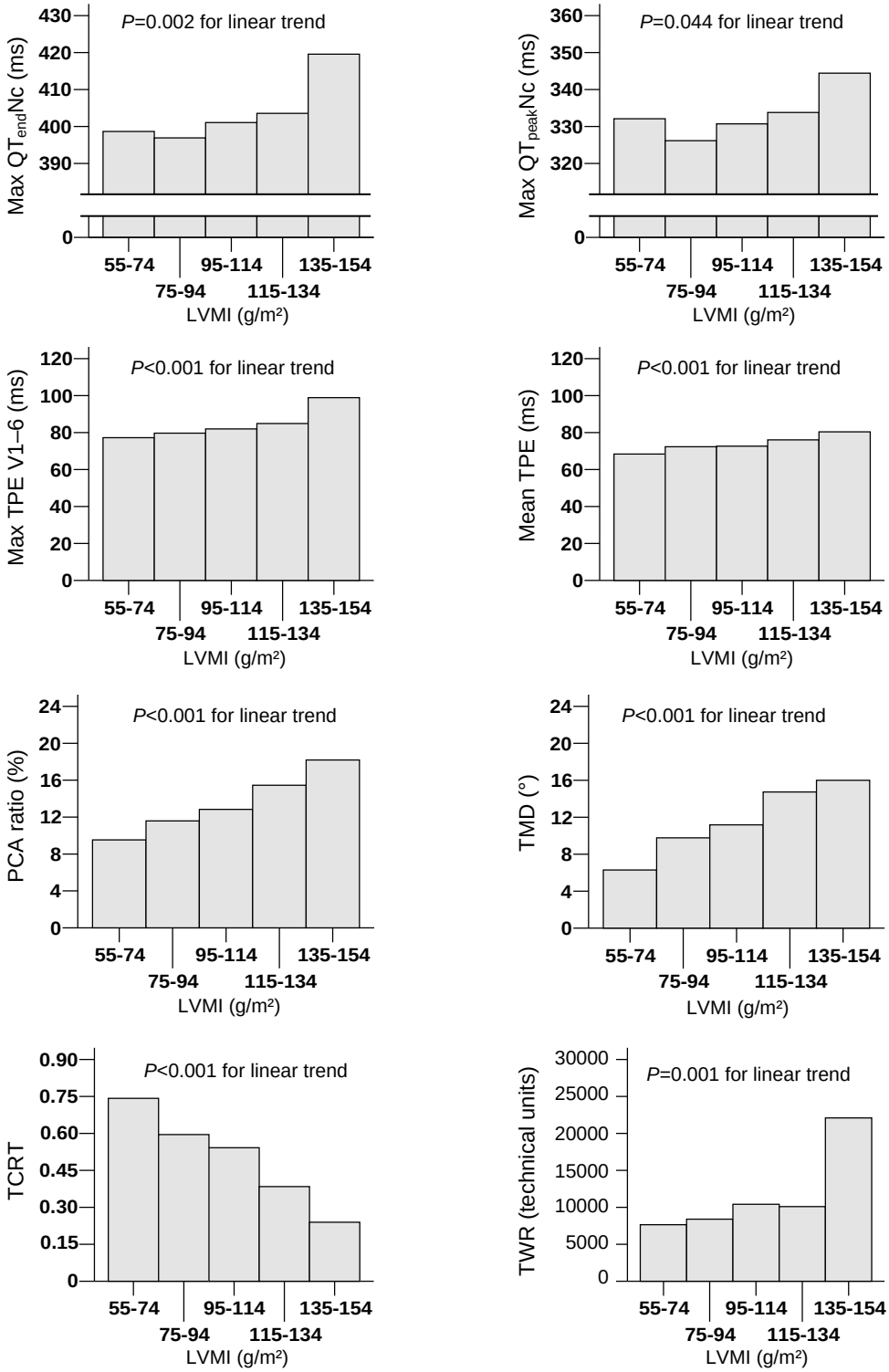

Figure 6 Linear trends for repolarization parameters in five ordered LVMI categories with equal ranges of LVMI values in study I (LVMI 55–74 g/m^2, n=18; 75–94 g/m^2, n=85; 95–114 g/m^2, n=76; 115–134 g/m^2, n=33; 135–154 g/m^2, n=8). Bars represent medians. *P* values from the Jonckheere-Terpstra test. LVMI=left ventricular mass index, Max=maximum, Nc=nomogram-corrected for heart rate, PCA=principal component analysis, QT_{end}=QT interval to the T-wave end, QT_{peak}=QT interval to the T-wave peak, TCRT=total cosine R-to-T, TMD=T-wave morphology dispersion, TPE=T-wave peak to T-wave end interval, TWR=T-wave residuum. With permission, from Porthan et al. 2007, Relationship of electrocardiographic repolarization measures to echocardiographic left ventricular mass in men with hypertension, J Hypertens 2007;25(9):1951–7.

Table 4 Effects of 4-week antihypertensive drug treatment (drug compared to placebo) on clinical and ECG parameters in study II (n=183).

	Placebo§	Losartan	Bisoprolol	Amlodipine	HCTZ
Systolic blood pressure (mmHg)	152±13	143±16‡	139±15‡	144±13‡	147±15‡
Diastolic blood pressure (mmHg)	100±7	92±9‡	90±9‡	93±8‡	97±8‡
Heart rate (beats per minute)	62±7	63±8†	53±7‡	65±10‡	65±9‡
QRS area sum (μV×s)	370±81	362±84‡	372±83	370±84	379±84‡
QRS (ms)	87±8	87±10	88±9	87±10	88±9
Serum potassium¶ (mmol/L)	4.4±0.2	4.4±0.3	4.6±0.3‡	4.4±0.3	4.1±0.3‡
Maximum QT_{end}Nc (ms)	401±20	395±20‡	394±21‡	399±19	402±20
Mean QT_{end}Nc (ms)	387±16	382±16‡	381±19‡	386±16	388±17
Maximum QT_{peak}Nc (ms)	331±16	327±17‡	325±18‡	330±17	330±18
Mean QT_{peak}Nc (ms)	314±13	311±14‡	309±17‡	314±15	314±16
Maximum TPE V1–6 (ms)	83±10	81±12†	82±12	82±11	85±12‡
Mean TPE (ms)	72±7	70±7‡	71±7*	72±7	75±8‡
PCA ratio (%)	14.4±5.4	13.9±5.8*	14.2±5.7	14.1±5.7	14.4±5.9
TMD (°)	13.3±9.2	12.9±9.6‡	12.6±10.5†	13.0±9.3	13.3±10.1
TCRT (unitless)	0.41±0.45	0.44±0.48†	0.45±0.45†	0.41±0.49	0.41±0.50

Values are expressed as mean±standard deviation. *P<0.05, †P<0.01, ‡P<0.001. §Mean of measurements from all placebo periods. ¶n=91 for bisoprolol and losartan, n=92 for amlodipine and HCTZ. P values from the Wilcoxon signed ranks test (TMD, TCRT), the paired samples t test (serum potassium), or from the repeated measures analysis of variance (all other variables). ECG=electrocardiographic, HCTZ=hydrochlorothiazide, Nc=nomogram-corrected for heart rate, PCA=principal component analysis, QT_{end}=QT interval to the T-wave end, QT_{peak}=QT interval to the T-wave peak, TCRT=total cosine R-to-T, TMD=T-wave morphology dispersion, TPE=T-wave peak to T-wave end interval.

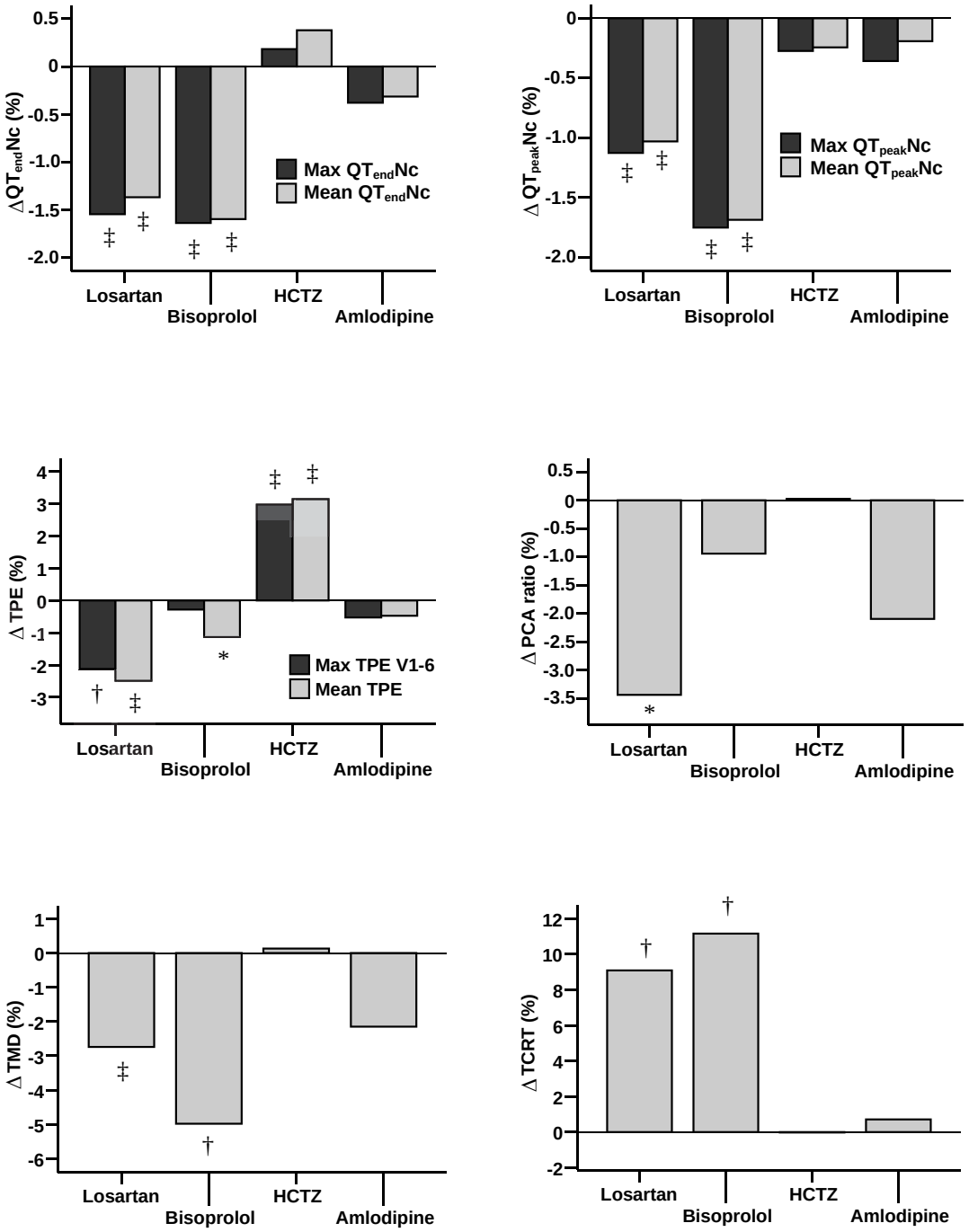

Figure 7 Percentage changes in repolarization measures after 4-week drug treatment in study II. *P* values (drug compared to placebo) as in Table 4. **P*<0.05, †*P*<0.01, ‡*P*<0.001. HCTZ=hydrochlorothiazide, Max=maximum, Nc=nomogram-corrected for heart rate, PCA=principal component analysis, QT_{end}=QT interval to the T-wave end, QT_{peak}=QT interval to the T-wave peak, TCRT=total cosine R-to-T, TMD=T-wave morphology dispersion, TPE=T-wave peak to T-wave end interval. Reproduced with permission of Informa Medical and Pharmaceutical Science - journals, from Annals of Medicine, Short-term electrophysiological effects of losartan, bisoprolol, amlodipine, and hydrochlorothiazide in hypertensive men, Porthan et al. 2009a, Volume 41, Pages 29–37, Copyright 2009; Permission conveyed through Copyright Clearance Center, Inc.

with shortening of QT_{end} intervals (r from 0.14 to 0.16, P<0.001) and TPE intervals (r from 0.17 to 0.28, P<0.001). A decrease in serum potassium level was associated with prolongation of QT intervals (r from –0.12 to –0.27, P from 0.020 to <0.001) and TPE intervals (r from –0.28 to –0.37, P<0.001). In multivariate analyses, a decrease in systolic blood pressure and QRS area sum as well as an increase in serum potassium level remained weak but significant independent predictors of the shortening of QT_{end} and TPE intervals. This was also obseved when the potential effects of baseline echocardiographic LVMI and placebo systolic blood pressure on these repolarization parameters were adjusted.

5.3 Relationship of common gene variants to repolarization parameters (studies III and IV)

In the population cohort of 5043 subjects (46.6% men) in study III, mean age was 51.4±14.1 years. Table 5 shows the relationship between SNPs and the QT_{end} interval. The *KCNE1* D85N minor allele was associated with a 10.5-ms prolongation of the age-, gender-, and heart rate-adjusted QT_{end} interval. When the study population was stratified according to the genotype, mean QT_{end}Nc interval was 393±20 ms in D85N major homozygotes (n=4684), 404±20 ms in D85N heterozygotes (n=127), and 415±23 ms in D85N minor homozygotes (n=3). Also the minor alleles of *KCNH2* rs3807375, *SCN5A* H558R, and the four strongly correlating *NOS1AP* SNPs (r^2 between 0.77 and 0.97) were associated with QT-interval prolongation. Table 5 also shows that minor alleles of *KCNH2* K897T and R1047L were associated with QT-interval shortening, although the effect was modest and the significance level was borderline for R1047L. To evaluate the impact of the four QT-interval altering SNPs of *KCNE1* D85N, *KCNH2* K897T and rs3807375, and *NOS1AP* rs2880058, a score calculating the additive effect of the SNPs was constructed for each study subject based on their genotype. Using this score, a linear association was found (P<0.00001) between the score and QT interval, with a one point increase in the score associated with a 0.89-ms prolongation of the age-, gender-, and heart rate-adjusted QT_{end} interval. For each quintile of the score, the adjusted QT_{end} interval increased by 2.4 ms (P<0.00001).

In the population cohort of 5890 subjects (45.2% men) in study IV, mean age was 52.1±14.3 years. Table 5 shows the relationship between SNPs and the TPE interval. The *KCNH2* K897T minor allele was associated with a 1.2-ms shortening and the *KCNH2* rs3807375 minor allele was associated with a 0.8-ms prolongation of TPE interval. For the four *NOS1AP* SNPs, minor alleles were associated with TPE-interval shortening, with the effect varying from –0.5 to –0.8 ms. When SNP effects on QT_{peak} and QT_{end} intervals were analysed, it was observed that, for *NOS1AP*, the association with QT_{peak} interval was stronger than with QT_{end} interval, resulting in TPE-interval shortening. For all SNPs in studies III and IV, the results for QT interval and TPE interval were tested also with the genotypic genetic models and this did not change the results.

The relationship of SNPs to T-wave morphology parameters (PCA ratio, TMD, TCRT, TWR) was analysed in study IV. The multivariate regression analyses showed that the *KCNH2* K897T minor allele was associated with a 0.03-unit decrease in normalized TWR (P=0.039), the *NOS1AP* rs4657139 minor allele was associated with a 0.04-unit decrease in normalized TCRT (P=0.042), and that the *NOS1AP* rs10494366 minor allele

Table 5 Relationship between SNPs and QT interval* in study III as well as between SNPs and TPE interval† in study IV.

