MOLECULAR AND FUNCTIONAL CHARACTERISATION OF LONG QT SYNDROME CAUSING GENES

By

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DECLARATION

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ABSTRACT

Ventricular arrhythmias are the most important cause of sudden cardiac death (SCD) among adults living in industrialised nations. Genetic factors have substantial effects in determining population-based risk for SCD and may also account for inter-individual variability in susceptibility. Great progress has been made in identifying genes underlying various Mendelian disorders associated with inherited arrhythmia susceptibility. The most well studied familial arrhythmia syndrome is the congenital long QT syndrome (LQTS) caused by mutations in genes encoding subunits of myocardial ion channels. Not all mutation carriers have equal risk for experiencing the clinical manifestations of disease (i.e. syncope, sudden death). This observation has raised the possibility that additional genetic factors may modify the risk of LQTS manifestations.

This study establishes the genetic aetiology of LQTS in South Africa and Denmark through the identification and characterisation of LQTS-causative mutations in five previously identified genes, as well as examining possible novel genetic causes of LQTS in a cohort comprising Danish and British probands. We have functionally characterised several of the mutations identified in this study and examined other cardiac phenotypes that may be explained by variants causing repolarisation disorders.

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OPSOMMING

Ventrikulêre aritmie bly die enkele belangrikste oorsaak van skielike hart dood (SCD) onder volwassenes wat in geïndustrialiseerde lande woon. Genetiese faktore het aansienlike gevolge in die bepaling van bevolking-gebaseerde risiko vir SCD en kan ook verantwoordelik wees vir die inter-individuele variasie in vatbaarheid. Groot vordering is gemaak in die identifisering van gene onderliggende verskeie Mendeliese siektes wat verband hou met geërf aritmie vatbaarheid. Die mees goed bestudeerde familie aritmie sindroom is die aangebore lang QT-sindroom (LQTS) wat veroorsaak word deur mutasies in gene kode subeenhede van miokardiale ioonkanale. Nie alle mutasie draers het 'n gelyke risiko vir die ervaring van die kliniese manifestasies van die siekte (dws sinkopee, skielike dood). Hierdie waarneming het die moontlikheid genoem dat genetiese faktore anders as die primêre siekte-verwante mutasie kan die risiko van LQTS manifestasies verander.

Hierdie studie stel die genetiese oorsake van LQTS in Suid-Afrika en Denemarke deur die identifisering en karakterisering van LQTS-veroorsakende mutasies in vyf voorheen geïdentifiseer gene, asook die behandeling van moontlike nuwe genetiese oorsake van LQTS in 'n groep wat bestaan uit van die Deense en die Britse probands. Ons het funksioneel gekenmerk verskeie van die mutasies wat in hierdie studie ondersoek en ander kardiovaskulêre fenotipes wat deur variante veroorsaak repolarisasie versteurings verduidelik word.

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PREFACE

Long QT syndrome (LQTS) will be presented here as a paradigm of an inherited arrhythmia disorder. Such disorders are interesting as they increase the risk of developing syncope and sudden death. The problems associated with inherited arrhythmia disorders are that the genetic basis is not fully established, the knowledge of phenotype modifiers is lacking and clinical inference is not evidence based.

In Chapter 1, I will provide a general over view of the clinical definitions and genetic bases of LQTS, short QT syndrome and Brugada syndrome (BrS) in the form of two reviews [1, 2]. Additionally, I have included an editorial highlighting the implications and evidence base of LQTS testing in clinical practice [3].

In Chapters 2 and 3, the genetic aetiology of LQTS in South Africa and Denmark is described in two papers which document the results of comprehensive genetic screening in these populations [4, 5].

In Chapter 4, *CAV3* (encoding caveolin-3)was examined in a cohort of Danish and British LQTS probands and a genetic variant, which was identified in a family known to carry a *KCNH2* (LQT2-causing gene) LQTS-causing mutation, was evaluated with respect to its aetiological and clinical significance [6]. Additionally, the role of microRNAs in LQTS was examined in a subset of this LQTS cohort [7].

In Chapter 5, the role that the *SCN5A* gene plays in the development of mixed/overlapping phenotypes is discussed in two papers. The first paper presents a complex phenotype (LQTS/BrS) [8]; and the second paper deals with *SCN5A* mutations in atrial fibrillation patients and the prevalence of known LQT3-causing mutations in this group [9].

Patients with hypertrophic cardiomyopathy (HCM) are at risk of atrial and ventricular arrhythmias and minK (encoded by *KCNE1*) is reported to form part of an "electro-mechanical feed-back" system which links cardiomyocyte stretching to changes in ion channel function. Consequently, in Chapter 6, genes which encode cardiac ion channel subunits were examined as possible causes of HCM [10].

These studies have substantially enhanced our understanding of LQTS and inherited arrhythmia disorders, and have contributed to the much needed evidence base for LQTS and, in doing so, expanded our understanding of SCD.

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CHAPTER 1: INTRODUCTION

1.1 THE HEART

The human heart is a muscular organ composed of four-chambers (Figure 1A) which, by rhythmic contractions, is responsible for pumping blood through the pulmonary and systemic circulation. Efficient functioning of the heart requires strict coordination of the particular functions of the pacemaker cells and cardiomyocytes within the myocardium (Figure 1B). These cells exhibit the characteristics of automaticity, excitability, conductivity and contractility. Automaticity, the ability to initiate an impulse (measured as an action potential (AP)), is a characteristic of pacemaker cells. Conductivity is the ability to propagate the AP to other areas of the heart. Excitability is the ability to respond to an impulse by depolarising and repolarising, thus propagating the AP. Finally, contractility is the ability of cardiomyocytes to mechanically respond to an electrical impulse by contracting.

Cardiac muscle is structurally different to skeletal muscle in that cardiomyocytes are connected to one another through interlocking, porous membranes (intercalated discs) at the ends of adjacent cells. Gap junctions within the intercalated discs create a continuous electromechanical syncytium, which allows the movement of ions and consequently the propagation of an AP throughout the myocardium [11, 12].

1.1.1 CONTRACTILITY

During a contraction (systole) both atria contract simultaneously, pumping blood to the ventricles – which subsequently contract, pumping the blood out of the heart. The right ventricle receives oxygen-poor venous blood from the right atrium and pumps it into the pulmonary circulation for gaseous exchange in the lungs. The left ventricle receives oxygen-rich arterial blood from the left atrium and pumps it into the systemic circulation (Figure 1). The four chambers of the heart each have a one-way valve to prevent blood from flowing backwards (Figure 1). To complete the contraction cycle (heartbeat), the heart muscle relaxes (diastole) which allows blood to fill the heart ready for the next contraction. The myocardium (layer of cardiac muscle within the heart wall) and the cardiac valves (tricuspid, pulmonary, mitral and aortic valves) (Figure 1) are key elements in ensuring the efficient pumping of blood through the heart. The contractility of the myocardium generates the force needed to circulate the blood and the valves ensure blood flows in the correct direction based on pressure differences across the valves.

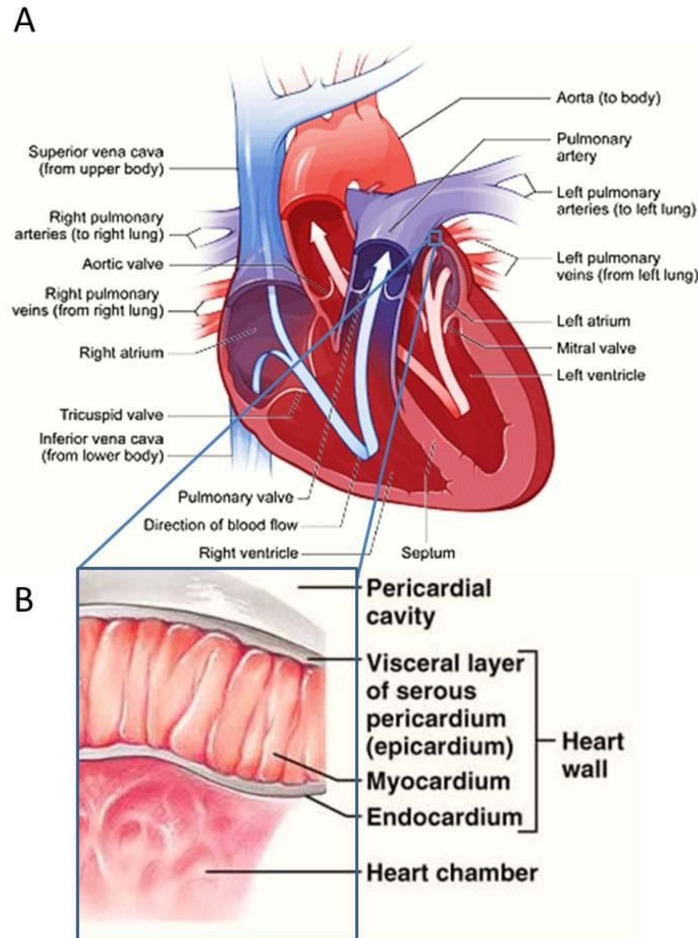

*Figure 1: **A**. A schematic diagram depicting the chambers, valves and great vessels of the heart, blood flow is indicated by blue and red arrows. Blue arrows indicate the flow of de-oxygenated blood and the red arrows indicate the flow of oxygenated blood. **B**. A diagrammatic representation of the layers of the heart wall, the myocardium is the thick middle layer. Figures adapted from http://www.nhlbi.nih.gov/health//dci/Diseases/hhw/hhw_anatomy.html and http://anatomyandphysiologyi.com/heart-anatomy/*

1.1.2 AUTOMATICITY, EXCITABILITY AND CONDUCTIVITY

The efficient coordination of a synchronous heartbeat is maintained by the heart's electrical system (Figure 2). All cardiomyocytes are excitable cells with respect to their ability to transmit an AP (Figure 2); while, automaticity is a specific characteristic of pacemaker cells. The cardiac conduction system (CCS) is responsible for the conduction of electrical impulses around the heart and is composed of several distinct anatomical structures that consist of highly specialised cardiomyocytes and conduction fibres (Figure 2). The sinoatrial node (SAN) is the pacemaker of the heart; electrical impulses are generated in the SAN more rapidly than any other part of the CCS. The propagation of the electrical impulse through the atrial wall initiates waves of depolarisation that causes the atria to contract. The atrioventricular node (AVN) conducts the depolarising wave through to the ventricles. The impulse is then propagated through the bundle of His which is a band of atypical cardiac muscle fibres specialised for conduction that originates

at the AVN, and then passes along the interventricular septum to the ventricles where it branches into the left and right bundle branches (LBB and RBB, respectively). From the LBB and RBB the impulse is propagated on to the Purkinje fibre network which results in depolarisation of the ventricular cardiomyocytes and excitation-contraction [13].

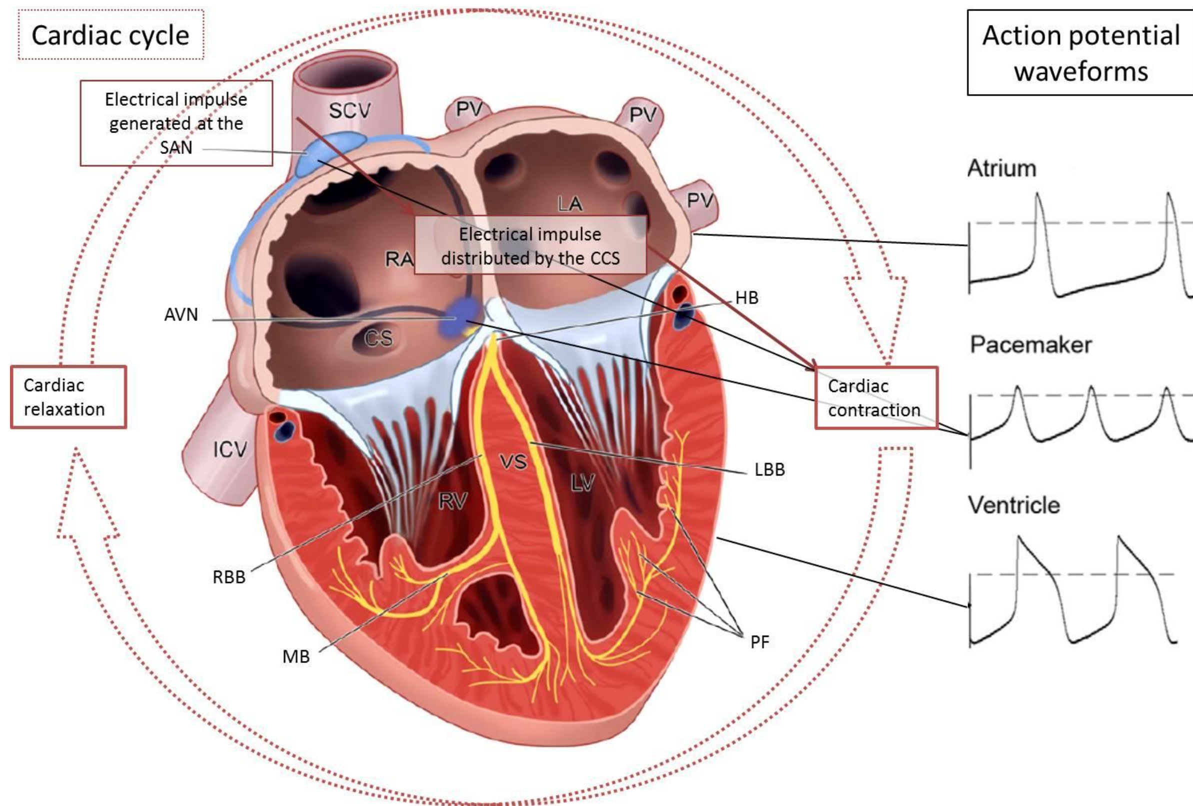

Figure 2: A schematic representation of the cardiac cycle, components of the cardiac conduction system and action potential wave forms from particular areas of the heart.

***AVN**: atrioventricular node, **CCS**: cardiac conduction system, **CS**: coronary sinus, **HB**: bundle of His, **IVC**: inferior vena cava, **LBB**: left bundle branch, **LV**: left ventricle, **MB**: moderator band, **PF**: Purkinje fibres, **PV**: pulmonary vein, **RA**: right atrium, **RBB**: right bundle branch, **RV**: right ventricle, **SAN**: sinoatrial node, **SCV**: superior caval vein, **VS**: ventricular septum. Figure adapted from [13, 14].*

The human cardiac excitatory process was first mapped in normal hearts by Durrer *et al.* (1970) [15]. Understanding this physiological process was the first step towards understanding abnormal electrical activity in arrhythmic diseases.

1.2 ELECTROCARDIOGRAM

The invention of Eithoven's string galvanometer at the beginning of the 20th century provided the opportunity to assess the heart's electrical activity in a non-invasive manner. The galvanometer greatly improved the sensitivity of recording electrical potentials and led to the development of the ECG technology [16]. It became possible to classify clinically occurring arrhythmias as to their clinical presentation, mechanism and focal origin [17].

The ECG uses twelve leads, each of which connects a surface-electrode to an amplifier within the ECG apparatus. The electrical activity from particular parts of the heart is measured based on the position of the lead on the body as shown in Figure 3. As the heart beats, the electrical information is translated into wave patterns; Einthoven labelled the ECG waves: P, Q, R, S and T; these labels are still the standard nomenclature when referring to ECG waves (Figure 3). The cardiac electrical impulse is generated in the SAN and spreads through both atria (Figure 2). This electrical activity is represented by the P-wave on an ECG trace (Figure 3). When the impulse reaches the AVN, the bundle of His is activated and the impulse spreads via the RBB and LBB (Figure 2) – depolarising the ventricles. Ventricular depolarisation is represented by the QRS complex on an ECG trace (Figure 3). The coordinated conduction of the impulse to both ventricles results in ventricular contraction. The repolarisation of the ventricles (return to normal resting potential) is represented by the T-waves on the ECG. Thus the QT interval represents the duration of the ventricular action potential (Figure 3).

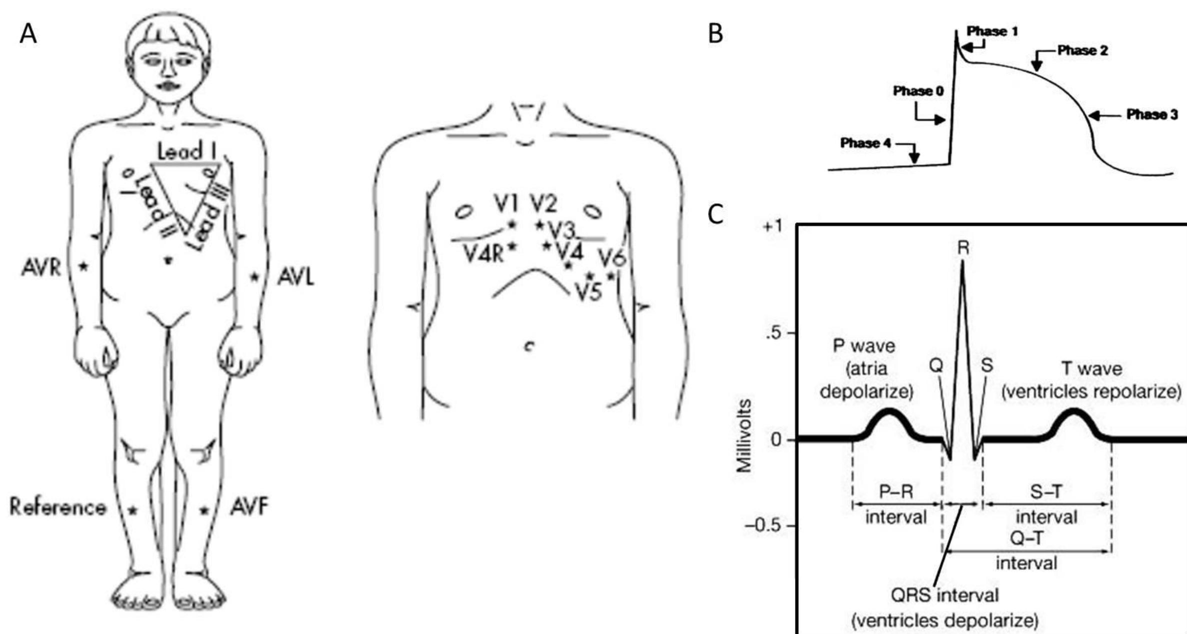

*Figure 3: **A**. Positions of ECG electrodes are indicated and labelled appropriately [18]. **B**. A schematic representation of the ventricular cardiomyocyte action potential which corresponds to the QT interval on an ECG trace. The phases of the action potential are indicated, these represent shifts in ion channel activity and consequent changes in membrane potential [19]. **C**. A representative diagram of single cycle ECG pattern, the P wave corresponds to the firing of the SA node and depolarization of the atria; the PR interval represents the delay of the electrical impulse at the AV node. The QRS complex corresponds to ventricular depolarization while the T Wave represents ventricular repolarisation. Figure adapted from http://www.as.miami.edu/chemistry/2086/NEW-Chap20/NEW-Chapter%2020_part2.htm*

As the QT interval is strongly influenced by the heart rate (RR interval), it is necessary to correct for this influence when reporting a QT interval. Several mathematical formulas have been developed in order to calculate the corrected QT (QT_c) interval, QT_c intervals reported throughout this thesis have been calculated using Bazett's formula ($QTc = QT/\sqrt{RR}$) [20].

1.3 CARDIAC ARRHYTHMIAS

Cardiac arrhythmias pose a significant health burden. The approximate ratios of people affected by particular heart rhythm disturbances are indicated in Table 1. Heart rhythm disturbances may abolish the synchronicity of the heartbeat, causing myocardial fibres to contract in a random, uncoordinated fashion resulting in atrial fibrillation (AF) or ventricular fibrillation (VF) depending on which chambers are affected. As a consequence of the frequency and societal impact of cardiac arrhythmias, the molecular mechanisms involved in arrhythmogenesis or the molecular determinants of disease have become a focus of cardiac research [21].

Table 1: The proportion of the US population hospitalised or deceased as a result of arrhythmias in 2003, data taken from Rosamond et al. (2007) [22]. The ratios were calculated from the number of people hospitalised as a result of an arrhythmia as well as the number of people whose deaths were caused by arrhythmias in 2003. The number of people who developed arrhythmias or died as a result of an arrhythmia, which was a secondary complication to their condition, was not included.

Heart rhythm disturbance	***Approximate proportion of US population***
Arrhythmia	1:300
AF	1:600
VT	1:3500
VF	1:40000

***AF**: atrial fibrillation, **VF**: ventricular fibrillation, **VT**: ventricular tachycardia.*

1.3.1 TERMINOLOGY OF RHYTHM DISTURBANCES

The normal heart rhythm is referred to as the sinus rhythm (because is it paced by the SAN), which results in the regular (60-100 bpm) rhythm characteristic of a normal ECG pattern (Figure 3). A slower than normal heart rate is called a sinus bradycardia and a faster than normal heart rate is called a sinus tachycardia. An aberrant heart rhythm is referred to as an arrhythmia; if the arrhythmia coincides with a slow or fast heart rate, it is referred to as a bradyarrhythmia or a tachyarrhythmia, respectively.

Supraventricular tachycardias (SVTs) are atrial in origin and interfere with the efficient flow of blood into the ventricles. The most common SVTs are AF, atrioventricular nodal reentrant tachycardia (AVNRT) and atrioventricular reentrant tachycardia (AVRT). SVTs can be described as paroxysmal or incessant. A paroxysmal arrhythmia is an irregular rhythm that occurs infrequently, comes and goes, lasts a few minutes to hours and then stops on its own. An incessant arrhythmia is a continuous arrhythmia which affects atrial contractions. This in turn affects the ventricles.

Ventricular tachycardias (VTs) are ventricular in origin; mild cases can effectively pump blood and maintain blood pressure. Serious cases, however, lead to VF, which leads to cardiac arrest,

hypoxia and, without medical intervention will result in death. VTs can be described as monomorphic or polymorphic. Monomorphic VT (MVT) manifests as a regular rhythm with similar QRS complexes in each ECG lead. Polymorphic VT (PVT) manifests as frequent changes morphology and axis of the QRS complexes on ECG. Furthermore, the duration of the VT episodes is another means of classification. Episodes of VT which last more than 30 seconds are called sustained VTs, while those which self-terminate before 30 seconds are called non-sustained VTs. These abnormalities often occur in people with structural heart problems, such as an infarct from a previous heart attack or rare inherited heart defects. Although the functional substrate, transient initiating event and arrhythmia mechanism may differ in these two cases understanding the development of arrhythmias in inherited arrhythmic diseases may shed light on the mechanisms by which arrhythmias develop in coronary artery disease (CAD) and myopathy patients.

1.3.2 MECHANISMS OF ARRHYTHMIAS

There are three basic mechanisms of arrhythmias as illustrated in Figure 4: namely, increased automaticity, triggered activity and re-entry. Automaticity is a measure of the propensity of a fibre to initiate an impulse spontaneously. Increased automaticity causes arrhythmias by the inappropriate spontaneous depolarisation of cardiac tissue. Triggered activity relates to the occurrence of 'afterdepolarisations'. These can occur before full repolarisation – early-afterdepolarisations (EADs), or after full repolarisation - delayed-afterdepolarisations (DADs). These afterdepolarisations can become self-perpetuating if they reach threshold potential and trigger another afterdepolarisation, thereby generating an arrhythmia. Re-entry is the most common arrhythmia mechanism whereby a block of the electrical impulse causes a feedback "loop".

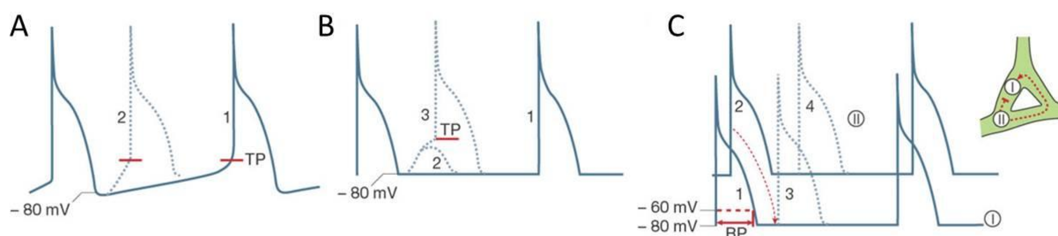

Figure 4: Mechanisms of arrhythmia generation. In all panels Trace 1 (solid line) represents the normal condition ***A.*** *Trace 2 (dotted line) represents increased automaticity, caused by inappropriate depolarisation and abnormally rapid firing.* ***B.*** *Trace 2 represents an afterdepolarisation which has not reached the threshold necessary to become self-perpetuating. Trace 3 represents an afterdepolarisation which has reached threshold to result in a premature ectopic AP before the next expected normal AP.* ***C.*** *Re-entry occurring between two connected zones of tissue (I and II) - as shown on the right. Trace 2 represents a premature activation in zone II. This activation current fails to initiate firing in zone I because zone I is still refractory. Trace 3: This current may conduct back (red dashed line) to zone I at a time when it can respond with an AP. Trace 4: This AP may propagate to initiate in zone II, and the process can continue indefinitely. Image taken from [23].*
AP*: action potential,* ***RP****: refractory period,* ***TP****: threshold potential.*

1.4 SUDDEN CARDIAC DEATH

Sudden cardiac death (SCD), defined as death by cardiac causes occurring within one hour from the onset of symptoms [24], accounts for the worldwide loss of more than three million people per annum [25]. While the true incidence of SCD is difficult to determine [26], it has been estimated to range from 180,000- >450,000 [27] within the USA, this indicates that ~7-18% of mortality in the USA is attributed to SCD. Epidemiological studies suggest that ~90 % of SCD cases are a consequence of arrhythmias [28], consequently, a better understanding of arrhythmogenesis is required to address this issue [29].

Regardless of the underlying disease state, progression to SCD typically follows the same course: VT degenerates to VF and circulatory arrest and death [25, 30] as indicated in Figure 5.

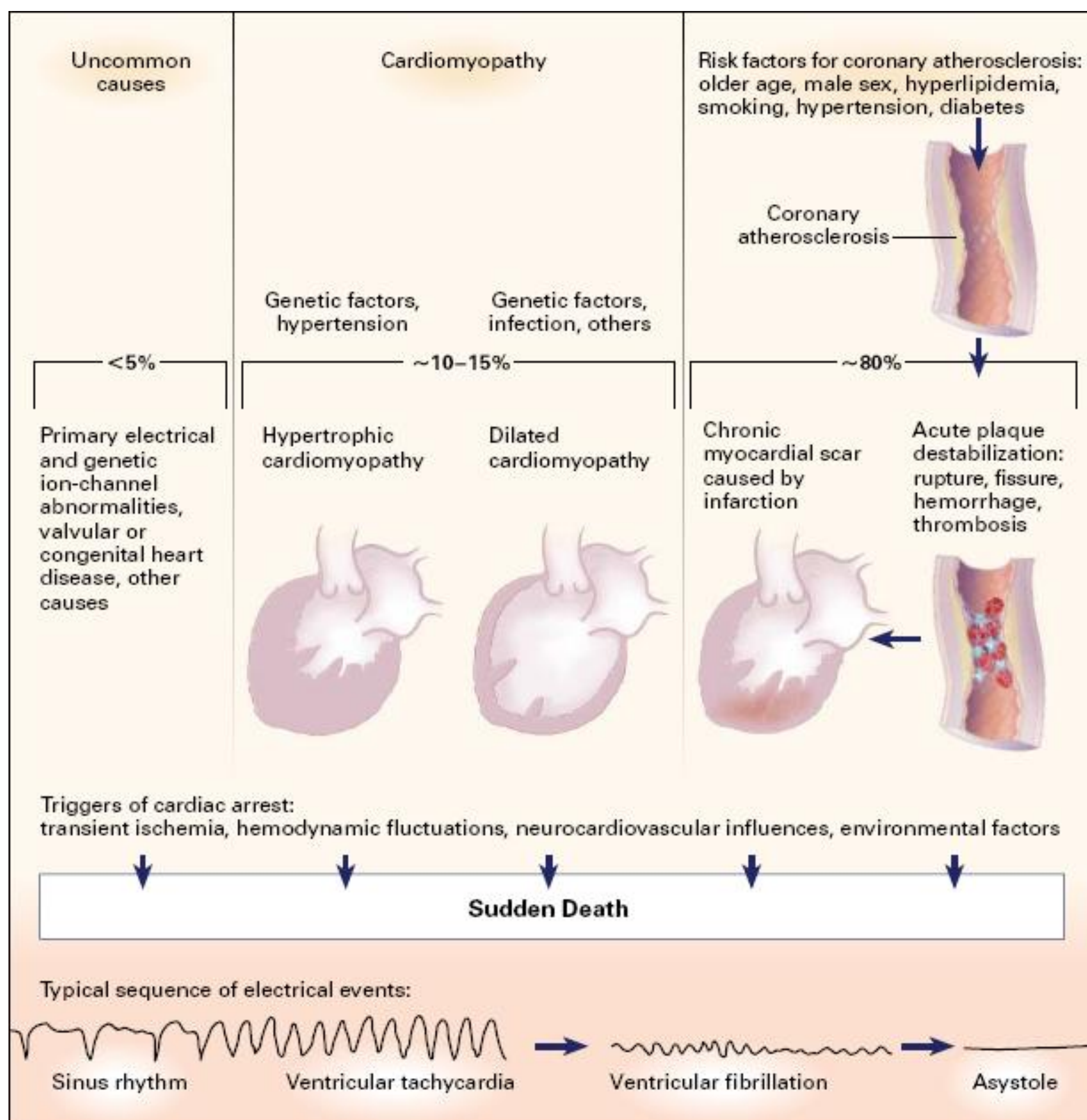

Figure 5: Pathophysiology and epidemiology of SCD [31].

To date, clinical surveys have failed to define risk markers which accurately predict the risk of SCD for individuals in the general population. Those risk factors which have been identified include: abnormal cardiovascular function, e.g. left ventricular ejection fraction (LVEF) [30]; electrocardiographic variables, e.g. QT and QRS measurements as well as T wave alternans [29]; abnormalities in electrophysiological testing [31]; variation in cardiac autonomic function e.g. heart rate variability and baroreflex sensitivity [24] and finally, ambient ventricular arrhythmias [29, 30]. These predictors are appropriate for identifying risk of SCD in highly selected groups, such as those with CAD, congestive heart failure (CHF) and myocardial infarction (MI) survivors. However, as shown in Figure 6, these high risk groups make up a small proportion of total SCD cases per annum. Only 10% of SCD victims have a high risk profile [17]. Members of the general population have a low relative risk of SCD, but the absolute number of deaths is high [24, 29]. This indicates a need for more specific markers to identify risk in the general population.

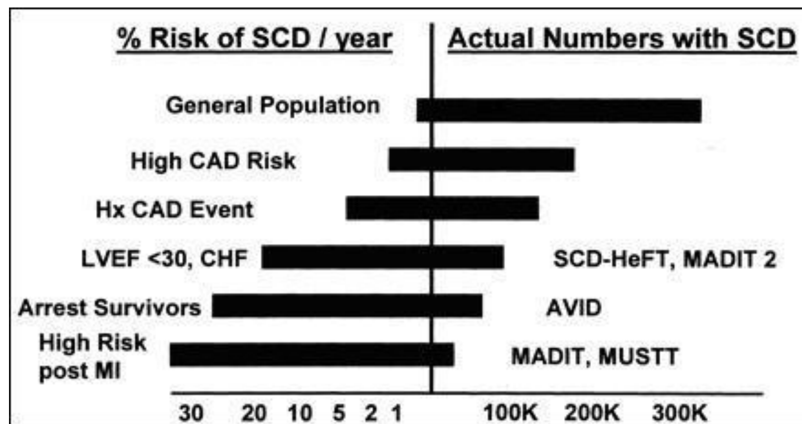

Figure 6: SCD incidence and total events for various population pools, showing an inverse relationship between risk and total number of events [25].

AVID: *Antiarrhythmics vs. Implantable Defibrillators Trial,* ***CAD***: *coronary artery disease,* ***CHF***: *congestive heart failure,* ***Hx***: *history,* ***LVEF***: *left ventricular ejection fraction,* ***MADIT***: *Multicentre Automatic Defibrillator Implantation Trial,* ***MI***: *myocardial infarction,* ***MUSTT***: *Multicentre Unsustained Tachycardia Trial,* ***SCD-HeFT***: *Sudden cardiac death in Heart Failure Trial*

A popular hypothesis is that SCD is an electrical mishap, whereby functional and structural substrates, modulated intrinsic or extrinsic triggers such as electrolyte imbalance or drug intake, impact on the potential arrhythmic mechanisms universal to all hearts (Figure 7).

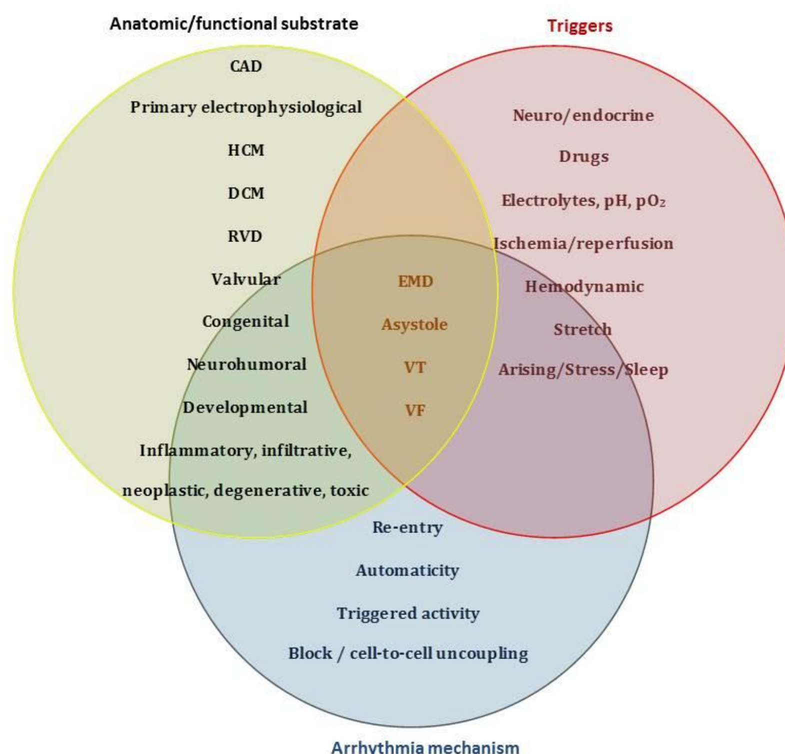

Figure 7: This Venn diagram indicates the potential interactions of various cardiac factors. The factors may modulate latent arrhythmogenic mechanisms capable of causing sudden death [29].
***CAD** – Coronary Artery Disease, **DCM** – Dilated Cardiomyopathy, **EMD** – Electromechanical dissociation,*
***HCM** - Hypertrophic Cardiomyopathy, **RVD** – Right Ventricular Dysplasia, **VT** – Ventricular Tachycardia,*
***VF** - Ventricular Fibrillation.*

1.5 INHERITED ARRHYTHMIA DISORDERS

Primary arrhythmogenic disorders remain the single most important cause of SCD among young, healthy individuals living in industrialised nations [32]. Genetic factors have substantial effects in determining population-based risk for SCD and may also account for inter-individual variability in susceptibility [33]. Inherited arrhythmia disorders encompass a group of syndromes with unique genetic defects and presentations but with very similar clinical outcomes and complications. Much progress has been made in identifying genes underlying various Mendelian disorders associated with inherited arrhythmia susceptibility.

1.5.1 LONG QT SYNDROME

Ventricular repolarisation as represented by the QT interval on an ECG recording (Figure 3 and Figure 8) is subject to a great deal of physiological variability with an upper limit of the QT_c interval of 470ms in post-pubertal males and 480ms in post-pubertal females [34]. Pathologically prolonged QT_c intervals are characteristic ECG findings in long QT syndrome (LQTS). QT_c interval prolongation is a consequence of delayed cardiac repolarization which may be caused by mutations in the genes encoding cardiac ion channels or proteins which modulate

their function; this form of LQTS is called congenital LQTS [1]. Alternatively, and more frequently, delayed cardiac repolarisation and consequent QT prolongation may be acquired.

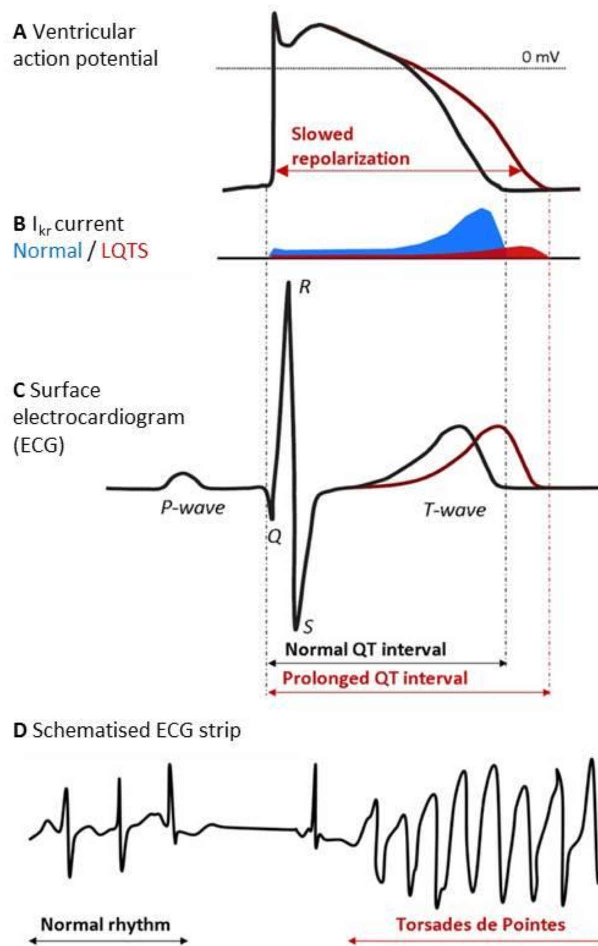

*Figure 8: **A.** Normal and prolonged ventricular action potential. **B.** Normal and reduced I_{Kr} currents. **C.** Normal and prolonged QT interval on an ECG. **D.** A schematic representation of Torsade de Pointes. Figure taken from [35].*

The most frequent mechanism of acquired QT prolongation is the drug-induced inhibition of the I_{Kr} current (Figure 8). In both congenital and acquired LQTS, abnormal cardiac repolarisation predisposes to the development of *torsades de pointes* (TdP), a PVT frequently associated with LQTS and SCD (Figure 8). QT_c prolongation is considered a marker of the arrhythmogenic potential of a drug. However, the relationship between QT_c and SCD is unknown with no clearly defined QT_c length which could be considered the “tipping point” of the risk for developing a potentially fatal arrhythmia [36]. A better understanding of the congenital form of LQTS will undoubtedly also improve our understanding of acquired LQTS.

Congenital LQTS is the focus of the work reported here and the acronym (LQTS) has been used to define the congenital form of LQTS, throughout. Reviews of the genetic bases of LQTS, SQTS and BrS, as well as an editorial describing the implications which LQTS genetic testing have for personalised medicine, follow.

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1.5.2 REVIEW 1: THE GENETIC BASIS OF LONG QT AND SHORT QT SYNDROMES: A MUTATION UPDATE

Paula L. Hedley, Poul Jørgensen, Sarah Schlamowitz, Romilda Wangari, Johanna Moolman-Smook, Paul A. Brink, Valerie A. Corfield and Michael Christiansen.

Human Mutation 30:1486-1511, **2009**.

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MUTATION UPDATE

Human Mutation

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The Genetic Basis of Long QT and Short QT Syndromes: A Mutation Update

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ABSTRACT: Long QT and short QT syndromes (LQTS and SQTS) are cardiac repolarization abnormalities that are characterized by length perturbations of the QT interval as measured on electrocardiogram (ECG). Prolonged QT interval and a propensity for ventricular tachycardia of the torsades de pointes (TdP) type are characteristic of LQTS, while SQTS is characterized by shortened QT interval with tall peaked T-waves and a propensity for atrial fibrillation. Both syndromes represent a high risk for syncope and sudden death. LQTS exists as a congenital genetic disease (cLQTS) with more than 700 mutations described in 12 genes (LQT1–12), but can also be acquired (aLQTS). The genetic forms of LQTS include Romano-Ward syndrome (RWS), which is characterized by isolated LQTS and an autosomal dominant pattern of inheritance, and syndromes with LQTS in association with other conditions. The latter includes Jervell and Lange-Nielsen syndrome (JLNS), Andersen syndrome (AS), and Timothy syndrome (TS). The genetics are further complicated by the occurrence of double and triple heterozygotes in LQTS and a considerable number of nonpathogenic rare polymorphisms in the involved genes. SQTS is a very rare condition, caused by mutations in five genes (SQTS1–5). The present mutation update is a comprehensive description of all known LQTS- and SQTS-associated mutations.
Hum Mutat 30:1486–1511, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: arrhythmia; action potential; cardiac repolarization; sudden death; syncope; ion channel; LQTS; SQTS

Introduction

Long QT and short QT syndromes (LQTS and SQTS) are genetic conditions characterized by perturbed QT intervals on an electrocardiogram (ECG). LQTS is characterized by prolonged QT intervals, syncope, T-wave abnormalities, ventricular tachycardia of the torsades de pointes (TdP) type (see Supp. Figure S1), and an increased risk of sudden death [Crotti et al., 2008]. SQTS, on the other hand, is associated with shortened QT intervals, tall peaked T-waves (see Supp. Figure S2), a propensity for atrial fibrillation (AF), and increased risk of sudden death [Algra et al., 1993; Gussak et al., 2000].

The population prevalence of LQTS (1:2,000 to 1:5,000) [Goldenberg et al., 2008; Crotti et al., 2008] is higher than that of SQTS, which was determined to be very low in population screening studies using shortened rate-corrected-QT (QT_c) interval as a screening parameter [Rautaharju et al., 1992; Funada et al., 2008; Moriya et al., 2007; Anttonen et al., 2007]. LQTS is also associated with highly variable expressivity [Vincent et al., 1992] and incomplete penetrance [Priori et al., 1999]; but the small number of SQTS families precludes a detailed assessment of penetrance patterns. Importantly, the QT interval increases with decreasing heart rate [Surawicz, 1995], making it necessary to use a rate-corrected QT interval ($QT_c = QT/\sqrt{RR}$) when assessing whether the interval is prolonged or shortened [Bazett, 1920].

QT_c-interval prolongation, reflecting prolonged cardiac repolarization (Fig. 1) can also be caused by intake of particular drugs, electrolyte disturbances, and by structural heart diseases [Surawicz, 1995]. It is also seen, albeit rarely, associated with a number of other noncardiac conditions [Surawicz, 1995]. Shortened QT_c-intervals may also be caused by nongenetic causes, such as sinus tachycardia, hyperthermia, hypercalcemia, acidosis, hyperkalemia, and digoxin therapy [Brugada et al., 2005; Cheng, 2004].

The clinical diagnosis of LQTS is made by the use of the diagnostic criteria [Schwartz et al., 1993] given in Table 1. These criteria do not presently involve the results of genetic testing, but such testing is necessary to identify asymptomatic carriers that may otherwise present clinically with sudden death [Schwartz, 2006]. In contrast, there are currently no well-defined criteria for SQTS. It has been established that a short QT_c alone does not mean that the patient has SQTS [Anttonen et al., 2007]. At present, the SQTS diagnosis requires that the condition is familial and that syncope or cardiac events are present in the history of the patient.

Four clinical types of congenital LQTS (cLQTS) have been defined, including: (1) Romano-Ward syndrome (RWS) [Romano et al., 1963; Ward, 1964], with autosomal dominant inheritance and a prevalence of ~1:2,500, where LQTS is present alone; (2) Jervell-Lange Nielsen syndrome (JLNS) [Jervell and Lange-Nielsen, 1957], a much rarer type, with a prevalence of ~1:50.000, where LQTS is associated with congenital deafness and the pattern of inheritance is autosomal recessive [Morita et al.,

Additional Supporting Information may be found in the online version of this article.

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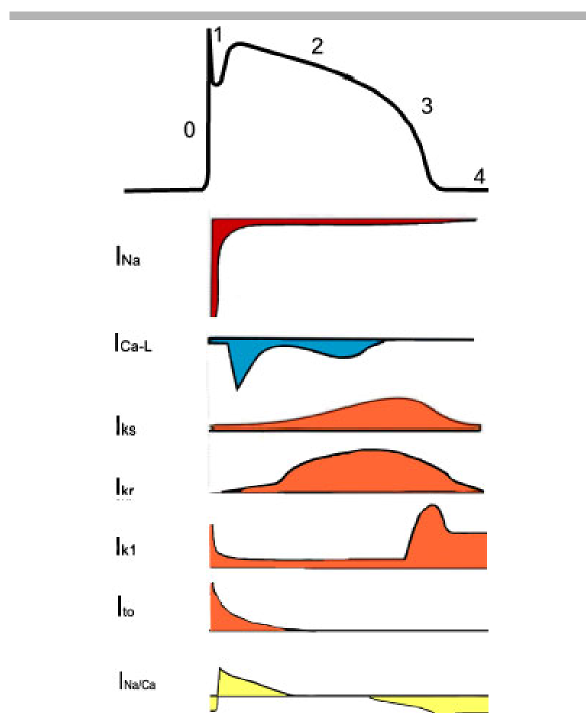

Figure 1. The action potential (AP) and schematic of the currents involved in creating it. The red current represents the sodium current (I_{Na}), which is formed by the Na^+-channel. The blue current represents the long-lasting calcium current ($I_{Ca\text{-}L}$), which is formed by the long-lasting Ca^{2+} channel. The orange currents represent the potassium currents (I_K), which are formed by the K^+-channels: Kv4.3 conducts the I_{to}-current, Kv11.1 conducts the I_{Kr}-current, Kv7.1 conducts the I_{Ks}-current, and Kir2.1 conducts the I_{K1}-current. The yellow current represents the sodium calcium exchange current ($I_{Na/Ca}$), which is formed by the Na^+/Ca^{2+}-exchange channel. The duration of the AP (APD) increases with decreasing repolarization; e.g., by loss-of-function mutations in the repolarizing potassium channels or gain-of-function mutations in genes coding for depolarizing Na^+ and Ca^{2+} channels.

2008]; (3) an even rarer syndromic form, Andersen syndrome (AS), where LQTS is variably present together with other arrhythmias, periodic paralysis, and malformations [Tawil et al., 1994; Tristani-Firouzi et al., 2002]; and (4) the very rare Timothy syndrome (TS), characterized by severe LQTS, cardiac and other somatic malformations, and autism [Splawski et al., 2004]. Furthermore, acquired LQTS (aLQTS), which is much more frequent than cLQTS, and is caused by drug-intake [Kannankeril and Roden, 2007] or structural heart disease [Saffitz, 2005], may have a genetic element.

Presently, mutations in 13 genes involved in the correct execution of the cardiac action potential have been associated with LQTS and SQTS. Four genes have been associated with both LQTS and SQTS (*KCNQ1*, *KCNH2*, *KCNJ2*, and *CACNA1C*), and it appears that the functional effect of the mutation on the protein (viz. loss-of-function or gain-of-function) determines the type of channelopathy that develops. LQTS has been shown to be an ion channelopathy associated with loss-of-function mutations in genes encoding repolarizing K^+-ion channels, their subunits, and certain interacting proteins; i.e., *KCNQ1* [Wang et al., 1996b], *KCNH2* [Itoh et al., 1998a], *ANK2* [Mohler et al., 2003], *KCNE1* [Splawski et al., 1997a,b; Tyson et al., 1997], *KCNE2* [Abbott et al., 1999],

Table 1. Diagnostic Criteria of Long QT Syndrome

		Points
ECG findings[a]		
A.	QT_c[b]	
	≥ 480 ms$^{1/2}$	
	460–470 ms$^{1/2}$	3
	450 (males)	2
		1
B.	Torsades de pointes[c]	2
C.	T-wave alternans	1
D.	Notched T-wave in three leads	1
E.	Low heart rate for age[d]	0.5
Clinical history		
A.	Syncope[c]	
	With stress	2
	Without stress	1
B.	Congenital deafness	0.5
Family history[e]		
A.	Family members with definite LQTS[f]	1
B.	Unexplained sudden cardiac death below age 30 years; among immediate family members	0.5

[a]In the absence of medications or disorders known to affect these electrocardiographic features.
[b]QTc calculated by Bazett's formula, where $QT_c = QT/\sqrt{RR}$.
[c]Mutually exclusive.
[d]Resting heart rate below the second percentile for age [Davignon et al., 1980].
[e]The same family member can not be counted in A and B. Low probability of LQTS is defined by an LQTS score ≤1 point; an intermediate probability of LQTS is defined by an LQTS score of 2 to 3 points; ≥4 points, high probability of LQTS. Modified from Schwartz et al. [1993].
[f]LQTS, long QT syndrome.

KCNJ2 [Plaster et al., 2001], *CAV3* [Vatta et al., 2006], *AKAP9* [Chen et al., 2007], and *SNTA1* [Ueda et al., 2008]. Gain-of-function mutations in genes encoding depolarizing Na^+- and Ca^{2+}-ion channels; i.e., *SCN5A* [Wang et al., 1995a] and *CACNA1C* [Splawski et al., 2005], as well as loss-of-function mutations in interacting proteins; i.e., *SCN4B* [Medeiros-Domingo et al., 2007], have also been associated with LQTS. LQTS genes are listed in Table 2. SQTS has been shown to be an ion channelopathy associated with gain-of-function mutations in the genes encoding repolarizing K^+- ion channels; i.e., *KCNQ1* [Bellocq et al., 2004a; Hong et al., 2005], *KCNH2* [Brugada et al., 2004; Cordeiro et al., 2005], and *KCNJ2* [Priori et al., 2005]. Loss-of-function mutations in *CACNA1C* [Antzelevitch et al., 2007d] and *CACNB2* [Antzelevitch et al., 2007d], encoding the α- and β2-subunits of the Cav1.2 channel, which is responsible for conducting the depolarizing long-lasting Ca^{2+} current ($I_{L,Ca}$), have also been associated with SQTS. SQTS genes are listed in Table 3.

This mutation update reviews the genetics of LQTS and SQTS and presents a comprehensive list of known mutations associated with these two conditions.

Overview of the Pathogenetic Mechanism

As the QT interval represents the combined cardiac depolarization and repolarization, a perturbation of the QT interval may be caused by changes in the function of the ion channels responsible for the timely execution of the cardiac action potential (AP). The AP is the potential registered over the cardiomyocyte plasma membrane during one contraction; i.e., one systole and the following diastole (Fig. 1). The AP is the result of a carefully orchestrated activity of a number of ion channels influenced by various effector systems; e.g., the β-adrenergic system. The ion channels of major significance for the AP and their individual time–voltage relationship are shown in Figure 1. A decrease in the

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Table 2. The Genes Associated With Long QT Syndrome

Type	Syndrome	Gene	Chromosomal localization	Protein	Function	Mechanism	Characteristics and triggers	Prevalence in LQTS patients (%)
LQT1	RWS, JLNS	*KCNQ1*	11p15.5	Kv7.1	α-subunit I_{Ks}	Loss-of-function	Arrhythmia triggered by exercise, swimming and emotion	40–55
LQT2	RWS	*KCNH2*	7q35–7q36	Kv11.1	α-subunit I_{Kr}	Loss-of-function	Arrhythmia triggered by sound or emotion	35–45
LQT3	RWS	*SCN5A*	3p21	Nav1.5	α-subunit I_{Na}	Gain-of-function	Arrhythmia triggered by sleep, rest and emotion	2–8
LQT4	RWS	*ANK2*	4q25–4q27	Ankyrin B	Adaptor (I_{Na-K}, I_{Na-Ca}, I_{Na})	Loss-of-function	Arrhythmia triggered by exercise	<1
LQT5	RWS, JLNS	*KCNE1*	21p22	minK	β-subunit I_{Ks}	Loss-of-function	Arrhythmia triggered by exercise and emotion	<1
LQT6	RWS	*KCNE2*	21p22	MiRP1	β-subunit I_{Kr}	Loss-of-function	Arrhythmia triggered by rest and exercise	<1
LQT7	AS	*KCNJ2*	17q23.1–17q24.2	Kir2.1	α-subunit I_{K1}	Loss-of-function	Syndromic, arrhythmia triggered by rest and exercise, frequent ectopy	<1
LQT8	TS	*CACNA1C*	12p13.3	Cav1.2	α-subunit I_{Ca}	Gain-of-function	Syndromic, early onset and death from arrhythmia	<1
LQT9	RWS	*CAV3*	3p25	M-Caveolin	Adaptor (I_{Na})	Loss-of-function	Rest and sleep triggers arrhythmia	<1
LQT10	RWS	*SCN4B*	11q23	Navβ4	β-subunit I_{Na}	Loss-of-function	Exercise triggers arrhythmia	<0.1
LQT11	RWS	*AKAP9*	7q21–7q22	Yotiao	Adaptor(I_{Ks})	Loss-of-function	Exercise triggers arrhythmia	<0.1
LQT12	RWS	*SNTA1*	20q11.2	α1-Syntrophin	Scaffolding protein (I_{Na})	Loss-of-function	Rest triggers arrhythmia	<0.1

RWS, Romano-Ward syndrome; JLNS, Jervell and Lange-Nielsen syndrome; AS, Andersen syndrome; TS, Timothy syndrome.

repolarizing outward K^+ currents or an increase in the depolarizing inward Na^+ and Ca^{2+} currents involved in the AP will lead to a prolongation of the QT interval. Conversely, an increase in the repolarizing outward K^+ currents or a decrease in the Ca^{2+} currents involved in the AP will lead to a shortening of the QT interval. The prolongation of the QT interval is associated with increased refractoriness and the occurrence of early-after-depolarizations (EADs) due to enhancement of the Na^+/Ca^{2+} exchange current and reactivation of the L-type Ca^{2+} channel [Antzelevitch, 2007b]; these EADs may trigger arrhythmias. However, in LQT7, where the late-acting I_{K1} current is decreased, delayed-after-depolarizations trigger arrhythmias [Tristani-Firouzi et al., 2002]. The shortening of the QT interval, on the other hand, is associated with a decreased period of refractoriness due to the shorter AP duration and an increase spatial heterogeneity of repolarization. Both mechanisms increase the risk of reentry arrhythmia [Antzelevitch, 2007b].

Whereas the trigger for TdP in LQTS is considered to be EADs, the substrate for the arrhythmia is believed to be an increase in transmural dispersion of repolarization (TDR) disposing to reentrant arrhythmias [Antzelevitch and Shimizu, 2002]. The increased TDR is the result of a differential distribution in the heart of the ion channels involved in the AP. Thus, endo- and epicardial cardiomyocytes have a more pronounced repolarizing I_{Ks} than the mid-myocardial (M) cells [Sicouri et al., 1994; Liu and Antzelevitch, 1995]. On the contrary, the depolarizing late I_{Na} [Zygmunt et al., 2001] and I_{Na-Ca} currents [Zygmunt et al., 2000] are more pronounced in M-cells. Consequently, the decreased repolarization results in a longer AP duration in M-cells as compared to endo- and epicardial cells [Antzelevitch, 2007a]. In accordance, animal model studies have demonstrated a more pronounced increase in repolarization in the M-cells in canine arterially perfused ventricular wedge preparation models of LQT1, LQT2, and LQT3 [Shimizu and Antzelevitch, 2000; Antzelevitch, 2007b]. In addition, as the spatial dispersion of the repolarization increases, the dispersion of refractoriness increases [Antzelevitch, 2007a], thereby providing a substrate for reentry tachycardia and TdP. The increased TDR may be accentuated by increased β-adrenergic activity in some forms of cLQTS (predominantly LQT1) [Tanabe et al., 2001] and by electrolyte changes or as a result of intake of drugs resulting in a reduction of the "repolarization reserve" [Roden, 2006]. An indirect confirmation of the role of TDR in TdP is the value of the $T_{peak}-T_{end}$ interval (an expression of the dispersion of repolarization) in predicting TdP in aLQTS [Yamaguchi et al., 2003b]. For instance, the power of $T_{peak}-T_{end}$ is even better than that of the QT_c in the prediction of TdP in troponin I-associated hypertrophic cardiomyopathy [Shimizu et al., 2002]. However, the predictive power of $T_{peak}-T_{end}$ has not been significant when the different genetic etiologies were also included in a multivariate prognostic algorithm [Kanters et al., 2008].

Transmural dispersion has also been shown to be significantly higher in SQTS patients than in normal controls [Anttonen et al., 2008]. Indeed, a computer simulation study showed that V307L in Kv7.1 increases the transmural heterogeneity and reduces the period of refractoriness [Zhang et al., 2008a]. A zebrafish model of SQTS based on the *reggae* mutation, where the zebrafish ether-a-go-go-related gene (zERG) is mutated so that the repolarizing ERG potassium channel exhibits increased activation and defective inactivation, is associated with a shortened QT interval and increased propensity for cardiac fibrillation. Pharmacological inhibition of the zERG channel normalized the phenotype. This model confirms the association between increased repolarization

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Table 3. The Genes Associated With Short QT Syndrome

SQTS type	Gene name	Chromosomal location	Protein name	Function	SQTS mechanism
SQT1	*KCNH2*	7q35–7q36	Kv11.1	α-subunit I_{Kr}	Gain-of-function
SQT2	*KCNQ1*	11p15.5	Kv7.1	α-subunit I_{Ks}	Gain-of-function
SQT3	*KCNJ2*	17q23.1–17q24.2	Kir2.1	α-subunit I_{K1}	Gain-of-function
SQT4	*CACNA1C*	12p13.3	Cav1.2	α-subunit $I_{L,Ca}$	Loss-of-function
SQT5	*CACNB2*	10p12	Cavβ2	β2-subunit $I_{L,Ca}$	Loss-of-function

and SQTS and the application of the model may aid in defining a pharmacological treatment of SQTS [Hassel et al., 2008]. A spontaneous animal model of SQTS that is currently not genetically characterized is seen in the red kangaroo, where a short QT_c interval is found and sudden death is very common [Campbell, 1989].

Furthermore, the increased propensity for arrhythmia in LQTS is also present in the atria and may result in AF or "atrial TdP" in LQTS [Kirchhof et al., 2000]. In SQTS, the co-occurrence of SQTS and AF can be explained by the reduced action potential duration (APD) and the resulting stability of wavelets in the atria [Moe, 1975; Moe et al., 1964]. However, some familial/genetic forms of AF caused by mutations in the genes *KCNE2* [Yang et al., 2004], *KCNQ1* [Chen et al., 2003b], and *KCNE5* [Ravn et al., 2008] are not associated with sudden death, despite the fact that their electrophysiological phenotype is increased repolarization.

The particular pathophysiological mechanism associated with mutations in each particular gene is given below. It should be noted that the mere finding of a sequence variant in any of the genes associated with LQTS and SQTS is not sufficient to claim that this is the cause of either disease. The number of polymorphisms in these genes is considerable, so suspected disease-associated variants should also be shown not to be present in 200 relevant population controls. Even this may not be sufficient to avoid associating a rare variant, with no functional significance, to disease in a particular case. Only very rarely is it possible to support the pathological significance of a mutation by genetic linkage; mostly cosegregation with disease has to suffice. A strong support for pathogenicity is obtained if functional analysis of the effect of a particular mutation, in the case of LQTS and SQTS most often through electrophysiological studies of mutated ion channel subunits expressed in vitro, is compatible with an already established pathophysiological mechanism. Far from all mutations described in this mutation update have been functionally analyzed or are linkage-supported, but they still represent a comprehensive collection of the LQTS and SQTS mutations that are reasonably substantiated. Moreover, as will be described later, sometimes the disease phenotype is the result of a complicated interaction between mutations in several genes and an even larger number of disease-modifying alleles.

Therapeutic Options

The main therapeutic option for cLQTS is antiadrenergic intervention with β-blockers [Moss et al., 2000] and, in some cases, insertion of an implantable cardioverter defibrillator (ICD) or sympathetic denervation [Schwartz et al., 2004; Goldenberg et al., 2008]. The effect of β-blockers is dependent on the genotype [Moss et al., 2000; Kass and Moss, 2006; Priori et al., 2004]. The standard treatment of SQTS, on the other hand, is implantation of an ICD [Boriani et al., 2006], which has been shown to save patients from sudden arrhythmic death, despite a considerable risk of inappropriate discharges due to misinterpretation of frequent short-coupled tall T-waves as an arrhythmia [Schimpf et al., 2003, 2005]. The use of pharmacological treatment, e.g., quinidine, which inhibits both I_{Kr} and I_{Ks} [Patel and Antzelevitch, 2008], or disopyramide, which may be effective for SQT1 [Schimpf et al., 2007], is normally only considered in cases where ICD treatment is difficult to institute, e.g., in young children [Patel and Antzelevitch, 2008].

Mutations in *KCNQ1* (LQT1 and SQT2)

The potassium voltage-gated channel, KQT-like subfamily, member 1 gene (*KCNQ1*; MIM♯ 607542) encodes the α-subunit of the slow producing voltage-gated potassium channel (Kv7.1, previously known as KvLQT1) (Fig. 2A) conducting the potassium channel current I_{Ks} (Fig. 1). The *KCNQ1* gene is encompassed by 404 kb at 11p15.5 [Wang et al., 1996b] and encodes a 75-kDa protein containing 676 amino acids [Yang et al., 1997] that is strongly expressed in the heart. Hydrophobicity analysis predicts a classic voltage-dependent potassium channel topology with six transmembrane segments (of the Shaker type) and a long unique C-terminal cytoplasmic domain (Fig. 2A) [Barhanin et al., 1996]. By genomic sequence analysis, Splawski et al. [1998] found that the *KCNQ1* gene contains 16 exons ranging in size from 47 to 1,122 bp (Fig. 2C).

In order to define the function of *KCNQ1*, Sanguinetti et al. [1996] and Barhanin et al. [1996] performed transfection studies with *KCNQ1* cDNA using Chinese hamster ovary (CHO) cells and COS cells, respectively. Both groups found cotransfection with *KCNE1* was required to form the I_{Ks} current. Melman et al. [2002] showed that multiple segments of Kv7.1, including the pore and C-terminus, bind the accessory proteins encoded by *KCNE1* and *KCNE3*. They demonstrated that all KCNE-binding sites of Kv7.1 are required for proper regulation by the accessory subunit.

Over 250 mutations in *KCNQ1* have been reported to cause LQTS type 1 (LQT1; MIM♯ 192500) (Supp. Table S1; and www.ssi.dk/lqt) [Morita et al., 2008]. Only two mutations have been reported to cause SQTS type 2 (SQT2; MIM♯ 609621) (Table 4) [Bellocq et al., 2004a]. Functional studies of mutated Kv7.1 indicate multiple biophysical consequences as a result of altering the function of I_{Ks}. Functional investigation of *KCNQ1* mutations have shown them to result in defective trafficking [Gouas et al., 2004] and dominant-negative (loss-of-function) effects [Shalaby et al., 1997]. Moreover, several mutations have been reported to affect binding of interacting proteins. Park et al. [2005] electrophysiologically characterized the LQT1-causing mutations, p.R243H, p.R539W, and p.R555C. These mutations increase the rate of dissociation of phosphatidylinositol-4,5-bisphosphate (PIP2) from the Kv7.1-minK channel, which decreases the number of open-state channels in the membrane. In addition to reducing the channel affinity for PIP2, the R243C mutation alters the voltage sensor [Park et al., 2005]. The p.G589D mutation, on the other hand, has been shown by coimmunoprecipitation experiments to prevent the binding of a macromolecular

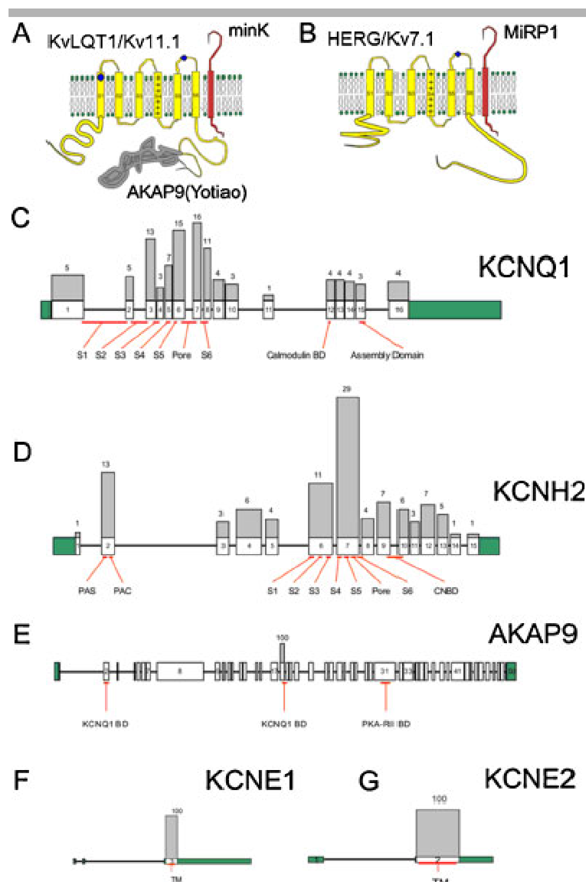

Figure 2. A,B: Schematic drawings of the ion channel complexes involving K^+-ion channels. The locations of the mutations known to cause SQTS are indicated by blue dots. The genomic structure with the basic structural elements is indicated, as well as the relative distribution of mutations per exon (in percent of total number of LQTS mutations in the gene) is shown for the involved genes, *KCNQ1* (**C**), *KCNH2* (**D**), *AKAP9* (**E**), *KCNE1* (**F**), and *KCNE2* (**G**). BD: binding domain; CNBD: cyclic nucleotide binding domain; PAC: PAS-associated C-terminal domain; PAS: domain containing the Per-ARNT-Sim motif; S1–6: transmembrane segments 1–6; TM: transmembrane domain.

signaling complex that is required for the cAMP-dependent regulation of the Kv7.1-minK channel [Marx et al., 2002].

Expression of the SQT2-causing mutation, p.V307L (Fig. 2), in COS-7 cells indicated that the mutation causes a gain-of-function of the channel. The I_{Ks} current displayed accelerated activation kinetics and a positive voltage shift in the tail current. Computer modeling demonstrated that this change in electrophysiology parameters would result in enhanced repolarization and shortened QT interval [Bellocq et al., 2004a]. A second SQT2 mutation, p.V141M (Fig. 2), was expressed in oocytes, with minK; the I_{Ks} current developed instantly, which indicated that the Kv7.1-minK channel was constitutively open; suggesting that this mutation causes a gain-of-function of the channel. This effect was less when mutant Kv7.1 was expressed with WT Kv7.1, suggesting the existence of heteromultimer s in the tetrameric Kv7.1 ion channel. Computer modeling showed that the effect of the p.V141M mutation would be shortened repolarization and SQTS [Hong et al., 2005].

In addition to causing LQT1 and SQT2, mutations in *KCNQ1* have been associated with familial AF [Chen et al., 2003a], sudden infant and adult death syndromes (SIDS/SADS) [Ackerman, 2005; Arnestad et al., 2007], and aLQTS. Additionally, homozygous or compound heterozygous mutations of *KCNQ1* cause JLNS type 1 (JLN1).

Discrepancies in the codon numbers of the allelic variants exist because of changes in information about the sequence of *KCNQ1*. Thus, for example, the p.A341V mutation, one of the most frequent causes of type 1 LQTS, was denoted p.A212V by Wang et al. [1996b] and p.A246V by Li et al. [1998]. LQTS-associated mutations in *KCNQ1* are listed in Supp. Table S1 using a single genomic reference.