		Allele‡ and respective amino acid change	Δ QT interval		Δ TPE interval	
Gene	SNP		Per minor allele (ms)	*P*	Per minor allele (ms)	*P*
KCNH2	rs1805123	A→C, K897T	–2.6	<0.00001	–1.2	0.00005
	rs3807375	G→A	+1.6	0.00005	+0.8	0.001
	rs36210421	G→T, R1047L	–1.5	0.049		
SCN5A		C→G, R190G	–0.5	0.85		
	rs1805124	A→G, H558R	+1.5	0.002		
		C→A, A572D	+0.5	0.66		
KCNE1	rs1805128	G→A, D85N	+10.5	<0.00001	–1.3	0.202
	rs1805127	G→A, G38S	+0.4	0.29		
KCNE2	rs2234916	A→G, T8A	+0.1	0.98		
NOS1AP	rs2880058	A→G	+4.0	<0.00001	–0.6	0.013
	rs4657139	T→A	+4.0	<0.00001	–0.5	0.032
	rs10918594	C→G	+3.9	<0.00001	–0.8	0.002
	rs10494366	T→G	+3.5	<0.00001	–0.6	0.018

*Mean QT_{end} interval of all leads, heart rate nomogram-correction. †Maximum TPE interval of precordial leads. ‡Major→minor allele. Using a stepwise linear regression analysis, QT interval and TPE interval were adjusted for the clinical covariates and the output residuals were saved as a variable. Age and gender were used as covariates for QT interval. Age, gender, Cornell voltage-duration product, systolic blood pressure, history of coronary heart disease, and history of previous myocardial infarction were used as covariates for TPE interval. In the second linear regression analysis, the output residual was used as the dependent and the genotype as the independent variable. Results shown are from additive models (see description in the Statistical analyses section). Data values represent differences from major homozygotes, which were used as a reference. SNP=single nucleotide polymorphism, TPE=T-wave peak to T-wave end interval.

was associated with a 0.04-unit decrease in normalized TCRT (*P*=0.034). For all other SNPs, the results were non-significant (both allelic and genotypic genetic models tested). When the three SNPs with significant results were tested with genotypic models, only the relationship between *KCNH2* K897T and TWR was found to be statistically significant (*P*=0.040).

5.4 Predictive value of repolarization parameters for mortality (study V)

Table 6 shows the clinical characteristics of the study population in study V. In this population cohort, mean follow-up time was 71±9 months (5.9±0.8 years). Of the 5917 study subjects, 335 (5.7%) died during the follow-up (9.6 deaths per 1000 person years of follow-up). In men and women, all-cause mortality was 6.5% and 5.0% (*P*=0.015), and cardiovascular mortality was 2.8% and 1.7% (*P*=0.006), respectively. Of all 335 deaths, 131 (39%) were cardiovascular deaths.

Table 6 Clinical characteristics of the study population in study V.

	Men (n=2674)	Women (n=3243)
Age (years)	50.9±13.3	53.1±14.9
Current smoking (%)	27.8	17.2
Body mass index (kg/m²)	27.1±4.1	26.7±5.1
Total cholesterol/HDL ratio	5.3±1.8	4.3±1.4
ECG LVH (%)	20.2	15.7
Systolic blood pressure (mmHg)	135.4±18.8	133.3±22.5
Diastolic blood pressure (mmHg)	84.4±10.7	79.8±10.8
Hypertension (%)	50.0	44.2
Diabetes mellitus (%)	6.8	4.6
Coronary heart disease (%)	7.6	5.7
Previous myocardial infarction (%)	3.4	1.4

Values are expressed as mean±standard deviation for continuous variables and percentages for categoric variables. For all variables, differences between the genders were statistically significant (*P*<0.05). ECG=electrocardiographic, HDL=high density lipoprotein, LVH=left ventricular hypertrophy.

Table 7 shows the ECG repolarization parameters for men and women according to survival status. Study subjects who died had longer ECG repolarization duration (QT interval) and higher degree of ECG repolarization heterogeneity (TWR) than those who survived. In addition, those who died had further changes in T-wave morphology (higher values of PCA ratio and TMD, lower values of TCRT), except that in men the differences were statistically non-significant for TCRT (all-cause and cardiovascular mortality) and TWR (all-cause mortality). In univariate Cox analyses, QT interval and with a few exceptions all T-wave morphology parameters were significant univariate mortality predictors in both genders. The only non-significant univariate predictors were TCRT in men (all-cause and cardiovascular mortality) and TWR in men (all-cause mortality). Kaplan-Meier curves for TMD in men and TWR in women are shown in Figures 8 and 9, respectively.

Table 7 ECG repolarization parameters in men and women according to survival status in study V.

	All-cause mortality			Cardiovascular mortality		
	Alive (Men: n=2501, Women: n=3081)	Dead (Men: n=173, Women: n=162)	*P*	Alive or non-cardiovascular death (Men: n=2599, Women: n=3187)	Dead (Men: n=75, Women: n=56)	*P*
QT interval* (ms)						
Men	400±22	410±22	<0.0001	400±22	415±25	<0.0001
Women	412±21	420±23	<0.0001	412±21	425±23	<0.0001
PCA ratio (%)						
Men	13.3±6.8	17.9±9.9	<0.0001	13.5±7.0	19.4±10.5	<0.0001
Women	16.9±8.7	20.9±12.2	<0.0001	17.0±8.7	23.7±16.1	0.0002
TMD (°)						
Men	13.0±12.3	21.4±18.1	<0.0001	13.2±12.5	25.4±20.1	<0.0001
Women	15.0±13.1	23.6±20.5	<0.0001	15.2±13.3	28.4±23.8	<0.0001
TCRT (unitless)						
Men	0.32±0.56	0.26±0.59	0.262	0.32±0.56	0.27±0.55	0.306
Women	0.41±0.47	0.26±0.61	0.036	0.41±0.47	0.00±0.68	<0.0001
TWR (technical units)						
Men	18083±24872	18439±15932	0.071	17998±24508	21871±19714	0.002
Women	15505±21379	19484±16168	<0.0001	15583±21192	22539±18456	<0.0001

Values are expressed as mean±standard deviation. *Maximum QT_{end} interval of all leads, heart rate nomogram-correction. ECG=electrocardiographic, PCA=principal component analysis, TCRT=total cosine R-to-T, TMD=T-wave morphology dispersion, TWR=T-wave residuum. Reprinted from Heart Rhythm, Volume 6, Porthan et al. 2009b, Predictive value of electrocardiographic QT interval and T-wave morphology parameters for all-cause and cardiovascular mortality in a general population sample, Pages 1202–8, Copyright 2009, with permission from Elsevier.

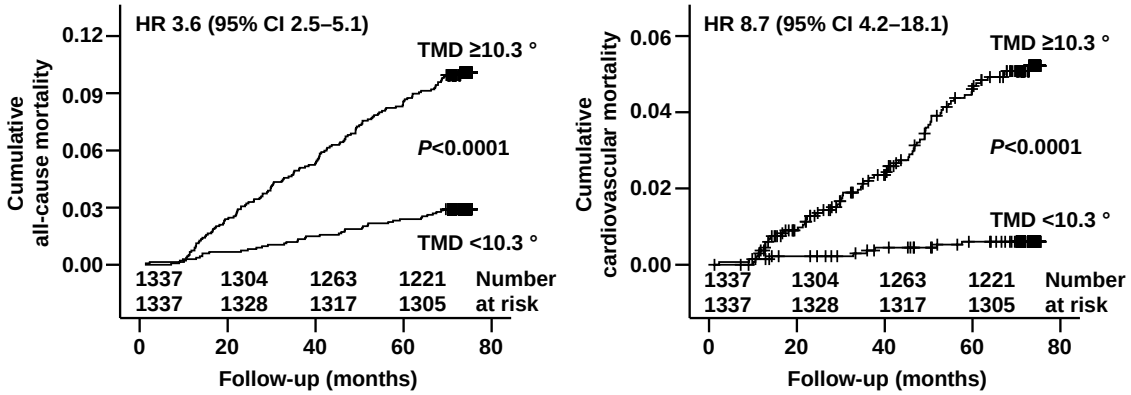

Figure 8 Kaplan-Meier curves (with log-rank tests) for TMD in men. Groups are stratified by above and below median values. Hazard ratios (HR) with 95% confidence intervals (CI) are also shown. Plus symbols indicate censoring times: a study subject has experienced non-cardiovascular death (in the panel showing cardiovascular mortality), or a study subject has not died by the end of the follow-up (both panels). TMD=T-wave morphology dispersion. Reprinted from Heart Rhythm, Volume 6, Porthan et al. 2009b, Predictive value of electrocardiographic QT interval and T-wave morphology parameters for all-cause and cardiovascular mortality in a general population sample, Pages 1202–8, Copyright 2009, with permission from Elsevier.

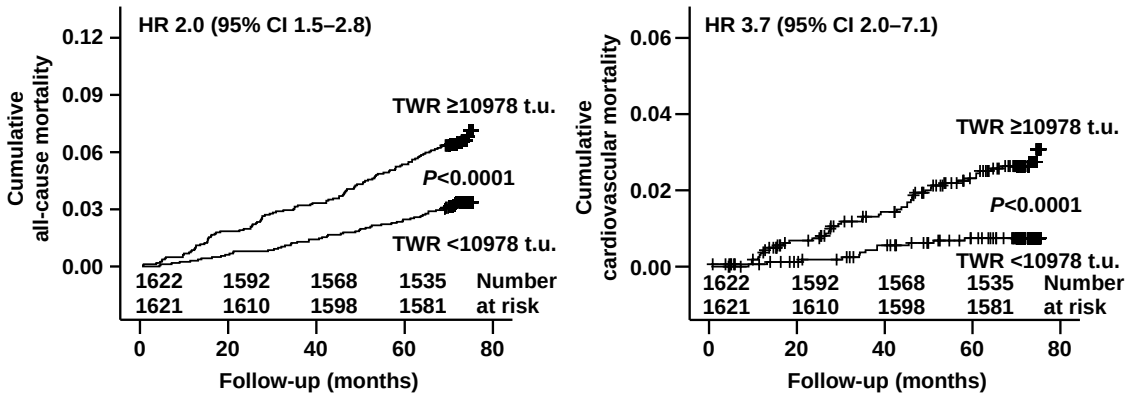

Figure 9 Kaplan-Meier curves (with log-rank tests) for TWR in women. Groups are stratified by above and below median values. Hazard ratios (HR) with 95% confidence intervals (CI) are also shown. Plus symbols indicate censoring times: a study subject has experienced non-cardiovascular death (in the panel showing cardiovascular mortality), or a study subject has not died by the end of the follow-up (both panels). TWR=T-wave residuum, t.u.=technical units. Reprinted from Heart Rhythm, Volume 6, Porthan et al. 2009b, Predictive value of electrocardiographic QT interval and T-wave morphology parameters for all-cause and cardiovascular mortality in a general population sample, Pages 1202–8, Copyright 2009, with permission from Elsevier.

Table 8 shows mortality hazard ratios (HRs) from multivariate Cox models for ECG repolarization parameters. After adjusting for covariates in men, each 1-SD increase in $\log_e$ TMD was associated with a 1.3-fold [95% confidence interval (CI) 1.1–1.6] risk of cardiovascular death, and TMD above the median value was associated with a 4.4-fold (95% CI 2.1–9.4) risk of cardiovascular death when compared to TMD below the median value. After adjusting for covariates in women, each 1-SD increase in $\log_e$ TWR was associated with a 1.3-fold (95% CI 1.01–1.6) risk of cardiovascular death, and TWR above the median value was associated with a 2.2-fold (95% CI 1.1–4.2) risk of cardiovascular death when compared to TWR below the median value. The QT interval and TCRT in men, as well as QT interval, PCA ratio, and TMD in women did not remain as independent mortality predictors. Multivariate Cox models using non-cardiovascular mortality (excluding cardiovascular deaths from all-cause mortality) as the dependent variable were also performed, and it was found that QT interval and T-wave morphology parameters were not independent predictors of non-cardiovascular mortality.

In comparative respective univariate Cox models using the QT interval with Bazett's formula adjustment for heart rate, the QTc interval was a significant univariate predictor of all-cause and cardiovascular mortality in both genders, with longer QTc interval indicating higher risk. In multivariate Cox models in men, the QTc interval was an independent predictor of all-cause mortality, with each 1-SD increase in the QTc interval associated with a 1.2-fold (95% CI 1.02–1.4, P=0.030) risk of all-cause death. In contrast, the QTc interval was not an independent predictor of cardiovascular mortality in men (P from 0.087 to 0.219). In women, the QTc interval did not remain as an independent mortality predictor in multivariate Cox models (P from 0.116 to 0.500). In both genders, the TPE interval was not a significant mortality predictor in univariate or multivariate Cox models (multivariate Cox model P from 0.404 to 0.992).

Table 8 Mortality hazard ratios from the multivariate Cox proportional hazards models for ECG repolarization parameters in men and women in study V.

	All-cause mortality				Cardiovascular mortality			
	Continuous variables*		Median-dichotomized variables†		Continuous variables*		Median-dichotomized variables†	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Men								
QT interval‡	1.02 (0.9–1.2)	0.782	1.01 (0.7–1.4)	0.953	1.1 (0.9–1.3)	0.549	1.2 (0.7–2.2)	0.449
PCA ratio	1.2 (1.1–1.4)	0.007	1.4 (1.02–2.0)	0.037	1.2 (1.02–1.5)	0.034	2.3 (1.2–4.1)	0.008
TMD	1.1 (1.0–1.3)	0.055	2.1 (1.4–3.0)	0.0001	1.3 (1.1–1.6)	0.014	4.4 (2.1–9.4)	0.0001
TCRT	0.97 (0.8–1.1)	0.625	0.8 (0.6–1.1)	0.272	0.97 (0.8–1.2)	0.798	0.9 (0.6–1.4)	0.584
TWR	1.05 (0.9–1.2)	0.525	1.1 (0.8–1.5)	0.655	1.2 (1.01–1.5)	0.044	1.5 (0.9–2.4)	0.120
Women								
QT interval‡	1.03 (0.9–1.2)	0.732	1.1 (0.8–1.6)	0.505	1.2 (0.9–1.6)	0.138	1.4 (0.8–2.6)	0.261
PCA ratio	0.98 (0.8–1.1)	0.767	1.03 (0.7–1.4)	0.857	1.1 (0.9–1.4)	0.424	1.1 (0.6–2.0)	0.675
TMD	1.1 (0.9–1.3)	0.254	1.3 (0.9–1.8)	0.143	1.1 (0.9–1.4)	0.367	1.4 (0.7–2.6)	0.297
TCRT	0.9 (0.8–1.1)	0.270	0.9 (0.7–1.3)	0.708	0.7 (0.5–0.9)	0.006	0.6 (0.3–1.2)	0.131
TWR	1.2 (1.01–1.3)	0.039	1.4 (0.97–1.9)	0.070	1.3 (1.01–1.6)	0.040	2.2 (1.1–4.2)	0.018

*Hazard ratio was calculated per standard deviation increment in the repolarization parameter, and variable distributions were normalized with $\log_e$ transformation (PCA ratio, TMD, TWR) or with the Blom method (TCRT). †Hazard ratio was calculated as the risk in the above-median group compared to the below-median group. ‡Maximum QT_{end} interval of all leads, heart rate nomogram-correction. Significant clinical predictors from univariate Cox models were entered into each multivariate model as covariates if significant univariate correlation existed (together with one repolarization measure at a time). In men, significant predictors of all-cause and cardiovascular mortality were age, current smoking (all-cause mortality only), systolic blood pressure, diastolic blood pressure (all-cause mortality only), hypertension, coronary heart disease, myocardial infarction, diabetes mellitus, and ECG LVH (cardiovascular mortality only). Respectively in women, significant predictors were age, body mass index (all-cause mortality only), systolic blood pressure, total cholesterol/high density lipoprotein ratio, hypertension, coronary heart disease, myocardial infarction, diabetes mellitus, and ECG LVH. CI=confidence interval, ECG=electrocardiographic, HR=hazard ratio, LVH=left ventricular hypertrophy, PCA=principal component analysis, TCRT=total cosine R-to-T, TMD=T-wave morphology dispersion, TWR=T-wave residuum.

6 DISCUSSION

6.1 Left ventricular mass as a repolarization modifier (study I)

Epidemiological evidence demonstrates that LVH is an independent risk factor for cardiovascular morbidity and mortality (Levy et al. 1990, Vakili et al. 2001, Bombelli et al. 2009) as well as for sudden cardiac death (Haider et al. 1998). Furthermore, experimental studies imply that marked LVH is associated with arrhythmogenic structural and electric myocardial changes (Kowey et al. 1991, Ben-David et al. 1992, Yan et al. 2001, Kozhevnikov et al. 2002, Jin et al. 2010). These epidemiological and experimental findings together strongly indicate that in advanced LVH, sudden cardiac death may be mediated, at least in part, by arrhythmogenic repolarization mechanisms. Based on both experimental and simulation studies, repolarization changes at the cellular level in marked LVH are also expected to prolong the QT interval and change T-wave morphology in body surface ECG (Zabel et al. 1995, Yan et al. 1998, Bacharova et al. 2010). In agreement with this, QT-interval prolongation and T-wave morphology changes have been observed in patients with high LVMI values (Oikarinen et al. 2001a, 2002).