Mutations in *KCNH2* (LQT2 and SQT1)

The potassium voltage-gated channel, subfamily H, member2 gene (*KCNH*; MIM♯ 15427) encodes the α-subunit of the voltage-gated potassium ion channel (Kv11.1, previously known as HERG) (Fig. 2B), conducting the cardiac rapidly activating delayed rectifier K^+ current (I_{Kr}) (Fig. 1) [Trudeau et al., 1995; Sanguinetti et al., 1995]. *KCNH2* encompasses 33 kb at 7q35–7q36 and encodes a protein of 1159 amino acids that is mainly expressed in the heart [Curran et al., 1995]. The genomic structure of *KCNH2* consists of 15 exons (Fig. 2D) [Itoh et al., 1998a].

Table 4. Mutations Causing SQT1–SQT5*

mRNA	AA	Mutation type	Genomic region	Protein region	References
SQT1: KCNH2/Kv11.1; NM_000238.2/NP_000229.1					
c.1764C>G	p.N588K	Missense	Exon_7	S5-pore	Brugada et al. [2004]
c.1764C>A	p.N588K	Missense	Exon_7	S5-pore	Brugada et al. [2004]
SQT2: KCNQ1/Kv7.1; NM_000218.2/NP_000209.2					
c.421G>A	p.V141M	Missense	Exon_2	S1	Hong et al. [2005]
c.919G>C	p.V307L	Missense	Exon_6	Pore	Bellocq et al. [2004a]
SQT3: KCNJ2/Kir2.1 α-subunit; NM_000891.2/NP_000882.1					
c.514G>A	p.D172N	Missense	Exon_2	M2	Priori et al. [2005]
SQT4: CACNA1C/Cav1.2 α-subunit; NM_000719.5/NP_000710.5					
c.116C>T	p.A39V	Missense	Exon_2	N-terminus	Antzelevitch et al. [2007d]
c.1468G>A	p.G490R	Missense	Exon_10	DI–DII	Antzelevitch et al. [2007d]
SQT5: CACNB2/Cavβ2; NM_000724.3/NP_000715.2					
c.1442C>T	p.S481L	Missense	Exon_13	C-terminus	Antzelevitch et al. [2007d]

*Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

The observation that Kv11.1 alone produced a K^+ current similar to I_{Kr}, except for drug sensitivity [Sanguinetti et al., 1995], led to a search for a partner protein that could modulate the current properties. Coexpression studies, in transformed CHO cells, indicated that minK and Kv11.1 coassemble; however, the resulting current is not different from that produced by Kv11.1 alone [McDonald et al., 1997]. MiRP1, encoded by *KCNE2*, was also shown to coassemble with Kv11.1 and to modulate the resulting current kinetics to closely resemble I_{Kr} [Abbott et al., 1999].

Over 300 mutations in *KCNH2* have been reported to cause LQTS type 2 (LQT2; MIM♯ 152427) [Curran et al., 1995] (Supp. Table S2; and www.ssi.dk/lqt). To date, two mutations have been reported to cause SQTS type 1 (SQT1; MIM♯ 609620) (Table 4) [Brugada et al., 2004]. Many of the LQT2-causing mutations cause trafficking deficiencies, thereby causing a decrease in I_{Kr} [Anderson et al., 2006], while others form nonfunctional channels or channels with abnormal gating properties [Zhou et al., 1998]. The p.K28E mutation was determined to affect both trafficking and gating [Rossenbacker et al., 2005]. Choe et al. [2006] reported that three C-terminal truncating mutations (p.G965X, p.R1014fs, p.V1038fs) displayed dominant-negative behavior. The mutated channels interacted with 14-3-3ε, an adaptor protein involved in β-adrenergic stimulation of Kv11.1 function.

In a genetic analysis of three unrelated SQTS families, Brugada et al. [2004] identified missense mutations in *KCNH2* in two families (see Fig. 2). The two different nucleotide substitutions occurred within the same codon and resulted in the same amino acid change (p.N588K). In both families, several affected family members were heterozygous mutation carriers. Expression in TSA201 cells revealed that the mutated ion channel had much higher current amplitude and the rectification characteristic of I_{Kr} was absent. This indicates that p.N588K is a gain-of-function mutation. Interestingly, the sensitivity to I_{Kr}-blocking drugs was much reduced [Brugada et al., 2004]. A metabolic-based gain-of-function of Kv11.1 has been noted in myocardial ischemia and is considered to be the basis of the shortened QT interval and the associated propensity for arrhythmia in ischemic heart disease [Bai et al., 2007].

Mutations in *KCNH2* have also been associated with SIDS [Christiansen et al., 2005] and 9.5% of SIDS victims have been shown to harbor mutations in LQTS-related genes; of these 19% were *KCNH2* mutations [Arnestad et al., 2007]. Furthermore, it has been demonstrated that SIDS-related *KCNH2* mutations cause functional defects similar to those exhibited in LQTS [Rhodes et al., 2008].

Many common polymorphisms (see below) have been identified in *KCNH2*, most of which are thought to have no functional impact on I_{Kr}. However, it has been shown that common polymorphisms can act to modify the clinical expression of latent LQTS mutations, in some cases to exaggerate an otherwise modest effect of a mutation on I_{Kr} [Crotti et al., 2005]. Additionally, it has been shown that common polymorphisms, although they apparently undergo wild-type (WT)-like biochemical processing, in some cases, exhibit small detectable differences in their physiological properties [Anson et al., 2004]. The most frequent cause of aLQTS is inhibition of the I_{Kr} by Kv 11.1 blockers [Roden and Viswanathan, 2005].

Mutations in *SCN5A* (LQT3)

The sodium voltage-gated channel, type V, alpha subunit gene (*SCN5A*; MIM♯ 600163) encodes the α-subunit of the cardiac sodium channel, Nav1.5, conducting the depolarizing sodium inward current (I_{Na}) (Fig. 1) [Gellens et al., 1992]. The genomic structure of *SCN5A* contains 28 exons spanning approximately 80 kb (Fig. 3B) [Wang et al., 1996c]; the gene has been mapped to chromosome 3p21 using fluorescent in situ hybridization [George et al., 1995]. Several splice forms exist and this should be taken into account when mutations are located. The deduced 2,016–amino acid protein has a structure similar to that of other depolarizing sodium channels [Gellens et al., 1992].

Nav1.5 contains four homologous domains, DI–DIV, each of which has six putative membrane-spanning regions, S1–S6 (see Fig. 3A) [Gellens et al., 1992]. The DI–DIV domains are connected via cytoplasmic linkers. S5 and S6 in each domain are connected via a pore segment that lines the outer pore of the ion channel [Fozzard and Hanck, 1996]. Three fundamental gating processes characterize the Nav1.5 channel [Yang et al., 1996]: activation, fast inactivation, and slow inactivation. Activation is a process whereby the channel opens and Na^+ enters the cell; it is the basic biophysical process behind cardiac depolarization. Fast inactivation is a process whereby the channel closes quickly (sometimes so quickly that some channels are inactivated prior to

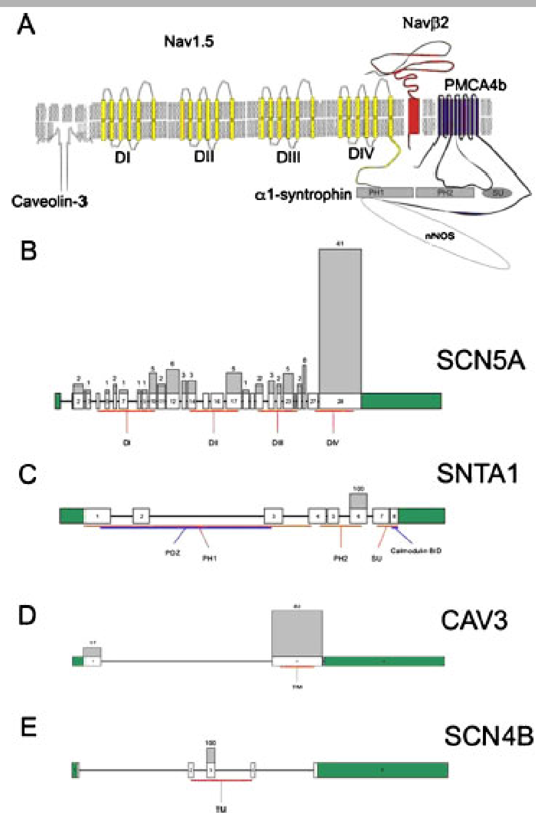

Figure 3. **A:** Schematic drawing of the ion channel complex conducting the I_{Na} current. The genomic structure with the basic structural elements is indicated, as well as the relative distribution of mutations per exon (in percent of total number of LQTS mutations in the gene) is shown for the involved genes, *SCN5A* (**B**), *SNTA1* (**C**), *CAV3* (**D**), and *SCN4B* (**E**). BD: binding domain; DI–IV: domains 1–4; PDZ: PDZ structural domain; PH1: pleckstrin homology domain1; PH2: pleckstrin homology domain2; SU: syntrophin unique domain; TM: transmembrane domain.

closing: "closed-state-inactivation") and then quickly opens again ("fast reactivation"). Slow inactivation is a process whereby the channel closes for >100 milliseconds and is only slowly reactivated. The S4 segments are paramount for fast inactivation [Kontis and Goldin, 1997; Kontis et al., 1997]. The DIII–S4 and DIV–S4 segments, the DIII–DIV cytoplasmic linker, and C-terminal segments are important for fast inactivation and closed-state inactivation [Cha et al., 1999; McPhee et al., 1998]. The P-segments and the DIV–S4 segment are involved in slow inactivation [Balser, 2001].

Mutations in *SCN5A* were initially found in four families with LQT linked to chromosome 3 [Wang et al., 1995b]. Since then, many mutations in all domains of Nav1.5 have been associated with LQTS (LQT3; MIM♯ 603830) (Supp. Table S3; and www.ssi.dk/lqt). Mutations causing LQT3 are characterized by producing increased late I_{Na} and consequently prolonged repolarization [Bennett et al., 1995; Napolitano et al., 2003]. The functional characteristics of *SCN5A* mutations have been analyzed in mammalian cell lines, as well as in mouse model systems [Nuyens et al., 2001; Papadatos et al., 2002]. Frequently LQTS-associated mutations interfere with fast inactivation [Bennett et al., 1995; Dumaine et al., 1996; Wang et al., 1996a]. A good example of this is the p.1505–1507del mutation in the DIII–DIV linker [Wang et al., 1995b], which interferes with this region's function in fast inactivation. Furthermore, the p.T1304M mutation in DIII–S4 [Wattanasirichaigoon et al., 1999] and p.R1623Q mutation in the DIV–S4 [Kambouris et al., 1998] both interfere with the attachment of the DIII–DIV linker to the pore and result in reduced fast inactivation. All three of these mutations cause LQT3. Sixty-five percent of LQT3-causing mutations are found in exons 20–28 of *SCN5A* (Fig. 3B), reflecting the importance of reduced fast inactivation in the pathogenesis of LQT3.

Following the identification of LQT3-causing mutations in *SCN5A*, several other genetically heterogenous disorders were associated with *SCN5A*-mutations; i.e., sick sinus node syndrome [Makita et al., 2008], conduction disease [Tan et al., 2001], Brugada syndrome (BrS) [Remme et al., 2008; Baroudi and Chahine, 2000; Hedley et al., 2009], AF [Darbar et al., 2008], arrhythmia in association with acute myocardial infarction [Hu et al., 2007], dilated cardiomyopathy (DCM) [Olson et al., 2005], and mixed clinical phenotypes, including structural heart disease, have been identified [Makita et al., 2008; Olson et al., 2005; McNair et al., 2004; Saffitz, 2005]. Furthermore, polymorphisms (see below) in *SCN5A* have been described that modify the clinical phenotype of *SCN5A*-caused disease [Viswanathan et al., 2003; Niu et al., 2006].

Mutations in *ANK2* (LQT4)

The Ankyrin-2 gene (*ANK2*; MIM♯ 600919) codes for ankyrin B, a member of a family of adaptor proteins (ankyrin-R, ankyrin-B, and ankyrin-G, and tissue-specific splice forms) involved in the targeting of proteins to, and maintenance of membrane protein complexes at, specialized membrane microdomains associated with the actin/spectrin cytoskeleton [Cunha and Mohler, 2006]. Ankyrins transport several ion channels and transporters, including the macromolecular complex of the IP3 receptor, Na^+/K^+ ATPase, and the Na^+/Ca^{2+} exchanger [Mohler and Bennett, 2005] to excitable membrane domains, and are of major importance for normal cardiac excitability [Cunha and Mohler, 2006; Mohler and Bennett, 2005]. The *ANK2* gene is located at chromosome 4q25–4q27 [Tse et al., 1991] and is composed of 46 exons, of which exon 38 is brain-specific (Fig. 4) [Cunha et al., 2008]. Several splice forms exist of each ankyrin and in the heart the predominant splice form is a 220-kDa protein composed of an N-terminal spectrin-binding domain (SBD), a death domain (DD), and a C-terminus [Cunha and Mohler, 2006].

The first LQTS-associated mutation in *ANK2* (LQT4; MIM♯ 600919) was described in a family with LQT, AF, and sinus node dysfunction, where the disease previously had been linked to 4q25–4q27 [Schott et al., 1995; Mohler et al., 2003]. This mutation and others found later (Table 5; and www.ssi.dk/lqt) were shown to result in loss-of-function [Mohler et al., 2004], and carriers of these mutations exhibit a complex and varied set of phenotypes, including bradycardia, sinus arrhythmia, idiopathic ventricular fibrillation, catecholaminergic polymorphic ventricular tachycardia, and sudden death [Le et al., 2008; Mohler et al., 2007]. This is most likely due to the abnormal coordination of multiple functionally-interconnected proteins; e.g., Na^+/Ca^+ exchanger, involved in Ca^{2+}-release cardiac excitability [Mohler et al., 2003]. Particularly, the inconsistent presence of a prolonged QT_c-interval prompted the definition of a more complex syndrome, "ankyrin-b syndrome" [Mohler et al., 2007]. The degree of functional loss of ankyrin B function seems correlated with clinical severity of disease [Mohler et al., 2007].

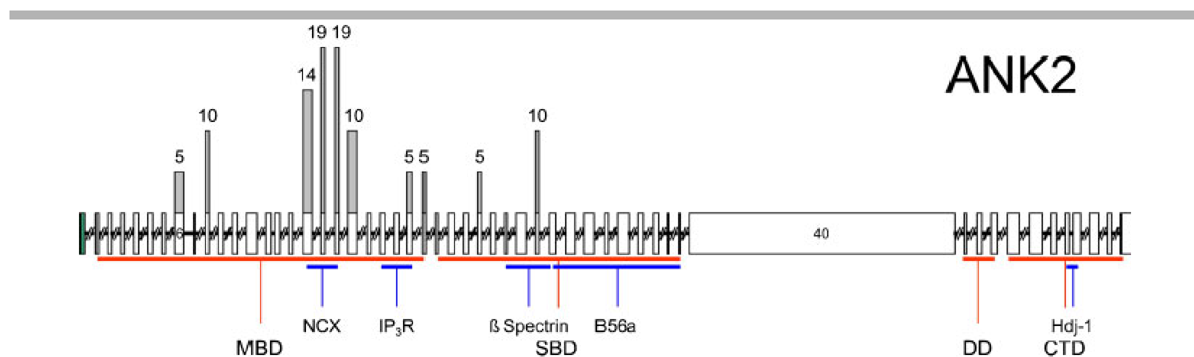

Figure 4. The genomic structure of *ANK2*, with the basic structural elements indicated and a relative distribution of mutations per exon (percent of total number of mutations in the gene). B56a: protein phosphatase 2A regulatory subunit B56α binding domain; CTD: carboxyl terminal domain; DD: death domain; Hdj-1: molecular chaperone HSP40/Hdj-1 binding domain; IP_3R: inositol 1,4,5-triphosphate receptor binding domain; MBD: membrane binding domain; NCX: sodium calcium exchanger binding domain; SBD: spectrin binding domain.

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Table 5. LQT4 Mutations in *ANK2**

mRNA: NM_020977.3	AA: NP_066187.2	Mutation type	Genomic region	Protein region	Phenotype	References
c.1423G>A	p.G475R	Missense	13	ANK13	TdP	Mank-Seymour et al. [2006]
c.2060A>G	p.N687S	Missense	18	ANK19	TdP	Mank-Seymour et al. [2006]
c.2122G>A	p.V708M	Missense	18	ANK20	TdP	Mank-Seymour et al. [2006]
c.4310_4311delinsTA	p.T1437I	Missense	34	Spectrin-BD	RWS/bradycardia/DITdP/AF	Mohler et al. [2007]
c.4373A>G	p.E1458G	Missense	35	Spectrin-BD	RWS	Mohler et al. [2003]
c.4453G>A	p.E1485K	Missense	35	DD	syncope/U-waves/bradycardia	Sherman et al. [2005]
c.4606C>G	p.L1536V	Missense	39	DD	syncope/U-waves/bradycardia	Sherman et al. [2005]
c.4645G>A	p.V1549I	Missense	39	DD	RWS/syncope/CA	Sherman et al. [2005]
c.4754C>A	p.T1585N	Missense	40	DD	AF/DITdP/exercise-induced VT/bradycardia/syncope/BrS/CPVT	Mohler et al. [2007]
c.4831G>A	p.E1611K	Missense	40	DD	AF/DITdP/exercise-induced VT/bradycardia/syncope/BrS/CPVT	Mohler et al. [2007]
c.4963C>A	p.L1655I	Missense	41	DD	AF/DITdP/exercise-induced VT/bradycardia/syncope/BrS/CPVT	Mohler et al. [2007]
c.4976C>A	p.T1659N	Missense	41	DD	AF/DITdP/exercise-induced VT/bradycardia/syncope/BrS/CPVT	Mohler et al. [2007]; Sherman et al. [2005]
c.5261G>C	p.S1754T	Missense	42	DD	AF/DITdP/exercise-induced VT/bradycardia/syncope/BrS/CPVT	Mohler et al. [2007]
c.5428G>A	p.V1810M	Missense	42	C-term	AF/DITdP/exercise-induced VT/bradycardia/syncope/BrS/CPVT	Mohler et al. [2007]
c.5461C>T	p.R1821W	Missense	43	C-term	syncope/RWS/bradycardia	Mohler et al. [2004]; Sherman et al. [2005]
c.5536G>A	p.E1846K	Missense	43	C-term	AF/DITdP/TdP/syncope	Mohler et al. [2007]

*Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. AF: atrial fibrillation; BD: binding domain; BrS: Brugada's syndrome; CA: cardiac arrest; CPVT: catecholaminergic polymorphic ventricular tachycardia; DD: death domain; DITdP: drug induced *torsades de pointes*; pVT: polymorphic ventricular tachycardia; RWS: Romano-Ward syndrome; SCD: sudden cardiac death; SUNDS: sudden unexpected nocturnal death syndrome; TdP: *torsades de pointes*; VT: ventricular tachycardia.

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Discrepancies in the codon numbers of the allelic variants exist because of changes in information about the sequence of *ANK2*. For example, the p.E1425G mutation, the first LQT4-causing mutation to be reported [Mohler et al., 2003] has been reannotated as p.E1458G using the most recent reference sequence. LQT4-associated mutations in *ANK2* are listed in Table 5.

Mutations in *KCNE1* (LQT5)

The potassium voltage-gated channel, IsK-related subfamily, member 1 gene (*KCNE1*; MIM♯ 176261) encodes the minimal potassium ion channel (minK), which is an ancillary single transmembrane β-subunit that associates with the α-subunits of voltage-gated potassium (Kv) ion channels and modulates conductance of these channels (Fig. 2A) [Murai et al., 1989; Abbott et al., 1999; Abbott and Goldstein, 2001]. The *KCNE1* gene comprises three exons (Fig. 2F) mapped to 21q22.1–21q22.2, the third exon encodes the 129–amino acid protein [Chevillard et al., 1993; Malo et al., 1995; Splawski et al., 1998].

In the heart, the minK subunit coassembles with the α-subunit of the Kv7.1 ion channel, which is encoded by the *KCNQ1* gene, resulting in channels that generate the slowly activating delayed rectifier (I_{Ks}) current (Fig. 1) [Sanguinetti et al., 1996]. The I_{Ks} current is important in the inner ear [Lundquist et al., 2006; Nicolas et al., 2001], and homozygous mutations in *KCNE1* may cause deafness due to interference with the K^+ content of the endolymph [Schulze-Bahr et al., 1997a].

Mutations and polymorphisms in the *KCNE1* gene have been shown to result in a reduction of the potassium flux generated by the I_{Ks} current, such that myocellular repolarization is delayed, thus producing a prolonged QT interval on an ECG (LQT5; MIM♯ 176261) [Splawski et al., 1997b]. Seebohm et al. [2008] determined that I_{Ks} current impairment, as a result of the minK p.D76N mutation, was a consequence of disrupted channel recycling. The minK p.D76 residue is essential for the regulation of serum/glucocorticoid-regulated kinase 1, which in turn is crucial for *RAB11*-dependent recycling. Homozygous loss-of-function mutations in *KCNE1* or compound heterozygotes of *KCNQ1* and *KCNE1* are associated with both LQTS, due to the effect of reduced I_{Ks} in the heart, and deafness due to a much reduced I_{Ks} in the inner ear [Wang et al., 2002]. This combination of deafness and LQTS is called JLNS and has a very poor prognosis [Richter and Brugada, 2006; Jacobson et al., 1990; Goldenberg et al., 2006]. See Table 6 and www.ssi.dk for a list of disease-causing mutations in *KCNE1*.

Mutations in *KCNE2* (LQT6)

The potassium voltage-gated channel, IsK-related subfamily, member gene (*KCNE2*; MIM♯ 603796) encodes the minimum potassium ion channel related peptide 1 (MiRP1), which is an ancillary single-transmembrane β-subunit associated with the α-subunits of voltage-gated potassium (Kv) ion channels and modulates the potassium ion conductance of these channels (Fig. 2B) [Murai et al., 1989; Abbott et al., 1999]. The *KCNE2* gene comprises two exons mapped to 21q22.1 (Fig. 2G); the second exon encodes the 123–amino acid protein expressed in heart and muscle. The two KCNE genes (*KCNE2* and *KCNE1*) are separated by 79 kb and share 34% identity in their coding regions, which suggests that they are related by gene duplication [Abbott et al., 1999].

In the heart, the MiRP1 peptide coassembles with Kv11.1, encoded by *KCNH2*, resulting in channels that generate the rapidly activating delayed rectifier (I_{Kr}) current (Fig. 1) [Abbott et al., 1999; Sanguinetti et al., 1995].

Mutations and polymorphisms in the *KCNE2* gene have been shown to result in a reduction of the potassium flux generated by the I_{Kr} current, such that myocellular repolarization is delayed, thus giving a prolonged QT interval on an ECG (LQT6; MIM♯ 603796) [Splawski et al., 1997b; Abbott et al., 1999]. Cases of aLQTS are frequently caused by dysfunction of the I_{Kr}, consequently, mutations in *KCNE2* may cause aLQTS [Roden and Viswanathan, 2005]. See mutation Table 6 and www.ssi.dk/lqt for a list of disease-causing mutations in *KCNE2*.

Mutations in *KCNJ2* (LQT7 and SQT3)

The potassium inward rectifying channel, subfamily J, member 2 gene (*KCNJ2*; MIM♯ 600681) codes for the voltage-dependent inwardly rectifying potassium ion channel (Kir2.1) (Fig. 5A), which conducts a significant part of the inwardly rectifying I_{K1} current. This current is important in regulating the membrane potential and membrane excitability by stabilizing the resting membrane potential, defining the excitation threshold and modulating the repolarization (Fig. 1) [Nichols et al., 1996; Shimoni et al., 1992]. The Kir ion channels constitute a large group of channels, where the Kir1.x, Kir2.x, Kir3.x, and Kir6.x are expressed in the heart [Reimann and Ashcroft, 1999]. Of the four Kir2.x proteins, Kir2.1, Kir2.2, and Kir2.3 are expressed in cardiac and skeletal muscle [Kubo et al., 1993; Liu et al., 2001; Wang et al., 1998].

The I_{K1} current represents the major ion conductance in the later part of repolarization and during diastole [Lopatin and Nichols, 2001]. A Kir2.1$^{-/-}$ knockout transgenic mouse exhibited nearly no I_{K1}, whereas a Kir2.2$^{-/-}$ knockout exhibited a 50% decrease in I_{K1} [Zaritsky et al., 2001]. The importance of Kir2.3 for I_{K1} has not been clarified. The *KCNJ2* gene contains two exons (Fig. 5B) and spans 10 kb at 17q23.1–17q24.2 [Derst et al., 2001]. The Kir2.1 ion channel is composed of 427 amino acids organized in a C-terminus, two transmembrane domains (M1 and M2) separated by a pore-associated extracellular domain H5, and an N-terminus (Fig. 5A). The I_{K1} current is conducted by a tetrameric assembly of Kir2.1 [Yang et al., 1995] and, possibly, other Kir2.x subunits [Preisig-Muller et al., 2002]. The inward rectification is caused by interaction with intracellular Mg^{2+} [Vandenberg, 1987] and polyamines [Lopatin et al., 1994]. The Kir2.1 channel protein is critically dependent upon interaction with PIP2, and the residues involved in this interaction have been defined [Lopes et al., 2002].

Through genetic linkage in a large family, mutations in *KCNJ2* were found to be associated with AS (LQT7; MIM♯ 170390) [Plaster et al., 2001], an autosomal dominant multisystem disease characterized by a combination of potassium-sensitive periodic paralysis, cardiac arrhythmia, and distinctive facial or skeletal dysmorphic features, with marked intrafamilial variability and incomplete penetrance [Andersen et al., 1971; Tawil et al., 1994; Davies et al., 2005]. *KCNJ2* mutations were found in 9 out of 13 AS families and two mutations were found to result in dominant-negative loss-of-function [Plaster et al., 2001]. The clinical presentation of AS is highly variable, with LQTS shown to be present in 71% of a large series of patients with AS and *KCNJ2* mutations, while 64% of the patients exhibited ventricular arrhythmias [Tristani-Firouzi et al., 2002]. These features have also been found in another study of five AS-affected kindreds [Sansone et al., 1997], although in some families LQT is not present [Lu et al., 2006]. However, a considerable number of mutations in *KCNJ2* have been associated with LQTS (see Table 7 and www.ssi.dk/lqt).

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Table 6. LQT5 Mutations in *KCNE1* and LQT6 Mutations in *KCNE2*

mRNA	AA	Mutation type	Genomic region	Protein region	Phenotype	References
KCNE1/minK; NM_000219.2/NP_000210.2						
c.20C>T	p.T7I	Missense	Exon_03	Extracellular	JLNS	Schulze-Bahr et al. [1997b, 2001]; Splawski et al. [2000]; Bianchi et al. [1999]
c.23C>T	pA8V	Missense	Exon_03	Extracellular	RWS	Ohno et al. [2007]
c.83C>T	p.S28L	Missense	Exon_03	Extracellular	RWS/AV block	Shim et al. [2005]
c.95G>A	p.R32H	Missense	Exon_03	Extracellular	RWS	Splawski et al. [2000]; Schulze-Bahr et al. [2001]
c.107G>A	p.R36H	Missense	Exon_03	Transmembrane	RWS	Napolitano et al. [2005]
c.139G>T	p.V47F	Missense	Exon_03	Transmembrane	JLNS	Bianchi et al. [1999]; Splawski et al. [2000]; Schulze-Bahr et al. [2001]
c.152_153delinsAT	p.L51H	Missense	Exon_03	Transmembrane	JLNS	Bianchi et al. [1999]; Schulze-Bahr et al. [2001]
c.154G>A	p.G52R	Missense	Exon_03	Transmembrane	RWS	Ma et al. [2003]
c.158T>C	p.F53S	Missense	Exon_03	Transmembrane	RWS	Napolitano et al. [2005]
c.172A>C; c.176_177delinsCT	p.T58P; L59P	Missense	Exon_03	Transmembrane	JLNS	Splawski et al. [2000]; Tyson et al. [1997]; Bianchi et al. [1999]; Schulze-Bahr et al. [2001]
c.199C>T	p.R67C	Missense	Exon_03	Transmembrane	RWS	This work
c.210G>C	p.K70N	Missense	Exon_03	Transmembrane	RWS	Lai et al. [2005]
c.221C>T	p.S74L	Missense	Exon_03	Cytoplasmatic	RWS	Splawski et al. [1997b, 2000]; Bianchi et al. [1999]; Schulze-Bahr et al. [2001]; Wu et al. [2006]
c.226G>A	p.D76N	Missense	Exon_03	Cytoplasmatic	JLNS/RWS	Splawski et al. [1997b]; Duggal et al. [1998]; Schulze-Bahr et al. [1997b]; Bianchi et al. [1999]; Wu et al. [2006]
c.242A>G	p.Y81C	Missense	Exon_03	Cytoplasmatic	RWS	Wu et al. [2006]
c.259T>C	p.W87R	Missense	Exon_03	Cytoplasmatic	RWS	Bianchi et al. [1999]; Splawski et al. [2000]; Schulze-Bahr et al. [2001]; Wu et al. [2006]
c.292C>T	p.R98W	Missense	Exon_03	Cytoplasmatic	RWS	Splawski et al. [2000]; Wu et al. [2006]; Ohno et al. [2007]
c.379C>A	p.P127T	Missense	Exon_03	Cytoplasmatic	RWS	Splawski et al. [2000]; Schulze-Bahr et al. [2001]
KCNE2/MiRP1; NM_172201.1/NP_751951.1						
c.79C>T	p.R27C	Missense	Exon_02	Extracellular	AF	Yang et al. [2004]
c.161T>C	p.M54T	Missense	Exon_02	Transmembrane	RWS/aLQTS	Abbott et al. [1999]; Sesti et al. [2000]; Isbrandt et al. [2002]; Paulussen et al. [2004]
c.156_161del	p.L51fs	Frameshift; deletion	Exon_02	Transmembrane	RWS	Napolitano et al. [2005]
c.166A>G; 166_168dup	p.M56V; M65dup	Missense; insertion	Exon_02	Transmembrane	RWS	Napolitano et al. [2005]
c.170T>C	p.I57T	Missense	Exon_02	Transmembrane	RWS/aLQTS	Abbott et al. [1999]; Sesti et al. [2000]; Isbrandt et al. [2002]; Paulussen et al. [2004]; Millat et al. [2006]
c.193G>A	p.V65M	Missense	Exon_02	Transmembrane	RWS	Isbrandt et al. [2002]
c.229C>T	p.R77W	Missense	Exon_02	Cytoplasmic	AV block/LQTS	Millat et al. [2006]; Chevalier et al. [2007]
c.347C>T	p.A116V	Missense	Exon_02	Cytoplasmic	aLQTS	Sesti et al. [2000]; Paulussen et al. [2004]

*Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.
AF, atrial fibrillation; aLQTS, acquired long QT syndrome; AV, atrioventricular; JLNS, Jervell Lange-Nielsen syndrome; RWS, Romano-Ward syndrome.

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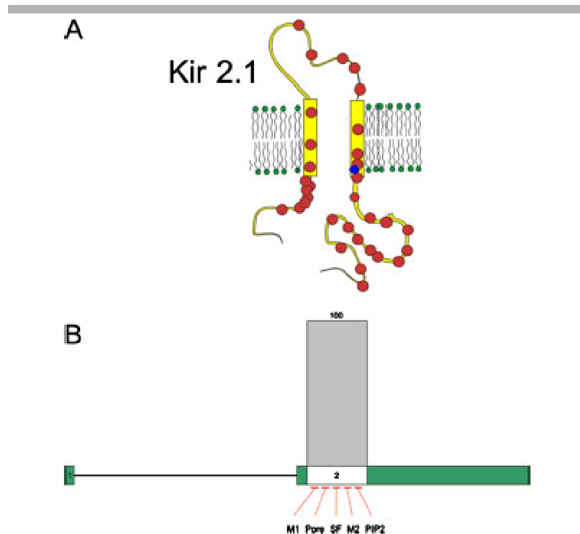

Figure 5. A: Schematic drawing of Kir2.1, the location of known LQTS-associated and SQTS-associated mutations are indicated by red and blue dots, respectively. B: The genomic structure with the basic structural elements indicated. Kir2.1 is encoded by a single exon of *KCNJ2*, and all mutations identified to date are contained within this exon. M1: transmembrane region 1; M2: transmembrane region 2; PIP2: phosphatidylinositol 4,5-bisphosphate binding domain; SF: selectivity filter.

Functional assessment of the p.R67W mutation showed that there was no detectable I_{K1} activity when mutated Kir2.1 was expressed in *Xenopus* oocytes and tsA201 cells [Andelfinger et al., 2002], which suggests that AS may be caused by reduced I_{K1} current. Lopes et al. [2002] indicated that two AS-causing mutations (p.R218Q and p.R218W) reduce the interaction of Kir2.1 with PIP2, and reduced the whole-cell current. Consequently, AS may be the result of loss-of-function mutations in *KCNJ2*.

However, an analysis of the ECGs from an AS patient cohort with 24 different *KCNJ2* mutations showed that the majority had normal QT_c time (only 17% had $QT_c > 460$ ms), but prolonged QU_c-time and a particular T-U wave pattern, while the median QT_c time was normal [Zhang et al., 2005]. Likewise, in both a single family [Lu et al., 2006] and 13 Japanese families, many mutation carriers had normal QT_c time [Haruna et al., 2007]. This is in contrast to the QU_c-time, where the overlap between normal controls and *KCNJ2* patients is much smaller [Zhang et al., 2005]. The particular ECG pattern with enlarged U-wave, prolonged T-wave down-slope, and a wide T-U junction exhibited an 84% sensitivity and 97% specificity for *KCNJ2*-positive genotype prediction in AS patients [Zhang et al., 2005]. Even though syncope, sudden death, and ventricular tachycardia (VT) are seen in AS patients with *KCNJ2* mutations, their occurrence is not linked to the QT_c prolongation [Haruna et al., 2007]. The differences between RWS and AS has resulted in the suggestion that mutations in *KCNJ2* should not be designated LQT7 [Zhang et al., 2005]. However, as patients with *KCNJ2* mutations can present with prolonged QT_c- and QU_c-time as single signs, we suggest that LQT7 remains as the name of the LQT type caused by *KCNJ2* mutations. It should be recognized, however, that LQT7 is associated with bidirectional VT, premature ventricular contraction, and extra systoles, and that sudden death is rare as compared to other LQT-types [Zhang et al., 2005].

Digenic inheritance involving *KCNJ2* and *KCNQ1* has been described; in one case with QT_c time of 460 ms and prominent U-wave [Kobori et al., 2004], and in another with only prolonged QT_c-time associated with syncope [Haruna et al., 2007].

The link between mutations in *KCNJ2* and SQTS was established in a 5-year old girl in whom a short QT_c (315 ms) and tall T-waves were identified as an accidental finding. Her father had a QT_c interval of 320 ms and a history of repeated seizures and episodes of palpitations. Genetic analysis revealed that both father and daughter were heterozygous carriers of the missense mutation p.D172N in *KCNJ2*. Expression of the mutated Kir2.1 ion channel in CHO cells revealed markedly increased current at high potentials, whereas the inward rectification was not seen above an AP of −80 mV [Priori et al., 2005]. In a mathematical simulation, the p.D172N was compared to known SQTS-associated mutations in *KCNQ1* and *KCNH2*; the change in repolarization was less pronounced in the *KCNJ2* mutant [Priori et al., 2005]. Gain-of-function mutations in *KCNJ2* can cause both isolated and familial AF and SQTS. The SQTS-associated mutations are shown in Figure 5.

Mutations in *CACNA1C* (LQT8 and SQT4)

The calcium voltage-dependent channel, L type, alpha-1C subunit gene (*CACNA1C*; MIM# 114205) encodes the α-subunit of the long-lasting (L-type) voltage-gated calcium channel (Cav1.2) that is activated upon depolarization of the cardiomyocyte. The Cav1.2 channel is responsible for the influx of Ca^{2+} that is of significance for the plateau phase of the AP. Furthermore, it represents a coupling between excitation and contraction by inducing release of Ca^{2+} from the sarcoplasmic reticulum. The Cav1.2 channel is composed of four domains (DI–DIV), each comprised of six transmembrane segments (S1–S6) (Fig. 6A). The molecular topology is thus very similar to that of Nav1.5. The *CACNA1C* gene spans 640 kb at chromosome 12p13.33 and contains 50 exons (Fig. 6B) [Soldatov, 1994]. The transcription of *CACNA1C* is subject to extensive variation and 40 splice variations occurring in 19 exons have been identified [Tang et al., 2004]. The extent of splice variation in the heart is subject to considerable interindividual variation [Wang et al., 2006]. The extensive variation in the composition of *CACNA1C* mRNA results in the translation of many different Cav1.2 sequence variants (isoforms) with differing electrophysiological and pharmacological properties [Tang et al., 2004]. This heterogeneity can explain the phenotypical variation in patients with TS. The predominant splice form contains 2,138 amino acids. The $I_{L,Ca}$ current (Fig. 1) is conducted by a complex composed of the pore-forming α1-subunit, a β2-subunit, an α2δ subunit, and—at least in skeletal muscle—a γ-subunit [Singer et al., 1991]. The Cav1.2 channel α1-subunit is composed of four homologous domains (DI–DIV), each composed of six transmembrane segments (S1–S6). Segments S5 and S6 in each domain are connected via a P-segment. The four domains constitute the wall of the Cav1.2 ion channel, with the P-segments lining the Ca^{2+} conducting pore [Bers, 2001]. The S4 segments are negatively charged and function as charge sensors. Cav1.2 interacts with the ryanodine receptor and calmodulin at the C-terminus, which also contains an EF-hand domain. Cavβ2 interacts with Cav1.2 at the DI–DII linker and the ryanodine receptor at the DII–DIII linker, in addition to the C-terminus [Bers, 2004]. The interaction with calmodulin is responsible for the Ca^{2+} dependence of inactivation [Grandi et al., 2007].

Mutations in *CACNA1C* have been described in patients with TS (LQT8; MIM# 601005) [Splawski et al., 2004, 2005], a condition characterized by prolonged QT interval, syndactyly, abnormal teeth, cardiac malformations, immune deficiency,

Table 7. LQT7 Mutations in *KCNJ2*[*]

mRNA: NM_000891.2	AA: NP_000882.1	Mutation type	Genomic region	Protein region	Phenotype	References
c.200G>A	p.R67Q	Missense	Exon_02	N-term	AS	Eckhardt et al. [2007]
c.199C>T	p.R67W	Missense	Exon_02	N-term	AS/pVT	Andelfinger et al. [2002]; Donaldson et al. [2003]
c.211G>A	p.D71A	Missense	Exon_02	N-term	AS	Donaldson et al. [2003]
c.212A>T	p.D71W	Missense	Exon_02	N-term	AS	Plaster et al. [2001]
c.220A>G	p.T74A	Missense	Exon_02	N-term	AS	Zhang et al. [2005]; Ballester et al. [2006]
c.223A>G	p.T75A	Missense	Exon_02	N-term	RWS	Fodstad et al. [2004]
c.224C>T	p.T75M	Missense	Exon_02	N-term	AS	Eckhardt et al. [2007]
c.224C>G	p.T75R	Missense	Exon_02	N-term	AS	Donaldson et al. [2003]
c.233A>G	p.D78G	Missense	Exon_02	N-term	AS	Davies et al. [2005]
c.245G>A	p.R82Q	Missense	Exon_02	M1	AS	Davies et al. [2005]
c.244C>T	p.R82W	Missense	Exon_02	M1	CPVT/RWS	Tester et al. [2006]; Eckhardt et al. [2007]
c.283_294del	p.95_98del	Deletion	Exon_02	M1	AS	Tristani-Firouzi et al. [2002]
c.301T>C	p.C101R	Missense	Exon_02	M1	AS	Zhang et al. [2005]
c.368T>G	p.V123G	Missense	Exon_02	H5 (pore-forming helix)	AS	Davies et al. [2005]
c.407C>T	p.S136F	Missense	Exon_02	H5 (pore-forming helix)	AS	Tristani-Firouzi et al. [2002]
c.430G>A	p.G144S	Missense	Exon_02	Selectivity filter	AS	Tristani-Firouzi et al. [2002]
c.431G>C	p.G144A	Missense	Exon_02	Selectivity filter	AS	Zhang et al. [2005]; Ballester et al. [2006]
c.437G>A	p.G146D	Missense	Exon_02	Selectivity filter	AS	Donaldson et al. [2003]
c.488_493del	p.163_164del	Missense	Exon_02	M2-region	RWS	Fodstad et al. [2004]
c.557C>T	p.P186L	Missense	Exon_02	PIP2 BM	RBBB/pVT/AS	Tristani-Firouzi et al. [2002]
c.566G>T	p.R189I	Missense	Exon_02	PIP2 BM	AS	Donaldson et al. [2003]
c.574A>G	p.T192A	Missense	Exon_02	PIP2 BD	AS	Ai et al. [2002]
c.644G>A	p.G215D	Missense	Exon_02	C-term	AS	Hosaka et al. [2003]
c.646A>C	p.N216H	Missense	Exon_02	C-term	AS/bidirectional VT/CA	Tristani-Firouzi et al. [2002]
c.650T>C	p.L217P	Missense	Exon_02	C-term	AS	Davies et al. [2005]
c.652C>T	p.R218W	Missense	Exon_02	C-term	AS/bigeminy/pVT	Plaster et al. [2001]
c.653G>A	p.R218Q	Missense	Exon_02	C-term	AS	Tristani-Firouzi et al. [2002]
c.679G>T	p.V227F	Missense	Exon_02	C-term	AS/NSVT	Tester et al. [2006]
c.899G>A	p.G300D	Missense	Exon_02	C-term	AS/bigeminy	Donaldson et al. [2003]
c.899G>T	p.G300V	Missense	Exon_02	C-term	AS/bigeminy/pVT	Tristani-Firouzi et al. [2002]
c.904G>A	p.V302M	Missense	Exon_02	C-term	AS/RBBB/bigeminy	Tristani-Firouzi et al. [2002]
c.907G>A	p.E303K	Missense	Exon_02	C-term	AS	Tristani-Firouzi et al. [2002]
c.913A>G	p.T305A	Missense	Exon_02	C-term	AS	Eckhardt et al. [2007]
c.926C>T	p.T309I	Missense	Exon_02	C-term	AS	Davies et al. [2005]
c.934C>T	p.R312C	Missense	Exon_02	C-term	AS	Donaldson et al. [2003]
c.940_945del	p.314_315del	Deletion	Exon_02	C-term	AS	Tristani-Firouzi et al. [2002]

*Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.
AS, Andersen's syndrome; CA, cardiac arrest; CPVT, catecholaminergic polymorphic ventricular tachycardia; NSVT, nonsustained ventricular tachycardia; pVT, polymorphic ventricular tachycardia; RBBB, right bundle branch block; RWS, Romano-Ward syndrome; VT, ventricular tachycardia.

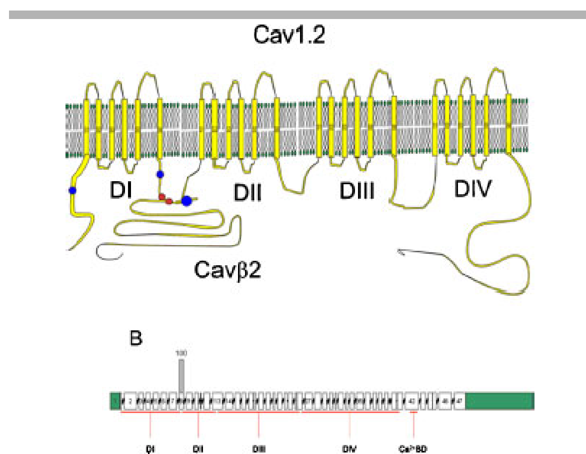

Figure 6. Schematic drawing of Cav1.2 ion channel α-subunit and the Cavβ2-subunit. **A:** The locations of the two LQT8 mutations are indicated by red dots while the locations of the SQTS-causing mutations are indicated by blue dots. **B:** The genomic structure with the basic structural elements indicated. Both mutations are located in exon 8 of *CANCA1C*. Ca^{2+}BD: calcium binding domain; DI–IV: domains 1–4.

intermittent hypoglycemia, cognitive abnormalities, and autism and baldness at birth. The condition is very severe, with most affected patients dying in early childhood from cardiac arrhythmia. The condition occurs sporadically, but has been described in a pair of siblings [Splawski et al., 2004]. However, the dominant pattern of inheritance and lethality of the mutations precludes the propagation of the gene defect. The described mutations (Table 8; and www.ssi.dk/lqt) result in complete loss of voltage-dependent Cav1.2 inactivation, causing Ca^{2+} overload and delayed repolarization due to prolonged Ca^{2+} inward current during the plateau phase of the AP [Splawski et al., 2004]. The mutations described are located in the differentially-spliced exons 8 and 8A. While both locations give rise to gain-of-function mutations; the mutation located in exon 8A is associated with a much lower arrhythmia risk than the mutation located in exon 8.

The association between *CACNA1C* and SQTS was established by the finding that 2 out of 82 patients previously diagnosed with BrS carried missense mutations in *CACNA1C*. The first index patient had a QT_c of 346 ms, very little rate-dependence of QT on heart rate, AF, and a brother who had died from cardiac arrest at the age of 45 years. The second patient had a QT_c of 360 ms, a type I BrS pattern on the ECG, and a mother who had died suddenly at the age of 48 years following repeated syncopal episodes. These two index patients were found to be heterozygous carriers of the

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Table 8. LQT8-, LQT9-, LQT10-, LQT11-, and LQT12-Causing Mutations in *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, *SNTA1*, Respectively*

mRNA	AA	Mutation type	Genomic region	Protein region	Phenotype	References
LQT8: CACNA1C/Cav1.2; NM_000719.6/NP_000710.5						
c.1204G>A	p.G402S	Missense	Exon_08	DI/S6	TS	Splawski et al. [2005]
c.1216G>A	p.G406R	Missense	Exon_08	DI/S6	TS	Splawski et al. [2005]
LQT9: CAV3/M-Caveolin; NM_001234.3/NP_001225.1						
c.40G>C	p.V14L	Missense	Exon_01	N-term	SIDS	Cronk et al. [2007]
c.233C>T	p.T78M	Missense	Exon_02	N-term	Nonexertional syncope	Vatta et al. [2006]; Cronk et al. [2007]; Arnestad et al. [2007]
c.239T>G	p.L79R	Missense	Exon_02	N-term	SIDS	Cronk et al. [2007]
c.253G>A	p.A85T	Missense	Exon_02	Intramembrane	SUNDS	Vatta et al. [2006]
c.290T>G	p.F97C	Missense	Exon_02	Intramembrane	dyspnea/chest pain/RWS	Vatta et al. [2006]
c.423C>G	p.S141R	Missense	Exon_02	C-term	Nonexertional syncope/RWS	Vatta et al. [2006]
LQT10: SCN4B/NavB4; NM_174934.2/NP_777594.1						
c.535C>T	p.L179F	Missense	Exon_03	TM	RWS/2:1 AV-block	Medeiros-Domingo et al. [2007]
LQT11: AKAP9/Yotiao; NM_005751.3/NP_005742.4						
c.4709C>T	p.S1570L	Missense	Exon_18	KCNQ1 BD	RWS	Chen et al. [2007]
LQT12: SNTA1/a1-Syntrophin; NM_003098.2/NP_003089.1						
c.1169C>T	p.A390V	Missense	Exon_06	PMCA4b ID	RWS	Ueda et al. [2008]

*Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. AV, atrioventricular; TS, Timothy's syndrome; RWS, Romano-Ward syndrome; SCD, sudden cardiac death; SIDS, sudden infant death syndrome; SUNDS, sudden unexpected nocturnal death syndrome; TM, transmembrane domain; BD, binding domain; ID, interacting domain.

mutations p.G490R and p.A39V, respectively. When expressed with Cav1.2 in CHO cells, a clearly reduced $I_{L,Ca}$ was found in both cases and confocal microscopy showed no signs of a trafficking defect [Antzelevitch et al., 2007d]. Thus, *CACNA1C* mutations can present with a phenotype that is a combination of BrS and SQTS.

Mutations in *CAV3* (LQT9)

The caveolins-3 gene (*CAV3*; MIM♯ 601253) encodes M-caveolin (CAV3), a principal protein in caveolae, 50- to 100-nm membrane invaginations involved in the localization of membrane proteins at the membrane surface (Fig. 3A) [Minetti et al., 1998; Song et al., 1996]. CAV3 is the muscle-specific form of caveolins, a group also comprising caveolin-1 and -2 [Minetti et al., 1998]. *CAV3* is a gene with two exons (Fig. 3D) spanning 13 kb at chromosome 3p25 [Tang et al., 1996; McNally et al., 1998; Minetti et al., 1998]. Expressed solely in heart and skeletal muscle, CAV3 is composed of 196 amino acids, and comprises an N-terminal domain, a scaffolding domain (SD), a hydrophobic domain, and a C-terminal domain. Through the SD, the protein interacts with many membrane components, including the α-subunit of Nav1.5. It interacts with ion channels and stimulatory G-protein subunits [Martinez-Marmol et al., 2008; Ye et al., 2008; Chini and Parenti, 2004; Martens et al., 2004] and modulates the I_{Na} current [Palygin et al., 2008]. It is also involved in intracellular sorting of proteins and lipids [Sprong et al., 2001] and the fine-tuning of the cell signaling machinery [Simons and Toomre, 2000].

Mutations in *CAV3* have been associated with limb-girdle muscular dystrophy (LGMD) [Minetti et al., 1998; McNally et al., 1998], dystrophin-glycoprotein-complex-associated muscular dystrophy [Batchelor and Winder, 2006], idiopathic hyperCKemia [Carbone et al., 2000], and rippling muscle disease [Betz et al., 2001; Fulizio et al., 2005]. Mutations resulting in overexpression of CAV3 result in LGMD, and truncating mutations cause rippling muscle disease; it has also been demonstrated that Duchenne muscular dystrophy patients overexpress CAV3, leading to a consequent increase in the number of caveolae [Repetto et al., 1999; Galbiati et al., 2001]. *CAV3* mutations were found in 6 out of 905 unrelated patients with LQT (LQT9; MIM♯ 611818) [Vatta et al., 2006] and have also been found to be a rare cause of SIDS in African American children (see Table 8 and www.ssi.dk/lqt) [Cronk et al., 2007]. The *CAV3* mutations associated with LQT and SIDS interfere with the association between CAV3 and the Nav1.5 ion channel, resulting in a two- to five-fold increase in the late I_{Na}—the same pathological mechanism that underlies LQT3.

Mutations in *SCN4B* (LQT10)

The sodium voltage-gated channel, type IV, beta subunit gene (*SCN4B*; MIM♯ 608256) encodes the β4-subunit of the cardiac sodium channel conducting the I_{Na} current (Fig. 1) [Yu et al., 2003]; it spans 19.5 kb at chromosome 11q23 [Yu et al., 2003] and is composed of five exons (Fig. 3C). The *SCN4B* gene is translated into a 228–amino acid protein that is composed of an N-terminal extracellular Ig-like domain, a transmembrane domain, and a C-terminal intracellular domain (Fig. 3A). The *SCN4B* gene is a member of the gene family *SCN1B*, *SCN2B*, *SCN3B*, and *SCN4B*, products of which encode small β-subunits, β1, β1A, β2, β3, and β4, of the Nav complexes [Meadows and Isom, 2005]. The β-subunits have several functions, including interaction with ankyrin-B and -G. The biophysical function of the β4-subunit is

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to modify the function of Nav1.5, slightly inhibiting the I_{Na} [Yu et al., 2003].

A mutation in *SCNB4* was recently found associated with LQTS (LQT10; MIM♯ 611819) in a Mexican-mestizo family with LQT (see Table 8 and www.ssi.dk/lqt). When the mutation was transiently expressed in HEK293 cells stably expressing the Nav1.5 ion channel, it caused an 8-fold increase in the late I_{Na} [Medeiros-Domingo et al., 2007].

Mutations in *AKAP9* (LQT11)

The A-kinase anchor protein 9 gene (*AKAP9*; MIM♯ 604001) encodes a scaffolding A-kinase anchor protein (AKAP) involved in the subcellular localization of protein kinase A (PKA); AKAPs are molecules regulating the PKA pathway, other kinases, and target molecules [McConnachie et al., 2006]. Thus, *AKAP9* is involved in macromolecular complexes controlling the correct phosphorylation of a number of PKA substrates [Smith and Scott, 2006]. *AKAP9*, coding for a deduced protein of 3,908 amino acids, actually codes for two proteins, the yotiao protein of 1,626 amino acids, and a human homolog of the rat protein, AKAP120 (Fig. 2A). The *AKAP9* gene is located on chromosome 7q21–7q22 and consists of 51 exons spanning more than 170 kb (Fig. 2E) [Witczak et al., 1999]. In the heart, yotiao is involved in phosphorylation of at least the ryanodine receptor [Marx et al., 2000], the L-type Ca^{2+} channel [Hulme et al., 2006], and the K^{+}-channel conducting the slow repolarization current, I_{Ks} [Marx et al., 2002]. The macromolecular complex controlling the I_{Ks} current consists of the Kv7.1 α-subunit, the minK β-subunit, and the AKAP yotiao protein that binds both to the N- and C-terminus of Kv7.1, PKA, and protein phosphatase 1 [Westphal et al., 1999]. The integrity of the complex is necessary for the functional modification of the I_{Ks} current by cAMP-dependent regulation mediated via PKA phosphorylation of Ser-27 in Kv7.1 [Kurokawa et al., 2004].

In 50 LQTS families, with no mutations in other LQTS-associated genes, a single *AKAP9* mutation, p.S1570L, was found (LQT11; MIM♯ 611820). The mutation is located in close proximity to the C-terminal Kv7.1 binding site and reduces the interaction between Kv7.1 and yotiao, as well as the cAMP-dependent phosphorylation of Kv7.1. It causes a reduction of the functional response of the I_{Ks} current to cAMP stimulation [Chen et al., 2007]. Mathematical modeling suggests that the impact of the S1570L mutation would be a significant prolongation of the QT-time on the ECG (see Table 8 and www.ssi.dk/lqt) [Chen et al., 2007].

Mutations in *SNTA1* (LQT12)

The syntrophin, alpha-1 gene (*SNTA1*; MIM♯ 601017) encodes α1-syntrophin, a 505–amino acid dystrophin-associated membrane-associated scaffolding protein with multiple molecular interactions, including neuronal nitric oxide synthase (nNOS), plasma membrane Ca-ATPase (PMCA), and Nav1.5 [Gavillet et al., 2006; Miyagoe-Suzuki and Takeda, 2001; Brenman et al., 1996]. It connects Nav1.5 to the nNOS/PMCA complex in the heart (Fig. 3A) [Brenman et al., 1996]. Thus, α1-syntrophin is involved in macromolecular complexes controlling the function of Nav1.5. *SNTA1* is composed of eight exons (Fig. 3C) covering 36 kb of chromosome 20 [Ahn et al., 1996].

In 1 out of 50 LQTS probands in whom no mutations were identified in screening of LQT1–11-causative genes, a missense mutation, p.A390V, in *SNTA1* was identified in a proband with very prolonged QT_c interval (LQT12; MIM♯ 601017) (see Table 8 and www.ssi.dk/lqt) [Ueda et al., 2008]. The mutation is located in a conserved part of α1-syntrophin, where it interacts with cardiac plasma membrane Ca^{2+}-ATPase (PMCA4b). Functional assessment showed that the mutation results in increased direct nitrosylation of Nav1.5 and increased late I_{Na} current—similar to the LQT3-phenotype [Ueda et al., 2008]. Furthermore, the mutation results in an uncoupling of the link between Ca^{2+}-transporting, PMCA subunit 4 and the Nav1.5/α1-syntrophin complex. Due to the complex effects of the mutation, the mechanism underlying the increased I_{Na} has not been established and a contribution to arrhythmogenesis from interrupted interactions with other ion channels cannot be excluded [Ueda et al., 2008].

Mutations in *CACNB2* (SQT5)

The calcium voltage-dependent channel, beta-2 subunit gene (*CACNB2*; MIM♯ 600003) encodes the 660–amino acid β2-subunit (Cavβ2) of Cav1.2 [Van Petegem et al., 2004], which modifies gating, increasing the $I_{L,Ca}$ current [Catterall, 2000]. Cavβ2 functions as a chaperone for the α-subunit of Cav1.2, ensuring its transport to the plasma membrane [Cornet et al., 2002]. The phosphatidylinositol 3-kinase pathway—via interaction with Cavβ2—is involved in ensuring a sufficient expression of Cav1.2 on the plasma membrane [Viard et al., 2004]. *CACNB2* consists of 14 exons covering 421 kb at chromosome 10p12.

A missense mutation, S481L, in *CACNB2* was identified in 1 out of 82 index patients previously diagnosed as having BrS, and who were subjected to comprehensive ion channel mutation screening. The patient had experienced an aborted sudden cardiac death and had a QT_c of 330 ms, with an ECG indicative of BrS type II. Ten of his family members were investigated; six of these were phenotype-positive and had a shortened QT_c (range, 330–370 ms). However, only the proband was symptomatic. When the mutation was expressed in CHO cells together with Cav1.2, the $I_{L,Ca}$ current was severely reduced, but was not the consequence a trafficking defect. The mutation is located in the C-terminal part of Cavβ2, downstream of the Cav1.2 binding domain [Antzelevitch et al., 2007d]; consequently, the mechanism by which the mutation causes this functional defect is most likely a disruption of the interaction of the α- and β-subunits and the supportive effect of Cavβ2 on the $I_{L,Ca}$. Thus, mutations in *CACNB2* can present with a phenotype that is a mixture of BrS and SQTS.

Polymorphisms in LQTS and SQTS Genes

In addition to a catalogue of mutations identified in LQTS- and SQTS-causing genes, the polymorphisms identified in these genes have also been cataloged at www.ssi.dk/lqt. In several studies, a number of these variants have been associated with repolarization and cardiac conduction defects (CCD) and have been proposed as disease-modifying factors. A French study investigating polymorphisms within the LQTS-causing genes in a healthy population found that the minor alleles of the Kv11.1 p.K897T and Nav1.5 (p.D1819D; c.5651T>C) variants were more frequent in the group with the shortest QT_c intervals, while the minor alleles of the minK p.D85N, Nav1.5 p.H558R, and *SCN5A* (c.1141–3C>A) variants were more frequent in the group with the longest QT_c intervals [Gouas et al., 2005]. Associations between particular polymorphisms in LQTS-causing genes and drug-induced LQT, as well as risk for developing TdP, have also been reported [Paulussen et al., 2004; Mank-Seymour et al., 2006].

The minor allele of the Kv11.1 variant p.K897T has been reported to have a protective effect when inherited *in cis* with the disease-causing allele of the p.A490T mutation [Zhang et al., 2008b]; it has

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also been reported to have a harmful effect when inherited *in trans* with the disease-causing allele of the p.A1116V mutation [Crotti et al., 2005]. Additionally, the p.K897T polymorphism has also been associated with AF [Sinner et al., 2008].

Cellular electrophysiology studies of the Nav1.5 channel indicate that the coinheritance of Nav1.5 p.H558R attenuates the electrophysiological effects of a conduction disease mutation—Nav1.5 p.T512I and a BrS mutation Nav1.5 p.R282H [Viswanathan et al., 2003; Poelzing and Rosenbaum, 2005]. In both cases, it appears that the 558R allele needs to be inherited *in trans* from the disease-causing allele in order to exert an effect.

Rare nonsynonymous polymorphisms with no known pathological significance are found in some of the ion channel genes [Anson et al., 2004] and it is imperative that polymorphisms are identified as such when counseling LQTS families, particularly in families with compound heterozygotes or digenic inheritance [Grunnet et al., 2005]. Some polymorphisms have a racial-ethnic specific distribution [Modell and Lehmann, 2006], necessitating particular focus on population affiliation when interpreting the significance of novel mutations.

Methods of Mutation Screening

Disease-causing mutations may take several forms, including substitutions, insertions, deletions, inversions, and duplications of various sizes. A variety of techniques exist to detect these genetic variants, of which direct sequencing by capillary array electrophoresis (CAE) is considered the gold standard. However, direct sequencing has been considered too expensive to be used as a first line mutation detection technique. Mobility shift assays such as single-strand conformation polymorphism (SSCP) [Hestekin and Barron, 2006] and denaturing high-performance liquid chromatography (DHPLC) [Yu et al., 2006] are able to analyze multiple genes and multiple gene regions for sequence variants at a reasonable cost [Frueh and Noyer-Weidner, 2003]. In addition to being inexpensive, mutation detection techniques need to be rapid and sensitive in order to be applicable in a clinical setting. To be of clinical significance, both the sensitivity and specificity should exceed 97% [Hestekin and Barron, 2006].

SSCP is a simple and inexpensive technique that detects mutations based on observable differences in the sequence specific, electrophoretic mobility of single-stranded DNA fragments. SSCP analysis of LQTS-associated genes has developed over time from a laborious and time-consuming slab gel electrophoresis approach [Larsen et al., 1999a, 2001a] to a CAE approach [Larsen et al., 2001b; Andersen et al., 2003a], which has improved the applicability of this technique as a screening method by improving throughput and creating a potential to multiplex [Hofman-Bang et al., 2006] and to use microsystems [Tian et al., 2005]. CAE-SSCP can be refined by combining it with dideoxysequencing [Larsen et al., 2001c] and cleavase treatment [Andersen et al., 1998]. A calculated sensitivity of 98% with a specificity of 100% using CAE-SSCP with two temperature conditions has been reported [Andersen et al., 2003b].

DHPLC detects mutations based on the decreased retention time of heteroduplexes [Yu et al., 2006], as a result of the reduced thermal stability displayed by heteroduplexes compared to homoduplexes. Several groups have described DHPLC methods for the identification of LQTS-associated mutations [Jongbloed et al., 2002; Lai et al., 2005]; a number of publications report sensitivity and specificity values <97% [Crepin et al., 2006; Fasano et al., 2005; Holinski-Feder et al., 2001]. Considerable optimization of the analysis temperature is required, and in many cases samples were investigated at multiple temperatures in order to improve sensitivity and specificity. However, a consequence of using multiple analysis temperatures is a significant increase in the time of analysis.

Strategies for efficient mutation identification by judicious selection of genes—which would reduce the resources required in screening—based on the relative frequencies of different genetic etiologies have been proposed [Napolitano et al., 2005], but the occurrence of compound heterozygotes [Berge et al., 2008], digenic inheritance [Larsen et al., 1999b; Priori et al., 1998; Ning et al., 2003; Fodstad et al., 2006; Schwartz et al., 2003], and modifying genes [Westenskow et al., 2004; Crotti et al., 2005; Ye et al., 2003] would seem to necessitate a comprehensive screening strategy comprising all genes. This also seems to be the case even in populations where founder mutations are frequent; e.g., the white South African population [Hedley et al., unpublished data].

Results of Population Screening

Four large LQTS population surveys have been published [Splawski et al., 2000; Tester et al., 2005; Napolitano et al., 2005; Berge et al., 2008]. All four surveys involved the three most frequently implicated LQTS-causing genes (*KCNQ1*, *KCNH2*, and *SCN5A*), as well as *KCNE1* and *KCNE2*. Splawski et al. [2000] investigated 262 unrelated patients recruited from clinics in North America and Europe; these patients were diagnosed with LQTS based on ECG characteristics and presence of symptoms. Mutations were defined as sequence variants that altered amino acid residues or splice sites that were not present in 400 control chromosomes. This study identified 177 mutations in 134 patients. Tester et al. [2005] performed a mutation screen on 541 consecutively collected, unrelated LQTS patients; this study identified 211 mutations in 272 patients. Napolitano et al. [2005] identified 235 mutations in 310 probands from a screening population of 430 consecutively collected probands. The most recent survey was performed on 182 unrelated RWS and JLN patients from Norway [Berge et al., 2008]. Thirty-seven different mutations were identified and the detection rate in RWS was 71%. The results of these surveys are summarized in Table 9. Population screening has documented compound heterozygotes and digenic inheritance [Berge et al., 2008]. Furthermore, the existence of recessive variants of RWS makes it likely that the prevalence of potential LQTS-causing mutations is around 0.3% to 1% [Berge et al., 2008; Priori et al., 1998; Larsen et al., 1999b].

Population screening for SQTS based on shortened QT_c interval alone was not possible [Anttonen et al., 2007], and to date, no population-based genetic screening has been performed.

Genotype–Phenotype Correlation

Genotype–phenotype correlations in LQTS and SQTS are complicated because the total number of mutations identified in several of the genes is fairly small and the intraallelic heterogeneity is considerable. However, for LQT1, LQT2, and LQT3, it has been possible to correlate the genotype to prognosis, i.e., risk of cardiac event or death, treatment response to adrenergic β-blockade, the physiological triggers of arrhythmia, and the ECG morphology [Schwartz et al., 2001; Zareba et al., 1998; Priori et al., 2003]. Within SQTS, very few patients have been reported; however, it appears SQT3 has a milder phenotype than the other SQT types. This has been corroborated by a milder electrophysiological phenotype; i.e., a smaller increase in repolarization [Priori et al., 2005]. There also does seem to be a higher propensity for AF in

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Table 9. Summary of Various Population Screening Surveys

	Studies			
	Splawski et al. [2000]	Tester et al. [2005]	Napolitano et al. [2005]	Berge et al. [2008]
Percentage of mutations identified				
Detection rate (%)	50.4	50.3	72.0	70.8
KCNQ1 (%)	39.4	44.0	48.6	42.6
KCNH2 (%)	51.5	34.0	38.8	46.3
SCN5A (%)	6.1	9.5	4.7	9.3
KCNE1 (%)	2.3	1.1	1.7	1.9
KCNE2 (%)	2.3	0.3	0.7	0.0
Mutation type				
Missense (%)	72.3	75.0	72.0	64.9
Nonsense (%)	6.2	5.7	5.1	13.5
Deletion (%)	5.0	2.3	14.1	2.7
Frameshift (%)	9.6	11.4	6.1	13.5
Splice site (%)	6.7	5.4	2.7	5.4
Mutation position				
N-terminal (%)	13.6	16.1	8.0	24.3
Transmembrane (%)	61.2	49.8	64.0	54.1
C-terminal (%)	25.2	35.1	28.0	21.6

SQT1 as compared to the other types. One might speculate that SQTS is one phenotypic presentation of what might be called an "increased repolarization" syndrome, with BrS and AF as other manifestations. This is also compatible with the known increased propensity for AF in BrS [Morita et al., 2002]. The deciding factor for the phenotypic presentation might be the gene involved, the extent of increased repolarization, and the occurrence of the increase during the APD; e.g., in phase II or phase III.

The risk of cardiac events as a function of genotype has been analyzed in three studies [Schwartz et al., 2001; Zareba et al., 1998; Priori et al., 2003]; in the latest and largest survey conducted by Priori et al. [2003], which comprised 647 LQTS patients from 193 families, it was found that the risk of cardiac events was considerably smaller in LQT1 carriers than in LQT2 and LQT3 carriers. Correspondingly, more than 30% of LQT1 mutation carriers were asymptomatic [Priori et al., 2003]. Limited data are available for the other LQT types, but JLNS patients [Goldenberg et al., 2006; Jervell and Lange-Nielsen, 1957] and patients with TS (LQT8) [Splawski et al., 2005] seem to carry a particularly high risk of sudden cardiac death from early childhood. Even when the mutations occur within the same gene, the risk of cardiac events varies, depending on the precise location of the mutation [Moss et al., 2007; Donger et al., 1997] and this complicates the risk estimation based on the genetic findings. Likewise, the effect of modifying factors [Schwartz, 2006] is just beginning to be clarified, so that it is not possible to use genetic information alone for risk stratification. However, this does not preclude the use of clinical risk stratification based on the length of the QT interval, gender, and genotype [Priori et al., 2003].