In the general population, subjects with marked LVH form a group with high relative cardiovascular risk. However, an even larger group is formed by mildly or moderately hypertensive individuals with minor structural remodelling, which nonetheless is also associated with increased risk (Schillaci et al. 2000). Consequently, the significance of minor structural remodelling and mild LVH at the population level may be high, although the risk increase in individuals may be relatively low. Of note, in a hypertensive population, the absolute number of subjects developing new-onset ischemic heart disease was higher in subjects with minor, non-specific ECG repolarization changes than in those with ECG repolarization strain pattern (Schillaci et al. 2004).

ECG repolarization changes were assessed in study I and were seen to be already detectable in hypertensive men with minor LV structural changes. All ECG repolarization parameters were significantly different in LVH subjects compared to non-LVH subjects when using a dichotomous echocardiographic LVH cutoff value. In addition, when using continuous variables, LVMI correlated linearly with all repolarization parameters despite the relatively narrow LVMI range. The independent relationship of LVMI to QT_{end} interval, TPE intervals, and T-wave morphology parameters was verified with multivariate analyses.

Study I suggests that in subjects with hypertensive mild LVH, a reduced repolarization reserve and an increased ventricular repolarization heterogeneity may already be present. This may indicate that, in hypertension, the correlation between mild LVM increase and increased cardiovascular risk (Schillaci et al. 2000) may in part be explained by unfavourable repolarization changes. These repolarization changes are probably not directly arrhythmogenic, however they may modify arrhythmia vulnerability and become significant in conjuction with other factors affecting repolarization unfavourably, for example during ischemia, electrolyte imbalances, or use of QT-interval prolonging drugs. Mechanisms of the changes observed in T-wave morphology parameters may only be speculated, but they may be related to alterations in ion channel function, intercellular uncoupling, or unidentified subtle cardiac structural changes.

6.2 Antihypertensive drugs as repolarization modifiers (study II)

In study II, an angiotensin II blocker losartan and a ß-blocker bisoprolol showed beneficial effects on ECG repolarization. A thiazide diuretic hydrochlorothiazide prolonged the TPE interval, potentially reflecting an adverse repolarization effect, whilst the effects of calcium-channel blocker amlodipine on ECG repolarization were nonsignificant. There are no previous studies on the short-term repolarization effects of these drugs.

Activation of the RAAS plays a central role in the adverse outcomes of hypertension including LVH, myocardial fibrosis, and heart failure (Weber and Brilla 1991, Kim and Iwao 2000, Mehta and Griendling 2007, Tomaschitz et al. 2010). Angiotensin II and aldosterone exert several potentially direct proarrhythmic mechanisms, such as promoting myocardial fibrosis, hypertrophy, and ischemia; increasing intracellular calcium concentration; causing depletion of potassium and magnesium; increasing the activity of the sympathetic nervous system; and modulating voltage-dependent potassium channels (Delpón et al. 2005). Experimentally, drugs that inhibit the RAAS have shown potentially rapid antiarrhythmic actions by blunting these mechanisms, and losartan with its metabolite also have direct effects on cardiac potassium channels (Caballero et al. 2000, Delpón et al. 2005). Previous clinical studies have found that long-term antihypertensive treatment, particularly with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, is associated with beneficial changes in ventricular repolarization (González-Juanatey et al. 1998, Malmqvist et al. 2002). Antihypertensive drugs inhibiting RAAS may also be more efficient in LVH regression compared to other drugs (Ruilope and Schmieder 2008). Thus, the beneficial repolarization changes in the previous studies are probably in part explained by LV structural reverse remodelling (Rials et al. 1998, Rials et al. 2001). In study II, however, the favourable repolarization changes with losartan treatment occurred rapidly. The magnitude of blood pressure reduction was associated weakly and only with some of the studied ECG repolarization measures, suggesting that repolarization changes were only partly explained by blood pressure reduction. The results suggest that in hypertension, losartan may also have direct clinically beneficial modifying effects on ventricular repolarization that are likely to be independent of structural reverse remodelling.

ß-blockers are generally considered to be antiarrhythmic because they decrease the incidence of presumably arrhythmic sudden cardiac death, for example in congestive heart failure and congenital LQTS (Huikuri et al. 2001, Zicha et al. 2006). ß-blockers are particularly effective in preventing arrhythmias in LQTS type 1 (Priori et al. 2004), and in these patients use of ß-blockers is associated with beneficial ECG repolarization effects (Viitasalo et al. 2006). In addition to preventing arrhythmia-promoting actions of ß-adrenergic stimulation, ß-blockers may also decrease RAAS activity (Blumenfeld et al. 1999). Study II indicates that, also in hypertensive patients, bisoprolol may have short-term protective repolarization effects which are not dependent on LV reverse remodelling.

Hydrochlorothiazide treatment was associated with an increase in QRS area sum, which was used as an estimate for changes in LVM and has performed better than standard ECG criteria in LVH detection (Okin et al. 1998, Oikarinen et al. 2004a). Although blood pressure decreased with all treatments, QRS area sum increased during hydrochlorothiazide and decreased during losartan, whereas bisoprolol and amlodipine had no significant effects, suggesting that direct blood pressure-dependent effects cannot explain this finding. In addition, no changes in QRS duration were observed,

suggesting that none of the drugs influenced ventricular conduction. QRS amplitudes are determined by LVM, but also by intracardiac blood volume, distance of the heart from the chest wall, and the relation between LV wall thickness and chamber dilation (Surawicz 1986). The short-term increase in QRS area sum with hydrochlorothiazide was surprising because significant short-term LVM changes were not expected. This finding may reflect volume changes (Surawicz 1986). However, since losartan antagonizes and hydrochlorothiazide activates the RAAS (Burnier and Brunner 1992, Mehta and Griendling 2007), whilst angiotensin II has cardiotrophic effects, it is possible that the observed changes in QRS area sum may also reflect true changes in electrically active LVM. Hydrochlorothiazide treatment was also associated with a decrease in serum potassium, which in turn was independently associated with TPE-interval prolongation. Hypokalemia prolongs QT interval and is a well-known risk factor for arrhythmias (Roden 2006). Additionally, in epidemiological studies, relatively short-term hydrochlorothiazide treatment, with much higher drug doses, has been associated with an increased incidence of sudden cardiac death in hypertensive patients (Siscovick et al. 1994, Hoes et al. 1995). It is suggested in the present study that in addition to RAAS activation, the hypokalemic effect that increases ventricular repolarization heterogeneity may partly mediate the increased risk of sudden death with high-dose hydrochlorothiazide treatment. Thus, a potential mechanism whereby high dose of hydrochlorothiazide causes TPE-interval prolongation may partly explain the previous epidemiological observations. However, increased incidence of sudden death due to low-dose hydrochlorothiazide therapy has not been reported. In addition, although the observed TPE-interval prolongation during hydrochlorothiazide is potentially an unfavourable modifying effect, the clinical significance of prolonged TPE interval needs to be studied further.

6.3 Common gene variants as repolarization modifiers (studies III and IV)

6.3.1 *KCNH2* K897T and rs3807375

Experimental studies indicate that the *KCNH2* K897T substitution may have multiple effects on cellular electrophysiology (Bezzina et al. 2003, Paavonen et al. 2003, Anson et al. 2004, Gentile et al. 2008), suggesting that predicting the net effect of this SNP on ECG ventricular repolarisation may prove difficult. In several previous population studies, K897T has been shown to slightly shorten the ECG QT interval (Bezzina et al. 2003, Gouas et al. 2005, Pfeufer et al. 2005, Newton-Cheh et al. 2007), but also neutral (Raitakari et al. 2009) and opposite associations have been reported (Pietilä et al. 2002, Koskela et al. 2008). In a few reports with small sample sizes in LQTS patients, both protective and harmful impacts have been suggested for the minor variant 897T (Laitinen et al. 2000, Crotti et al. 2005, Zhang et al. 2008). In two recent studies (Newton-Cheh et al. 2009, Pfeufer et al. 2009), K897T was not among the genome-wide significant variants associated with QT interval. However, K897T is strongly correlated with two *KCNH2* variants (rs2968863 and rs2968864) which were associated with QT-interval shortening (Newton-Cheh et al. 2009, Pfeufer et al. 2009). Recently, it has been suggested that the effect of K897T may vary, depending on whether it is inherited *in cis* or *in trans* with the disease-causing allele (Hedley et al. 2009). In line with the majority of the previous studies, it was shown in study III that the 897T variant is associated with

QT-interval shortening in the general population. In study IV, this variant also shortened the TPE interval but did not consistently alter T-wave morphology parameters.

There are no experimental studies that characterize the functional properties of *KCNH2* intronic SNP rs3807375 in cardiac repolarization. Previously, the minor allele of this variant has been reported to prolong QT interval in a general population sample (Newton-Cheh et al. 2007). In line with this, the minor allele of rs3807375 was shown to be associated with QT-interval prolongation in study III. However, results from recent genome-wide studies suggest that this association may not be independent of other SNPs (Newton-Cheh et al. 2009, Pfeufer et al. 2009). In study IV, the minor allele of rs3807375 had no effects on T-wave morphology parameters but prolonged the TPE interval. In LQTS type 2, which is caused by mutation of *KCNH2* resulting in reduced I_{Kr}, the typical feature of resting ECG is the prolongation of QT and TPE intervals (Shimizu and Antzelevitch 1998, Gima and Rudy 2002, Viitasalo et al. 2002). Thus, the findings of study III and IV suggest that that the minor allele of rs3807375 may be associated with reduced I_{Kr} and with reduced repolarization reserve.

6.3.2 *KCNE1* D85N

The *KCNE1* D85N polymorphism causes variation in minK, the regulatory subunit of the ion channel mediating I_{Ks} (Lehmann-Horn and Jurkat-Rott 1999). Given previous experimental studies, the minor 85N variant most likely reduces potassium current in this channel (Westenskow et al. 2004), and a reduction also in I_{Kr} was recently reported (Nishio et al. 2009). The 85N variant may thus predispose to acquired LQTS and modify the phenotype of congenital LQTS (Nishio et al. 2009). In experimental LQTS type 1 models, I_{Ks} block prolongs action potential duration but does not increase transmural dispersion at rest (Shimizu and Antzelevitch 1998). However, transmural dispersion is increased in conjunction with ß-adrenergic stimulation (Shimizu and Antzelevitch 1998). Previous clinical studies have suggested that the 85N variant is associated with drug-induced TdP (Paulussen et al. 2004, Mank-Seymour et al. 2006) and with QT-interval prolongation in healthy individuals (Gouas et al. 2005), and is more common in LQTS patients than in healthy controls (Nishio et al. 2009). A few studies in healthy individuals did not find association between the D85N polymorphism and the QT interval (Aydin et al. 2005, Friedlander et al. 2005). Genome-wide association analysis supports the independent association between the 85N variant and QT-interval prolongation (Newton-Cheh et al. 2009). Study III, showing a substantial 10.5-ms prolongation in the QT interval for each D85N minor allele, also supports the association with QT-interval prolongation and was the first study to show the association in a large general population sample. Despite the marked QT-interval prolongation, the 85N variant had no effect on ECG TPE interval or T-wave morphology measures (study IV). In ambulatory ECG recordings from patients with LQTS type 1, the TPE interval was only prolonged significantly at high heart rates (Viitasalo et al. 2002). Thus, the findings from resting ECGs showing that the 85N variant prolongs the QT but not the TPE interval are consistent with a reduction in I_{Ks}. Whether this polymorphism is associated with TPE-interval prolongation during physical exercise remains to be studied.

6.3.3 *NOS1AP* variants

Overexpression of CAPON, which is the product of *NOS1AP* and functions as a ligand of nitric oxide synthase 1, results in up-regulation of nitric oxide synthase 1-nitric oxide signalling pathways, leading to decreased $I_{Ca,L}$ and increased I_{Kr}, and to action potential shortening in guinea pigs (Chang et al. 2008). If one postulated that genetic *NOS1AP* variants reduce CAPON levels, then increased $I_{Ca,L}$, decreased I_{Kr}, and prolonged action potential are expected (Crotti et al. 2009). However, species differences in CAPON function may occur, such as for the zebrafish where the opposite effect is observed with the loss of *NOS1AP* expression that leads to action potential shortening (Milan et al. 2009). In addition to the effects on repolarization, nitric oxide synthase has a role in a number of cardiac physiological processes including contractility and calcium cycle (Xu et al. 1999, Barouch et al. 2002, Burkard et al. 2007, Oceandy et al. 2007, Asada et al. 2009).

The minor alleles of several *NOS1AP* variants have been associated with a longer QT interval in several population studies (Arking et al. 2006, Aarnoudse et al. 2007, Post et al. 2007, Lehtinen et al. 2008, Tobin et al. 2008, Raitakari et al. 2009). Genome-wide association studies have recently reported four independent SNPs (rs12029454, rs12143842, rs16857031, rs4657178) located in or near *NOS1AP* (Newton-Cheh et al. 2009, Pfeufer et al. 2009). Furthermore, recent studies have reported the disease-modifying effect of *NOS1AP* variants in LQTS (Crotti et al. 2009, Tomás et al. 2010), and that *NOS1AP* variants associate with sudden cardiac death in the general population (Eijgelsheim et al. 2009, Kao et al. 2009). In a large population cohort of white adults (Kao et al. 2009), two variants (rs16847548, rs12567209) were associated with sudden cardiac death, but the effect was independent of QT-interval duration in multivariate analyses. The authors suggested that the QT interval might not be a sufficient measure of ventricular repolarization or the prognostic value of *NOS1AP* variants might be mediated by some other mechanisms than repolarization.

The association of minor alleles of four strongly correlating *NOS1AP* SNPs with longer QT interval was confirmed in study III. In study IV, these *NOS1AP* variants were associated with a shorter TPE interval but had no effects on T-wave morphology. Together with the QT-interval prolongation effect, TPE-interval shortening was an unexpected finding. Interestingly, the results suggest a stronger prolongation effect of *NOS1AP* SNPs on the QT_{peak} in comparison to the QT_{end} interval. Whether the TPE-interval shortening is mediated by increased $I_{Ca,L}$ or by some other mechanism remains to be studied.

6.3.4 Other gene variants

The *SCN5A* R190G and A572D have been found to be enriched in LQTS (Paulussen et al. 2003, Fodstad et al. 2004). For *KCNE1* G38S, both QT-interval prolonging (Friedlander et al. 2005) and null effects have been reported (Aydin et al. 2005, Akyol et al. 2007, Gouas et al. 2007). The *KCNE2* T8A has been found to be enriched in drug-induced LQTS (Sesti et al. 2000, Paulussen et al. 2004), and an association with QT-interval prolongation was observed in healthy individuals (Aydin et al. 2005). However, no association with the QT interval was observed in a larger general population sample (Pfeufer et al. 2005). For all these four SNPs, the association with QT interval was non-significant in study III, and thus these variants seem not to modify resting ECG

repolarization duration in Finns. In *in vitro* studies, *KCNH2* R1047L has been reported to impair I_{Kr} density (Chevalier et al. 2007), but on the other hand, Anson et al. (2004) found no functional differences between R1047L and wild type *KCNH2* channel. Sun et al. (2004) reported *KCNH2* R1047L to be associated with drug-induced LQTS. In study III, R1047L was associated with a slight QT-interval shortening. However, the statistical significance was borderline (*P*=0.049), and it seems unlikely that this SNP is a true modifier of QT interval in this population. In agreement with the results of study III, genome-wide association was not found between R190G, A572D, G38S, T8A, and R1047L, and the QT interval in individuals of European ancestry (Newton-Cheh et al. 2009, Pfeufer et al. 2009).

The *SCN5A* gene is associated with various types of cardiac diseases (Hedley et al. 2009), and the *SCN5A* H558R polymorphism has also been associated with an extensive list of cardiac abnormalities. The H558R has disease-modifying effects in *in vitro* models of a conduction disease (Viswanathan et al. 2003), LQTS type 3 (Ye et al. 2003), and sick sinus syndrome (Gui et al. 2010). Clinically, H558R has been found to be a genetic modifier in Brugada syndrome (Lizotte et al. 2009), complete atrioventricular block (Chevalier et al. 2007), and to be associated with early onset atrial fibrillation (Chen et al. 2007). The 558R variant prolonged the QT interval in healthy twins (Aydin et al. 2005) and in a general population sample (Gouas et al. 2005). Recent studies did not find significant genome-wide association between H558R and the QT interval (Newton-Cheh et al. 2009, Pfeufer et al. 2009). In study III, a modest QT-interval prolongation for this SNP with a *P* value of 0.002 was seen. However, considering the number of previous population studies and the number of SNPs tested, the evidence of the association between the H558R polymorphism and the QT interval in the general population was considered to be unresolved in study III.