The standard prophylactic treatment for LQTS has been adrenergic β-blockade [Schwartz, 1985; Moss et al., 2000], but it seems only to be effective in patients with LQT1 or LQT2, whereas it seems to confer no, or even reduced, protection to LQT3 patients [Schwartz et al., 2001; Priori et al., 2004]. Several specific arrhythmia triggers have been associated with particular genotypes, i.e., physical exercise is an arrhythmia trigger in LQT1, emotional distress or loud noises are arrhythmia triggers in LQT2 [Wilde et al., 1999], while rest or sleep are arrhythmia triggers in LQT3 [Schwartz et al., 2001].

The morphology and location of T-waves in the ECG exhibits characteristic differences between LQT1, LQT2, and LQT3 patients [Moss et al., 1995; Zhang et al., 2000], and derived parameters can be used to distinguish between LQT1 and LQT2 [Struijk et al., 2006; Kanters et al., 2004], as well as to identify drug effects [Graff et al., 2009]. Likewise, patients with LQT7 exhibit a distinct ECG pattern with characteristic U-waves [Zhang et al., 2005]. However, the intrafamilial and intraallelic variation of the ECG pattern is considerable [Moss et al., 1995], making it impossible to use the ECG as a substitute for genotyping [Kanters et al., 2006; Kaufman et al., 2001].

In some cases, the phenotype of LQTS-gene associated disease varies between affected family members. Additionally, complex phenotypes involving LQTS, BrS, CCD [Makita et al., 2008], and also structural heart disease, e.g., DCM, have been reported [Olson et al., 2005]. All of the mentioned conditions may present as sudden cardiac death in adult- or childhood [Ackerman, 2005; Wang et al., 2007; Brugada et al., 2007], which is important to remember when designing molecular-genetic screening programs in families with diverse cardiac phenotypes.

Clinical Significance of Mutation Identification

The major significance of genetic screening in LQTS is the identification of mutation carriers and noncarriers. The first group may be asymptomatic but still at risk of sudden death [Schwartz, 2006], while the second group may benefit from certain knowledge that they are not at risk. In particular, in neonates, where the ECG assessment is difficult, it is important to perform a genetic analysis, which can be performed using the Guthrie card used for neonatal screening [Norgaard-Pedersen and Simonsen, 1999].

Despite the many advances in the understanding of genetics of LQTS, it is important to realize that the diagnosis is still a clinical one and that mutation screening will normally only result in identification of a disease-causing mutation in 70 to 80% of cases. The institution of relevant prophylactic treatment in the index case and any symptomatic relatives should not await the results of the genetic analysis. Furthermore, it may be extended to asymptomatic gene carriers when the genetic background has been established and cascade-screening has become possible.

It must be emphasized that the genetic complexity of cLQTS, with an appreciable number of families with digenic inheritance, currently makes it necessary to screen at least the five most

frequently involved genes before the molecular-genetic workup is finished. Future advances in technology are likely to extend this approach to include more of the genetic modifiers of phenotype. Genetic analysis—or "molecular autopsy"—may also identify LQTS as the underlying cause in cases of sudden infant and adult death [Tester and Ackerman, 2009] and, in turn, make it possible to perform cascade screening to identify similarly at-risk gene carriers that may benefit from prophylactic β-adrenergic blockade. Prior to the molecular-genetic era it was suggested that LQTS—based on neonatal ECG measurements—could be a significant contributor to SIDS [Maron et al., 1976], and a large prospective study comprising >30,000 children showed that one-half of SIDS cases had prolonged QT-time [Schwartz et al., 1998]. However, neonatal ECG recordings are difficult to interpret due to spurious, clinically insignificant, QT interval prolongations [Schwartz et al., 1998] and the positive predictive value of a prolonged QT interval would be less than 1% [Zupancic et al., 2000]. A recent genetic study comprising 201 Norwegian SIDS cases that were genotyped in seven LQTS-associated genes, showed that 26 (13%) cases had an unusual—possibly disease-causing—variant in one of these genes [Arnestad et al., 2007]. Five variants exhibited functional characteristics expected in LQTS-associated mutations [Wang et al., 2007]. As genetic arrhythmia-associated disease in younger adults is fairly common (up to 50%) as a cause of sudden death [Behr et al., 2008], and seems to be involved in ~10% of SIDS cases, it would seem that genetic screening in SIDS and SADS cases would be beneficial. However, the cost–benefit relationship of such an approach has not been established and will depend greatly on the price and quality of the screening and the expertise in interpretation and risk assessment, as well as availability of relevant treatment, e.g., ICD units, and may thus vary greatly between different countries [Berul and Perry, 2007]. At present, it must be expected that all physicians involved in sudden death cases are knowledgeable of the possibility of performing testing, but the assessment of cases should be left to clinicians with a special interest.

Clinical assessment of SQTS is hindered by the lack of diagnostic criteria. In the absence of clear clinical parameters, it is difficult to document the efficiency of genetic screening in SQTS. However, although genetic testing in SQTS is an important tool in identifying mutation carriers that are at risk of sudden death, there is no evidence-based knowledge of the efficiency of screening based on different clinical criteria [Anttonen et al., 2007]. Despite the scant experience and small number of genotyped cases, it would seem that cascade-screening is important due to the intrafamilial phenotypic variability of mutation carriers. Furthermore, the association of SQTS with AF suggests that genetic testing could be relevant in all young people with lone AF [Antzelevitch et al., 2007c]. However, clinical studies assessing the efficiency of such a screening are lacking, and currently, genetic screening in the general population for both LQTS and SQTS must be considered a research activity [Priori and Napolitano, 2006].

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References

Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA. 1999. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97:175–187.

Abbott GW, Goldstein SA. 2001. Potassium channel subunits encoded by the KCNE gene family: physiology and pathophysiology of the MinK-related peptides (MiRPs). Mol Interv 1:95–107.

Abriel H, Cabo C, Wehrens XH, Rivolta I, Motoike HK, Memmi M, Napolitano C, Priori SG, Kass RS. 2001. Novel arrhythmogenic mechanism revealed by a long-QT syndrome mutation in the cardiac Na(+) channel. Circ Res 88:740–745.

Ackerman MJ, Porter CJ. 1998. Identification of a family with inherited long QT syndrome after a pediatric near-drowning. Pediatrics 101:306–308.

Ackerman MJ, Schroeder JJ, Berry R, Schaid DJ, Porter CJ, Michels VV, Thibodeau SN. 1998. A novel mutation in KVLQT1 is the molecular basis of inherited long QT syndrome in a near-drowning patient's family. Pediatr Res 44:148–153.

Ackerman MJ, Tester DJ, Porter CJ. 1999a. Swimming, a gene-specific arrhythmogenic trigger for inherited long QT syndrome. Mayo Clin Proc 74:1088–1094.

Ackerman MJ, Tester DJ, Porter CJ, Edwards WD. 1999b. Molecular diagnosis of the inherited long-QT syndrome in a woman who died after near-drowning. N Engl J Med 341:1121–1125.

Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, Towbin JA. 2001. Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. JAMA 286:2264–2269.

Ackerman MJ. 2005. Cardiac causes of sudden unexpected death in children and their relationship to seizures and syncope: genetic testing for cardiac electropathies. Semin Pediatr Neurol 12:52–58.

Ahn AH, Freener CA, Gussoni E, Yoshida M, Ozawa E, Kunkel LM. 1996. The three human syntrophin genes are expressed in diverse tissues, have distinct chromosomal locations, and each bind to dystrophin and its relatives. J Biol Chem 271:2724–2730.

Ai T, Fujiwara Y, Tsuji K, Otani H, Nakano S, Kubo Y, Horie M. 2002. Novel KCNJ2 mutation in familial periodic paralysis with ventricular dysrhythmia. Circulation 105:2592–2594.

Aizawa Y, Ueda K, Scornik F, Cordeiro JM, Wu Y, Desai M, Guerchicoff A, Nagata Y, Iesaka Y, Kimura A, Hiraoka M, Antzelevitch C. 2007. A novel mutation in KCNQ1 associated with a potent dominant negative effect as the basis for the LQT1 form of the long QT syndrome. J Cardiovasc Electrophysiol 18:972–977.

Albert CM, Nam EG, Rimm EB, Jin HW, Hajjar RJ, Hunter DJ, MacRae CA, Ellinor PT. 2008. Cardiac sodium channel gene variants and sudden cardiac death in women. Circulation 117:16–23.

Algra A, Tijssen JGP, Roelandt JRTC, Pool J, Lubsen J. 1993. QT interval variables from 24-hour electrocardiography and the 2-year risk of sudden death. Br Heart J 70:43–48.

An RH, Wang XL, Kerem B, Benhorin J, Medina A, Goldmit M, Kass RS. 1998. Novel LQT-3 mutation affects Na+ channel activity through interactions between alpha- and beta1-subunits. Circ Res 83:141–146.

Andelfinger G, Tapper AR, Welch RC, Vanoye CG, George Jr AL, Benson DW. 2002. KCNJ2 mutation results in Andersen syndrome with sex-specific cardiac and skeletal muscle phenotypes. Am J Hum Genet 71:663–668.

Andersen ED, Krasilnikoff PA, Overvad H. 1971. Intermittent muscular weakness, extrasystoles, and multiple developmental anomalies. A new syndrome? Acta Paediatr Scand 60:559–564.

Andersen PS, Larsen LA, Kanters Jr J, Havndrup O, Bundgaard H, Brandt NJ, Vuust J, Christiansen M. 1998. Mutation detection by cleavase in combination with capillary electrophoresis analysis: application to mutations causing hypertrophic cardiomyopathy and Long-QT syndrome. Mol Diagn 3:105–111.

Andersen PS, Jespersgaard C, Vuust J, Christiansen M, Larsen LA. 2003a. Capillary electrophoresis-based single strand DNA conformation analysis in high-throughput mutation screening. Hum Mutat 21:455–465.

Andersen PS, Jespersgaard C, Vuust J, Christiansen M, Larsen LA. 2003b. High-throughput single strand conformation polymorphism mutation detection by automated capillary array electrophoresis: validation of the method. Hum Mutat 21:116–122.

Anderson CL, Delisle BP, Anson BD, Kilby JA, Will ML, Tester DJ, Gong Q, Zhou Z, Ackerman MJ, January CT. 2006. Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. Circulation 113:365–373.

Anson BD, Ackerman MJ, Tester DJ, Will ML, Delisle BP, Anderson CL, January CT. 2004. Molecular and functional characterization of common polymorphisms in HERG (KCNH2) potassium channels. Am J Physiol Heart Circ Physiol 286:H2434–H2441.

Anttonen O, Junttila MJ, Rissanen H, Reunanen A, Viitasalo M, Huikuri HV. 2007. Prevalence and prognostic significance of short QT interval in a middle-aged finnish population. Circulation 116:714–720.

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

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

Antzelevitch C. 2007a. Ionic, molecular, and cellular bases of QT interval prolongation and torsade de pointes. Europace 9(Suppl 4):iv4–iv15.

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Antzelevitch C. 2007b. Role of spatial dispersion of repolarization in inherited and acquired sudden cardiac death syndromes. Am J Physiol Heart Circ Physiol 293:H2024–H2038.

Antzelevitch C. 2007c. Heterogeneity and cardiac arrhythmias: an overview. Heart Rhythm 4:964–972.

Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeifer R, Oliva A, Wollnik B, Gelber P, Bonaros Jr EP, Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu L-F, Haissaguerre M, Schimpf R, Borggrefe M, Wolpert C. 2007d. Loss-of-function mutations in the cardiac calcium channel underlie new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation 115:442–449.

Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George Jr AL, Schwartz PJ. 2007. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. Circulation 115:361–367.

Bai Y, Wang J, Lu Y, Shan H, Yang B, Wang Z. 2007. Phospholipid lysophosphatidylcholine as a metabolic trigger and HERG as an ionic pathway for extracellular K^+ accumulation and "short QT syndrome" in acute myocardial ischemia. Cell Physiol Biochem 20:417–428.

Ballester LY, Benson DW, Wong B, Law IH, Mathews KD, Vanoye CG, George Jr AL. 2006. Trafficking-competent and trafficking-defective KCNJ2 mutations in Andersen syndrome. Hum Mutat 27:388.

Balser JR. 2001. The cardiac sodium channel: gating function and molecular pharmacology. J Mol Cell Cardiol 33:599–613.

Bankston JR, Sampson KJ, Kateriya S, Glaaser IW, Malito DL, Chung WK, Kass RS. 2007a. A novel LQT-3 mutation disrupts an inactivation gate complex with distinct rate-dependent phenotypic consequences. Channels (Austin) 1:273–280.

Bankston JR, Yue M, Chung W, Spyres M, Pass RH, Silver E, Sampson KJ, Kass RS. 2007b. A novel and lethal de novo LQT-3 mutation in a newborn with distinct molecular pharmacology and therapeutic response. PLoS ONE 2:e1258.

Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. 1996. K(V)LQT1 and IsK (minK) proteins associate to form the I(Ks) cardiac potassium current. Nature 384:78–80.

Baroudi G, Chahine M. 2000. Biophysical phenotypes of SCN5A mutations causing long QT and Brugada syndromes. FEBS Lett 487:224–228.

Batchelor CL, Winder SJ. 2006. Sparks, signals and shock absorbers: how dystrophin loss causes muscular dystrophy. Trends Cell Biol 16:198–205.

Bazett H. 1920. An analysis of the time relationship of electrocardiograms. Heart 7:353–370.

Beery TA, Dyment M, Shooner K, Knilans TK, Benson DW. 2003. A candidate locus approach identifies a long QT syndrome gene mutation. Biol Res Nurs 5:97–104.

Behr ER, Dalageorgou C, Christiansen M, Syrris P, Hughes S, Tome Esteban MT, Rowland E, Jeffery S, McKenna WJ. 2008. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. Eur Heart J 29:1670–1680.

Bellocq C, van Ginneken AC, Bezzina CR, Alders M, Escande D, Mannens MM, Baró I, Wilde AA. 2004a. Mutation in the KCNQ1 gene leading to the short QT interval syndrome. Circulation 109:2394–2397.

Bellocq C, Wilders R, Schott JJ, Louerat-Oriou B, Boisseau P, Le MH, Escande D, Baro I. 2004b. A common antitussive drug, clobutinol, precipitates the long QT syndrome 2. Mol Pharmacol 66:1093–1102.

Benhorin J, Goldmit M, MacCluer JW, Blangero J, Goffen R, Leibovitch A, Rahat A, Wang Q, Medina A, Towbin J, Kerem B. 1998. Identification of a new SCN5A mutation, D1840G, associated with the long QT syndrome. Mutations in brief no. 153. Online. Hum Mutat 12:72.

Benito B, Brugada R, Perich RM, Lizotte E, Cinca J, Mont L, Berruezo A, Tolosana JM, Freixa X, Brugada P, Brugada J. 2008. A mutation in the sodium channel is responsible for the association of long QT syndrome and familial atrial fibrillation. Heart Rhythm 5:1434–1440.

Bennett PB, Yazawa K, Makita N, George Jr AL. 1995. Molecular mechanism for an inherited cardiac arrhythmia. Nature 376:683–685.

Benson DW, MacRae CA, Vesely MR, Walsh EP, Seidman JG, Seidman CE, Satler CA. 1996. Missense mutation in the pore region of HERG causes familial long QT syndrome. Circulation 93:1791–1795.

Berge KE, Haugaa KH, Fruh A, Anfinsen OG, Gjesdal K, Siem G, Oyen N, Greve G, Carlsson A, Rognum TO, Hallerud M, Kongsgard E, Amlie JP, Leren TP. 2008. Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers. Scand J Clin Lab Invest 68:362–368.

Bers DM. 2001. Excitation-contraction coupling and cardiac contractile force. Berlin: Springer. 456p.

Bers DM. 2004. Macromolecular complexes regulating cardiac ryanodine receptor function. J Mol Cell Cardiol 37:417–429.

Berthet M, Denjoy I, Donger C, Demay L, Hammoude H, Klug D, Schulze-Bahr E, Richard P, Funke H, Schwartz K, Coumel P, Hainque B, Guicheney P. 1999. C-terminal HERG mutations: the role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. Circulation 99:1464–1470.

Berul CI, Perry JC. 2007. Contribution of long-QT syndrome genes to sudden infant death syndrome: is it time to consider newborn electrocardiographic screening? Circulation 115:294–296.

Betz RC, Schoser BG, Kasper D, Ricker K, Ramirez A, Stein V, Torbergsen T, Lee YA, Nothen MM, Wienker TF, Malin JP, Propping P, Reis A, Mortier W, Jentsch TJ, Vorgerd M, Kubisch C. 2001. Mutations in CAV3 cause mechanical hyperirritability of skeletal muscle in rippling muscle disease. Nat Genet 28:218–219.

Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van Der Hout AH, Mannens MM, Wilde AA. 1999. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. Circ Res 85:1206–1213.

Bianchi L, Shen Z, Dennis AT, Priori SG, Napolitano C, Ronchetti E, Bryskin R, Schwartz PJ, Brown AM. 1999. Cellular dysfunction of LQT5-minK mutants: abnormalities of IKs, IKr and trafficking in long QT syndrome. Hum Mol Genet 8:1499–1507.

Boriani G, Biffi M, Valzani C, Bronzetti G, Martignani C. 2006. Short QT syndrome and arrhythmogenic cardiac diseases in the young: The challenge of implantable cardioverter-defibrillator therapy for children. Eur Heart J 27:2382–2384.

Brenman JE, Chao DS, Gee SH, McGee AW, Craven SE, Santillano DR, Wu Z, Huang F, Xia H, Peters MF, Froehner SC, Bredt DS. 1996. Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. Cell 84:757–767.

Brink PA, Crotti L, Corfield V, Goosen A, Durrheim G, Hedley P, Heradien M, Geldenhuys G, Vanoli E, Bacchini S, Spazzolini C, Lundquist AL, Roden DM, George Jr AL, Schwartz PJ. 2005. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. Circulation 112:2602–2610.

Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu YS, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C. 2004. Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation 109:30–35.

Brugada R, Hong K, Cordeiro JM, Dumaine R. 2005. Short QT syndrome. CMAJ 173:1349–1354.

Brugada J, Brugada R, Brugada P. 2007. Channelopathies: a new category of diseases causing sudden death. Herz 32:185–191.

Campbell TJ. 1989. Characteristics of cardiac action potentials in marsupials. J Comp Physiol B 158:759–762.

Carbone I, Bruno C, Sotgia F, Bado M, Broda P, Masetti E, Panella A, Zara F, Bricarelli FD, Cordone G, Lisanti MP, Minetti C. 2000. Mutation in the CAV3 gene causes partial caveolin-3 deficiency and hyperCKemia. Neurology 54:1373–1376.

Catterall WA. 2000. Structure and regulation of voltage-gated Ca^{2+} channels. Annu Rev Cell Dev Biol 16:521–555.

Cha A, Ruben PC, George Jr AL, Fujimoto E, Bezanilla F. 1999. Voltage sensors in domains III and IV, but not I and II, are immobilized by Na+ channel fast inactivation. Neuron 22:73–87.

Chang CC, Acharfi S, Wu MH, Chiang FT, Wang JK, Sung TC, Chahine M. 2004. A novel SCN5A mutation manifests as a malignant form of long QT syndrome with perinatal onset of tachycardia/bradycardia. Cardiovasc Res 64:268–278.

Chen J, Zou A, Splawski I, Keating MT, Sanguinetti MC. 1999a. Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. J Biol Chem 274:10113–10118.

Chen Q, Zhang D, Gingell RL, Moss AJ, Napolitano C, Priori SG, Schwartz PJ, Kehoe E, Robinson JL, Schulze-Bahr E, Wang Q, Towbin JA. 1999b. Homozygous deletion in KVLQT1 associated with Jervell and Lange-Nielsen syndrome. Circulation 99:1344–1347.

Chen S, Zhang L, Bryant RM, Vincent GM, Flippin M, Lee JC, Brown E, Zimmerman F, Rozich R, Szafranski P, Oberti C, Sterba R, Marangi D, Tchou PJ, Chung MK, Wang Q. 2003a. KCNQ1 mutations in patients with a family history of lethal cardiac arrhythmias and sudden death. Clin Genet 63:273–282.

Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, Jin HW, Sun H, Su XY, Zhuang QN, Yang YQ, Li YB, Liu Y, Xu HJ, Li XF, Ma N, Mou CP, Chen Z, Barhanin J, Huang W. 2003b. KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 299:251–254.

Chen L, Marquardt ML, Tester DJ, Sampson KJ, Ackerman MJ, Kass RS. 2007. Mutation of an A-kinase-anchoring protein causes long-QT syndrome. Proc Natl Acad Sci USA 104:20990–20995.

Cheng TO. 2004. Digitalis administration: an underappreciated but common cause of short QT interval. Circulation 109:e152.

Chevalier P, Bellocq C, Millat G, Piqueras E, Potet F, Schott JJ, Baro I, Lemarec H, Barhanin J, Rousson R, Rodriguez-Lafrasse C. 2007. Torsades de pointes complicating atrioventricular block: evidence for a genetic predisposition. Heart Rhythm 4:170–174.

Chevillard C, Attali B, Lesage F, Fontes M, Barhanin J, Lazdunski M, Mattei MG. 1993. Localization of a potassium channel gene (KCNE1) to 21q22.1–q22.2 by in situ hybridization and somatic cell hybridization. Genomics 15:243–245.

Stellenbosch University http://scholar.sun.ac.za

Chini B, Parenti M. 2004. G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there? J Mol Endocrinol 32:325–338.
Choe CU, Schulze-Bahr E, Neu A, Xu J, Zhu ZI, Sauter K, Bahring R, Priori S, Guicheney P, Monnig G, Neapolitano C, Heidemann J, Clancy CE, Pongs O, Isbrandt D. 2006. C-terminal HERG (LQT2) mutations disrupt IKr channel regulation through 14-3-3epsilon. Hum Mol Genet 15:2888–2902.
Choi G, Kopplin LJ, Tester DJ, Will ML, Haglund CM, Ackerman MJ. 2004. Spectrum and frequency of cardiac channel defects in swimming-triggered arrhythmia syndromes. Circulation 110:2119–2124.
Chouabe C, Neyroud N, Guicheney P, Lazdunski M, Romey G, Barhanin J. 1997. Properties of KvLQT1 K+channel mutations in Romano-Ward and Jervell and Lange-Nielsen inherited cardiac arrhythmias. EMBO J 16:5472–5479.
Chouabe C, Neyroud N, Richard P, Denjoy I, Hainque B, Romey G, Drici MD, Guicheney P, Barhanin J. 2000. Novel mutations in KvLQT1 that affect Iks activation through interactions with Isk. Cardiovasc Res 45:971–980.
Christiansen M, Tonder N, Larsen LA, Andersen PS, Simonsen H, Oyen N, Kanters JK, Jacobsen JR, Fosdal I, Wettrell G, Kjeldsen K. 2005. Mutations in the HERG K+-ion channel: a novel link between long QT syndrome and sudden infant death syndrome. Am J Cardiol 95:433–434.
Chugh SS, Senashova O, Watts A, Tran PT, Zhou Z, Gong Q, Titus JL, Hayflick SJ. 2004. Postmortem molecular screening in unexplained sudden death. J Am Coll Cardiol 43:1625–1629.
Chung SK, MacCormick JM, McCulley CH, Crawford J, Eddy CA, Mitchell EA, Shelling AN, French JK, Skinner JR, Rees MI. 2007. Long QT and Brugada syndrome gene mutations in New Zealand. Heart Rhythm 4:1306–1314.
Cordeiro JM, Brugada R, Wu YS, Hong K, Dumaine R. 2005. Modulation of I(Kr) inactivation by mutation N588K in KCNH2: A link to arrhythmogenesis in short QT syndrome. Cardiovasc Res 67:498–509.
Cornet VD, Bichet G, Sandoz I, Marty J, Brocard E, Bourinet Y, Mori M, Villaz M, De Waard M. 2002. Multiple determinants in voltage-dependent P/Q calcium channels control their retention in the endoplasmic reticulum. Eur J Neurosci 16:883–895.
Crepin M, Pigny P, Escande F, Bauters CC, Calender A, Lefevre S, Buisine MP, Porchet N, Odou MF. 2006. Evaluation of denaturing high performance liquid chromatography for the mutational analysis of the MEN1 gene. J Mol Endocrinol 36:369–376.
Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, Ackerman MJ. 2007. Novel mechanism for sudden infant death syndrome: persistent late sodium current secondary to mutations in caveolin-3. Heart Rhythm 4:161–166.
Crotti L, Lundquist AL, Insolia R, Pedrazzini M, Ferrandi C, De Ferrari GM, Vicentini A, Yang P, Roden DM, George Jr AL, Schwartz PJ. 2005. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. Circulation 112:1251–1258.
Crotti L, Pedrazzini M, Ferrandi C, Insolia R, Tosin L, Vicentini A, Turco A, De Ferrari GM, Schwartz PJ. 2007. Gene symbol: KCNH2. Hum Genet 120:912.
Crotti L, Celano G, Dagradi F, Schwartz PJ. 2008. Congenital long QT syndrome. Orphanet J Rare Dis 3:18.
Cunha SR, Mohler PJ. 2006. Cardiac ankyrins: essential components for development and maintenance of excitable membrane domains in heart. Cardiovasc Res 71:22–29.
Cunha SR, Le SS, Schott JJ, Mohler PJ. 2008. Exon organization and novel alternative splicing of the human ANK2 gene: implications for cardiac function and human cardiac disease. J Mol Cell Cardiol 45:724–734.
Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. 1995. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 80:795–803.
Dahimene S, Alcolea S, Naud P, Jourdon P, Escande D, Brasseur R, Thomas A, Baro I, Merot J. 2006. The N-terminal juxtamembranous domain of KCNQ1 is critical for channel surface expression: implications in the Romano-Ward LQT1 syndrome. Circ Res 99:1076–1083.
Darbar D, Kannankeril PJ, Donahue BS, Kucera G, Stubblefield T, Haines JL, George Jr AL, Roden DM. 2008. Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation. Circulation 117:1927–1935.
Davies NP, Imbrici P, Fialho D, Herd C, Bilsland LG, Weber A, Mueller R, Hilton-Jones D, Ealing J, Boothman BR, Giunti P, Parsons LM, Thomas M, Manzur AY, Jurkat-Rott K, Lehmann-Horn F, Chinnery PF, Rose M, Kullmann DM, Hanna MG. 2005. Andersen-Tawil syndrome: new potassium channel mutations and possible phenotypic variation. Neurology 65: 1083–1089.
de Jager T, Corbett CH, Badenhorst JC, Brink PA, Corfield VA. 1996. Evidence of a long QT founder gene with varying phenotypic expression in South African families. J Med Genet 33:567–573.
Denjoy I, Lupoglazoff JM, Donger C, Berthet M, Richard P, Neyroud N, Villain E, Lucet V, Coumel P, Guicheney P. 1999. [Congenital long QT syndrome. The value of genetics in prognostic evaluation]. Arch Mal Coeur Vaiss 92:557–563.
Derst C, Karschin C, Wischmeyer E, Hirsch JR, Preisig-Muller R, Rajan S, Engel H, Grzeschik K, Daut J, Karschin A. 2001. Genetic and functional linkage of Kir5.1 and Kir2.1 channel subunits. FEBS Lett 491:305–311.
Deschenes D, Acharfi S, Pouliot V, Hegele R, Krahn A, Daleau P, Chahine M. 2003. Biophysical characteristics of a new mutation on the KCNQ1 potassium channel (L251P) causing long QT syndrome. Can J Physiol Pharmacol 81:129–134.
Donaldson MR, Jensen JL, Tristani-Firouzi M, Tawil R, Bendahhou S, Suarez WA, Cobo AM, Poza JJ, Behr E, Wagstaff J, Szepetowski P, Pereira S, Mozaffar T, Escolar DM, Fu YH, Ptacek LJ. 2003. PIP2 binding residues of Kir2.1 are common targets of mutations causing Andersen syndrome. Neurology 60:1811–1816.
Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, Chivoret G, Schwartz K, Coumel P, Guicheney P. 1997. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. Circulation 96:2778–2781.
Duggal P, Vesely MR, Wattanasirichaigoon D, Villafane J, Kaushik V, Beggs AH. 1998. Mutation of the gene for IsK associated with both Jervell and Lange-Nielsen and Romano-Ward forms of Long-QT syndrome. Circulation 97:142–146.
Dumaine R, Wang Q, Keating MT, Hartmann HA, Schwartz PJ, Brown AM, Kirsch GE. 1996. Multiple mechanisms of Na+ channel–linked long-QT syndrome. Circ Res 78:916–924.
Eckhardt LL, Farley AL, Rodriguez E, Ruwaldt K, Hammill D, Tester DJ, Ackerman MJ, Makielski JC. 2007. KCNJ2 mutations in arrhythmia patients referred for LQT testing: a mutation T305A with novel effect on rectification properties. Heart Rhythm 4:323–329.
Ellinor PT, Nam EG, Shea MA, Milan DJ, Ruskin JN, MacRae CA. 2008. Cardiac sodium channel mutation in atrial fibrillation. Heart Rhythm 5:99–105.
Fasano T, Bocchi L, Pisciotta L, Bertolini S, Calandra S. 2005. Denaturing high-performance liquid chromatography in the detection of ABCA1 gene mutations in familial HDL deficiency. J Lipid Res 46:817–822.
Ficker E, Thomas D, Viswanathan PC, Dennis AT, Priori SG, Napolitano C, Memmi M, Wible BA, Kaufman ES, Iyengar S, Schwartz PJ, Rudy Y, Brown AM. 2000. Novel characteristics of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. Am J Physiol Heart Circ Physiol 279:H1748–H1756.
Fodstad H, Swan H, Auberson M, Gautschi I, Loffing J, Schild L, Kontula K. 2004. Loss-of-function mutations of the K(+) channel gene KCNJ2 constitute a rare cause of long QT syndrome. J Mol Cell Cardiol 37:593–602.
Fodstad H, Bendahhou S, Rougier JS, Laitinen-Forsblom PJ, Barhanin J, Abriel H, Schild L, Kontula K, Swan H. 2006. Molecular characterization of two founder mutations causing long QT syndrome and identification of compound heterozygous patients. Ann Med 38:294–304.
Fozzard HA, Hanck DA. 1996. Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. Physiol Rev 76:887–926.
Franqueza L, Lin M, Shen J, Splawski I, Keating MT, Sanguinetti MC. 1999. Long QT syndrome-associated mutations in the S4-S5 linker of KvLQT1 potassium channels modify gating and interaction with minK subunits. J Biol Chem 274:21063–21070.
Frueh FW, Noyer-Weidner M. 2003. The use of denaturing high-performance liquid chromatography (DHPLC) for the analysis of genetic variations: impact for diagnostics and pharmacogenetics. Clin Chem Lab Med 41:452–461.
Fulizio L, Nascimbeni AC, Fanin M, Piluso G, Politano L, Nigro V, Angelini C. 2005. Molecular and muscle pathology in a series of caveolinopathy patients. Hum Mutat 25:82–89.
Funada A, Hayashu K, Ino H, Fujino N, Uchiyama K, Sakata K, Masuta E, Sakamoto Y, Tsubokawa T, Yamagishi M. 2008. Assessment of QT intervals and prevalence of short QT syndrome in Japan. Clin Cardiol 31:270–274.
Galbiati F, Razani B, Lisanti MP. 2001. Caveolae and caveolin-3 in muscular dystrophy. Trends Mol Med 7:435–441.
Gavillet B, Rougier JS, Domenighetti AA, Behar R, Boixel C, Ruchat P, Lehr HA, Pedrazzini T, Abriel H. 2006. Cardiac sodium channel Nav1.5 is regulated by a multiprotein complex composed of syntrophins and dystrophin. Circ Res 99:407–414.
Gellens ME, George Jr AL, Chen LQ, Chahine M, Horn R, Barchi RL, Kallen RG. 1992. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proc Natl Acad Sci USA 89:554–558.
George Jr AL, Varkony TA, Drabkin HA, Han J, Knops JF, Finley WH, Brown GB, Ward DC, Haas M. 1995. Assignment of the human heart tetrodotoxin-resistant voltage-gated Na+ channel alpha-subunit gene (SCN5A) to band 3p21. Cytogenet Cell Genet 68:67–70.
Goldenberg I, Moss AJ, Zareba W, McNitt S, Robinson JL, Qi M, Towbin JA, Ackerman MJ, Murphy L. 2006. Clinical course and risk stratification of patients affected with the Jervell and Lange-Nielsen syndrome. J Cardiovasc Electrophysiol 17:1161–1168.
Goldenberg I, Zareba W, Moss AJ. 2008. Long QT Syndrome. Curr Probl Cardiol 33:629–694.
Gouas L, Bellocq C, Berthet M, Potet F, Demolombe S, Forhan A, Lescasse R, Simon F, Balkau B, Denjoy I, Hainque B, Baro I, Guicheney P. 2004. New KCNQ1 mutations

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leading to haploinsufficiency in a general population; Defective trafficking of a KvLQT1 mutant. Cardiovasc Res 63:60–68.
Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B, Guicheney P. 2005. Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population. Eur J Hum Genet 13:1213–1222.
Graff C, Andersen MP, Xue JQ, Hardahl TB, Kanters JK, Toft E, Christiansen M, Jensen HK, Struijk JJ. 2009. Identifying drug-induced repolarization abnormalities from distinct ECG patterns in congenital long QT syndrome: a study of sotalol effects on t-wave morphology. Drug Saf 32:599–611.
Grandi E, Puglisi JL, Wagner S, Maier LS, Severi S, Bers DM. 2007. Simulation of Ca-calmodulin-dependent protein kinase II on rabbit ventricular myocyte ion currents and action potentials. Biophys J 93:3835–3847.
Grant AO, Carboni MP, Neplioueva V, Starmer CF, Memmi M, Napolitano C, Priori S. 2002. Long QT syndrome, Brugada syndrome, and conduction system disease are linked to a single sodium channel mutation. J Clin Invest 110:1201–1209.
Grunnet M, Behr ER, Calloe K, Hofman-Bang J, Till J, Christiansen M, McKenna WJ, Olesen SP, Schmitt N. 2005. Functional assessment of compound mutations in the KCNQ1 and KCNH2 genes associated with long QT syndrome. Heart Rhythm 2:1238–1249.
Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, Chaitman BR, Bjerregaard P. 2000. Idiopathic short QT interval: a new clinical syndrome. Cardiology 94:99–102.
Haruna Y, Kobori A, Maakiyama T, Yoshida H, Akao M, Doi T, Tsuji K, Ono S, Nishio Y, Shimizu W, Inoue T, Murakami T, Tsuboi N, Yamanouchi H, Ushinohama H, Nakamura Y, Yoshinaga M, Horigome H, Aizawa Y, Kita T, Horie M. 2007. Genotype-phenotype correlations of KCNJ2 mutations in japanese patients with Andersen-Tawil syndrome. Hum Mutat 28:208.
Hassel D, Scholz EP, Trano N, Friedrich O, Just S, Meder B, Weiss DL, Zitron E, Marquart S, Vogel B, Karle CA, Seemann G, Fishman MC, Katus HA, Rottbauer W. 2008. Deficient zebrafish *ether-a-go-go*-related gene channel gating causes short-QT syndrome in zebrafish mutants. Circulation 117:866–875.
Hayashi K, Shimizu M, Ino H, Yamaguchi M, Mabuchi H, Hoshi N, Higashida H. 2002. Characterization of a novel missense mutation E637K in the pore-S6 loop of HERG in a patient with long QT syndrome. Cardiovasc Res 54:67–76.
Hayashi K, Shimizu M, Ino H, Yamaguchi M, Terai H, Hoshi N, Higashida H, Terashima N, Uno Y, Kanaya H, Mabuchi H. 2004. Probucol aggravates long QT syndrome associated with a novel missense mutation M124T in the N-terminus of HERG. Clin Sci (Lond) 107:175–182.
Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA, Christiansen M. 2009. The genetic basis of Brugada syndrome: a mutation update. Hum Mutat 30:1256–1266.
Hestekin CN, Barron AE. 2006. The potential of electrophoretic mobility shift assays for clinical mutation detection. Electrophoresis 27:3805–3815.
Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, McKenna WJ, Christiansen M. 2006. High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia. Clin Genet 69:504–511.
Holinski-Feder E, Muller-Koch Y, Friedl W, Moeslein G, Keller G, Plaschke J, Ballhausen W, Gross M, Baldwin-Jedele K, Jungck M, Mangold E, Vogelsang H, Schackert HK, Lohsea P, Murken J, Meitinger T. 2001. DHPLC mutation analysis of the hereditary nonpolyposis colon cancer (HNPCC) genes hMLH1 and hMSH2. J Biochem Biophys Methods 47:21–32.
Hong K, Piper DR, Diaz-Valdecantos A, Brugada J, Oliva A, Burashnikov E, Santos-de-Soto J, Grueso-Montero J, Diaz-Enfante E, Brugada P, Sachse F, Sanguinetti MC, Brugada R. 2005. De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. Cardiovasc Res 68:433–440.
Hoorntje T, Alders M, van TP, van der Lip K, Sreeram N, van der Wal A, Mannens M, Wilde A. 1999. Homozygous premature truncation of the HERG protein: the human HERG knockout. Circulation 100:1264–1267.
Hosaka Y, Hanawa H, Washizuka T, Chinushi M, Yamashita F, Yoshida T, Komura S, Watanabe H, Aizawa Y. 2003. Function, subcellular localization and assembly of a novel mutation of KCNJ2 in Andersen's syndrome. J Mol Cell Cardiol 35:409–415.
Howard RJ, Clark KA, Holton JM, Minor Jr DL. 2007. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. Neuron 53:663–675.
Hu D, Viskin S, Oliva A, Carrier T, Cordeiro JM, Barajas-Martinez H, Wu Y, Burashnikov E, Sicouri S, Brugada R, Rosso R, Guerchicoff A, Pollevick GD, Antzelevitch C. 2007. Novel mutation in the SCN5A gene associated with arrhythmic storm development during acute myocardial infarction. Heart Rhythm 4:1072–1080.
Huang L, Bitner-Glindzicz M, Tranebjaerg L, Tinker A. 2001. A spectrum of functional effects for disease causing mutations in the Jervell and Lange-Nielsen syndrome. Cardiovasc Res 51:670–680.
Hulme JT, Westenbroek RE, Scheuer T, Catterall WA. 2006. Phosphorylation of serine 1928 in the distal C-terminal domain of cardiac CaV1.2 channels during beta1-adrenergic regulation. Proc Natl Acad Sci USA 103:16574–16579.
Ikrar T, Hanawa H, Watanabe H, Okada S, Aizawa Y, Ramadan MM, Komura S, Yamashita F, Chinushi M, Aizawa Y. 2008. A double-point mutation in the selectivity filter site of the KCNQ1 potassium channel results in a severe phenotype, LQT1, of long QT syndrome. J Cardiovasc Electrophysiol 19:541–549.
Isbrandt D, Friederich P, Solth A, Haverkamp W, Ebneth A, Borggrefe M, Funke H, Sauter K, Breithardt G, Pongs O, Schulze-Bahr E. 2002. Identification and functional characterization of a novel KCNE2 (MiRP1) mutation that alters HERG channel kinetics. J Mol Med 80:524–532.
Itoh T, Tanaka T, Nagai R, Kamiya T, Sawayama T, Nakayama T, Tomoike H, Sakurada H, Yazaki Y, Nakamura Y. 1998a. Genomic organization and mutational analysis of HERG, a gene responsible for familial long QT syndrome. Hum Genet 102:435–439.
Itoh T, Tanaka T, Nagai R, Kikuchi K, Ogawa S, Okada S, Yamagata S, Yano K, Yazaki Y, Nakamura Y. 1998b. Genomic organization and mutational analysis of KVLQT1, a gene responsible for familial long QT syndrome. Hum Genet 103:290–294.
Iwasa H, Itoh T, Nagai R, Nakamura Y, Tanaka T. 2000. Twenty single nucleotide polymorphisms (SNPs) and their allelic frequencies in four genes that are responsible for familial long QT syndrome in the Japanese population. J Hum Genet 45:182–183.
Jacobson J, Jacobson C, Francis P. 1990. Congenital hearing loss in Jervell and Lange-Nielsen syndrome. J Am Acad Audiol 1:171–173.
Jahr S, Lewalter T, Hesch RD, Luderitz B, Englisch S. 2000. New missense mutation (G626V) in the predicted selectivity filter of the HERG channel associated with familial long QT syndrome. Hum Mutat 15:584.
Jervell A, Lange-Nielsen F. 1957. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J 54:59–68.
Johnson Jr WH, Yang P, Yang T, Lau YR, Mostella BA, Wolff DJ, Roden DM, Benson DW. 2003. Clinical, genetic, and biophysical characterization of a homozygous HERG mutation causing severe neonatal long QT syndrome. Pediatr Res 53:744–748.
Jongbloed RJ, Wilde AA, Geelen JL, Doevendans P, Schaap C, Van I L, van Tintelen JP, Cobben JM, Beaufort-Krol GC, Geraedts JP, Smeets HJ. 1999. Novel KCNQ1 and HERG missense mutations in Dutch long-QT families. Hum Mutat 13:301–310.
Jongbloed R, Marcelis C, Velter C, Doevendans P, Geraedts J, Smeets H. 2002. DHPLC analysis of potassium ion channel genes in congenital long QT syndrome. Hum Mutat 20:382–391.
Kambouris NG, Nuss HB, Johns DC, Tomaselli GF, Marban E, Balser JR. 1998. Phenotypic characterization of a novel long-QT syndrome mutation (R1623Q) in the cardiac sodium channel. Circulation 97:640–644.
Kannankeril PJ, Roden DM. 2007. Drug-induced long QT and torsade de pointes: recent advances. Curr Opin Cardiol 22:39–43.
Kanters JK, Larsen LA, Orholm M, Agner E, Andersen PS, Vuust J, Christiansen M. 1998. Novel donor splice site mutation in the KVLQT1 gene is associated with long QT syndrome. J Cardiovasc Electrophysiol 9:620–624.
Kanters JK, Fanoe S, Larsen LA, Bloch Thomsen PE, Toft E, Christiansen M. 2004. T wave morphology analysis distinguishes between KvLQT1 and HERG mutations in long QT syndrome. Heart Rhythm 1:285–292.
Kanters JK, Graff C, Andersen MP, Hardahl T, Toft E, Christiansen M, Bloch Thomsen PE, Struijk JJ. 2006. Long QT syndrome genotyping by electrocardiography: fact, fiction, or something in between? J Electrocardiol 39:S119–S122.
Kanters JK, Haarmark C, Vedel-Larsen E, Andersen MP, Graff C, Struijk JJ, Thomsen PE, Christiansen M, Jensen HK, Toft E. 2008. T(peak)T(end) interval in long QT syndrome. J Electrocardiol 41:603–608.
Kass RS, Moss AJ. 2006. Mutation-specific pharmacology of the long QT syndrome. Handb Exp Pharmacol 117:287–304.
Kaufman ES, Priori SG, Napolitano C, Schwartz PJ, Iyengar S, Elston RC, Schnell AH, Gorodeski EZ, Rammohan G, Bahhur NO, Connuck D, Verrilli L, Rosenbaum DS, Brown AM. 2001. Electrocardiographic prediction of abnormal genotype in congenital long QT syndrome: experience in 101 related family members. J Cardiovasc Electrophysiol 12:455–461.
Kehl HG, Haverkamp W, Rellensmann G, Yelbuz TM, Krasemann T, Vogt J, Schulze-Bahr E. 2004. Images in cardiovascular medicine. Life-threatening neonatal arrhythmia: successful treatment and confirmation of clinically suspected extreme long QT-syndrome-3. Circulation 109:e205–e206.
Keller DI, Acharfi S, Delacretaz E, Benammar N, Rotter M, Pfammatter JP, Fressart V, Guicheney P, Chahine M. 2003. A novel mutation in SCN5A, delQKP 1507–1509, causing long QT syndrome: role of Q1507 residue in sodium channel inactivation. J Mol Cell Cardiol 35:1513–1521.
Kirchhof P, Eckardt L, Monnig G, Johna R, Loh P, Schulze-Bahr E, Breithardt G, Borggrefe M, Haverkamp W. 2000. A patient with "atrial torsades de pointes". J Cardiovasc Electrophysiol 11:806–811.
Ko YL, Tai DY, Chen SA, Lee-Chen GJ, Chu CH, Lin MW. 2001. Linkage and mutation analysis in two Taiwanese families with long QT syndrome. J Formos Med Assoc 100:767–771.

Stellenbosch University http://scholar.sun.ac.za

Kobori A, Sarai N, Shimizu W, Nakamura Y, Murakami Y, Makiyama T, Ohno S, Takenaka K, Ninomiya T, Fujiwara Y, Matsuoka S, Takano M, Noma A, Kita T, Horie M. 2004. Additional gene variants reduce effectiveness of beta-blockers in the LQT1 form of long QT syndrome. J Cardiovasc Electrophysiol 15:190–199.
Kontis KJ, Goldin AL. 1997. Sodium channel inactivation is altered by substitution of voltage sensor positive charges. J Gen Physiol 110:403–413.
Kontis KJ, Rounaghi A, Goldin AL. 1997. Sodium channel activation gating is affected by substitutions of voltage sensor positive charges in all four domains. J Gen Physiol 110:391–401.
Koopmann TT, Alders M, Jongbloed RJ, Guerrero S, Mannens MM, Wilde AA, Bezzina CR. 2006. Long QT syndrome caused by a large duplication in the KCNH2 (HERG) gene undetectable by current polymerase chain reaction-based exon-scanning methodologies. Heart Rhythm 3:52–55.
Krahn AD, Wang J, Spindler B, Skanes AC, Yee R, Klein GJ, Hegele RA. 2000. A novel mutation in KVLQT1, L122P, found in a family with autosomal dominant long QT syndrome. Am Heart J 140:146–149.
Kubo Y, Baldwin TJ, Jan YN, Jan LY. 1993. Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature 362:127–133.
Kubota T, Shimizu W, Kamakura S, Horie M. 2000. Hypokalemia-induced long QT syndrome with an underlying novel missense mutation in S4–S5 linker of KCNQ1. J Cardiovasc Electrophysiol 11:1048–1054.
Kurokawa J, Motoike HK, Rao J, Kass RS. 2004. Regulatory actions of the A-kinase anchoring protein Yotiao on a heart potassium channel downstream of PKA phosphorylation. Proc Natl Acad Sci USA 101:16374–16378.
Lai LP, Su YN, Hsieh FJ, Chiang FT, Juang JM, Liu YB, Ho YL, Chen WJ, Yeh SJ, Wang CC, Ko YL, Wu TJ, Ueng KC, Lei MH, Tsao HM, Chen SA, Lin TK, Wu MH, Lo HM, Huang SK, Lin JL. 2005. Denaturing high-performance liquid chromatography screening of the long QT syndrome-related cardiac sodium and potassium channel genes and identification of novel mutations and single nucleotide polymorphisms. J Hum Genet 50:490–496.
Laitinen P, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kaprio J, Kontula K. 2000. 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 15:580–581.
Larsen LA, Andersen PS, Kanters JK, Jacobsen JR, Vuust J, Christiansen M. 1999a. A single strand conformation polymorphism/heteroduplex (SSCP/HD) method for detection of mutations in 15 exons of the KVLQT1 gene, associated with long QT syndrome. Clin Chim Acta 280:113–125.
Larsen LA, Fosdal I, Andersen PS, Kanters JK, Vuust J, Wettrell G, Christiansen M. 1999b. Recessive Romano-Ward syndrome associated with compound heterozygosity for two mutations in the KVLQT1 gene. Eur J Hum Genet 7:724–728.
Larsen LA, Svendsen IH, Jensen AM, Kanters JK, Andersen PS, Moller M, Sorensen SA, Sandoe E, Jacobsen JR, Vuust J, Christiansen M. 2000. Long QT syndrome with a high mortality rate caused by a novel G572R missense mutation in KCNH2. Clin Genet 57:125–130.
Larsen LA, Andersen PS, Kanters J, Svendsen IH, Jacobsen JR, Vuust J, Wettrell G, Tranebjaerg L, Bathen J, Christiansen M. 2001a. Screening for mutations and polymorphisms in the genes KCNH2 and KCNE2 encoding the cardiac HERG/MiRP1 ion channel: implications for acquired and congenital long Q-T syndrome. Clin Chem 47:1390–1395.
Larsen LA, Christiansen M, Vuust J, Andersen PS. 2001b. Recent developments in high-throughput mutation screening. Pharmacogenomics 2:387–399.
Larsen LA, Johnson M, Brown C, Christiansen M, Frank-Hansen R, Vuust J, Andersen PS. 2001c. Automated mutation screening using dideoxy fingerprinting and capillary array electrophoresis. Hum Mutat 18:451–457.
Le SS, Bhasin N, Vieyres C, Hund TJ, Cunha SR, Koval O, Marionneau C, Chen B, Wu Y, Demolombe S, Song LS, Le MH, Probst V, Schott JJ, Anderson ME, Mohler PJ. 2008. Dysfunction in ankyrin-B-dependent ion channel and transporter targeting causes human sinus node disease. Proc Natl Acad Sci USA 105:15617–15622.
Lees-Miller JP, Duan Y, Teng GQ, Thorstad K, Duff HJ. 2000. Novel gain-of-function mechanism in K(+) channel-related long-QT syndrome: altered gating and selectivity in the HERG1 N629D mutant. Circ Res 86:507–513.
Lehtonen A, Fodstad H, Laitinen-Forsblom P, Toivonen L, Kontula K, Swan H. 2007. Further evidence of inherited long QT syndrome gene mutations in antiarrhythmic drug-associated torsades de pointes. Heart Rhythm 4:603–607.
Li H, Chen Q, Moss AJ, Robinson J, Goytia V, Perry JC, Vincent GM, Priori SG, Lehmann MH, Denfield SW, Duff D, Kaine S, Shimizu W, Schwartz PJ, Wang Q, Towbin JA. 1998. New mutations in the KVLQT1 potassium channel that cause long-QT syndrome. Circulation 97:1264–1269.
Liang L, Du ZD, Cai LL, Wu JX, Zheng T, Qi TX. 2003. [A novel KCNQ1 mutation in Chinese with congenital long QT syndrome]. Zhonghua Er Ke Za Zhi 41:724–727.
Liu DW, Antzelevitch C. 1995. Characteristics of the delayed rectifier current (IKr and IKs) in canine ventricular epicardial, midmyocardial, and endocardial myocytes. A weaker IKs contributes to the longer action potential of the M cell. Circ Res 76:351–365.
Liu GX, Derst C, Schlichthorl G, Heinen S, Seebohm G, Bruggemann A, Kummer W, Veh RW, Daut J, Preisig-Muller R. 2001. Comparison of cloned Kir2 channels with native inward rectifier K+ channels from guinea-pig cardiomyocytes. J Physiol 532:115–126.
Liu W, Yang J, Hu D, Kang C, Li C, Zhang S, Li P, Chen Z, Qin X, Ying K, Li Y, Li Y, Li Z, Cheng X, Li L, Qi Y, Chen S, Wang Q. 2002. KCNQ1 and KCNH2 mutations associated with long QT syndrome in a Chinese population. Hum Mutat 20:475–476.
Liu WL, Hu DY, Li P, Li CL, Qin XG, Li YT, Li L, Li ZM, Dong W, Qi Y, Wang Q. 2006. [Novel mutations of potassium channel KCNQ1 S145L and KCNH2 Y475C genes in Chinese pedigrees of long QT syndrome]. Zhonghua Nei Ke Za Zhi 45:463–466.
Lopatin AN, Makhina EN, Nichols CG. 1994. Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. Nature 372:366–369.
Lopatin AN, Nichols CG. 2001. Inward rectifiers in the heart: an update on I(K1). J Mol Cell Cardiol 33:625–638.
Lopes CM, Zhang H, Rohacs T, Jin T, Yang J, Logothetis DE. 2002. Alterations in conserved Kir channel-PIP2 interactions underlie channelopathies. Neuron 34:933–944.
Lu CW, Lin JH, Rajawat YS, Jerng H, Rami TG, Sanchez X, DeFreitas G, Carabello B, DeMayo F, Kearney DL, Miller G, Li H, Pfaffinger PJ, Bowles NE, Khoury DS, Towbin JA. 2006. Functional and clinical characterization of a mutation in KCNJ2 associated with Andersen-Tawil syndrome. J Med Genet 43:653–659.
Lundby A, Ravn LS, Svendsen JH, Olesen SP, Schmitt N. 2007. KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT interval. Heart Rhythm 4:1532–1541.
Lundquist AL, Turner CL, Ballester LY, George Jr AL. 2006. Expression and transcriptional control of human KCNE genes. Genomics 87:119–128.
Lupoglazoff JM, Cheav T, Baroudi G, Berthet M, Denjoy I, Cauchemez B, Extramiana F, Chahine M, Guicheney P. 2001a. Homozygous SCN5A mutation in long-QT syndrome with functional two-to-one atrioventricular block. Circ Res 89:E16–E21.
Lupoglazoff JM, Denjoy I, Berthet M, Neyroud N, Demay L, Richard P, Hainque B, Vaksmann G, Klug D, Leenhardt A, Maillard G, Coumel P, Guicheney P. 2001b. Notched T waves on Holter recordings enhance detection of patients with LQt2 (HERG) mutations. Circulation 103:1095–1101.
Lupoglazoff JM, Denjoy I, Villain E, Fressart V, Legall-Petit I, Bozio A, Berthet M, Benammar N, Hainque B, Guicheney P. 2004a. [Neonatal forms of congenital long QT syndrome]. Arch Mal Coeur Vaiss 97:479–483.
Lupoglazoff JM, Denjoy I, Villain E, Fressart V, Simon F, Bozio A, Berthet M, Benammar N, Hainque B, Guicheney P. 2004b. Long QT syndrome in neonates: conduction disorders associated with HERG mutations and sinus bradycardia with KCNQ1 mutations. J Am Coll Cardiol 43:826–830.
Ma L, Lin C, Teng S, Chai Y, Bahring R, Vardanyan V, Li L, Pongs O, Hui R. 2003. Characterization of a novel Long QT syndrome mutation G52R-KCNE1 in a Chinese family. Cardiovasc Res 59:612–619.
Makita N, Shirai N, Nagashima M, Matsuoka R, Yamada Y, Tohse N, Kitabatake A. 1998. A de novo missense mutation of human cardiac Na+channel exhibiting novel molecular mechanisms of long QT syndrome. FEBS Lett 423:5–9.
Makita N, Horie M, Nakamura T, Ai T, Sasaki K, Yokoi H, Sakurai M, Sakuma I, Otani H, Sawa H, Kitabatake A. 2002. Drug-induced long-QT syndrome associated with a subclinical SCN5A mutation. Circulation 106:1269–1274.
Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, Schulze-Bahr E, Fukuhara S, Mochizuki N, Makiyama T, Itoh H, Christiansen M, McKeown P, Miyamoto K, Kamakura S, Tsutsui H, Schwartz PJ, George Jr AL, Roden DM. 2008. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. J Clin Invest 118:2219–2229.
Makiyama T, Akao M, Shizuta S, Doi T, Nishiyama K, Oka Y, Ohno S, Nishio Y, Tsuji K, Itoh H, Kimura T, Kita T, Horie M. 2008. A novel SCN5A gain-of-function mutation M1875T associated with familial atrial fibrillation. J Am Coll Cardiol 52:1326–1334.
Malo MS, Srivastava K, Ingram VM. 1995. Gene assignment by polymerase chain reaction: localization of the human potassium channel IsK gene to the Down's syndrome region of chromosome 21q22.1–q22.2. Gene 159:273–275.
Mank-Seymour AR, Richmond JL, Wood LS, Reynolds JM, Fan YT, Warnes GR, Milos PM, Thompson JF. 2006. Association of torsades de pointes with novel and known single nucleotide polymorphisms in long QT syndrome genes. Am Heart J 152:1116–1122.
Maron BJ, Clark CE, Goldstein RE, Epstein SE. 1976. Potential role of QT interval prolongation in sudden infant death syndrome. Circulation 54:423–430.
Marquez MF, Ramos-Kuri M, Hernandez-Pacheco G, Estrada J, Fabregat JR, Perez-Vielma N, Gomez-Flores J, Gonzalez-Hermosillo A, Cardenas M, Vargas-Alarcon G. 2006. [KCNQ 1 (KvLQT1) missense mutation causing congenital long QT syndrome (Jervell-Lange-Nielsen) in a Mexican family]. Arch Cardiol Mex 76:257–262.

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Martens JR, O'Connell K, Tamkun M. 2004. Targeting of ion channels to membrane microdomains: localization of KV channels to lipid rafts. Trends Pharmacol Sci 25:16–21.

Martinez-Marmol R, Villalonga N, Sole L, Vicente R, Tamkun MM, Soler C, Felipe A. 2008. Multiple Kv1.5 targeting to membrane surface microdomains. J Cell Physiol 217:667–673.

Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR. 2000. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 101:365–376.

Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS. 2002. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. Science 295:496–499.

McConnachie G, Langeberg LK, Scott JD. 2006. AKAP signaling complexes: getting to the heart of the matter. Trends Mol Med 12:317–323.

McDonald TV, Yu Z, Ming Z, Palma E, Meyers MB, Wang KW, Goldstein SA, Fishman GI. 1997. A minK-HERG complex regulates the cardiac potassium current I(Kr). Nature 388:289–292.

McNair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, Mestroni L. 2004. SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. Circulation 110:2163–2167.

McNally EM, de Sa ME, Duggan DJ, Bonnemann CG, Lisanti MP, Lidov HG, Vainzof M, Passos-Bueno MR, Hoffman EP, Zatz M, Kunkel LM. 1998. Caveolin-3 in muscular dystrophy. Hum Mol Genet 7:871–877.

McPhee JC, Ragsdale DS, Scheuer T, Catterall WA. 1998. A critical role for the S4–S5 intracellular loop in domain IV of the sodium channel alpha-subunit in fast inactivation. J Biol Chem 273:1121–1129.

Meadows LS, Isom LL. 2005. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. Cardiovasc Res 67:448–458.

Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, Valdivia C, Ueda K, Canizales-Quinteros S, Tusie-Luna MT, Makielski JC, Ackerman MJ. 2007. SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. Circulation 116:134–142.

Melman YF, Krummerman A, McDonald TV. 2002. KCNE regulation of KvLQT1 channels: structure-function correlates. Trends Cardiovasc Med 12:182–187.

Millat G, Chevalier P, Restier-Miron L, Da CA, Bouvagnet P, Kugener B, Fayol L, Gonzalez AC, Oddou B, Chanavat V, Froidefond E, Perraudin R, Rousson R, Rodriguez-Lafrasse C. 2006. Spectrum of pathogenic mutations and associated polymorphisms in a cohort of 44 unrelated patients with long QT syndrome. Clin Genet 70:214–227.

Miller TE, Estrella E, Myerburg RJ, Garcia d V, Moreno N, Rusconi P, Ahearn ME, Baumbach L, Kurlansky P, Wolff G, Bishopric NH. 2004. Recurrent third-trimester fetal loss and maternal mosaicism for long-QT syndrome. Circulation 109:3029–3034.

Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, Masetti E, Mazzocco M, Egeo A, Donati MA, Volonte D, Galbiati F, Cordone G, Bricarelli FD, Lisanti MP, Zara F. 1998. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. Nat Genet 18:365–368.

Miyagoe-Suzuki Y, Takeda SI. 2001. Association of neuronal nitric oxide synthase (nNOS) with alpha1-syntrophin at the sarcolemma. Microsc Res Tech 55:164–170.

Modell SM, Lehmann MH. 2006. The long QT syndrome family of cardiac ion channelopathies: a HuGE review. Genet Med 8:143–155.

Moe GK, Rheinholdt WC, Abildskov JA. 1964. A computer model of atrial fibrillation. Am Heart J 67:200–220.

Moe GK. 1975. Evidence for reentry as a mechanism of cardiac arrhythmias. Rev Physiol Biochem Pharmacol 72:55–81.

Mohammad-Panah R, Demolombe S, Neyroud N, Guicheney P, Kyndt F, van den Hoff M, Baro I, Escande D. 1999. Mutations in a dominant-negative isoform correlate with phenotype in inherited cardiac arrhythmias. Am J Hum Genet 64:1015–1023.

Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, Song LS, Haurogne K, Kyndt F, Ali ME, Rogers TB, Lederer WJ, Escande D, Le MH, Bennett V. 2003. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature 421:634–639.

Mohler PJ, Splawski I, Napolitano C, Bottelli G, Sharpe L, Timothy K, Priori SG, Keating MT, Bennett V. 2004. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. Proc Natl Acad Sci USA 101:9137–9142.

Mohler PJ, Bennett V. 2005. Ankyrin-based cardiac arrhythmias: a new class of channelopathies due to loss of cellular targeting. Curr Opin Cardiol 20:189–193.

Mohler PJ, Le SS, Denjoy I, Lowe JS, Guicheney P, Caron L, Driskell IM, Schott JJ, Norris K, Leenhardt A, Kim RB, Escande D, Roden DM. 2007. Defining the cellular phenotype of "ankyrin-B syndrome" variants: human ANK2 variants associated with clinical phenotypes display a spectrum of activities in cardiomyocytes. Circulation 115:432–441.

Morita H, Kusano-Fukushima K, Nagase S, Fujimoto Y, Hisamatsu K, Fujio H, Haraoka K, Kobayashi M, Morita ST, Nakamura K, Emori T, Matsubara H, Hina K, Kita T, Fukatani M, Ohe T. 2002. Atrial fibrillation and atrial vulnerability in patients with Brugada syndrome. J Am Coll Cardiol 40:1437–1444.

Morita H, Wu J, Zipes DP. 2008. The QT syndromes: long and short. Lancet 372:750–763.

Moriya M, Seto S, Yano K, Akahoshi M. 2007. Two cases of short QT interval. Pacing Clin Electrophysiol 30:1522–1526.

Moss AJ, Zareba W, Benhorin J, Locati EH, Hall WJ, Robinson JL, Schwartz PJ, Towbin JA, Vincent GM, Lehmann MH. 1995. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. Circulation 92:2929–2934.

Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, Medina A, Zhang L, Robinson JL, Timothy K, Towbin JA, Andrews ML. 2000. Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. Circulation 101:616–623.

Moss AJ, Zareba W, Kaufman ES, Gartman E, Peterson DR, Benhorin J, Towbin JA, Keating MT, Priori SG, Schwartz PJ, Vincent GM, Robinson JL, Andrews ML, Feng C, Hall WJ, Medina A, Zhang L, Wang Z. 2002. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. Circulation 105:794–799.

Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S. 2007. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. Circulation 115:2481–2489.

Murai T, Kakizuka A, Takumi T, Ohkubo H, Nakanishi S. 1989. Molecular cloning and sequence analysis of human genomic DNA encoding a novel membrane protein which exhibits a slowly activating potassium channel activity. Biochem Biophys Res Commun 161:176–181.

Murray A, Donger C, Fenske C, Spillman I, Richard P, Dong YB, Neyroud N, Chevalier P, Denjoy I, Carter N, Syrris P, Afzal AR, Patton MA, Guicheney P, Jeffery S. 1999. Splicing mutations in KCNQ1: a mutation hot spot at codon 344 that produces in frame transcripts. Circulation 100:1077–1084.

Nakajima T, Furukawa T, Hirano Y, Tanaka T, Sakurada H, Takahashi T, Nagai R, Itoh T, Katayama Y, Nakamura Y, Hiraoka M. 1999. Voltage-shift of the current activation in HERG S4 mutation (R534C) in LQT2. Cardiovasc Res 44:283–293.

Nakajima T, Kurabayashi M, Ohyama Y, Kaneko Y, Furukawa T, Itoh T, Taniguchi Y, Tanaka T, Nakamura Y, Hiraoka M, Nagai R. 2000. Characterization of S818L mutation in HERG C-terminus in LQT2. Modification of activation-deactivation gating properties. FEBS Lett 481:197–203.

Napolitano C, Schwartz PJ, Brown AM, Ronchetti E, Bianchi L, Pinnavaia A, Acquaro G, Priori SG. 2000. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. J Cardiovasc Electrophysiol 11:691–696.

Napolitano C, Rivolta I, Priori SG. 2003. Cardiac sodium channel diseases. Clin Chem Lab Med 41:439–444.

Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S. 2005. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. JAMA 294:2975–2980.

Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, Barhanin J, Faure S, Gary F, Coumel P, Petit C, Schwartz K, Guicheney P. 1997. A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nat Genet 15:186–189.

Neyroud N, Denjoy I, Donger C, Gary F, Villain E, Leenhardt A, Benali K, Schwartz K, Coumel P, Guicheney P. 1998. Heterozygous mutation in the pore of potassium channel gene KvLQT1 causes an apparently normal phenotype in long QT syndrome. Eur J Hum Genet 6:129–133.

Neyroud N, Richard P, Vignier N, Donger C, Denjoy I, Demay L, Shkolnikova M, Pesce R, Chevalier P, Hainque B, Coumel P, Schwartz K, Guicheney P. 1999. Genomic organization of the KCNQ1 K+channel gene and identification of C-terminal mutations in the long-QT syndrome. Circ Res 84:290–297.

Nichols CG, Makhina EN, Pearson WL, Sha Q, Lopatin AN. 1996. Inward rectification and implications for cardiac excitability. Circ Res 78:1–7.

Nicolas M, Dememes D, Martin A, Kupershmidt S, Barhanin J. 2001. KCNQ1/KCNE1 potassium channels in mammalian vestibular dark cells. Hear Res 153:132–145.

Ning L, Moss AJ, Zareba W, Robinson J, Rosero S, Ryan D, Qi M. 2003. Novel compound heterozygous mutations in the KCNQ1 gene associated with autosomal recessive long QT syndrome (Jervell and Lange-Nielsen syndrome). Ann Noninvasive Electrocardiol 8:246–250.