6.3.5 Impact of the findings in common gene variants

Study III confirmed several common gene variants to be associated with the duration of QT interval. Consistent with previous studies, the association was relatively modest for most of the SNPs, and these SNPs are unlikely to play a major role in arrhythmogenesis in individuals. However, even a small increase in individual risk may eventually contribute significantly to the absolute number of arrhythmias at the population level. The association for *KCNE1* D85N was markedly stronger and possibly indicates that carriers of 85N variant(s) may be more susceptible to repolarization-related arrhythmias than non-carriers.

The SNPs showing a significant association with QT interval in study III were, for the first time in a large study population, further analysed in study IV for a possible association with the TPE interval and T-wave morphology parameters. The minor allele of *KCNH2* K897T shortened, and the minor allele of *KCNH2* rs3807375 prolonged, both the QT and TPE intervals. Thus, the results indicate that these SNPs have a modifying effect on I_{Kr}. Consistent with a reduction in I_{Ks}, *KCNE1* D85N was not associated with the TPE interval. None of the studied SNPs showed reliable association with ECG T-wave morphology parameters, suggesting that these polymorphisms do not mediate the observed prognostic value of T-wave morphology parameters in the general population (study V). Also, neither the TPE interval nor the reported T-wave morphology parameters seem to clarify the previously observed association between *NOS1AP* variants and sudden cardiac death (Eijgelsheim et al. 2009, Kao et al. 2009).

6.4 Repolarization parameters as mortality predictors (study V)

The QT interval is a measure of repolarization duration and is clinically used to detect short QT syndrome and LQTS, and to evaluate disease- and drug-related repolarization effects. The majority of previous epidemiological studies in the general population suggest that QT-interval prolongation may be, at the most, a weak risk factor for mortality (Montanez et al. 2004). The observed prognostic value in the previous studies may be in part affected by study methods, for example different heart rate-adjustment formulas. In the present study, QT intervals were adjusted for heart rate with gender-specific nomograms derived from the same study population, and a valid QT-interval measurement technique was used. With this robust methodology, rate-adjusted QT interval was a significant univariate predictor of mortality, but when adjusted for other clinical predictors, the QT interval did not contain independent prognostic value. To compare these results to previous epidemiological studies, analyses using Bazett-corrected QT interval were also performed, and the QTc interval remained only in men as a weak independent predictor of all-cause mortality. Thus, the results suggest that in the general population, QT-interval prolongation may be considered as a surrogate for, and no more informative than, the other clinical risk factors.

As the QT interval measures only temporal variation in repolarization duration, it may not detect changes in T-wave morphology. In contrast, T-wave morphology parameters measure temporospatial changes of the ventricular repolarization. As ventricular repolarization, T-wave morphology, and susceptibility to arrhythmias are interrelated (Burgess 1979, Kuo et al. 1983), non-invasive quantification of changes in T-wave morphology could be useful in risk evaluation. Several studies have shown the potential prognostic value of T-wave morphology parameters in various patient groups including patients with cardiovascular diseases (Zabel et al. 2002), myocardial infarction (Zabel et al. 2000, Batchvarov et al. 2004, Malik et al. 2004, Perkiömäki et al. 2006), systolic heart failure (Huang et al. 2009), diabetes mellitus (Okin et al. 2004b), and end-stage renal disease (Lin et al. 2007). The prognostic value of these parameters in the general population has also been reported in a few previous epidemiological cohort studies, performed in US Indians (Okin et al. 2002), elderly Dutch inhabitants, and postmenopausal US women (Rautaharju et al. 2006a,b).

Study V extends the previous findings on T-wave morphology to a large, mainly Caucasian representation of the Finnish population aged 30 years and over. Concerning the T-wave morphology parameters used in the present study, study V is at present the largest on their predictive value in the general population. As a methodological strength, all analyses were performed separately for both genders, and the results suggest that T-wave morphology parameters have gender differences in their numerical values. With a few exceptions all T-wave morphology parameters were significant mortality predictors in univariate analyses in both genders. However, when other significant clinical predictors were used as covariates in multivariate analyses, PCA ratio and TMD were the only significant independent predictors of both all-cause and cardiovascular mortality in men. In contrast in women, the PCA ratio and TMD lost their prognostic value in multivariate analyses, and the independent mortality predictors were TCRT (cardiovascular mortality) and TWR (all-cause and cardiovascular mortality). Thus, study V suggests that T-wave morphology parameters have gender-related differences also in their predictive value, implicating that in risk evaluation these measures should probably be assessed separately in men and women. Also an important finding of study V was that T-wave morphology parameters were not independent predictors of non-

cardiovascular mortality, indicating that these measures are specifically related to cardiovascular mortality.

6.5 Study strengths and limitations

Study strengths. *Studies I and II:* Comorbidities were thoroughly screened and excluded. Consequently, the study population represented subjects with essential hypertension and without other diseases which potentially could have modified repolarization measures. Echocardiograms were performed by a single, experienced cardiologist, and the recordings were analysed by a single observer in a blinded fashion. Study protocol with cross-over design and intervening placebo periods was suitable to minimize both the subject-specific drug responses and the potential carryover effects from previous antihypertensive treatment.

Studies III–V: Study population was large and is representative of the entire Finnish adult population. The clinical and genetic data were collected prospectively and systematically.

Studies I–V: ECGs were high-quality digital recordings and all leads were recorded simultaneously. ECG measurements were performed in a blinded fashion with validated measurement techniques by a single observer. The intraobserver variability of the measurements was low.

Study limitations. *Studies I and II:* The studies included Caucasian male study subjects, and the results may not be applicable to women or other races. LVM was determined using echocardiography, which has limitations compared to other techniques, such as magnetic resonance imaging. The prevalence of LVH was low, and we cannot exclude the possibility that previous antihypertensive treatments may have caused reverse structural remodelling without reverse electric remodelling. Study II does not provide information on the repolarization effects of antihypertensive drug combinations or repolarization effects of long-term treatment, and the results are directly applicable only to the drugs and doses used. The antihypertensive drug doses used in study II did not lower blood pressure equally, and a decrease in systolic blood pressure may directly affect ventricular repolarization favourably by stretch-mediated mechanisms (Eckardt et al. 2001). On the other hand, blood pressure change was associated weakly and only with some of the studied ECG repolarization measures.

Studies III–V: Study subjects in these studies were derived from the Finnish general population, which consists almost totally of Caucasians. The distribution of studied SNPs may differ between populations. Hence, conclusions drawn from these studies may not be applicable to other races or populations with different genetic backgrounds. Our analyses included four strongly correlating *NOS1AP* SNPs, and some other SNPs not examined in the present study may have different repolarization effects and prognostic value.

Studies I–V: These studies used ECG surrogate markers of arrhythmia vulnerability and do not provide direct arrhythmia data. The exact electrophysiological basis of T-wave morphology parameters is currently unknown.

7 CONCLUSIONS

Mild LVM increase in essential hypertension is associated with potentially adverse repolarization changes. This finding may carry risk implications at the population level for the large number of hypertensive patients.

Antihypertensive drugs have divergent short-term ECG repolarization effects. Losartan and bisoprolol have beneficial effects on the QT interval, TPE interval, and T-wave morphology parameters. Hydrochlorothiazide may have unfavourable effects on the TPE interval, and the effects of amlodipine on ECG repolarization seem to be neutral. Therefore, ventricular repolarization may be modified by the type of antihypertensive drug also in the beginning of the therapy, before LVH regression with potentially beneficial repolarization reverse remodelling of long-term treatment occurs.

The *KCNE1* D85N SNP is associated with a marked QT-interval prolongation in the Finnish general population. This variant may potentially independently increase susceptibility to repolarization-related arrhythmias. In addition, *KCNH2* K897T and rs3807375, as well as the studied *NOS1AP* variants are associated with modest alterations in the durations of QT and TPE intervals, confirming their roles as mild repolarization modulators. In contrast, the studied SNPs seem not to be consistently associated with T-wave morphology parameters, suggesting that these polymorphisms do not mediate the prognostic value of T-wave morphology parameters.

In the general population, when other risk factors of mortality are taken into account, T-wave morphology parameters, but not the QT interval or TPE interval, provide independent and thus potentially clinically useful risk assessment data. The independent prognostic value of these parameters seems to be gender-specific and is specifically related to cardiovascular mortality.

ACKNOWLEDGEMENTS

This thesis is based on the studies carried out at the Division of Cardiology, Department of Medicine, Helsinki University Central Hospital. In part, the studies were carried out in the following collaborative institutions in Finland: Biomedicum Helsinki; Department of Biomedical Engineering and Computational Science, Aalto University; Department of Medicine, Helsinki University Central Hospital; Finnish Genome Center; National Institute for Health and Welfare. The studies were also partly carried out in collaboration with Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA, and with Massachusetts General Hospital, Boston, Massachusetts, USA.

I am very grateful to Professor Markku S. Nieminen, former head of the Division of Cardiology and the current head of the Department of Medicine, for providing the research facilities and for the possibility to start the project. I also express my thanks to Professor Markku Kupari, current head of the Division of Cardiology, for providing the research facilities. My sincere thanks belong to Professors Kimmo Kontula and Veikko Salomaa for providing study material and for giving precious comments on the manuscripts.

I express my deepest gratitude to my main supervisor, Docent Lasse Oikarinen. He inspired me to begin this study, and he had a clear vision of the whole project already at the very beginning of the project. He was always ready to give his support and time for the project, and his deep knowledge of the study field and thorough guidance was invaluable in performing the analyses and completing the studies.

I am also deeply grateful to my second supervisor, Docent Matti Viitasalo. He gave patient and friendly advice and support over the years. His wide clinical and scientific experience was very useful for the project, and he gave very valuable advice in preparing the manuscripts.

I express my thanks to Professor Lauri Toivonen, who was very helpful at the beginning when the study plan was constructed and also gave useful manuscript comments. I am very thankful to Juha Virolainen, MD PhD, for his experienced collaboration in the echocardiographic analyses, as well as to Docent Timo P. Hiltunen, Annukka Marjamaa, MD PhD, and Cristopher Newton-Cheh, MD MPH, for their friendly and distinguished collaboration in the analyses and manuscript preparation. My warm thanks also go to Heikki Väänänen, Lic Sc (Tech), and Juhani Dabek, M Sc (Tech), for their expertise in the development of analysis software.

I wish to sincerely thank the following collaborators: Professor Aarno Palotie, Docent Antti Jula, Professor Antti Reunanen, Hannu Karanko, MD, Docent Heikki Swan, Janne Rapola, MD PhD, Professor Leena Peltonen-Palotie (†), Päivi Lahermo, PhD, Timo Suonsyrjä, MD, Tuula Hannila-Handelberg, MD PhD.

I express warm thanks to Docent Antti Hedman and Docent Kari Ylitalo for their expertise in reviewing this thesis and their constructive comments. Doctor Jennifer Rowland is acknowledged for editing the language of the thesis. I owe gratitude to all patients and volunteers who participated in the studies.

Finally, I thank my dear wife Piia for her patience during the project. I thank our lovely children Laura, Fiona, and Linda, who all spent their first years of childhood during this project. The time spent together with the family has provided a refreshing counterbalance for the research work.

This work was supported financially by the Aarne and Aili Turunen Foundation, the Aarne Koskelo Foundation, the Finnish Foundation for Cardiovascular Research, the

Finnish Medical Foundation, the Ida Montin Foundation, the Maud Kuistila Memorial Foundation, the Orion-Farmos Research Foundation, and the Paavo and Eila Salonen Foundation. I warmly thank them for their support.

Helsinki, March 2011

LIST OF REFERENCES

Aarnoudse AJ, Newton-Cheh C, de Bakker PI, Straus SM, Kors JA, Hofman A, Uitterlinden AG, Witteman JC, Stricker BH. Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. Circulation 2007;116:10–6.

Abildskov JA, Burgess MJ, Urie PM, Lux RL, Wyatt RF. The unidentified information content of the electrocardiogram. Circ Res 1977;40:3–7.

Acar B, Yi G, Hnatkova K, Malik M. Spatial, temporal and wavefront direction characteristics of 12-lead T-wave morphology. Med Biol Eng Comput 1999;37:574–84.

Ackerman MJ, Mohler PJ. Defining a new paradigm for human arrhythmia syndromes: phenotypic manifestations of gene mutations in ion channel- and transporter-associated proteins. Circ Res 2010;107:457–65.

Akyol M, Jalilzadeh S, Sinner MF, Perz S, Beckmann BM, Gieger C, Illig T, Wichmann HE, Meitinger T, Kääb S, Pfeufer A. The common non-synonymous variant G38S of the KCNE1-(minK)-gene is not associated to QT interval in Central European Caucasians: results from the KORA study. Eur Heart J 2007;28:305–9.

Alfakih K, Reid S, Jones T, Sivananthan M. Assessment of ventricular function and mass by cardiac magnetic resonance imaging. Eur Radiol 2004;14:1813–22.

Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J. QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. Circulation 1991;83:1888–94.

Andersen MP, Xue JQ, Graff C, Kanters JK, Toft E, Struijk JJ. New descriptors of T-wave morphology are independent of heart rate. J Electrocardiol 2008;41:557–61.

Anson BD, Ackerman MJ, Tester DJ, Will ML, Delisle BP, Anderson CL, January CT. Molecular and functional characterization of common polymorphism in HERG (KCNH2) potassium channels. Am J Physiol Heart Circ Physiol 2004;286:H2434–41.

Anttonen O, Junttila J, Giustetto C, Gaita F, Linna E, Karsikas M, Seppänen T, Perkiömäki JS, Mäkikallio TH, Brugada R, Huikuri HV. T-Wave morphology in short QT syndrome. Ann Noninvasive Electrocardiol 2009;14:262–7.

Anttonen O, Väänänen H, Junttila J, Huikuri HV, Viitasalo M. Electrocardiographic transmural dispersion of repolarization in patients with inherited short QT syndrome. Ann Noninvasive Electrocardiol 2008;13:295–300.

Antzelevitch C. Drug-induced spatial dispersion of repolarization. Cardiol J 2008;15:100–21.

Antzelevitch C. Ionic, molecular, and cellular bases of QT-interval prolongation and torsade de pointes. Europace 2007;9 Suppl 4:iv4–15.

Antzelevitch C, Fish J. Electrical heterogeneity within the ventricular wall. Basic Res Cardiol 2001;96:517–27.

Antzelevitch C, Oliva A. Amplification of spatial dispersion of repolarization underlies sudden cardiac death associated with catecholaminergic polymorphic VT, long QT, short QT and Brugada syndromes. J Intern Med 2006;259:48–58.

Antzelevitch C, Shimizu W. Cellular mechanisms underlying the Long QT syndrome. Curr Opin Cardiol 2002;17:43–51.

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;10:1124–52.

Apkon M. Cellular physiology of skeletal, cardiac, and smooth muscle. In: Boron WF, Boulpaep EL, editors. Medical physiology. Philadelphia, Pennsylvania: Saunders/Elsevier Science 2003, pages 230–54.

Arking DE, Pfeufer A, Post W, Kao WH, Newton-Cheh C, Ikeda M, West K, Kashuk C, Akyol M, Perz S, Jalilzadeh S, Illig T, Gieger C, Guo CY, Larson MG, Wichmann HE, Marbán E, O'Donnell CJ, Hirschhorn JN, Kääb S, Spooner PM, Meitinger T, Chakravarti A. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. Nat Genet 2006;38:644–51.

Aromaa A, Koskinen S, editors. Health and functional capacity in Finland. Baseline results of the Health 2000 Health Examination Survey. Publication number B12/2004. Helsinki: Publications of the National Public Health Institute.

Asada K, Kurokawa J, Furukawa T. Redox- and calmodulin-dependent S-nitrosylation of the KCNQ1 channel. J Biol Chem 2009;284:6014–20.

Aydin A, Bähring S, Dahm S, Guenther UP, Uhlmann R, Busjahn A, Luft FC. Single nucleotide polymorphism map of five long-QT genes. J Mol Med 2005;83:159–65.