Niu DM, Hwang B, Hwang HW, Wang NH, Wu JY, Lee PC, Chien JC, Shieh RC, Chen YT. 2006. A common SCN5A polymorphism attenuates a severe cardiac

phenotype caused by a nonsense SCN5A mutation in a Chinese family with an inherited cardiac conduction defect. J Med Genet 43:817–821.
Norgaard-Pedersen B, Simonsen H. 1999. Biological specimen banks in neonatal screening. Acta Paediatr Suppl 88:106–109.
Nuyens D, Stengl M, Dugarmaa S, Rossenbacker T, Compernolle V, Rudy Y, Smits JF, Flameng W, Clancy CE, Moons L, Vos MA, Dewerchin M, Benndorf K, Collen D, Carmeliet E, Carmeliet P. 2001. Abrupt rate accelerations or premature beats cause life-threatening arrhythmias in mice with long-QT3 syndrome. Nat Med 7:1021–1027.
Ohno S, Zankov DP, Yoshida H, Tsuji K, Makiyama T, Itoh H, Akao M, Hancox JC, Kita T, Horie M. 2007. N- and C-terminal KCNE1 mutations cause distinct phenotypes of long QT syndrome. Heart Rhythm 4:332–340.
Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, Horton SC, Rodeheffer RJ, Anderson JL. 2005. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. JAMA 293:447–454.
Palygin OA, Pettus JM, Shibata EF. 2008. Regulation of caveolar cardiac sodium current by a single Gsalpha histidine residue. Am J Physiol Heart Circ Physiol 294:H1693–H1699.
Papadatos GA, Wallerstein PM, Head CE, Ratcliff R, Brady PA, Benndorf K, Saumarez RC, Trezise AE, Huang CL, Vandenberg JI, Colledge WH, Grace AA. 2002. Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene Scn5a. Proc Natl Acad Sci USA 99:6210–6215.
Park KH, Piron J, Dahimene S, Merot J, Baro I, Escande D, Loussouarn G. 2005. Impaired KCNQ1-KCNE1 and phosphatidylinositol-4,5-bisphosphate interaction underlies the long QT syndrome. Circ Res 96:730–739.
Patel C, Antzelevitch C. 2008. Pharmacological approach to the treatment of long and short QT syndromes. Pharmacol Ther 118:138–151.
Paulussen A, Yang P, Pangalos M, Verhasselt P, Marrannes R, Verfaille C, Vandenberk I, Crabbe R, Konings F, Luyten W, Armstrong M. 2000. Analysis of the human KCNH2(HERG) gene: identification and characterization of a novel mutation Y667X associated with long QT syndrome and a non-pathological 9 bp insertion. Hum Mutat 15:483.
Paulussen A, Raes A, Matthijs G, Snyders DJ, Cohen N, Aerssens J. 2002. A novel mutation (T65P) in the PAS domain of the human potassium channel HERG results in the long QT syndrome by trafficking deficiency. J Biol Chem 277:48610–48616.
Paulussen A, Matthijs G, Gewillig M, Verhasselt P, Cohen N, Aerssens J. 2003. Mutation analysis in congenital Long QT Syndrome–a case with missense mutations in KCNQ1 and SCN5A. Genet Test 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. 2004. Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. J Mol Med 82:182–188.
Pellegrino PL, Bafunno V, Ieva R, Brunetti ND, Mavilio G, Sessa F, Grimaldi M, Margaglione M, Di BM. 2007. A novel mutation in human ether-a-go-go-related gene, alanine to proline at position 490, found in a large family with autosomal dominant long QT syndrome. Am J Cardiol 99:1737–1740.
Piippo K, Laitinen P, Swan H, Toivonen L, Viitasalo M, Pasternack M, Paavonen K, Chapman H, Wann KT, Hirvela E, Sajantila A, Kontula K. 2000. Homozygosity for a HERG potassium channel mutation causes a severe form of long QT syndrome: identification of an apparent founder mutation in the Finns. J Am Coll Cardiol 35:1919–1925.
Piippo K, Swan H, Pasternack M, Chapman H, Paavonen K, Viitasalo M, Toivonen L, Kontula K. 2001. A founder mutation of the potassium channel KCNQ1 in long QT syndrome: implications for estimation of disease prevalence and molecular diagnostics. J Am Coll Cardiol 37:562–568.
Plaster NM, Tawil R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, Donaldson MR, Iannaccone ST, Brunt E, Barohn R, Clark J, Deymeer F, George Jr AL, Fish FA, Hahn A, Nitu A, Ozdemir C, Serdaroglu P, Subramony SH, Wolfe G, Fu YH, Ptacek LJ. 2001. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. Cell 105:511–519.
Poelzing S, Rosenbaum DS. 2005. Cellular mechanisms of Torsade de Pointes. Novartis Found Symp 266:204–217.
Preisig-Muller R, Schlichthorl G, Goerge T, Heinen S, Bruggemann A, Rajan S, Derst C, Veh RW, Daut J. 2002. Heteromerization of Kir2.x potassium channels contributes to the phenotype of Andersen's syndrome. Proc Natl Acad Sci USA 99:7774–7779.
Priori SG, Schwartz PJ, Napolitano C, Bianchi L, Dennis A, De FM, Brown AM, Casari G. 1998. A recessive variant of the Romano-Ward long-QT syndrome? Circulation 97:2420–2425.
Priori SG, Napolitano C, Schwartz PJ. 1999. Low penetrance in the long-QT syndrome: clinical impact. Circulation 99:529–533.
Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. 2003. Risk stratification in the long-QT syndrome. N Engl J Med 348:1866–1874.
Priori SG, Napolitano C, Schwartz PJ, Grillo M, Bloise R, Ronchetti E, Moncalvo C, Tulipani C, Veia A, Bottelli G, Nastoli J. 2004. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. JAMA 292:1341–1344.
Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dhamoon A, Napolitano C, Anumonwo J, di Barletta MR, Gudapakkam S, Bosi G, Stramba-Badiale M, Jalife J. 2005. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. Circ Res 96:800–807.
Priori SG, Napolitano C. 2006. Role of genetic analyses in cardiology: Part I: mendelian diseases: cardiac channelopathies. Circulations 113:1130–1135.
Rautaharju PM, Zhou SH, Wong S, Calhoun HP, Berenson GS, Prineas R, Davignon A. 1992. Sex differences in the evolution of the electrocardiographic QT intervals with age. Can J Cardiol 8:690–695.
Ravn LS, Aizawa Y, Pollevick GD, Hofman-Bang J, Cordeiro JM, Dixen U, Jensen G, Wu Y, Burashnikov E, Haunso S, Guerchicoff A, Hu D, Svendsen JH, Christiansen M, Antzelevitch C. 2008. Gain of function in IKs secondary to a mutation in KCNE5 associated with atrial fibrillation. Heart Rhythm 5:427–435.
Reimann F, Ashcroft FM. 1999. Inwardly rectifying potassium channels. Curr Opin Cell Biol 11:503–508.
Repetto S, Bado M, Broda P, Lucania G, Masetti E, Sotgia F, Carbonne I, Pavan A, Bonilla E, Cordone G, Lisanti MP, Minetti C. 1999. Increased number of caveolae and caveolin-3 overexpression in Duchenne muscular dystrophy. Biochem Biophys Res Commun 261:547–550.
Remme CA, Wilde AA, Bezzina CR. 2008. Cardiac sodium channel overlap syndromes: different faces of SCN5A mutations. Trends Cardiovasc Med 18:78–87.
Rhodes TE, Abraham RL, Welch RC, Vanoye CG, Crotti L, Arnestad M, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Rognum T, Roden DM, Schwartz PJ, George Jr AL. 2008. Cardiac potassium channel dysfunction in sudden infant death syndrome. J Mol Cell Cardiol 44:571–581.
Richter S, Brugada P. 2006. Risk-stratifying Jervell and Lange-Nielsen syndrome from clinical data. J Cardiovasc Electrophysiol 17:1169–1171.
Rivolta I, Abriel H, Tateyama M, Liu H, Memmi M, Vardas P, Napolitano C, Priori SG, Kass RS. 2001. Inherited Brugada and long QT-3 syndrome mutations of a single residue of the cardiac sodium channel confer distinct channel and clinical phenotypes. J Biol Chem 276:30623–30630.
Rivolta I, Clancy CE, Tateyama M, Liu H, Priori SG, Kass RS. 2002. A novel SCN5A mutation associated with long QT-3: altered inactivation kinetics and channel dysfunction. Physiol Genomics 10:191–197.
Roden DM, Viswanathan PC. 2005. Genetics of acquired long QT syndrome. J Clin Invest 115:2025–2032.
Roden DM. 2006. Long QT syndrome: reduced repolarization reserve and the genetic link. J Intern Med 259:59–69.
Romano C, Gemme G, Pongiglione R. 1963. Rare cardiac arrhythmias of the pediatric age II. Syncopal attacks due to paroxysmal ventricular fibrillation. (presentation of 1st case in Italian pediatric literature). Clin Pediatr (Bologna) 45:656–683.
Rossenbacker T, Mubagwa K, Jongbloed RJ, Vereecke J, Devriendt K, Gewillig M, Carmeliet E, Collen D, Heidbuchel H, Carmeliet P. 2005. Novel mutation in the Per-Arnt-Sim domain of KCNH2 causes a malignant form of long-QT syndrome. Circulation 111:961–968.
Ruan Y, Liu N, Bloise R, Napolitano C, Priori SG. 2007. Gating properties of SCN5A mutations and the response to mexiletine in long-QT syndrome type 3 patients. Circulation 116:1137–1144.
Russell MW, Dick M, Collins FS, Brody LC. 1996. KVLQT1 mutations in three families with familial or sporadic long QT syndrome. Hum Mol Genet 5:1319–1324.
Saarinen K, Swan H, Kainulainen K, Toivonen L, Viitasalo M, Kontula K. 1998. Molecular genetics of the long QT syndrome: two novel mutations of the KVLQT1 gene and phenotypic expression of the mutant gene in a large kindred. Hum Mutat 11:158–165.
Saffitz JE. 2005. Structural heart disease, SCN5A gene mutations, and Brugada syndrome: a complex menage a trois. Circulation 112:3672–3674.
Sanguinetti MC, Jiang C, Curran ME, Keating MT. 1995. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 81:299–307.
Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. 1996. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. Nature 384:80–83.
Sansone V, Griggs RC, Meola G, Ptacek LJ, Barohn R, Iannaccone S, Bryan W, Baker N, Janas SJ, Scott W, Ririe D, Tawil R. 1997. Andersen's syndrome: a distinct periodic paralysis. Ann Neurol 42:305–312.
Satler CA, Walsh EP, Vesely MR, Plummer MH, Ginsburg GS, Jacob HJ. 1996. Novel missense mutation in the cyclic nucleotide-binding domain of HERG causes long QT syndrome. Am J Med Genet 65:27–35.
Satler CA, Vesely MR, Duggal P, Ginsburg GS, Beggs AH. 1998. Multiple different missense mutations in the pore region of HERG in patients with long QT syndrome. Hum Genet 102:265–272.

Schimpf R, Wolpert C, Bianchi F, Giustetto C, Gaita F, Bauersfeld U, Borggrefe M. 2003. Congenital short QT syndrome and implantable cardioverter defibrillator treatment: Inherent risk for inappropriate shock delivery. J Cardiovasc Electrophysiol 14:1273–1277.

Schimpf R, Bauersfeld U, Gaita F, Borggrefe M, Wolpert C. 2005. Short QT syndrome: successful prevention of sudden cardiac death in an adolescent by implantable cardioverter defibrillator treatment for primary prophylaxis. Heart Rhythm 2:416–417.

Schimpf R, Veltmann C, Giustetto C, Gaita F, Borggrefe M, Wolpert C. 2007. In vivo effects of mutant HERG K+ channel inhibition by disopyramide in patients with a short QT-1 syndrome: A pilot study. J Cardiovasc Electrophysiol 18: 1157–1160.

Schmitt N, Calloe K, Nielsen NH, Buschmann M, Speckmann EJ, Schulze-Bahr E, Schwarz M. 2007. The novel C-terminal KCNQ1 mutation M520R alters protein trafficking. Biochem Biophys Res Commun 358:304–310.

Schott JJ, Charpentier F, Peltier S, Foley P, Drouin E, Bouhour JB, Donnelly P, Vergnaud G, Bachner L, Moisan JP. 1995. Mapping of a gene for long QT syndrome to chromosome 4q25-27. Am J Hum Genet 57:1114–1122.

Schulze-Bahr E, Haverkamp W, Wedekind H, Rubie C, Hordt M, Borggrefe M, Assmann G, Breithardt G, Funke H. 1997a. Autosomal recessive long-QT syndrome (Jervell Lange-Nielsen syndrome) is genetically heterogeneous. Hum Genet 100:573–576.

Schulze-Bahr E, Wang Q, Wedekind H, Haverkamp W, Chen Q, Sun Y, Rubie C, Hordt M, Towbin JA, Borggrefe M, Assmann G, Qu X, Somberg JC, Breithardt G, Oberti C, Funke H. 1997b. KCNE1 mutations cause jervell and Lange-Nielsen syndrome. Nat Genet 17:267–268.

Schulze-Bahr E, Schwarz M, Hauenschild S, Wedekind H, Funke H, Haverkamp W, Breithardt G, Pongs O, Isbrandt D. 2001. A novel long-QT 5 gene mutation in the C-terminus (V109I) is associated with a mild phenotype. J Mol Med 79:504–509.

Schwartz PJ. 1985. Idiopathic long QT syndrome: progress and questions. Am Heart J 109:399–411.

Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. 1993. Diagnostic criteria for the long QT syndrome. An update. Circulation 88:782–784.

Schwartz PJ, Stramba-Badiale M, Segantini A, Austoni P, Bosi G, Giorgetti R, Grancini F, Marni ED, Perticone F, Rosti D, Salice P. 1998. Prolongation of the QT interval and the sudden infant death syndrome. N Engl J Med 338:1709–1714.

Schwartz PJ, Priori SG, Dumaine R, Napolitano C, Antzelevitch C, Stramba-Badiale M, Richard TA, Berti MR, Bloise R. 2000. A molecular link between the sudden infant death syndrome and the long-QT syndrome. N Engl J Med 343:262–267.

Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R. 2001. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation 103:89–95.

Schwartz PJ, Priori SG, Napolitano C. 2003. How really rare are rare diseases?: the intriguing case of independent compound mutations in the long QT syndrome. J Cardiovasc Electrophysiol 14:1120–1121.

Schwartz PJ, Priori SG, Cerrone M, Spazzolini C, Odero A, Napolitano C, Bloise R, De Ferrari GM, Klersy C, Moss AJ, Zareba W, Robinson JL, Hall WJ, Brink PA, Toivonen L, Epstein AE, Li C, Hu D. 2004. Left cardiac sympathetic denervation in the management of high-risk patients affected by the long-QT syndrome. Circulation 109:1826–1833.

Schwartz PJ. 2006. The congenital long QT syndromes from genotype to phenotype: clinical implications. J Intern Med 259:39–47.

Schwartz PJ, Vanoli E, Crotti L, Spazzolini C, Ferrandi C, Goosen A, Hedley P, Heradien M, Bacchini S, Turco A, La Rovere MT, Bartoli A, George Jr AL, Brink PA. 2008. Neural control of heart rate is an arrhythmia risk modifier in long QT syndrome. J Am Coll Cardiol 51:920–929.

Seebohm G, Westenskow P, Lang F, Sanguinetti MC. 2005. Mutation of colocalized residues of the pore helix and transmembrane segments S5 and S6 disrupt deactivation and modify inactivation of KCNQ1 K+ channels. J Physiol 563:359–368.

Seebohm G, Strutz-Seebohm N, Ureche ON, Henrion U, Baltaev R, Mack AF, Korniychuk G, Steinke K, Tapken D, Pfeufer A, Kaab S, Bucci C, Attali B, Merot J, Tavare JM, Hoppe UC, Sanguinetti MC, Lang F. 2008. Long QT syndrome-associated mutations in KCNQ1 and KCNE1 subunits disrupt normal endosomal recycling of IKs channels. Circ Res 103:1451–1457.

Sesti F, Abbott GW, Wei J, Murray KT, Saksena S, Schwartz PJ, Priori SG, Roden DM, George Jr AL, Goldstein SA. 2000. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc Natl Acad Sci USA 97: 10613–10618.

Shalaby FY, Levesque PC, Yang WP, Little WA, Conder ML, Jenkins-West T, Blanar MA. 1997. Dominant-negative KvLQT1 mutations underlie the LQT1 form of long QT syndrome. Circulation 96:1733–1736.

Shang YP, Xie XD, Wang XX, Chen JZ, Zhu JH, Tao QM, Zheng LR. 2005. A novel splice mutation of HERG in a Chinese family with long QT syndrome. J Zhejiang Univ Sci B 6:626–630.

Sherman J, Tester DJ, Ackerman MJ. 2005. Targeted mutational analysis of ankyrin-B in 541 consecutive, unrelated patients referred for long QT syndrome genetic testing and 200 healthy subjects. Heart Rhythm 2:1218–1223.

Shi R, Zhang Y, Yang C, Huang C, Zhou X, Qiang H, Grace AA, Huang CL, Ma A. 2008. The cardiac sodium channel mutation delQKP 1507-1509 is associated with the expanding phenotypic spectrum of LQT3, conduction disorder, dilated cardiomyopathy, and high incidence of youth sudden death. Europace 10:1329–1335.

Shim SH, Ito M, Maher T, Milunsky A. 2005. Gene sequencing in neonates and infants with the long QT syndrome. Genet Test 9:281–284.

Shimizu W, Antzelevitch C. 2000. Effects of a K(+) channel opener to reduce transmural dispersion of repolarization and prevent torsade de pointes in LQT1, LQT2, and LQT3 models of the long-QT syndrome. Circulation 102: 706–712.

Shimizu M, Ino H, Okeie K, Yamaguchi M, Nagata M, Hayashi K, Itoh H, Iwaki T, Oe K, Konno T, Mabuchi H. 2002. 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 25:335–339.

Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, Washizuka T, Aizawa Y, Nakamura K, Ohe T, Aiba T, Miyamoto Y, Yoshimasa Y, Towbin JA, Priori SG, Kamakura S. 2004. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: multicenter study in Japan. J Am Coll Cardiol 44:117–125.

Shimoni Y, Clark RB, Giles WR. 1992. Role of an inwardly rectifying potassium current in rabbit ventricular action potential. J Physiol 448:709–727.

Shushi L, Kerem B, Goldmit M, Peretz A, Attali B, Medina A, Towbin JA, Kurokawa J, Kass RS, Benhorin J. 2005. Clinical, genetic, and electrophysiologic characteristics of a new PAS-domain HERG mutation (M124R) causing Long QT syndrome. Ann Noninvasive Electrocardiol 10:334–341.

Sicouri S, Fish J, Antzelevitch C. 1994. Distribution of M cells in the canine ventricle. J Cardiovasc Electrophysiol 5:824–837.

Simons K, Toomre D. 2000. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1:31–39.

Singer D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N. 1991. The roles of the subunits in the function of the calcium channel. Science 253:1553–1557.

Sinner MF, Pfeufer A, Akyol M, Beckmann BM, Hinterseer M, Wacker A, Perz S, Sauter W, Illig T, Nabauer M, Schmitt C, Wichmann HE, Schomig A, Steinbeck G, Meitinger T, Kaab S. 2008. The non-synonymous coding IKr-channel variant KCNH2-K897T is associated with atrial fibrillation: results from a systematic candidate gene-based analysis of KCNH2 (HERG). Eur Heart J 29:907–914.

Smith FD, Scott JD. 2006. Anchored cAMP signaling: onward and upward - a short history of compartmentalized cAMP signal transduction. Eur J Cell Biol 85: 585–592.

Smith JA, Vanoye CG, George Jr AL, Meiler J, Sanders CR. 2007. Structural models for the KCNQ1 voltage-gated potassium channel. Biochemistry 46:-14141–14152.

Soldatov NM. 1994. Genomic structure of human L-type Ca2+ channel. Genomics 22:77–87.

Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M, Chu C, Kohtz DS, Lisanti MP. 1996. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. J Biol Chem 271:15160–15165.

Splawski I, Timothy KW, Vincent GM, Atkinson DL, Keating MT. 1997a. Molecular basis of the long-QT syndrome associated with deafness. N Engl J Med 336:1562–1567.

Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, Keating MT. 1997b. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. Nat Genet 17:338–340.

Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. 1998. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. Genomics 51:86–97.

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

Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, Napolitano C, Schwartz PJ, Joseph RM, Condouris K, Tager-Flusberg H, Priori SG, Sanguinetti MC, Keating MT. 2004. Ca(V)1.2 calcium channel dysfunction

causes a multisystem disorder including arrhythmia and autism. Cell 119: 19–31.

Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, Sanguinetti MC, Keating MT. 2005. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. Proc Natl Acad Sci USA 102:8089–8096.

Sprong H, van der Sluijs P, van MG. 2001. How proteins move lipids and lipids move proteins. Nat Rev Mol Cell Biol 2:504–513.

St-Pierre J, Roy N, Blier L, Plante E, Cote JM, Gilbert M, Chahine M. 2000. The distinct HERG missense mutation L564P causes long QT syndrome in one French Canadian family. Can J Cardiol 16:307–312.

Struijk JJ, Kanters JK, Andersen MP, Hardahl T, Graff C, Christiansen M, Toft E. 2006. Classification of the long-QT syndrome based on discriminant analysis of T-wave morphology. Med Biol Eng Comput 44:543–549.

Surawicz B. 1995. Long QT interval, torsade de pointes, and early afterdepolarizations. In: Electrophysiologic basis of ECG and cardiac arrhythmias. Malvern, PA: Williams & Wilkins. p 191–229.

Surber R, Hensellek S, Prochnau D, Werner GS, Benndorf K, Figulla HR, Zimmer T. 2008. Combination of cardiac conduction disease and long QT syndrome caused by mutation T1620K in the cardiac sodium channel. Cardiovasc Res 77:740–748.

Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L. 1999. Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects. J Am Coll Cardiol 34:823–829.

Tan HL, Bink-Boelkens MT, Bezzina CR, Viswanathan PC, Beaufort-Krol GC, van Tintelen PJ, van Den Berg MP, Wilde AA, Balser JR. 2001. A sodium-channel mutation causes isolated cardiac conduction disease. Nature 409:1043–1047.

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. 2001. 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 37:911–919.

Tanaka T, Nagai R, Tomoike H, Takata S, Yano K, Yabuta K, Haneda N, Nakano O, Shibata A, Sawayama T, Kasai H, Yazaki Y, Nakamura Y. 1997. Four novel KVLQT1 and four novel HERG mutations in familial long-QT syndrome. Circulation 95:565–567.

Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, Nishimoto I, Lodish HF, Lisanti MP. 1996. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. J Biol Chem 271:2255–2261.

Tang ZZ, Liang MC, Lu S, Yu D, Yu CY, Yue DT, Soong TW. 2004. Transcript scanning reveals novel and extensive splice variations in human L-type voltage-gated calcium channel, Cav1.2 α_1 subunit. J Biol Chem 279:44335–44343.

Tawil R, Ptacek LJ, Pavlakis SG, DeVivo DC, Penn AS, Ozdemir C, Griggs RC. 1994. Andersen's syndrome: potassium-sensitive periodic paralysis, ventricular ectopy, and dysmorphic features. Ann Neurol 35:326–330.

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

Tester DJ, Arya P, Will M, Haglund CM, Farley AL, Makielski JC, Ackerman MJ. 2006. Genotypic heterogeneity and phenotypic mimicry among unrelated patients referred for catecholaminergic polymorphic ventricular tachycardia genetic testing. Heart Rhythm 3:800–805.

Tester DJ, Ackerman MJ. 2009. Cardiomyopathic and channelopathic causes of sudden unexplained death in infants and children. Annu Rev Med 60:69–84.

Tian H, Emrich CA, Scherer JR, Mathies RA, Andersen PS, Larsen LA, Christiansen M. 2005. High-throughput single-strand conformation polymorphism analysis on a microfabricated capillary array electrophoresis device. Electrophoresis 26:1834–1842.

Tranebjaerg L, Bathen J, Tyson J, Bitner-Glindzicz M. 1999. Jervell and Lange-Nielsen syndrome: a Norwegian perspective. Am J Med Genet 89:137–146.

Tristani-Firouzi M, Jensen JL, Donaldson MR, Sansone V, Meola G, Hahn A, Bendahhou S, Kwiecinski H, Fidzianska A, Plaster N, Fu YH, Ptacek LJ, Tawil R. 2002. Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome). J Clin Invest 110:381–388.

Trudeau MC, Warmke JW, Ganetzky B, Robertson GA. 1995. HERG, a human inward rectifier in the voltage-gated potassium channel family. Science 269:92–95.

Tse WT, Menninger JC, Yang-Feng TL, Francke U, Sahr KE, Lux SE, Ward DC, Forget BG. 1991. Isolation and chromosomal localization of a novel nonerythroid ankyrin gene. Genomics 10:858–866.

Tsuji K, Akao M, Ishii TM, Ohno S, Makiyama T, Takenaka K, Doi T, Haruna Y, Yoshida H, Nakashima T, Kita T, Horie M. 2007. Mechanistic basis for the pathogenesis of long QT syndrome associated with a common splicing mutation in KCNQ1 gene. J Mol Cell Cardiol 42:662–669.

Tsurugi T, Nagatomo T, Abe H, Oginosawa Y, Takemasa H, Kohno R, Makita N, Makielski JC, Otsuji Y. 2009. Differential modulation of late sodium current by protein kinase A in R1623Q mutant of LQT3. Life Sci 13 84:380–387.

Tyson J, Tranebjaerg L, Bellman S, Wren C, Taylor JF, Bathen J, Aslaksen B, Sorland SJ, Lund O, Malcolm S, Pembrey M, Bhattacharya S, Bitner-Glindzicz M. 1997. IsK and KvLQT1: mutation in either of the two subunits of the slow component of the delayed rectifier potassium channel can cause Jervell and Lange-Nielsen syndrome. Hum Mol Genet 6:2179–2185.

Tyson J, Tranebjaerg L, McEntagart M, Larsen LA, Christiansen M, Whiteford ML, Bathen J, Aslaksen B, Sorland SJ, Lund O, Pembrey ME, Malcolm S, Bitner-Glindzicz M. 2000. Mutational spectrum in the cardioauditory syndrome of Jervell and Lange-Nielsen. Hum Genet 107:499–503.

Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, Ackerman MJ, Makielski JC. 2008. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. Proc Natl Acad Sci USA 105:9355–9360.

Valdivia CR, Ackerman MJ, Tester DJ, Wada T, McCormack J, Ye B, Makielski JC. 2002. A novel SCN5A arrhythmia mutation, M1766L, with expression defect rescued by mexiletine. Cardiovasc Res 55:279–289.

van den Berg MH, Wilde AA, Robles de Medina EO, Meyer H, Geelen JL, Jongbloed RJ, Wellens HJ, Geraedts JP. 1997. The long QT syndrome: a novel missense mutation in the S6 region of the KVLQT1 gene. Hum Genet 100:356–361.

Vandenberg CA. 1987. Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. Proc Natl Acad Sci USA 84:2560–2564.

van Langen IM, Birnie E, Alders M, Jongbloed RJ, Le MH, Wilde AA. 2003. The use of genotype-phenotype correlations in mutation analysis for the long QT syndrome. J Med Genet 40:141–145.

Van Petegem F, Clark KA, Chatelain FC, Minor DL. 2004. Structure of a complex between a voltage-gated calcium channel beta-subunit and an alpha-subunit domain. Nature 429:671–675.

Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foell JD, Li Z, Kamp TJ, Towbin JA. 2006. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation 114:2104–2112.

Viard P, Butcher AJ, Halet G, Davies A, Nurnberg B, Heblich F, Dolphin AC. 2004. PI3K promotes voltage-dependent calcium channel trafficking to the plasma membrane. Nat Neurosci 7:939–946.

Vincent GM, Timothy KW, Leppert M, Keating M. 1992. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. N Engl J Med 327:846–852.

Viswanathan PC, Benson DW, Balser JR. 2003. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. J Clin Invest 111:341–346.

Wang Q, Shen J, Li Z, Timothy K, Vincent GM, Priori SG, Schwartz PJ, Keating MT. 1995a. Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia. Hum Mol Genet 4:1603–1607.

Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. 1995b. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 80:805–811.

Wang DW, Yazawa K, George Jr AL, Bennett PB. 1996a. Characterization of human cardiac Na+ channel mutations in the congenital long QT syndrome. Proc Natl Acad Sci USA 93:13200–13205.

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

Wang Q, Li Z, Shen J, Keating MT. 1996c. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 34:9–16.

Wang Z, Yue L, White M, Pelletier G, Nattel S. 1998. Differential distribution of inward rectifier potassium channel transcripts in human atrium versus ventricle. Circulation 98:2422–2428.

Wang Z, Li H, Moss AJ, Robinson J, Zareba W, Knilans T, Bowles NE, Towbin JA. 2002. Compound heterozygous mutations in KvLQT1 cause Jervell and Lange-Nielsen syndrome. Mol Genet Metab 75:308–316.

Wang D, Papp AC, Binkley PF, Johnson JA, Sadee W. 2006. Highly variable mRNA expression and slicing of L-type voltage-dependent calcium channel alpha subunit 1C in human heart tissues. Pharmacogenetics and Genomics 16:735–745.

Wang DW, Desai RR, Crotti L, Arnestad M, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Rognum T, Schwartz PJ, George Jr AL. 2007. Cardiac sodium channel dysfunction in sudden infant death syndrome. Circulation 115:368–376.

Ward OC. 1964. A new familial cardiac syndrome in children. J Ir Med Assoc 54:103–106.

Wattanasirichaigoon D, Vesely MR, Duggal P, Levine JC, Blume ED, Wolff GS, Edwards SB, Beggs AH. 1999. Sodium channel abnormalities are infrequent in patients with long QT syndrome: identification of two novel SCN5A mutations. Am J Med Genet 86:470–476.

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Wedekind H, Smits JP, Schulze-Bahr E, Arnold R, Veldkamp MW, Bajanowski T, Borggrefe M, Brinkmann B, Warnecke I, Funke H, Bhuiyan ZA, Wilde AA, Breithardt G, Haverkamp W. 2001. De novo mutation in the SCN5A gene associated with early onset of sudden infant death. Circulation 104:1158–1164.

Wehrens XH, Rossenbacker T, Jongbloed RJ, Gewillig M, Heidbuchel H, Doevendans PA, Vos MA, Wellens HJ, Kass RS. 2003. A novel mutation L619F in the cardiac Na+ channel SCN5A associated with long-QT syndrome (LQT3): a role for the I-II linker in inactivation gating. Hum Mutat 21:552.

Wei J, Wang DW, Alings M, Fish F, Wathen M, Roden DM, George Jr AL. 1999. Congenital long-QT syndrome caused by a novel mutation in a conserved acidic domain of the cardiac Na+ channel. Circulation 99:3165–3171.

Wei J, Fish FA, Myerburg RJ, Roden DM, George Jr AL. 2000. Novel KCNQ1 mutations associated with recessive and dominant congenital long QT syndromes: evidence for variable hearing phenotype associated with R518X. Hum Mutat 15:387–388.

Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. 2004. Compound mutations: a common cause of severe long-QT syndrome. Circulation 109:1834–1841.

Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser ID, Langeberg LK, Sheng M, Scott JD. 1999. Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. Science 285:93–96.

Wilde AA, Jongbloed RJ, Doevendans PA, Duren DR, Hauer RN, van Langen IM, van Tintelen JP, Smeets HJ, Meyer H, Geelen JL. 1999. Auditory stimuli as a trigger for arrhythmic events differentiate HERG-related (LQTS2) patients from KVLQT1-related patients (LQTS1). J Am Coll Cardiol 33:327–332.

Wilson AJ, Quinn KV, Graves FM, Bitner-Glindzicz M, Tinker A. 2005. Abnormal KCNQ1 trafficking influences disease pathogenesis in hereditary long QT syndromes (LQT1). Cardiovasc Res 67:476–486.

Witczak O, Skalhegg BS, Keryer G, Bornens M, Tasken K, Jahnsen T, Orstavik S. 1999. Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450. EMBO J 18:1858–1868.

Wollnik B, Schroeder BC, Kubisch C, Esperer HD, Wieacker P, Jentsch TJ. 1997. Pathophysiological mechanisms of dominant and recessive KVLQT1 K+channel mutations found in inherited cardiac arrhythmias. Hum Mol Genet 6:1943–1949.

Wu DM, Lai LP, Zhang M, Wang HL, Jiang M, Liu XS, Tseng GN. 2006. Characterization of an LQT5-related mutation in KCNE1, Y81C: implications for a role of KCNE1 cytoplasmic domain in IKs channel function. Heart Rhythm 3:1031–1040.

Yamaguchi M, Shimizu M, Ino H, Terai H, Hayashi K, Mabuchi H, Hoshi N, Higashida H. 2003a. Clinical and electrophysiological characterization of a novel mutation (F193L) in the KCNQ1 gene associated with long QT syndrome. Clin Sci (Lond) 104:377–382.

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

Yamaguchi M, Shimizu M, Ino H, Terai H, Hayashi K, Kaneda T, Mabuchi H, Sumita R, Oshima T, Hoshi N, Higashida H. 2005. Compound heterozygosity for mutations Asp611->Tyr in KCNQ1 and Asp609->Gly in KCNH2 associated with severe long QT syndrome. Clin Sci (Lond) 108:143–150.

Yamashita F, Horie M, Kubota T, Yoshida H, Yumoto Y, Kobori A, Ninomiya T, Kono Y, Haruna T, Tsuji K, Washizuka T, Takano M, Otani H, Sasayama S, Aizawa Y. 2001. Characterization and subcellular localization of KCNQ1 with a heterozygous mutation in the C terminus. J Mol Cell Cardiol 33:197–207.

Yang J, Jan YN, Jan LY. 1995. Determination of the subunit stoichiometry of an inwardly rectifying potassium channel. Neuron 15:1441–1447.

Yang N, George Jr AL, Horn R. 1996. Molecular basis of charge movement in voltage-gated sodium channels. Neuron 16:113–122.

Yang WP, Levesque PC, Little WA, Conder ML, Shalaby FY, Blanar MA. 1997. KvLQT1, a voltage-gated potassium channel responsible for human cardiac arrhythmias. Proc Natl Acad Sci USA 94:4017–4021.

Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, Murray KT, Norris K, George Jr AL, Roden DM. 2002. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. Circulation 105:1943–1948.

Yang Y, Xia M, Jin Q, Bendahhou S, Shi J, Chen Y, Liang B, Lin J, Liu Y, Liu B, Zhou Q, Zhang D, Wang R, Ma N, Su X, Niu K, Pei Y, Xu W, Chen Z, Wan H, Cui J, Barhanin J, Chen Y. 2004. Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. Am J Hum Genet 75:899–905.

Yang Y, Liu Y, Dong X, Kuang Y, Lin J, Su X, Peng L, Jin Q, He Y, Liu B, Pan Z, Li L, Zhu Q, Lin X, Zhou Q, Pan Q, Eurlings PM, Fei J, Wang Z, Chen YH. 2007. Human KCNQ1 S140G mutation is associated with atrioventricular blocks. Heart Rhythm 4:611–618.