Bacharova L, Szathmary V, Mateasik A. Secondary and primary repolarization changes in left ventricular hypertrophy: a model study. J Electrocardiol 2010;43:624–33.

Badilini F, Vaglio M, Dubois R, Roussel P, Sarapa N, Denjoy I, Extramiana F, Maison-Blanche P. Automatic analysis of cardiac repolarization morphology using Gaussian mesa function modeling. J Electrocardiol 2008;41:588–94.

Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JA, Berkowitz DE, Hare JM. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature 2002;416:337–9.

Batchvarov V, Kaski JC, Parchure N, Dilaveris P, Brown S, Ghuran A, Färbom P, Hnatkova K, Camm AJ, Malik M. Comparison between ventricular gradient and a new descriptor of the wavefront direction of ventricular activation and recovery. Clin Cardiol 2002(a);25:230–6.

Batchvarov VN, Ghuran A, Smetana P, Hnatkova K, Harries M, Dilaveris P, Camm AJ, Malik M. QT-RR relationship in healthy subjects exhibits substantial intersubject variability and high intrasubject stability. Am J Physiol Heart Circ Physiol 2002(b);282:H2356–63.

Batchvarov VN, Hnatkova K, Poloniecki J, Camm AJ, Malik M. Prognostic value of heterogeneity of ventricular repolarization in survivors of acute myocardial infarction. Clin Cardiol 2004;27:653–9.

Bazett HC. An analysis of the time-relations of electrocardiograms. Heart 1920;7:353–70.

Ben-David J, Zipes DP, Ayers GM, Pride HP. Canine left ventricular hypertrophy predisposes to ventricular tachycardia induction by phase 2 early afterdepolarizations after administration of BAY K 8644. J Am Coll Cardiol 1992;20:1576–84.

Benton RE, Sale M, Flockhart DA, Woosley RL. Greater quinidine-induced QTc interval prolongation in women. Clin Pharmacol Ther 2000;67:413–8.

Bezzina CR, Verkerk AO, Busjahn A, Jeron A, Erdmann J, Koopmann TT, Bhuiyan ZA, Wilders R, Mannens MM, Tan HL, Luft FC, Schunkert H, Wilde AA. A common polymorphism in KCNH2 (HERG) hastens cardiac repolarization. Cardiovasc Res 2003;59:27–36.

Bidoggia H, Maciel JP, Capalozza N, Mosca S, Blaksley EJ, Valverde E, Bertran G, Arini P, Biagetti MO, Quinteiro RA. Sex differences on the electrocardiographic pattern of cardiac repolarization: possible role of testosterone. Am Heart J 2000;140:678–83.

Blom G. Statistical estimates and transformed beta variables. New York: John Wiley & Sons, Inc. 1958.

Blumenfeld JD, Sealey JE, Mann SJ, Bragat A, Marion R, Pecker MS, Sotelo J, August P, Pickering TG, Laragh JH. Beta-adrenergic receptor blockade as a therapeutic approach for suppressing the renin-angiotensin-aldosterone system in normotensive and hypertensive subjects. Am J Hypertens 1999;12:451–9.

Bombelli M, Facchetti R, Carugo S, Madotto F, Arenare F, Quarti-Trevano F, Capra A, Giannattasio C, Dell'Oro R, Grassi G, Sega R, Mancia G. Left ventricular hypertrophy increases cardiovascular risk independently of in-office and out-of-office blood pressure values. J Hypertens 2009;27:2458–64.

Boulpaep EL. The heart as a pump. In: Boron WF, Boulpaep EL, editors. Medical physiology. Philadelphia, Pennsylvania: Saunders/Elsevier Science 2003, pages 508–33.

Burgess MJ. Relation of ventricular repolarization to electrocardiographic T wave-form and arrhythmia vulnerability. Am J Physiol 1979;236:H391–402.

Burkard N, Rokita AG, Kaufmann SG, Hallhuber M, Wu R, Hu K, Hofmann U, Bonz A, Frantz S, Cartwright EJ, Neyses L, Maier LS, Maier SK, Renné T, Schuh K, Ritter O. Conditional neuronal nitric oxide synthase overexpression impairs myocardial contractility. Circ Res 2007;100:e32–44.

Burnier M, Brunner HR. Neurohormonal consequences of diuretics in different cardiovascular syndromes. Eur Heart J 1992;13 Suppl G:28–33.

Caballero R, Delpón E, Valenzuela C, Longobardo M, Tamargo J. Losartan and its metabolite E3174 modify cardiac delayed rectifier K(+) currents. Circulation 2000;101:1199–205.

Carnethon MR, Anthony MS, Cascio WE, Folsom AR, Rautaharju PM, Liao D, Evans GW, Heiss G. A prospective evaluation of the risk of QT prolongation with hormone replacement therapy: the atherosclerosis risk in communities study. Ann Epidemiol 2003;13:530–6.

Castro Hevia J, Antzelevitch C, Tornés Bárzaga F, Dorantes Sánchez M, Dorticós Balea F, Zayas Molina R, Quiñones Pérez MA, Fayad Rodríguez Y. Tpeak-Tend and Tpeak-Tend dispersion as risk factors for ventricular tachycardia/ventricular fibrillation in patients with the Brugada syndrome. J Am Coll Cardiol 2006;47:1828–34.

Cerrone M, Noujaim S, Jalife J. The short QT syndrome as a paradigm to understand the role of potassium channels in ventricular fibrillation. J Intern Med 2006;259:24–38.

Chang KC, Barth AS, Sasano T, Kizana E, Kashiwakura Y, Zhang Y, Foster DB, Marbán E. CAPON modulates cardiac repolarization via neuronal nitric oxide synthase signaling in the heart. Proc Natl Acad Sci U S A 2008;105:4477–82.

Chen LY, Ballew JD, Herron KJ, Rodeheffer RJ, Olson TM. A common polymorphism in SCN5A is associated with lone atrial fibrillation. Clin Pharmacol Ther 2007;81:35–41.

Cheng J. Evidences of the gender-related differences in cardiac repolarization and the underlying mechanisms in different animal species and human. Fundam Clin Pharmacol 2006;20:1–8.

Chevalier P, Bellocq C, Millat G, Piqueras E, Potet F, Schott JJ, Baró I, Lemarec H, Barhanin J, Rousson R, Rodriguez-Lafrasse C. Torsades de pointes complicating atrioventricular block: evidence for a genetic predisposition. Heart Rhythm 2007;4:170–4.

Choudhury L, Mahrholdt H, Wagner A, Choi KM, Elliott MD, Klocke FJ, Bonow RO, Judd RM, Kim RJ. Myocardial scarring in asymptomatic or mildly symptomatic patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2002;40:2156–64.

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

Conrath CE, Wilders R, Coronel R, de Bakker JM, Taggart P, de Groot JR, Opthof T. Intercellular coupling through gap junctions masks M cells in the human heart. Cardiovasc Res 2004;62:407–14.

Couderc JP, Kaab S, Hinterseer M, McNitt S, Xia X, Fossa A, Beckmann BM, Polonsky S, Zareba W. Baseline values and sotalol-induced changes of ventricular repolarization duration, heterogeneity, and instability in patients with a history of drug-induced torsades de pointes. J Clin Pharmacol 2009;49:6–16.

Crotti L, Lundquist AL, Insolia R, Pedrazzini M, Ferrandi C, De Ferrari GM, Vicentini A, Yang P, Roden DM, George AL Jr, Schwartz PJ. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. Circulation 2005;112:1251–8.

Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, Greenberg DA, Schwartz PJ, George AL Jr. NOS1AP Is a Genetic Modifier of the Long-QT Syndrome. Circulation 2009;120:1657–63.

Darpo B, Nebout T, Sager PT. Clinical evaluation of QT/QTc prolongation and proarrhythmic potential for nonantiarrhythmic drugs: the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use E14 guideline. J Clin Pharmacol 2006;46:498–507.

De Ambroggi L, Bertoni T, Locati E, Stramba-Badiale M, Schwartz PJ. Mapping of body surface potentials in patients with the idiopathic long QT syndrome. Circulation 1986;74:1334–45.

de Bruyne MC, Hoes AW, Kors JA, Hofman A, van Bemmel JH, Grobbee DE. Prolonged QT interval predicts cardiac and all-cause mortality in the elderly. The Rotterdam Study. Eur Heart J 1999;20:278–84.

Dekker JM, Crow RS, Hannan PJ, Schouten EG, Folsom AR; ARIC Study. Heart rate-corrected QT interval prolongation predicts risk of coronary heart disease in black and white middle-aged men and women: the ARIC study. J Am Coll Cardiol 2004;43:565–71.

Dekker JM, Schouten EG, Klootwijk P, Pool J, Kromhout D. Association between QT interval and coronary heart disease in middle-aged and elderly men. The Zutphen Study. Circulation 1994;90:779–85.

Dekker JM, Schouten EG, Klootwijk P, Pool J, Kromhout D. ST segment and T wave characteristics as indicators of coronary heart disease risk: the Zutphen Study. J Am Coll Cardiol 1995;25:1321–6.

Delpón E, Caballero R, Gómez R, Núñez L, Tamargo J. Angiotensin II, angiotensin II antagonists and spironolactone and their modulation of cardiac repolarization. Trends Pharmacol Sci 2005;26:155–61.

Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450–8.

di Bernardo D, Murray A. Explaining the T-wave shape in the ECG. Nature 2000;403:40.

Di Diego JM, Cordeiro JM, Goodrow RJ, Fish JM, Zygmunt AC, Pérez GJ, Scornik FS, Antzelevitch C. Ionic and cellular basis for the predominance of the Brugada syndrome phenotype in males. Circulation 2002;106:2004–11.

Dogan A, Tunc E, Varol E, Ozaydin M, Ozturk M. Comparison of the four formulas of adjusting QT interval for the heart rate in the middle-aged healthy Turkish men. Ann Noninvasive Electrocardiol 2005;10:134–41.

Domenighetti AA, Boixel C, Cefai D, Abriel H, Pedrazzini T. Chronic angiotensin II stimulation in the heart produces an acquired long QT syndrome associated with IK1 potassium current downregulation. J Mol Cell Cardiol 2007;42:63–70.

Eckardt L, Kirchhof P, Breithardt G, Haverkamp W. Load-induced changes in repolarization: evidence from experimental and clinical data. Basic Res Cardiol 2001;96:369–80.

Eijgelsheim M, Newton-Cheh C, Aarnoudse AL, van Noord C, Witteman JC, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in NOS1AP is associated with sudden cardiac death: evidence from the Rotterdam Study. Hum Mol Genet 2009;18:4213–8.

Elming H, Holm E, Jun L, Torp-Pedersen C, Køber L, Kircshoff M, Malik M, Camm J. The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. Eur Heart J 1998;19:1391–400.

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;96:4392–9.

Emori T, Antzelevitch C. Cellular basis for complex T waves and arrhythmic activity following combined I(Kr) and I(Ks) block. J Cardiovasc Electrophysiol 2001;12:1369–78.

Erdogan D, Yildirim I, Ciftci O, Ozer I, Caliskan M, Gullu H, Muderrisoglu H. Effects of normal blood pressure, prehypertension, and hypertension on coronary microvascular function. Circulation 2007;115:593–9.

Extramiana F, Haggui A, Maison-Blanche P, Dubois R, Takatsuki S, Beaufils P, Leenhardt A. T-wave morphology parameters based on principal component analysis reproducibility and dependence on T-offset position. Ann Noninvasive Electrocardiol 2007;12:354–63.

Fischer R, Dechend R, Gapelyuk A, Shagdarsuren E, Gruner K, Gruner A, Gratze P, Qadri F, Wellner M, Fiebeler A, Dietz R, Luft FC, Muller DN, Schirdewan A. Angiotensin II-induced sudden arrhythmic death and electrical remodeling. Am J Physiol Heart Circ Physiol 2007;293:H1242–53.

Fodstad H, Swan H, Laitinen P, Piippo K, Paavonen K, Viitasalo M, Toivonen L, Kontula K. Four potassium channel mutations account for 73% of the genetic spectrum underlying long-QT syndrome (LQTS) and provide evidence for a strong founder effect in Finland. Ann Med 2004;36 Suppl 1:53–63.

Friedlander Y, Vatta M, Sotoodehnia N, Sinnreich R, Li H, Manor O, Towbin JA, Siscovick DS, Kark JD. Possible association of the human KCNE1 (minK) gene and QT interval in healthy subjects: evidence from association and linkage analyses in Israeli families. Ann Hum Genet 2005;69:645–56.

Furukawa T, Kurokawa J. Potassium channel remodeling in cardiac hypertrophy. J Mol Cell Cardiol 2006;41:753–61.

Gentile S, Martin N, Scappini E, Williams J, Erxleben C, Armstron DL. The human ERG1 channel polymorphism, K897T, creates a phosphorylation site that inhibits channel activity. Proc Natl Acad Sci U S A 2008;105:14704–8.

Gima K, Rudy Y. Ionic current basis of electrocardiographic waveforms: a model study. Circ Res 2002;90:889–96.

Goldberg RJ, Bengtson J, Chen ZY, Anderson KM, Locati E, Levy D. Duration of the QT interval and total and cardiovascular mortality in healthy persons (The Framingham Heart Study experience). Am J Cardiol 1991;67:55–8.

González-Juanatey JR, García-Acuña JM, Pose A, Varela A, Calvo C, Cabezas-Cerrato J, de la Peña MG. Reduction of QT and QTc dispersion during long-term treatment of systemic hypertension with enalapril. Am J Cardiol 1998;81:170–4.

Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B, Guicheney P; D.E.S.I.R. Study Group. Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population. Eur J Hum Genet 2005;13:1213–22.

Gouas L, Nicaud V, Chaouch S, Berthet M, Forhan A, Tichet J, Tiret L, Balkau B, Guicheney P. Confirmation of associations between ion channel gene SNPs and QTc interval duration in healthy subjects. Eur J Hum Genet 2007;15:974–9.

Graff C, Matz J, Christensen EB, Andersen MP, Kanters JK, Toft E, Pehrson S, Hardahl TB, Nielsen J, Struijk JJ. Quantitative analysis of T-wave morphology increases confidence in drug-induced cardiac repolarization abnormalities: evidence from the investigational IKr inhibitor Lu 35-138. J Clin Pharmacol 2009;49:1331–42.

Gui J, Wang T, Trump D, Zimmer T, Lei M. Mutation-specific effects of polymorphism H558R in SCN5A-related sick sinus syndrome. J Cardiovasc Electrophysiol 2010;21:564–73.

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;41:567–74.

Gökçe M, Karahan B, Yilmaz R, Orem C, Erdöl C, Ozdemir S. Long term effects of hormone replacement therapy on heart rate variability, QT interval, QT dispersion and frequencies of arrhythmia. Int J Cardiol 2005;99:373–9.

Haapalahti P, Viitasalo M, Kaartinen M, Väänänen H, Oikarinen L, Heliö T, Toivonen L. Electrocardiographic ventricular repolarization during cardiovascular autonomic function testing in patients with arrhythmogenic right ventricular cardiomyopathy. Scand Cardiovasc J 2008;42:375–82.

Haarmark C, Graff C, Andersen MP, Hardahl T, Struijk JJ, Toft E, Xue J, Rowlandson GI, Hansen PR, Kanters JK. Reference values of electrocardiogram repolarization variables in a healthy population. J Electrocardiol 2010;43:31–9.

Haarmark C, Hansen PR, Vedel-Larsen E, Pedersen SH, Graff C, Andersen MP, Toft E, Wang F, Struijk JJ, Kanters JK. The prognostic value of the Tpeak-Tend interval in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. J Electrocardiol 2009;42:555–60.

Haider AW, Larson MG, Benjamin EJ, Levy D. Increased left ventricular mass and hypertrophy are associated with increased risk for sudden death. J Am Coll Cardiol 1998;32:1454–9.

Haverkamp W, Breithardt G, Camm AJ, Janse MJ, Rosen MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A, Shah R. The potential for QT prolongation and proarrhythmia by non-antiarrhythmic drugs: clinical and regulatory implications. Report on a policy conference of the European Society of Cardiology. Eur Heart J 2000;21:1216–31.

Hedley PL, Jørgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, Kanters JK, Corfield VA, Christiansen M. The genetic basis of long QT and short QT syndromes: a mutation update. Hum Mutat 2009;30:1486–511.

Hill JA. Electrical remodeling in cardiac hypertrophy. Trends Cardiovasc Med 2003;13:316–22.

Hiltunen TP, Suonsyrjä T, Hannila-Handelberg T, Paavonen KJ, Miettinen HE, Strandberg T, Tikkanen I, Tilvis R, Pentikäinen PJ, Virolainen J, Kontula K. Predictors of antihypertensive drug responses: initial data from a placebo-controlled, randomized, cross-over study with four antihypertensive drugs (The GENRES Study). Am J Hypertens 2007;20:311–8.

Hoes AW, Grobbee DE, Lubsen J, Man in ’t Veld AJ, van der Does E, Hofman A. Diuretics, beta-blockers, and the risk for sudden cardiac death in hypertensive patients. Ann Intern Med 1995;123:481–7.

Huang B, Qin D, El-Sherif N. Early down-regulation of K^+ channel genes and currents in the postinfarction heart. J Cardiovasc Electrophysiol 2000;11:1252–61.

Huang HC, Lin LY, Yu HY, Ho YL. Risk stratification by T-wave morphology for cardiovascular mortality in patients with systolic heart failure. Europace 2009;11:1522–8.

Huikuri HV, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. N Engl J Med 2001;345:1473–82.

International HapMap Consortium. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449:851–61. (See the list of authors in the publication.)

Jeyaraj D, Abernethy DP, Natarajan RN, Dettmer MM, Dikshteyn M, Meredith DM, Patel K, Allareddy RR, Lewis SA, Kaufman ES. I(Kr) channel blockade to unmask occult congenital long QT syndrome. Heart Rhythm 2008;5:2–7.

Jin H, Chemaly ER, Lee A, Kho C, Hadri L, Hajjar RJ, Akar FG. Mechanoelectrical remodeling and arrhythmias during progression of hypertrophy. FASEB J 2010;24:451–63.

Kadish AH, Greenland P, Limacher MC, Frishman WH, Daugherty SA, Schwartz JB. Estrogen and progestin use and the QT interval in postmenopausal women. Ann Noninvasive Electrocardiol 2004;9:366–74.

Kanters JK, Fanoe S, Larsen LA, Bloch Thomsen PE, Toft E, Christiansen M. T wave morphology analysis distinguishes between KvLQT1 and HERG mutations in long QT syndrome. Heart Rhythm 2004;1:285–92.

Kanters JK, Haarmark C, Vedel-Larsen E, Andersen MP, Graff C, Struijk JJ, Thomsen PE, Christiansen M, Jensen HK, Toft E. T(peak)T(end) interval in long QT syndrome. J Electrocardiol 2008;41:603–8.

Kao WH, Arking DE, Post W, Rea TD, Sotoodehnia N, Prineas RJ, Bishe B, Doan BQ, Boerwinkle E, Psaty BM, Tomaselli GF, Coresh J, Siscovick DS, Marbán E, Spooner PM, Burke GL, Chakravarti A. Genetic variations in nitric oxide synthase 1 adaptor protein are associated with sudden cardiac death in US white community-based populations. Circulation 2009;119:940–51.

Kardys I, Kors JA, van der Meer IM, Hofman A, van der Kuip DA, Witteman JC. Spatial QRS-T angle predicts cardiac death in a general population. Eur Heart J 2003;24:1357–64.

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

Karjalainen J, Viitasalo M, Mänttäri M, Manninen V. Relation between QT intervals and heart rates from 40 to 120 beats/min in rest electrocardiograms of men and a simple method to adjust QT interval values. J Am Coll Cardiol 1994;23:1547–53.

Katra RP, Laurita KR. Cellular mechanism of calcium-mediated triggered activity in the heart. Circ Res 2005;96:535–42.

Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet 2005;365:217–23.

Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Worldwide prevalence of hypertension: a systematic review. J Hypertens 2004;22:11–9.

Kesek M, Jernberg T, Lindahl B, Xue J, Englund A. Principal component analysis of the T wave in patients with chest pain and conduction disturbances. Pacing Clin Electrophysiol 2004;27:1378–87.

Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacol Rev 2000;52:11–34.

King MK, Coker ML, Goldberg A, McElmurray JH 3rd, Gunasinghe HR, Mukherjee R, Zile MR, O'Neill TP, Spinale FG. Selective matrix metalloproteinase inhibition with developing heart failure: effects on left ventricular function and structure. Circ Res 2003;92:177–85.

Kors JA, de Bruyne MC, Hoes AW, van Herpen G, Hofman A, van Bemmel JH, Grobbee DE. T axis as an indicator of risk of cardiac events in elderly people. Lancet 1998;352:601–5.

Kors JA, Ritsema van Eck HJ, van Herpen G. The meaning of the Tp-Te interval and its diagnostic value. J Electrocardiol 2008;41:575–80.

Koskela J, Laiho J, KäHönen M, Rontu R, Lehtinen R, Viik J, Niemi M, Niemelä K, Kööbi T, Turjanmaa V, Pörsti I, Lehtimäki T, Nieminen T. Potassium channel KCNH2 K897T polymorphism and cardiac repolarization during exercise test: The Finnish Cardiovascular Study. Scand J Clin Lab Invest 2008;68:31–8.

Kowey PR, Friechling TD, Sewter J, Wu Y, Sokil A, Paul J, Nocella J. Electrophysiological effects of left ventricular hypertrophy. Effect of calcium and potassium channel blockade. Circulation 1991;83:2067–75.

Kozhevnikov DO, Yamamoto K, Robotis D, Restivo M, El-Sherif N. Electrophysiological mechanism of enhanced susceptibility of hypertrophied heart to acquired torsade de pointes arrhythmias: tridimensional mapping of activation and recovery patterns. Circulation 2002;105:1128–34.

Kreger BE, Cupples LA, Kannel WB. The electrocardiogram in prediction of sudden death: Framingham Study experience. Am Heart J 1987;113:377–82.

Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27:234–6.

Kuo CS, Munakata K, Reddy CP, Surawicz B. Characteristics and possible mechanism of ventricular arrhythmia dependent on the dispersion of action potential durations. Circulation 1983;67:1356–67.

Laitinen P, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kaprio J, Kontula K. Survey of the coding region of the HERG gene in long QT syndrome reveals six novel mutations and an amino acid polymorphism with possible phenotypic effects. Hum Mutat 2000;15:580–1.

Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440–63.

Lederer WJ. Cardiac electrophysiology and the electrocardiogram. In: Boron WF, Boulpaep EL, editors. Medical physiology. Philadelphia, Pennsylvania: Saunders/Elsevier Science 2003, pages 483–507.

Lehmann-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. Physiol Rev 1999;79:1317–72.

Lehnart SE, Ackerman MJ, Benson DW Jr, Brugada R, Clancy CE, Donahue JK, George AL Jr, Grant AO, Groft SC, January CT, Lathrop DA, Lederer WJ, Makielski JC, Mohler PJ, Moss A, Nerbonne JM, Olson TM, Przywara DA, Towbin JA, Wang LH, Marks AR. Inherited arrhythmias: a National Heart, Lung, and Blood Institute and Office of Rare Diseases workshop consensus report about the diagnosis, phenotyping, molecular mechanisms, and therapeutic approaches for primary cardiomyopathies of gene mutations affecting ion channel function. Circulation 2007;116:2325–45.

Lehtinen AB, Newton-Cheh C, Ziegler JT, Langefeld CD, Freedman BI, Daniel KR, Herrington DM, Bowden DW. Association of NOS1AP genetic variants with QT interval duration in families from the Diabetes Heart Study. Diabetes 2008;57:1108–14.

Lellouche N, De Diego C, Akopyan G, Boyle NG, Mahajan A, Cesario DA, Wiener I, Shivkumar K. Changes and predictive value of dispersion of repolarization parameters for appropriate therapy in patients with biventricular implantable cardioverter-defibrillators. Heart Rhythm 2007;4:1274–83.

Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990;322:1561–6.

Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA 1996;275:1557–62.

Levy MN. Electrical activity of the heart. In: Berne RM, Levy MN, Koeppen BM, Stanton BA, editors. Physiology. St. Louis, Missouri: Mosby/Elsevier Science 2004, pages 274–304.

Lin CY, Lin LY, Chen PC. Analysis of T-wave morphology from the 12-lead electrocardiogram for prediction of long-term prognosis in patients initiating haemodialysis. Nephrol Dial Transplant 2007;22:2645–52.

Lin JH, Lu AY. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet 1998;35:361–90.

Liu JE, Devereux RB. Clinical assessment of cardiac hypertrophy. In: Sheridan DJ, editor. Left ventricular hypertrophy. London: Churchill Livingstone 1998, pages 11–6.

Lizotte E, Junttila MJ, Dube MP, Hong K, Benito B, DE Zutter M, Henkens S, Sarkozy A, Huikuri HV, Towbin J, Vatta M, Brugada P, Brugada J, Brugada R. Genetic modulation of Brugada syndrome by a common polymorphism. J Cardiovasc Electrophysiol 2009;20:1137–41.

Lubinski A, Kornacewicz-Jach Z, Wnuk-Wojnar AM, Adamus J, Kempa M, Królak T, Lewicka-Nowak E, Radomski M, Swiatecka G. The terminal portion of the T wave: a new electrocardiographic marker of risk of ventricular arrhythmias. Pacing Clin Electrophysiol 2000;23:1957–9.

Lubinski A, Lewicka-Nowak E, Kempa M, Baczynska AM, Romanowska I, Swiatecka G. New insight into repolarization abnormalities in patients with congenital long QT syndrome: the increased transmural dispersion of repolarization. Pacing Clin Electrophysiol 1998;21:172–5.

Lux RL, Evans AK, Burgess MJ, Wyatt RF, Abildskov JA. Redundancy reduction for improved display and analysis of body surface potential maps. I. Spatial compression. Circ Res 1981;49:186–96.

Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. JAMA 1993;270:2590–7.

Malik M. Arrhythmic substrate. Heart 2008;94:402–3.

Malik M. Drug-induced changes in the T-wave morphology. Drug Saf 2009;32:613–7.

Malik M, Acar B, Gang Y, Yap YG, Hnatkova K, Camm AJ. QT dispersion does not represent electrocardiographic interlead heterogeneity of ventricular repolarization. J Cardiovasc Electrophysiol 2000;11:835–43.

Malik M, Batchvarov VN. Measurement, interpretation and clinical potential of QT dispersion. J Am Coll Cardiol 2000;36:1749–66.

Malik M, Hnatkova K, Batchvarov VN. Post infarction risk stratification using the 3-D angle between QRS complex and T-wave vectors. J Electrocardiol 2004;37 Suppl:201–8.

Malmqvist K, Kahan T, Edner M, Bergfedt L. Comparison of actions of irbesartan versus atenolol on cardiac repolarization in hypertensive left ventricular hypertrophy: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation Versus Atenolol (SILVHIA). Am J Cardiol 2002;90:1107–12.

Mank-Seymour AR, Richmond JL, Wood LS, Reynolds JM, Fan YT, Warnes GR, Milos PM, Thompson JF. Association of torsades de pointes with novel and known single nucleotide polymorphisms in long QT syndrome genes. Am Heart J 2006;152:1116–22.

Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 2007;292:C82–97.

Merri M, Benhorin J, Alberti M, Locati E, Moss AJ. Electrocardiographic quantitation of ventricular repolarization. Circulation 1989;80:1301–8.

Milan DJ, Kim AM, Winterfield JR, Jones IL, Pfeufer A, Sanna S, Arking DE, Amsterdam AH, Sabeh KM, Mably JD, Rosenbaum DS, Peterson RT, Chakravarti A, Kääb S, Roden DM, MacRae CA. Drug-sensitized zebrafish screen identifies multiple genes, including GINS3, as regulators of myocardial repolarization. Circulation 2009;120:553–9.

Moczydlowski EG. Electrical excitability and action potentials. In: Boron WF, Boulpaep EL, editors. Medical physiology. Philadelphia, Pennsylvania: Saunders/Elsevier Science 2003(a), pages 172–203.

Moczydlowski EG. Electrophysiology of the cell membrane. In: Boron WF, Boulpaep EL, editors. Medical physiology. Philadelphia, Pennsylvania: Saunders/Elsevier Science 2003(b), pages 145–71.

Molloy TJ, Okin PM, Devereux RB, Kligfield P. Electrocardiographic detection of left ventricular hypertrophy by the simple QRS voltage-duration product. J Am Coll Cardiol 1992;20:1180–6.

Montanez A, Ruskin JN, Hebert PR, Lamas GA, Hennekens CH. Prolonged QTc interval and risks of total and cardiovascular mortality and sudden death in the general population: a review and qualitative overview of the prospective cohort studies. Arch Intern Med 2004;164:943–8.

Mosteller RD. Simplified calculation of body-surface area. N Engl J Med 1987;317:1098.

Nakagawa M, Sekine Y, Ono M, Taniguchi Y, Takahashi N, Yonemochi H, Saikawa T. Gender differences in the effect of auditory stimuli on ventricular repolarization in healthy subjects. J Cardiovasc Electrophysiol 2009;20:653–7.

Nakagawa M, Takahashi N, Watanabe M, Ichinose M, Nobe S, Yonemochi H, Ito M, Saikawa T. Gender differences in ventricular repolarization: terminal T wave interval was shorter in women than in men. Pacing Clin Electrophysiol 2003;26:59–64.

Nerbonne JM. Studying cardiac arrhythmias in the mouse–a reasonable model for probing mechanisms? Trends Cardiovasc Med 2004;14:83–93.

Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PI, Yin X, Estrada K, Bis JC, Marciante K, Rivadeneira F, Noseworthy PA, Sotoodehnia N, Smith NL, Rotter JI, Kors JA, Witteman JC, Hofman A, Heckbert SR, O'Donnell CJ, Uitterlinden AG, Psaty BM, Lumley T, Larson MG, Stricker BH. Common variants at ten loci influence QT interval duration in the QTGEN Study. Nat Genet 2009;41:399–406.

Newton-Cheh C, Guo CY, Larson MG, Musone SL, Surti A, Camargo AL, Drake JA, Benjamin EJ, Levy D, D'Agostino RB Sr, Hirschhorn JN, O'Donnell CJ. Common genetic variation in KCNH2 is associated with QT interval duration: the Framingham Heart Study. Circulation 2007;116:1128–36.

Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, Yamamoto S, Ozawa T, Ding WG, Toyoda F, Kawamura M, Akao M, Matsuura H, Kimura T, Kita T, Horie M. D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome. J Am Coll Cardiol 2009;54:812–9.

O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, Mengden T, Myers M, Padfield P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, Verdecchia P; European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens 2003;21:821–48.

Oceandy D, Cartwright EJ, Emerson M, Prehar S, Baudoin FM, Zi M, Alatwi N, Venetucci L, Schuh K, Williams JC, Armesilla AL, Neyses L. Neuronal nitric oxide synthase signaling in the heart is regulated by the sarcolemmal calcium pump 4b. Circulation 2007;115:483–92.

Oikarinen L, Karvonen M, Viitasalo M, Takala P, Kaartinen M, Rossinen J, Tierala I, Hänninen H, Katila T, Nieminen MS, Toivonen L. Electrocardiographic assessment of left ventricular hypertrophy with time-voltage QRS and QRST-wave areas. J Hum Hypertens 2004(a);18:33–40.

Oikarinen L, Nieminen MS, Toivonen L, Viitasalo M, Wachtell K, Papademetriou V, Jern S, Dahlöf B, Devereux RB, Okin PM; LIFE Study Investigators. Relation of QT interval and QT dispersion to regression of echocardiographic and electrocardiographic left ventricular hypertrophy in hypertensive patients: the Losartan Intervention For Endpoint Reduction (LIFE) study. Am Heart J 2003;145:919–25.

Oikarinen L, Nieminen MS, Viitasalo M, Toivonen L, Jern S, Dahlöf B, Devereux RB, Okin PM; LIFE Study Investigators. QRS duration and QT interval predict mortality in hypertensive patients with left ventricular hypertrophy: the Losartan Intervention for Endpoint Reduction in Hypertension Study. Hypertension 2004(b);43:1029–34.

Oikarinen L, Nieminen MS, Viitasalo M, Toivonen L, Wachtell K, Papademetriou V, Jern S, Dahlöf B, Devereux RB, Okin PM. Relation of QT interval and QT dispersion to echocardiographic left ventricular hypertrophy and geometric pattern in hypertensive patients. The LIFE study. The Losartan Intervention For Endpoint Reduction. J Hypertens 2001(a);19:1883–91.

Oikarinen L, Paavola M, Montonen J, Viitasalo M, Mäkijärvi M, Toivonen L, Katila T. Magnetocardiographic QT interval dispersion in postmyocardial infarction patients with sustained ventricular tachycardia: validation of automated QT measurements. Pacing Clin Electrophysiol 1998;21:1934–42.

Oikarinen L, Viitasalo M, Korhonen P, Väänänen H, Hänninen H, Montonen J, Mäkijärvi M, Katila T, Toivonen L. Postmyocardial infarction patients susceptible to ventricular tachycardia show increased T wave dispersion independent of delayed ventricular conduction. J Cardiovasc Electrophysiol 2001(b);12:1115–20.

Oikarinen L, Väänänen H, Dabek J, Kaartinen M, Hänninen H, Katila T, Nieminen MS, Toivonen L, Viitasalo M. Relation of twelve-lead electrocardiographic T-wave morphology descriptors to left ventricular mass. Am J Cardiol 2002;90:1032–5.

Okin PM. Serial evaluation of electrocardiographic left ventricular hypertrophy for prediction of risk in hypertensive patients. J Electrocardiol 2009;42:584–8.

Okin PM, Devereux RB, Fabsitz RR, Lee ET, Galloway JM, Howard BV. Principal component analysis of the T wave and prediction of cardiovascular mortality in American Indians: the Strong Heart Study. Circulation 2002;105:714–9.

Okin PM, Devereux RB, Howard BV, Fabsitz RR, Lee ET, Welty TK. Assessment of QT interval and QT dispersion for prediction of all-cause and cardiovascular mortality in American Indians: The Strong Heart Study. Circulation 2000(a);101:61–6.

Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, Snapinn S, Harris KE, Aurup P, Edelman JM, Wedel H, Lindholm LH, Dahlöf B; LIFE Study Investigators. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive treatment and the prediction of major cardiovascular events. JAMA 2004(a);292:2343–9.

Okin PM, Devereux RB, Lee ET, Galloway JM, Howard BV; Strong Heart Study. Electrocardiographic repolarization complexity and abnormality predict all-cause and cardiovascular mortality in diabetes: the strong heart study. Diabetes 2004(b);53:434–40.

Okin PM, Malik M, Hnatkova K, Lee ET, Galloway JM, Best LG, Howard BV, Devereux RB. Repolarization abnormality for prediction of all-cause and cardiovascular mortality in American Indians: the Strong Heart Study. J Cardiovasc Electrophysiol 2005;16:945–51.

Okin PM, Roman MJ, Devereux RB, Pickering TG, Borer JS, Kligfield P. Time-voltage QRS area of the 12-lead electrocardiogram: detection of left ventricular hypertrophy. Hypertension 1998;31:937–42.

Okin PM, Xue Q, Reddy S, Kligfield P. Electrocardiographic quantitation of heterogeneity of ventricular repolarization. Ann Noninvasive Electrocardiol 2000(b);5:79–87.

Ono T, Saitoh H, Yi G, Hnatkova K, Kobayashi Y, Atarashi H, Katoh T, Takano T, Malik M. Clinical implication of T-wave morphology analysis as a new repolarization descriptor. Circ J 2005;69:666–70.

Opthof T, Coronel R, Wilms-Schopman FJ, Plotnikov AN, Shlapakova IN, Danilo P Jr, 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;4:341–8.

Paavonen KJ, Chapman H, Laitinen PJ, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kontula K, Pasternack M. Functional characterization of the common amino acid 897 polymorphism of the cardiac potassium channel KCNH2 (HERG). Cardiovasc Res 2003;59:603–11.

Paerson K. On lines and planes of closest fit to systems of points in space. Philosophical Magazine 1901:2:559–72.

Pajunen P, Koukkunen H, Ketonen M, Jerkkola T, Immonen-Räihä P, Kärjä-Koskenkari P, Mähönen M, Niemelä M, Kuulasmaa K, Palomäki P, Mustonen J, Lehtonen A, Arstila M, Vuorenmaa T, Lehto S, Miettinen H, Torppa J, Tuomilehto J, Kesäniemi YA, Pyörälä K, Salomaa V. The validity of the Finnish Hospital Discharge Register and Causes of Death Register data on coronary heart disease. Eur J Cardiovasc Prev Rehabil 2005;12:132–7.

Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, Borbély A, Edes I, Handoko ML, Heymans S, Pezzali N, Pieske B, Dickstein K, Fraser AG, Brutsaert DL. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007;28:2539–50.

Paulussen A, Matthijs G, Gewillig M, Verhasselt P, Cohen N, Aerssens J. Mutation analysis in congenital Long QT Syndrome–a case with missense mutations in KCNQ1 and SCN5A. Genet Test 2003;7:57–61.

Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ, Schulze-Bahr E, Haverkamp W, Breithardt G, Cohen N, Aerssens J. Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. J Mol Med 2004;82:182–8.

Perkiömäki JS, Hyytinen-Oinas M, Karsikas M, Seppänen T, Hnatkova K, Malik M, Huikuri HV. Usefulness of T-wave loop and QRS complex loop to predict mortality after acute myocardial infarction. Am J Cardiol 2006;97:353–60.

Pfeufer A, Jalilzadeh S, Perz S, Mueller JC, Hinterseer M, Illig T, Akyol M, Huth C, Schöpfer-Wendels A, Kuch B, Steinbeck G, Holle R, Näbauer M, Wichmann HE, Meitinger T, Kääb S. Common variants in myocardial ion channel genes modify the QT interval in the general population: results from the KORA study. Circ Res 2005;96:693–701.

Pfeufer A, Sanna S, Arking DE, Müller M, Gateva V, Fuchsberger C, Ehret GB, Orrú M, Pattaro C, Köttgen A, Perz S, Usala G, Barbalic M, Li M, Pütz B, Scuteri A, Prineas RJ, Sinner MF, Gieger C, Najjar SS, Kao WH, Mühleisen TW, Dei M, Happle C, Möhlenkamp S, Crisponi L, Erbel R, Jöckel KH, Naitza S, Steinbeck G, Marroni F, Hicks AA, Lakatta E, Müller-Myhsok B, Pramstaller PP, Wichmann HE, Schlessinger D, Boerwinkle E, Meitinger T, Uda M, Coresh J, Kääb S, Abecasis GR, Chakravarti A. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. Nat Genet 2009;41:407–14.

Pietilä E, Fodstad H, Niskasaari E, Laitinen P PJ, Swan H, Savolainen M, Kesäniemi YA, Kontula K, Huikuri HV. Association between HERG K897T polymorphism and QT interval in middle-aged Finnish women. J Am Coll Cardiol 2002;40:511–4.

Porthan K, Viitasalo M, Hiltunen TP, Väänänen H, Dabek J, Suonsyrjä T, Hannila-Handelberg T, Virolainen J, Nieminen MS, Toivonen L, Kontula K, Oikarinen L. Short-term electrophysiological effects of losartan, bisoprolol, amlodipine, and hydrochlorothiazide in hypertensive men. Ann Med 2009(a);41:29–37.

Porthan K, Viitasalo M, Jula A, Reunanen A, Rapola J, Väänänen H, Nieminen MS, Toivonen L, Salomaa V, Oikarinen L. Predictive value of electrocardiographic QT interval and T-wave morphology parameters for all-cause and cardiovascular mortality in a general population sample. Heart Rhythm 2009(b);6:1202–8.

Porthan K, Virolainen J, Hiltunen TP, Viitasalo M, Väänänen H, Dabek J, Hannila-Handelberg T, Toivonen L, Nieminen MS, Kontula K, Oikarinen L. Relationship of electrocardiographic repolarization measures to echocardiographic left ventricular mass in men with hypertension. J Hypertens 2007;25:1951–7.

Post W, Shen H, Damcott C, Arking DE, Kao WH, Sack PA, Ryan KA, Chakravarti A, Mitchell BD, Shuldiner AR. Associations between genetic variants in the NOS1AP (CAPON) gene and cardiac repolarization in the old order Amish. Hum Hered 2007;64:214–9.

Prineas, Crow RS, Blackburn H. The Minnesota Code Manual of Electrocardiographic Findings: Standards and Procedures for Measurement and Classification. Boston: John Wright 1982.

Priori SG, Mortara DW, Napolitano C, Diehl L, Paganini V, Cantù F, Cantù G, Schwartz PJ. Evaluation of the spatial aspects of T-wave complexity in the long-QT syndrome. Circulation 1997;96:3006–12.

Priori SG, Napolitano C, Schwartz PJ, Grillo M, Bloise R, Ronchetti E, Moncalvo C, Tulipani C, Veia A, Bottelli G, Nastoli J. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. JAMA 2004;292:1341–4.

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;348:1866–74.

Raitakari OT, Blom-Nyholm J, Koskinen TA, Kähönen M, Viikari JS, Lehtimäki T. Common variation in NOS1AP and KCNH2 genes and QT interval duration in young adults. The Cardiovascular Risk in Young Finns Study. Ann Med 2009;41:144–51.

Rautaharju PM. Why did QT dispersion die? Card Electrophysiol Rev 2002;6:295–301.

Rautaharju PM, Kooperberg C, Larson JC, LaCroix A. Electrocardiographic abnormalities that predict coronary heart disease events and mortality in postmenopausal women: the Women's Health Initiative. Circulation 2006(a);113:473–80.

Rautaharju PM, Kooperberg C, Larson JC, LaCroix A. Electrocardiographic predictors of incident congestive heart failure and all-cause mortality in postmenopausal women: the Women's Health Initiative. Circulation 2006(b);113:481–9.

Rautaharju PM, Zhou SH, Wong S, Calhoun HP, Berenson GS, Prineas R, Davignon A. Sex differences in the evolution of the electrocardiographic QT interval with age. Can J Cardiol 1992;8:690–5.

Rials SJ, Wu Y, Ford N, Pauletto FJ, Abramson SV, Rubin AM, Marinchak RA, Kowey PR. Effect of left ventricular hypertrophy and its regression on ventricular electrophysiology and vulnerability to inducible arrhythmia in the feline heart. Circulation 1995;91:426–30.

Rials SJ, Xu X, Wu Y, Liu T, Marinchack RA, Kowey PR. Restoration of normal ventricular electrophysiology in renovascular hypertensive rabbits after treatment with losartan. J Cardiovasc Pharmacol 2001;37:317–23.

Rials SJ, Xu X, Wu Y, Marinchak RA, Kowey PR. Regression of LV hypertrophy with captopril normalizes membrane currents in rabbits. Am J Physiol 1998;275:H1216–24.

Robbins J, Nelson JC, Rautaharju PM, Gottdiener JS. The association between the length of the QT interval and mortality in the Cardiovascular Health Study. Am J Med 2003;115:689–94.

Roden DM. Cellular basis of drug-induced torsades de pointes. Br J Pharmacol 2008;154:1502–7.

Roden DM. Drug-induced prolongation of the QT interval. N Engl J Med 2004;350:1013–22.
Roden DM. Long QT syndrome: reduced repolarization reserve and the genetic link. J Intern Med 2006;259:59–69.
Roden DM. Taking the "idio" out of "idiosyncratic": predicting torsades de pointes. Pacing Clin Electrophysiol 1998;21:1029–34.
Roden DM, Balser JR, George AL Jr, Anderson ME. Cardiac ion channels. Annu Rev Physiol 2002;64:431–75.
Rose G, Baxter PJ, Reid DD, McCartney P. Prevalence and prognosis of electrocardiographic findings in middle-aged men. Br Heart J 1978;40:636–43.
Ruilope LM, Schmieder RE. Left ventricular hypertrophy and clinical outcomes in hypertensive patients. Am J Hypertens 2008;21:500–8.
Sager PT. Key clinical considerations for demonstrating the utility of preclinical models to predict clinical drug-induced torsades de pointes. Br J Pharmacol 2008;154:1544–9.
Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072–83.
Salles GF, Cardoso CR, Leocadio SM, Muxfeldt ES. Recent ventricular repolarization markers in resistant hypertension: are they different from the traditional QT interval? Am J Hypertens 2008;21:47–53.
Schillaci G, Pirro M, Pasqualini L, Vaudo G, Ronti T, Gemelli F, Marchesi S, Reboldi G, Porcellati C, Mannarino E. Prognostic significance of isolated, non-specific left ventricular repolarization abnormalities in hypertension. J Hypertens 2004;22:407–14.
Schillaci G, Verdecchia P, Porcellati C, Cuccurullo O, Cosco C, Perticone F. Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension. Hypertension 2000;35:580–6.
Schouten EG, Dekker JM, Meppelink P, Kok FJ, Vandenbroucke JP, Pool J. QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. Circulation 1991;84:1516–23.
Scicluna BP, Wilde AA, Bezzina CR. The primary arrhythmia syndromes: same mutation, different manifestations. Are we starting to understand why? J Cardiovasc Electrophysiol 2008;19:445–52.
Selzer A, Wray HW. Paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias. Circulation 1964;30:17–26.
Sesti F, Abbott GW, Wei J, Murray KT, Saksena S, Schwartz PJ, Priori SG, Roden DM, George AL Jr, Goldstein SA. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc Natl Acad Sci U S A 2000;97:10613–8.
Shimizu M, Ino H, Okeie K, Yamaguchi M, Nagata M, Hayashi K, Itoh H, Iwaki T, Oe K, Konno T, Mabuchi H. T-peak to T-end interval may be a better predictor of high-risk patients with hypertrophic cardiomyopathy associated with a cardiac troponin I mutation than QT dispersion. Clin Cardiol 2002;25:335–9.
Shimizu W, Antzelevitch C. Cellular basis for the ECG features of the LQT1 form of the long QT-syndrome: effect of beta-adrenergic agonists and antagonists and sodium channel blockers on transmural dispersion of repolarization and torsades de pointes. Circulation 1998;98:2314–22.
Sicouri S, Antzelevitch C. A subpopulation of cells with unique electrophysiological properties in the deep subepicardium of the canine ventricle. The M cell. Circ Res 1991;68:1729–41.
Siscovick DS, Raghunathan TE, Psaty BM, Koepsell TD, Wicklund KG, Lin X, Cobb L, Rautaharju PM, Copass MK, Wagner EH. Diuretic therapy for hypertension and the risk of primary cardiac arrest. N Engl J Med 1994;330:1852–7.
Smetana P, Batchvarov V, Hnatkova K, Camm AJ, Malik M. Sex differences in the rate dependence of the T wave descending limb. Cardiovasc Res 2003;58:549–54.
Smetana P, Batchvarov VN, Hnatkova K, Camm AJ, Malik M. Sex differences in repolarization homogeneity and its circadian pattern. Am J Physiol Heart Circ Physiol 2002;282:H1889–97.

Smetana P, Batchvarov VN, Hnatkova K, Camm AJ, Malik M. Ventricular gradient and nondipolar repolarization components increase at higher heart rate. Am J Physiol Heart Circ Physiol 2004;286:H131–6.

Straus SM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MC, Stricker BH, Witteman JC. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. J Am Coll Cardiol 2006;47:362–7.

Sun Z, Milos PM, Thompson JF, Lloyd DB, Mank-Seymour A, Richmond J, Cordes JS, Zhou J. Role of a KCNH2 polymorphism (R1047 L) in dofetilide-induced Torsades de Pointes. J Mol Cell Cardiol 2004;37:1031–9.

Surawicz B. Electrocardiographic diagnosis of chamber enlargement. J Am Coll Cardiol 1986;8:711–24.

Szentadrassy N, Banyasz T, Biro T, Szabo G, Toth BI, Magyar J, Lazar J, Varro A, Kovacs L, Nanasi PP. Apico-basal inhomogeneity in distribution of ion channels in canine and human ventricular myocardium. Cardiovasc Res 2005;65:851–60.

Takenaka K, Ai T, Shimizu W, Kobori A, Ninomiya T, Otani H, Kubota T, Takaki H, Kamakura S, Horie M. Exercise stress test amplifies genotype-phenotype correlation in the LQT1 and LQT2 forms of the long-QT syndrome. Circulation 2003;107:838–44.

Tanabe Y, Inagaki M, Kurita T, Nagaya N, Taguchi A, Suyama K, Aihara N, Kamakura S, Sunagawa K, Nakamura K, Ohe T, Towbin JA, Priori SG, Shimizu W. Sympathetic stimulation produces a greater increase in both transmural and spatial dispersion of repolarization in LQT1 than LQT2 forms of congenital long QT syndrome. J Am Coll Cardiol 2001;37:911–9.

Tobin MD, Kähönen M, Braund P, Nieminen T, Hajat C, Tomaszewski M, Viik J, Lehtinen R, Ng GA, Macfarlane PW, Burton PR, Lehtimäki T, Samani NJ. Gender and effects of a common genetic variant in the NOS1 regulator NOS1AP on cardiac repolarization in 3761 individuals from two independent populations. Int J Epidemiol 2008;37:1132–41.

Tomás M, Napolitano C, De Giuli L, Bloise R, Subirana I, Malovini A, Bellazzi R, Arking DE, Marban E, Chakravarti A, Spooner PM, Priori SG. Polymorphisms in the NOS1AP gene modulate QT interval duration and risk of arrhythmias in the long QT syndrome. J Am Coll Cardiol 2010;55:2745–52.

Tomaschitz A, Pilz S, Ritz E, Meinitzer A, Boehm BO, März W. Plasma aldosterone levels are associated with increased cardiovascular mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Eur Heart J 2010;31:1237–47.

Topilski I, Rogowski O, Rosso R, Justo D, Copperman Y, Glikson M, Belhassen B, Hochenberg M, Viskin S. The morphology of the QT interval predicts torsade de pointes during acquired bradyarrhythmias. J Am Coll Cardiol 2007;49:320–8.

Truong QA, Ptaszek LM, Charipar EM, Taylor C, Fontes JD, Kriegel M, Irlbeck T, Toepker M, Schlett CL, Bamberg F, Blankstein R, Brady TJ, Nagurney JT, Hoffmann U. Performance of electrocardiographic criteria for left ventricular hypertrophy as compared with cardiac computed tomography: from the Rule Out Myocardial Infarction Using Computer Assisted Tomography trial. J Hypertens 2010;28:1959–67.

Turakhia MP, Schiller NB, Whooley MA. Prognostic significance of increased left ventricular mass index to mortality and sudden death in patients with stable coronary heart disease (from the Heart and Soul Study). Am J Cardiol 2008;102:1131–5.

Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. Am Heart J 2001;141:334–41.

Van Huysduynen BH, Swenne CA, Draisma HH, 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;16:1097–103.

Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, Devereux RB, Porcellati C. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. Am J Hypertens 2003;16:895–9.

Verkerk AO, Wilders R, Veldkamp MW, de Geringel W, Kirkels JH, Tan HL. Gender disparities in cardiac cellular electrophysiology and arrhythmia susceptibility in human failing ventricular myocytes. Int Heart J 2005;46:1105–18.

Viitasalo M, Oikarinen L, Swan H, Väänänen H, Glatter K, Laitinen PJ, Kontula K, Barron HV, Toivonen L, Scheinman MM. Ambulatory electrocardiographic evidence of transmural dispersion of repolarization in patients with long-QT syndrome type 1 and 2. Circulation 2002;106:2473–8.

Viitasalo M, Oikarinen L, Swan H, Väänänen H, Järvenpää J, Hietanen H, Karjalainen J, Toivonen L. Effects of beta-blocker therapy on ventricular repolarization documented by 24-h electrocardiography in patients with type 1 long-QT syndrome. J Am Coll Cardiol 2006;48:747–53.

Viitasalo M, Oikarinen L, Väänänen H, Kontula K, Toivonen L, Swan H. U-waves and T-wave peak to T-wave end intervals in patients with catecholaminergic polymorphic ventricular tachycardia, effects of beta-blockers. Heart Rhythm 2008;5:1382–8.

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

Viswanathan PC, Benson DW, Balser JR. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. J Clin Invest 2003;111:341–6.

Volders PG, Sipido KR, Carmeliet E, Spätjens RL, Wellens HJ, Vos MA. Repolarizing K+ currents ITO1 and IKs are larger in right than left canine ventricular midmyocardium. Circulation 1999;99:206–10.

Wall ME, Rechtsteiner A, Rocha LM. Singular value decomposition and principal component analysis. In: Berrar DP, Dubitzky W, Granzow M, editors. A practical approach to microarray data analysis. Norwell, Massachusetts: Kluwer 2003, pages 91–109.

Watanabe N, Kobayashi Y, Tanno K, Miyoshi F, Asano T, Kawamura M, Mikami Y, Adachi T, Ryu S, Miyata A, Katagiri T. Transmural dispersion of repolarization and ventricular tachyarrhythmias. J Electrocardiol 2004;37:191–200.

Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991;83:1849–65.

Wei S, Guo A, Chen B, Kutschke W, Xie YP, Zimmerman K, Weiss RM, Anderson ME, Cheng H, Song LS. T-tubule remodeling during transition from hypertrophy to heart failure. Circ Res 2010;107:520–31.

Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: a common cause of severe long-QT syndrome. Circulation 2004;109:1834–41.

Wu J, Kors JA, Rijnbeek PR, van Herpen G, Lu Z, Xu C. Normal limits of the electrocardiogram in Chinese subjects. Int J Cardiol 2003;87:37–51.

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;2:162–9.

Xu KY, Huso DL, Dawson TM, Bredt DS, Becker LC. Nitric oxide synthase in cardiac sarcoplasmic reticulum. Proc Natl Acad Sci U S A 1999;96:657–62.

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;105:671–6.

Yan GX, Antzelevitch C. Cellular basis for the normal T wave and the electrocardiographic manifestations of the long-QT syndrome. Circulation 1998;98:1928–36.

Yan GX, Lankipalli RS, Burke JF, Musco S, Kowey PR. Ventricular repolarization components on the electrocardiogram: cellular basis and clinical significance. J Am Coll Cardiol 2003;42:401–9.

Yan GX, Rials SJ, Wu Y, Liu T, Xu X, Marinchak RA, Kowey PR. Ventricular hypertrophy amplifies transmural repolarization dispersion and induces early afterdepolarization. Am J Physiol Heart Circ Physiol 2001;281:H1968–75.

Yang PC, Clancy CE. Effects of sex hormones on cardiac repolarization. J Cardiovasc Pharmacol 2010;56:123–9.

Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation. Physiol Genomics 2003;12:187–93.

Yi G, Prasad K, Elliott P, Sharma S, Guo X, McKenna WJ, Malik M. T wave complexity in patients with hypertrophic cardiomyopathy. Pacing Clin Electrophysiol 1998;21:2382–6.

Zabel M, Acar B, Klingenheben T, Franz MR, Hohnloser SH, Malik M. Analysis of 12-lead T-wave morphology for risk stratification after myocardial infarction. Circulation 2000;102:1252–7.

Zabel M, Malik M, Hnatkova K, Papademetriou V, Pittaras A, Fletcher RD, Franz MR. Analysis of T-wave morphology from the 12-lead electrocardiogram for prediction of long-term prognosis in male US veterans. Circulation 2002;105:1066–70.

Zabel M, Portnoy S, Franz MR. Electrocardiographic indexes of dispersion of ventricular repolarization: an isolated heart validation study. J Am Coll Cardiol 1995;25:746–52.

Zhang X, Chen S, Zhang L, Liu M, Redfearn S, Bryant RM, Oberti C, Vincent GM, Wang QK. Protective effect of KCNH2 single nucleotide polymorphism K897T in LQTS families and identification of novel KCNQ1 and KCNH2 mutations. BMC Med Genet 2008;9:87.

Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. Circulation 2001;104:2158–63.

Zicha S, Tsuji Y, Shiroshita-Takeshita A, Nattel S. Beta-blockers as antiarrhythmic agents. Handb Exp Pharmacol 2006:235–66.

APPENDICES

Appendix 1. Disease definitions in studies IV and V.

1. *Hypertension* was present if any of 1.a–1.c were fulfilled:
   - 1.a In the National Register of Rights to Reimbursements for Medical Costs,* a diagnosis of hypertension was present at the time of the study baseline
   - 1.b In the health examination, arterial mean (two measurements) systolic blood pressure ≥140 mmHg or mean (two measurements) diastolic blood pressure ≥90 mmHg
   - 1.c Use of antihypertensive medication prescribed by a physician

2. *Coronary heart disease* was present if any of 2.a–2.g were fulfilled:
   - 2.a In the physician's clinical examination,† a history of previous angina pectoris‡ was considered to be certain (other options, but not accepted here, were: no previous angina pectoris, possible previous angina pectoris)
   - 2.b In the National Hospital Discharge Register,§ a diagnosis code of myocardial infarction or other ischemic heart disease [International Classification of Diseases (ICD) -8 or ICD-9 codes 410–414 or ICD-10 codes I20–I25] was present at the time of the study baseline or before the study baseline
   - 2.c In the National Register of Rights to Reimbursements for Medical Costs,* a diagnosis of chronic coronary heart disease was present at the time of the study baseline or before the study baseline
   - 2.d In the physician's clinical examination,† there was history of a previous coronary artery bypass surgery
   - 2.e In the physician's clinical examination,† there was history of a previous coronary angioplasty
   - 2.f In the home interview,¶ there was history of a previous coronary artery bypass surgery
   - 2.g In the home interview,¶ there was history of a previous coronary angioplasty

3. Previous *myocardial infarction* was present if any of 3.a–3.c were fulfilled:
   - 3.a In the physician's clinical examination,† previous myocardial infarction was considered to be certain (other options, but not accepted here, were: no previous myocardial infarction, possible previous myocardial infarction)
   - 3.b In the ECG Minnesota coding, large Q waves indicating probable previous myocardial infarction were observed (Minnesota codes 1.1 or 1.2 together with codes 5.1–5.2)
   - 3.c In the National Hospital Discharge Register,§ a diagnosis code of a myocardial infarction (ICD-8 or ICD-9 code 410 or ICD-10 codes I21–I23) was present at the time of the study baseline or before the study baseline

4. *Diabetes mellitus* was present if 4.a or 4.b (or both) were fulfilled:
   - 4.a Use of blood glucose-lowering medication
   - 4.b Four-hour fasting venous plasma glucose ≥7.0 mmol/L

*In Finland, individuals with hypertension or coronary heart disease are entitled to reimbursement for medication costs. To obtain the right, they have to apply for it and append a medical certificate by a physician to show that they fulfill the objective criteria of the disease. †In the physician's clinical examination, the physician critically assessed clinical history and available documents and performed a structured clinical examination. ‡Defined as typical chest pain brought on by exertion and relieved by nitroglycerine or rest. §The Finnish hospital discharge register is valid for identifying major coronary heart disease events (Pajunen et al. 2005). ¶Performed by a trained interviewer (by Statistics Finland's interview organisation).

Appendix 2. Computation of TCRT, TMD, and TWR.

This appendix follows the guidelines previously published for TCRT and TMD (Acar et al. 1999), and for TWR (Malik et al. 2000). The original guidelines were modified by correcting the baseline wander (point 5), by using 90 ms for the epsilon value (point 5), and by modifying the T-wave detection to be more suitable for signal with silent ST segments (point 6). Upper-case bold letters are used for matrices and lower-case bold letters for vectors. Vector or matrix followed by time index in parenthesis, (t), refers respective index or column in the vector or matrix, respectively.

1. Create matrix **M** (size 8×nsamp; nsamp is the number of samples in the data) with leads I, II, V1, V2, V3, V4, V5, V6 in each row of the matrix. The matrix must contain ECG traces from one beat, and the traces have to start at least 50 ms before the P wave and last at least 50 ms after the T wave.

2. Correct baseline wander for each lead (rows in **M**):
   a Find a 40 ms window with the lowest SD 250–350 ms before the QRS complex (preceding TP interval) and another 400–500 ms after the QRS complex (following TP interval).
   b In both windows, calculate the point matching the average time and average amplitude of the window. Then, define a straight line through these two points.
   c Subtract the fitted line from the data (row in **M**).

3. Do singular value decomposition (Wall et al. 2003) for **M** and formulate the 2- and 3-dimensional subspaces (from the original 8 dimensional space):

   a $\Sigma = U^T M V$, where Σ is a 8×8 diagonal matrix (diag(σ1, σ2, … , σ8)) and σ1≤σ2≤σ8≤0.
   b $S_{3D}(t) = [s_1(t)\, s_2(t)\, s_3(t)]^T$ and $S_{2D}(t) = [s_1(t)\, s_2(t)]^T$, where $\mathbf{s_i}$ are signal vectors spanned by $\mathbf{u_i}$.

4. Define the energy for $\mathbf{S_{3D}(t)}$: $e_{3D}(t) = \sqrt{S_{3D}(t)^2}$.

5. Detect the QRS complex (t'_{RS}, t'_{RE}, $t_{RS,}$ t_{RE}) from $\mathbf{e_{3D}(t)}$:
   a Detect the maximum $\mathbf{e_{3D}(t)}$.
   b Define t'_{RS} as the time point where $\mathbf{e_{3D}(t)}$ decreases below 70% of the maximum value before the maximum $\mathbf{e_{3D}(t)}$, and t'_{RE} as the time point where $\mathbf{e_{3D}(t)}$ decreases below 70% after the maximum $\mathbf{e_{3D}(t)}$.
   c Define t_{RS} as the time point 90 ms before $t'_{RS,}$ and t_{RE} as the time point 90 ms after the t'_{RS}.

6. Detect the T wave (t_{TS}, $t_{TP,}$ t_{TE}) from $\mathbf{e_{3D}(t)}$:
   a Define t_{TP} as the time of the maximum value in $\mathbf{e_{3D}(t)}$ after the t_{RE}.
   b Define t_{TS}: $t_{TS} = t'_{RE} + (t_{TP} - t'_{RE})/3$.
   c Define t_{TE}:
      i Determine the maximum and minimum values for **s1** and **s2**.
      ii Divide both ranges to 10 bins, and create a 10×10 matrix.
      iii Compute the number of times that $\mathbf{S_{2D}(t)}$ hits each cell of the matrix.
      iv Determine the mean and SD of the number of hits in non-zero cells.
      v Determine the threshold value as mean + *mu* · SD of hits, where *mu* is set to *3*.

vi If the threshold value is below the maximum hit value, decrease *mu 0.2* at a time until the maximum value is reached.

vii Start from the beginning of $\mathbf{S_{2D}(t)}$ and count hits to the matrix cells until the threshold value is reached. If the threshold value is reached before t_{TP}, increase *mu* by *0.2* and formulate a new threshold value. Continue counting until the new threshold value is reached. If t_{TP} has not still been passed, keep repeating the last two phases until it has. Define t_{TE} as the time instant (after the t_{TP}) where the threshold value is reached.

7. Normalize the signals so that the maximum energy of the signals is 1:
$$S'_{3D}(t) = S_{3D}(t) / max(e_{3D}(t)) .$$

8. Subtract the direct-current vector from the signals: direct-current vector is defined as the average of amplitude values at t_{RS}, t_{RE}, $t_{TS,}$ and t_{TE}.

9. Define the energy $\mathbf{e'_{3D}(t)}$ from the direct-current corrected $\mathbf{S'_{3D}(t)}$: $e'_{3D}(t) = \sqrt{S'^{2}_{3D}(t)}$.

10. Define TCRT:
    a Define t'_{TP} as the time instant of maximum value in $\mathbf{e'_{3D}(t)}$ after the t_{TS}.
    b Define TCRT as the average cosine of the angle between QRS complex and T-wave:
$$TCRT = \frac{1}{t'_{RE} - t'_{RS}} \sum_{t'_{RS}}^{t'_{RE}} \frac{S'_{3D}(t) \cdot S'_{3D}(t'_{TP})}{|S'_{3D}(t)||S'_{3D}(t'_{TP})|} .$$

11. Define TMD:
    a Reconstruct $\mathbf{M'}$ from $\mathbf{S_{3D}}$ (by multiplying it left with $\mathbf{U}$), and do another singular value decomposition for signal between t_{TS} and t_{TE} only.
    b Normalize the two biggest left singular vectors by scaling them with singular values.
    c Formulate 21 pairs from the seven (I, II, V2, V3, V4, V5, V6; V1 is dropped off) 2D reconstruction vectors ({I, II}, {I, V2}, … , {V5, V6}).
    d Define TMD as the average angle [0°, 180°] between the 21 pairs.

12. Define TWR:
    a Do singular value decomposition for the original 8 leads from t_{TS} to t_{TE}.
    b Define TWR as the sum of the singular values from 4 to 8.

Calculation data in appendix 2 are given by Heikki Väänänen Lic Sc (Tech).