Ye B, Valdivia CR, Ackerman MJ, Makielski JC. 2003. A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation. Physiol Genomics 12:187–193.

Ye B, Balijepalli RC, Foell JD, Kroboth S, Ye Q, Luo YH, Shi NQ. 2008. Caveolin-3 Associates with and Affects the Function of Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel 4. Biochemistry 47:12312–12318.

Yoshida H, Horie M, Otani H, Takano M, Tsuji K, Kubota T, Fukunami M, Sasayama S. 1999. Characterization of a novel missense mutation in the pore of HERG in a patient with long QT syndrome. J Cardiovasc Electrophysiol 10:1262–1270.

Yoshida H, Horie M, Otani H, Kawashima T, Onishi Y, Sasayama S. 2001. Bradycardia-induced long QT syndrome caused by a de novo missense mutation in the S2–S3 inner loop of HERG. Am J Med Genet 98:348–352.

Yu FH, Westenbroek RE, Silos-Santiago I, McCormick KA, Lawson D, Ge P, Ferriera H, Lilly J, DiStefano PS, Catterall WA, Scheuer T, Curtis R. 2003. Sodium channel beta4, a new disulfide-linked auxiliary subunit with similarity to beta2. J Neurosci 23:7577–7585.

Yu B, Sawyer NA, Chiu C, Oefner PJ, Underhill PA. 2006. DNA mutation detection using denaturing high-performance liquid chromatography (DHPLC). Curr Protoc Hum Genet Chapter 7:Unit 7.10.

Zamorano-Leon JJ, Alonso-Org, Moreno J, Cinza R, Garcia-Torrent MJ, Perez-Castellano N, Perez-Villacastin J, Macaya C, Lopez-Farre AJ. 2009. Novel mutation (H402R) in the S1 domain of KCNH2-encoded gene associated with long QT syndrome in a Spanish family. Int J Cardiol [http://dx.doi.org/10.1016/j.ijcard.2008.11.166].

Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Robinson JL, Priori SG, Benhorin J, Locati EH, Towbin JA, Keating MT, Lehmann MH, Hall WJ. 1998. Influence of genotype on the clinical course of the long-QT syndrome. International Long-QT Syndrome Registry Research Group. N Engl J Med 339:960–965.

Zareba W, Moss AJ, Sheu G, Kaufman ES, Priori S, Vincent GM, Towbin JA, Benhorin J, Schwartz PJ, Napolitano C, Hall WJ, Keating MT, Qi M, Robinson JL, Andrews ML. 2003. Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. J Cardiovasc Electrophysiol 14:1149–1153.

Zaritsky JJ, Redell JB, Tempel BL, Schwarz TL. 2001. The consequences of disrupting cardiac inwardly rectifying K(+) current (I(K1)) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. J Physiol 533:697–710.

Zehelein J, Thomas D, Khalil M, Wimmer AB, Koenen M, Licka M, Wu K, Kiehn J, Brockmeier K, Kreye VA, Karle CA, Katus HA, Ulmer HE, Schoels W. 2004. Identification and characterisation of a novel KCNQ1 mutation in a family with Romano-Ward syndrome. Biochim Biophys Acta 1690:185–192.

Zehelein J, Kathoefer S, Khalil M, Alter M, Thomas D, Brockmeier K, Ulmer HE, Katus HA, Koenen M. 2006. Skipping of Exon 1 in the KCNQ1 gene causes Jervell and Lange-Nielsen syndrome. J Biol Chem 281:35397–35403.

Zhang L, Timothy KW, Vincent GM, Lehmann MH, Fox J, Giuli LC, Shen J, Splawski I, Priori SG, Compton SJ, Yanowitz F, Benhorin J, Moss AJ, Schwartz PJ, Robinson JL, Wang Q, Zareba W, Keating MT, Towbin JA, Napolitano C, Medina A. 2000. Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. Circulation 102:2849–2855.

Zhang L, Vincent GM, Baralle M, Baralle FE, Anson BD, Benson DW, Whiting B, Timothy KW, Carlquist J, January CT, Keating MT, Splawski I. 2004. An intronic mutation causes long QT syndrome. J Am Coll Cardiol 44:1283–1291.

Zhang L, Benson DW, Tristani-Firouzi M, Ptacek LJ, Tawil R, Schwartz PJ, George AL, Horie M, Andelfinger G, Snow GL, Fu YH, Ackerman MJ, Vincent GM. 2005. Electrocardiographic features in Andersen-Tawil syndrome patients with KCNJ2 mutations: characteristic T-U-wave patterns predict the KCNJ2 genotype. Circulation 111:2720–2726.

Zhang Y, Zhou N, Jiang W, Peng J, Wan H, Huang C, Xie Z, Huang CL, Grace AA, Ma A. 2007. A missense mutation (G604S) in the S5/pore region of HERG causes long QT syndrome in a Chinese family with a high incidence of sudden unexpected death. Eur J Pediatr 166:927–933.

Zhang H, Kharche S, Holden AV, Hancox JC. 2008a. Repolarisation and vulnerability to re-entry in the human heart with short QT syndrome arising from KCNQ1 mutation–a simulation study. Prog Biophys Mol Biol 96:112–131.

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

Zhou Z, Gong Q, Epstein ML, January CT. 1998. HERG channel dysfunction in human long QT syndrome. Intracellular transport and functional defects. J Biol Chem 273:21061–21066.

Zupancic JAF, Triedman JK, Alexander M, Walsh EP, Richardson DK, Berul CI. 2000. Cost-effectiveness and implications of newborn screening for prolongation of the QT interval for the prevention of sudden infant death syndrome. J Pediatr 136:481–489.

Zygmunt AC, Goodrow RJ, Antzelevitch C. 2000. I(NaCa) contributes to electrical heterogeneity within the canine ventricle. Am J Physiol Heart Circ Physiol 278:H1671–H1678.

Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C. 2001. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. Am J Physiol Heart Circ Physiol 281:H689–H697.

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1.5.3 REVIEW 2: THE GENETIC BASIS OF BRUGADA SYNDROME: A MUTATION UPDATE

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MUTATION UPDATE

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The Genetic Basis of Brugada Syndrome: A Mutation Update

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ABSTRACT: Brugada syndrome (BrS) is a condition characterized by a distinct ST-segment elevation in the right precordial leads of the electrocardiogram and, clinically, by an increased risk of cardiac arrhythmia and sudden death. The condition predominantly exhibits an autosomal dominant pattern of inheritance with an average prevalence of 5:10,000 worldwide. Currently, more than 100 mutations in seven genes have been associated with BrS. Loss-of-function mutations in *SCN5A*, which encodes the α-subunit of the $Na_v1.5$ sodium ion channel conducting the depolarizing I_{Na} current, causes 15–20% of BrS cases. A few mutations have been described in *GPD1L*, which encodes glycerol-3-phosphate dehydrogenase-1 like protein; *CACNA1C*, which encodes the α-subunit of the $Ca_v1.2$ ion channel conducting the depolarizing $I_{L,Ca}$ current; *CACNB2*, which encodes the stimulating β2-subunit of the $Ca_v1.2$ ion channel; *SCN1B and SCN3B*, which, in the heart, encodes β-subunits of the $Na_v1.5$ sodium ion channel, and *KCNE3*, which encodes the ancillary inhibitory β-subunit of several potassium channels including the Kv4.3 ion channel conducting the repolarizing potassium I_{to} current. BrS exhibits variable expressivity, reduced penetrance, and "mixed phenotypes," where families contain members with BrS as well as long QT syndrome, atrial fibrillation, short QT syndrome, conduction disease, or structural heart disease, have also been described.
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KEY WORDS: arrhythmia; cardiac repolarization; sudden death; syncope; ion channel

Introduction

Brugada's syndrome (BrS) is a genetic cardiac disease with an autosomal dominant pattern of inheritance and incomplete penetrance [Antzelevitch et al., 2003]. It is characterized by ST-segment elevation in the right precordial leads (V1–V3) of the electrocardiogram (ECG) (see Supp. Fig. S1), and a propensity for sudden death due to rapid polymorphic ventricular tachycardia (VT) or ventricular fibrillation (VF) [Brugada and Brugada, 1992]. Two types of ST-elevation have been described (see Supp. Fig. S1). Self-terminating VF results in symptoms of syncope, seizure, and sleep disturbance, as the arrhythmia is more frequent at night. The ECG characteristics exhibit day-to-day variation and may not always be present [Veltmann et al., 2006]. However, they may be unmasked by administration of sodium channel blockers, for example, ajmaline or flecainide [Brugada et al., 2000a,b; Shimizu et al., 2000], fever [Antzelevitch and Brugada, 2002; Dumaine et al., 1999], vagotonic agents [Mizumaki et al., 2004; Wichter et al., 2002], α-adrenergic agonists [Pastor et al., 2001], β-adrenergic blockers [Miyazaki et al., 1996], cyclic antidepressants [Goldgran-Toledano et al., 2002], hyper- and hypokalemia [Araki et al., 2003; Littmann et al., 2007a], hypercalcemia [Littmann et al., 2007b], alcohol [Pilz and Luft, 2003], and cocaine [Ortega-Carnicer et al., 2001]. The current diagnostic criteria for BrS are given in Box 1.

The mean age of BrS clinical debut is 40 years; however, the first occurrence of symptoms may occur in early childhood or old age [Antzelevitch et al., 2005]. The disease is much more common in men than in women [Brugada et al., 2002b; Priori et al., 2002; Smits et al., 2002], probably due to gender differences in the expression of the I_{to} and $I_{L,Ca}$ [Di Diego et al., 2002; Fish and Antzelevitch, 2003], two currents involved in the cardiac action potential (AP). BrS has been reported to be responsible for >4% of all sudden deaths and 20% of all sudden deaths in patients with structurally normal hearts [Antzelevitch et al., 2005]. The prevalence of the disease is difficult to estimate, as asymptomatic patients are only identified by accidental ECG recording, but it is estimated to be approximately 5:10,000 [Blangy et al., 2005; Bozkurt et al., 2006; Gervacio-Domingo et al., 2008; Ito et al., 2006] and higher in South East Asia, where BrS occurs endemically [Nademanee, 1997]. Among children, the occurrence is much lower than in adults, but the gender difference is still evident with more boys being affected than girls [Yamakawa et al., 2004].

During a mean follow-up of 24 months, BrS patients with no prior cardiac arrest were determined to have an 8% risk of cardiac arrest or VF. Additionally, a spontaneous type 1 ST-segment elevation pattern, male gender, and inducibility of sustained ventricular arrhythmia by programmed ventricular stimulation (PVS) were risk factors [Brugada et al., 2003]. Very strong risk factors are: a previous aborted cardiac arrest, a history of syncopal episodes and spontaneous BrS pattern on the ECG [Brugada et al., 2002a; Priori et al., 2002]. Other studies reported very low risks of

Additional Supporting Information may be found in the online version of this article.

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Box 1: Diagnostic Criteria for BRS

Definite Diagnosis
1.
Type 1 ST-segment elevation pattern (coved ST-segment elevation ≥ 2 mm followed by negative T-wave) in > 1 right precordial lead (V1–V3) in the presence or absence of Na^+ channel-blocking drug *or*
2.
a. Type 2 ST-segment elevation pattern (saddleback appearance with a high takeoff ST-segment elevation of ≥ 2 mm, a trough displaying ≥ 1 mm ST elevation followed by a positive or biphasic T-wave) *or* type 3 (saddleback or coved appearance of ST-elevation < 1 mm), present in > 1 right precordial lead (V1–V3) at baseline
b. AND conversion to type 1 pattern (ST-elevation ≥ 2 mm) following sodium blocker administration.
Both criterion 1 and 2 should be combined with one of the following conditions: ventricular fibrillation (VF); polymorphic ventricular tachycardia (VT); a family history of sudden death at < 45 years old; coved type ECGs in family members; inducibility of VT with programmed electrical stimulation (EPS); syncope or nocturnal agonal respiration.
The following conditions, which can account for the ECG changes and syncope, should be excluded: atypical right bundle branch block; left ventricular hypertrophy; early repolarization; acute pericarditis; acute myocardial ischemia or infarction; pulmonary embolism; Printzmetal angina; dissecting aortic aneurysm; central or peripheral nervous system abnormalities; Duchenne muscular dystrophy; thiamine deficiency; hyperkalemia; hypercalcemia; arrhythmogenic right ventricular cardiomyopathy; pectus excavatum; hypothermia, or mechanical compression of the right outflow tract (RVOT) as seen with mediastinal tumors or hemopericardium.
Adapted from the 2005 consensus paper [Antzelevitch et al., 2005].

arrhythmia in asymptomatic patients with a type 1 ST-segment elevation pattern [Eckardt et al., 2005; Priori et al., 2002]. In children (< 16 years of age) with BrS, fever is the most frequent precipitator of VF and the highest arrhythmia risk was seen in children with a spontaneous type 1 ST-segment elevation pattern and previous clinical symptoms [Probst et al., 2007].

The only proven effective treatment of BrS is prophylactic implantation of an automatic cardioverter-defibrillator (ICD) unit [Brugada et al., 2000a; Nademanee et al., 2003]. However, a recent study has shown that during a follow-up of > 3 years, only 2.6% of a cohort of BrS patients received appropriate shocks, whereas 8.9% experienced device-related complications [Sacher et al., 2006]. The frequency of adverse complications of ICD therapy in BrS underscores the need for better risk stratification. Quinidine has been suggested as an alternative to ICD implantation due to its I_{to} inhibitory effect (see Pathogenetic mechanism) and its VF inhibitory effect [Belhassen et al., 2004]. Due to the lack of prospective studies of treatment effects, quinidine cannot be used as first line therapy in BrS, but could be considered as an adjunctive therapy in high-risk patients with ICD complications [Mizusawa et al., 2006], or in children where ICD implantation is difficult. A recent cost-effectiveness analysis found ICD prophylaxis a cost-effective therapy in BrS, and suggested that quinidine could be used when this therapy is not available, for example, in developing countries [Wang et al., 2008]. It has also been proposed that empiric drug therapy with quinidine in asymptomatic patients with BrS could perform better than ICD implantation. Viskin et al. [2009] propose to initiate a prospective registry to evaluate the use of quinidine on asymptomatic patients with BrS.

Since 1998 [Chen et al., 1998], it has been established that 15–30% of BrS cases [Alings and Wilde, 1999] can be attributed to mutations in *SCN5A* (MIM# 600163). A further 11–12% of BrS cases can be attributed to *CACNA1C* (MIM# 114205) and *CACNB2* (MIM# 600003) [Antzelevitch and Nof, 2008]. Minor contributions to BrS cases are made from mutations in other genes (*GPD1L*; MIM# 611778, *SCN1B*; MIM# 600235, *KCNE3*; MIM# 604433, and *SCN3B*; MIM# 608214). These genes encode proteins involved, directly or indirectly, in the execution of the cardiac AP. Thus, at present, seven types of BrS (BrS1–BrS7) have been described (see Table 1). The present review reports detailed information on known mutations causing BrS.

Pathogenetic Mechanism—In Overview

The contraction of the heart is carefully controlled by the distribution of electrical depolarization from the sinus node to the ventricles. The cardiac AP is the time course of electrical activity over the plasmalemma of a single cardiomyocyte from depolarization, immediately prior to systole, to repolarization ending with diastole. The AP is the result of a concerted action of a number of ion channels (see Fig. 1), where some depolarize the membrane (Na^+ and Ca^{2+}-channels) and others repolarize it (K^+-channels). The AP is divided into five phases: phase 0 represents the initial depolarization; phase 1, an initial repolarization; phase 2, a plateau phase; phase 3, the final repolarization phase; and phase 4, the slow depolarization prior to the next depolarization. Each phase is characterized by the conducting activity of a particular set of channels (see Fig. 1).

Dysfunction of one of several of the ion channels can affect AP duration, and, depending on which particular ion channel is dysfunctional, the dispersion of repolarization may be increased [Antzelevitch, 2005]. These mechanisms are the pathophysiological basis of long QT syndrome (LQTS), short QT syndrome (SQTS), and some cases of AF. In BrS, it is believed that the arrhythmic substrate is the result of increased heterogeneity of the currents involved in the phase I repolarization (Supp. Fig. S2A), of the right ventricle [Antzelevitch, 2001]. Electrophysiological evidence indicates that the basis for arrhythmogenicity is the loss of the AP dome (see Supp. Fig. S2B, in some epi-cardial sites) [Antzelevitch et al., 2002; Kurita et al., 2002; Yan and Antzelevitch, 1999]. This leads to increased epicardial dispersion of repolarization, enabling local reexcitation via phase-2 reentry (Supp. Fig. S2B). This, in turn, may lead to the occurrence of a closely coupled complex that can capture the vulnerable window across the whole right ventricular wall, triggering a circular VT/VF reentry [Lukas and Antzelevitch, 1996; Yan and Antzelevitch, 1999]. The effect of quinidine in BrS is attributed to an inhibitory effect on I_{to}, and thus to a reduction of the electrical heterogeneity and reduced phase 2 reentrant activity [Antzelevitch, 1998; Yan and Antzelevitch, 1999]. This mechanism is illustrated in Supp. Figures S2A–C. Furthermore, quinidine is vagotonic, reducing the risk of arrhythmia in BrS [Nakajima et al., 1989]. An alternative, or supplementary, cause of the ST-elevation has been ascribed to selective conduction slowing in the right ventricular outflow tract [Meregalli et al., 2005]. Another contributor to the arrhythmic propensity may be the occurrence of structural changes in the heart tissue [Carlson et al., 1994; Meregalli et al., 2005]. The precise mechanism causing these has not, however, been clarified [Papavassiliu et al., 2004; Saffitz, 2005].

There is an association between BrS and an increased occurrence of AF [Eleftheriadis et al., 2004], indicating that the reentry mechanism is also possible in the atria. A number of cases with mixed phenotypes, that is, combinations of BrS with SQT

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Table 1. BrS Types and Associated Genes

BrS type	Gene name	Chromosomal location	Protein name	Ion channel	Effect of mutations	Reference
BrS1	*SCN5A*	3p21–23	$Na_v1.5$	α subunit I_{Na}	Loss of function	[Chen et al., 1998]
BrS2	*GPD1L*	3p24	G3PD1L	Interacts with α subunit I_{Na}	Loss of function	[London et al., 2007]
BrS3	*CACNA1C*	12p13.3	$Ca_v1.2$	α subunit I_{Ca}	Loss of function	[Antzelevitch et al., 2007]
BrS4	*CACNB2*	10p12.33	$Ca_v\beta2$	β subunit I_{Ca}	Loss of function	[Antzelevitch et al., 2007]
BrS5	*SCN1B*	19q13.1	$Na_v\beta$	β subunit I_{Na}	Loss of function	[Watanabe et al., 2008]
BrS6	*KCNE3*	11q13–q14	MiRP2	β subunit I_{Ks}/I_{to}	Gain of function	[Delpon et al., 2008]
BrS7	*SCN3B*	11q23.3	$Na_v\beta3$	β subunit I_{Na}	Loss of function	[Hu et al., 2009]

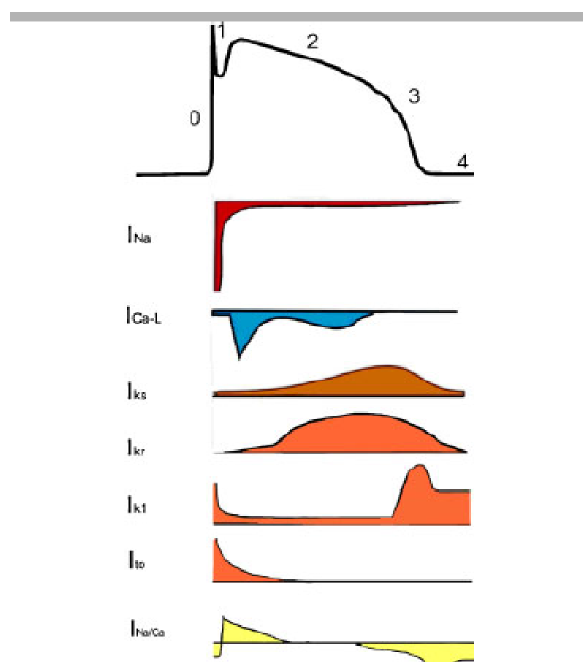

Figure 1. A schematic representation of the action potential (AP) with the ion channels of major significance for the AP, and the currents they conduct as a function of the AP. The red channel depicts the I_{Na} conducted by the $Na_v1.5$; the yellow channel represents the Na^+/Ca^{2+}-exchange ion channel; the blue channel represents the long lasting Ca^{2+} current $I_{L.Ca}$ conducted by the $Ca_v1.2$ ion channel; the orange channel represents the major repolarizing K^+ ion currents. Kv4.3 conducts the I_{to}; Kv11.1 conducts the I_{Kr} current; Kv7.1 conducts the I_{Ks}-current, and Kir2.1 conducts the I_{K1}-current.

[Antzelevitch et al., 2007], BrS with conduction disease (CCD) [Rossenbacker et al., 2004], BrS with LQTS, CCD, and dilated cardiomyopathy (DCM) and combinations thereof, have been described elsewhere [Makita et al., 2008; Olson et al., 2005].

The particular molecular mechanism of BrS associated with each gene is given below.

BrS1—Mutations in *SCN5A*

The gene *SCN5A* has been associated with BrS1 (MIM 601144). This gene encodes the α-subunit of the cardiac sodium channel conducting the depolarizing sodium inward current, I_{Na} [Gellens et al., 1992]. The genomic structure of *SCN5A* contains 28 exons spanning approximately 80 kb [Wang et al., 1996], and the gene has been mapped to chromosome 3p21 using fluorescence in situ hybridization [George et al., 1995]. The deduced 2,016 amino acid protein has a structure similar to that of other depolarizing sodium channels, that is, it contains four homologous domains, DI–DIV, each of which has six putative membrane-spanning regions, S1–S6 [Gellens et al., 1992] (see Fig. 2). The DI–DIV domains are connected via cytoplasmic linkers. S5 and S6 in each domain are connected via a P (pore)-segment that lines the outer pore of the ion channel [Fozzard and Hanck, 1996]. Three fundamental gating processes characterize the $Na_v1.5$ channel [Yang et al., 1996]: activation, fast inactivation, and slow inactivation. Activation is a process whereby the channel opens and Na^+ enters the cell; it is the basic biophysical process behind the cardiac depolarization. Fast inactivation is a process whereby the channel closes quickly (sometimes so quickly that some channels are inactivated prior to closing—*closed-state inactivation*) and then quickly opens again (*fast reactivation*). Slow inactivation is a process whereby the channel closes for 100s of milliseconds and is only slowly reactivated. The S4 segments are paramount for fast inactivation [Kontis and Goldin, 1997; Kontis et al., 1997]. The DIIIS4 and DIVS4 segments, the DIII–DIV cytoplasmic linker, and C-terminal segments are important for fast and closed-state inactivation [Cha et al., 1999; McPhee et al., 1998]. The P-segments and the DIVS4 segment are involved in slow inactivation [Balser, 2001].

Mutations in *SCN5A* were initially found in four families with LQT linked to chromosome 3 [Wang et al., 1995]. Since then, many mutations in all domains of *SCN5A* have been associated with LQTS [Ackerman et al., 2004; Hofman-Bang et al., 2006; Napolitano et al., 2005], some in CCD [Bezzina et al., 2003; Laitinen-Forsblom et al., 2006; Petitprez et al., 2008; Probst et al., 2006], AF [Darbar et al., 2008; Makiyama et al., 2008b], and DCM [Olson et al., 2005]. In the clinical sudden infant and adult death syndromes (SIDS and SADS), mutations are also found in *SCN5A* [Arnestad et al., 2007; Behr et al., 2008; Wang et al., 2007]. Mixed phenotypes, where *SCN5A* mutations are associated with a combination of hereditary arrhythmias and structural heart disease, have also been described [Makita et al., 2008; Olson et al., 2005; Rossenbacker et al., 2004].

In the late 1990s, *SCN5A* was associated with BrS1 when mutations were identified in a number of families with idiopathic ventricular fibrillation [Chen et al., 1998]. With time, BrS1 has been associated with almost 100 different mutations in *SCN5A* (see Supp. Table S1).

The functional characteristics of *SCN5A* mutations associated with BrS1 have been analyzed in mammalian cell lines and mouse models [Nuyens et al., 2001; Papadatos et al., 2002]. In most cases, *SCN5A* mutations found in BrS1 patients are loss-of-function types, that is, leading to reduced I_{Na} current, through various mechanisms such as decreased expression of the gene, a shift in voltage and time dependence of I_{Na} activation, inactivation or reactivation; entry of the sodium channel into an intermediate

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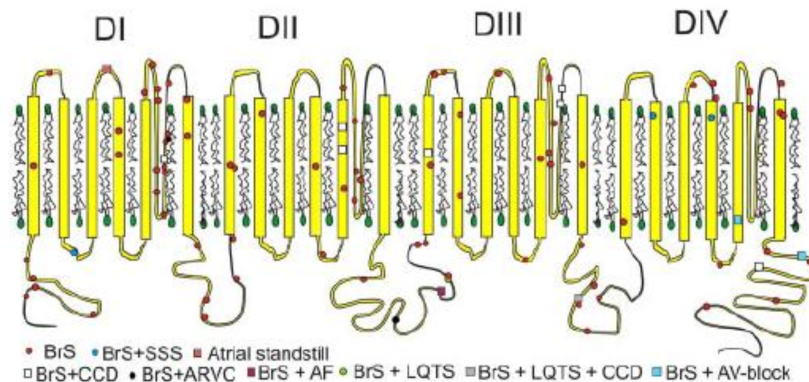

Figure 2. A schematic drawing of the $Na_v1.5$ ion channel with the BrS-associated mutations shown as red dots and phenotypes, color coded, as described in the table.

state of inactivation from which it recovers more slowly; or accelerated inactivation of the sodium channel [Antzelevitch, 2005]. The G351V mutation is a classic example of "typical" BrS1 electrophysiology, as the mutation results in increased I_{Na} current decay leading to reduced I_{Na} current density [Vatta et al., 2002a]. Other mutations lead to absolutely no I_{Na}, for example, R367H [Vatta et al., 2002b], G1406R [Kyndt et al., 2001], and R1432G [Deschenes et al., 2000]. In some cases, the picture is more complex, as with the Y1795H mutation with reduced current decay, increased late persistent I_{Na}, but increased inactivation and, as the total result, reduced I_{Na} current density [Rivolta et al., 2001]. In the latter case, the increased late persistent I_{Na} would be expected to lead to a LQT3 phenotype, and the clinical phenotypes associated with Y1795H are, in accordance with this, complex [Rivolta et al., 2001]. Likewise, the G514C mutation results in both a reduction in the voltage dependent I_{Na} current—which should result in a BrS1 phenotype, but at the same time the voltage-dependent inactivation is reduced, so that the BrS1 phenotype does not appear, consequently, the sole symptom is one of conduction disease [Tan et al., 2001]. The electrophysiological phenotype associated with a *SCN5A* mutation thus depends on which gating process is perturbed and to what extent. Despite the identification of segments of particular importance for different gating processes (see above), it is not possible to deduce the expected phenotype purely from the physical location of the mutation. Furthermore, some mutations, for example, G1406R, have a single distinct BrS phenotype in some carriers and a single distinct CCD phenotype in other carriers in the same family [Kyndt et al., 2001], suggesting that modifying factors are present defining the final phenotype. Such factors have not yet been identified.

It has been demonstrated that the electrophysiological phenotype of *SCN5A* mutations is temperature dependent [Rivolta et al., 2001], and this may explain why BrS can be precipitated by fever, particularly in children [Antzelevitch and Brugada, 2002; Dumaine et al., 1999].

Effective methods for mutation screening in the coding region of *SCN5A* using CAE-SSCP [Hofman-Bang et al., 2006], DHPLC [Lai et al., 2005; Ning et al., 2003], or Melting Curve Analysis [Millat et al., 2009] have been described. Irrespective of the method used, it is important that the sensitivity and specificity of the method used is validated in the implementing laboratory [Jespersgaard et al., 2006].

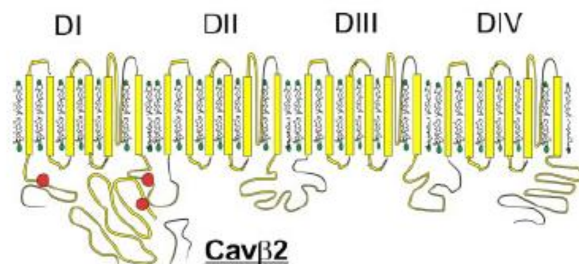

Figure 3. A schematic drawing of the $Ca_v1.2$ ion channel with its associated $Ca_v\beta2$-subunit. The three mutations associated with BrS are shown as red dots.

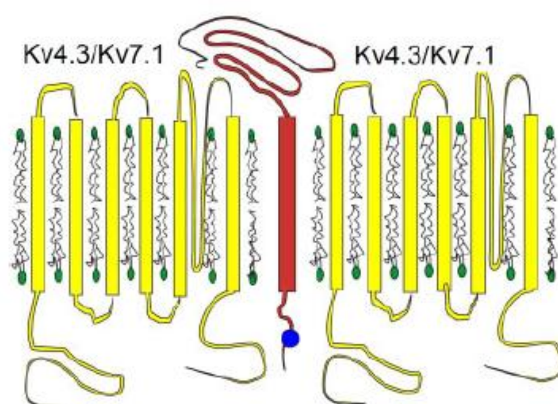

Figure 4. A schematic drawing of MiRP2 with the BrS-associated mutation marked as a red dot.

BrS2—Mutations in *GPD1L*

The *GPD1L* gene encodes glycerol-3-phosphate dehydrogenase 1-like protein (G3PD1L), and is associated with BrS2 (MIM#

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Table 2. Mutations in Genes Causing BrS2–BrS7

Syndrome *Gene Symbol*	mRNA	Predicted Protein Change (Ref Seq)	Type	Genomic region	Protein region	Functional effect	Reference
BrS2	(NM_015141.2)	(NP_055956.1)					
GPD1L	c.247G>A	p.E83K	Missense	exon 3	NAD binding domain	Loss of function	[Van Norstrand et al., 2007]
GPD1L	c.370A>G	p.I124V	Missense	exon 3	NAD binding domain	Loss of function	[Van Norstrand et al., 2007]
GPD1L	c.817C>T	p.R273C	Missense	exon 6	substrate binding domain	Loss of function	[Van Norstrand et al., 2007]
GPD1L	c.839C>T	p.A280V	Missense	exon 6	substrate binding domain	Loss of function	[London et al., 2007]
BrS3	(NM_000719.5)	(NP_000710.5)					
CACNA1C	c.116C>T	p.A39V	Missense	exon 2	C-term	Loss of function	[Antzelevitch et al., 2007]
CACNA1C	c.1468G>A	p.G490R	Missense	exon 10	DI–DII	Loss of function	[Antzelevitch et al., 2007]
BrS4	(NM_201590.2)	(NP_963884.2)					
CACNB2	c.32C>T	p.T11I	Missense	exon 1	N-term	Loss of function	[Cordeiro et al., 2009]
	c.1442C>T	p.S481L	Missense	exon 13	C-term	Loss of function	[Antzelevitch et al., 2007]
BrS5	(NM_001037.3 (β1))	(NP_001028.1 (β1))					
SCN1B	c.259G>C	p.E87Q	Missense	exon 3	Ig domain	Loss of function	[Watanabe et al., 2008]
	(NM_199037.2 (β1b))	(NP_950238.1 (β1b))					
SCN1B	c.536G>A	W179X	Nonsense	exon 3A	juxtamembrane domain	Loss of function	[Watanabe et al., 2008]
	c.537G>A	W179X	Nonsense	exon 3A	juxtamembrane domain	Loss of function	[Watanabe et al., 2008]
BrS6	(NM_005472.4)	(NP_005463.1)					
KCNE3	c.296G>A	p.R99H	Missense	exon 1	C-term	Loss of function	[Delpon et al., 2008]
BrS7	(NM_018400)	(NP_060870.1)					
SCN3B	c.29T>C	p.L10P	Missense	exon 2	N-term	Loss of function	[Hu et al., 2009]

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation is codon 1.

611777). The gene is composed of eight exons spanning 62 kb at chromosome 3p24–p22 [London et al., 2007; Nagase et al., 1995]; it is expressed predominantly in the heart, with lower levels in skeletal muscle and other organs [London et al., 2007]. The 351 amino acid protein is predicted to contain a NAD^+-binding site and a dehydrogenase catalytic site [London et al., 2007]. The function of the protein has not been established.

The link between BrS2 and *GPD1L* was established through linkage analysis in a large BrS family [Weiss et al., 2002], followed by candidate gene analysis [London et al., 2007]. Heterozygosity for a missense mutation, A280V, in *GPD1L*, was found in 46 family members of this family and the penetrance was 37%. Expression of mutated protein in HEK cells demonstrated a 50% reduction in I_{Na} and marked decrease in surface expression of $Na_v1.5$ [London et al., 2007]. In 304 unrelated SIDS cases, three missense mutations, E83K, I24V, and R273C, were identified (see Table 2). All mutations were coexpressed with *SCN5A* in HEK cells and neonatal cardiomyocytes, and found to reduce the I_{Na} current [Van Norstrand et al., 2007]. This suggests that the mechanism of GPD1L-caused BrS2 is related to BrS1, as the electrophysiological phenotype is the result of decreased *SCN5A* expression. A recent screening study in Japan comprising 220 BrS patients did not result in the identification of disease-causing mutations, so the contribution of *GPD1L* mutations to BrS is probably small, at least in Japan [Makiyama et al., 2008a].

BrS3—Mutations in *CACNA1C*

Defects in the *CACNA1C* gene, which encodes a number of isoforms of the pore-forming α_1-subunit of the long-lasting (L-type) voltage gated Ca^{2+} channel ($Ca_v1.2$) [Takimoto et al., 1997], cause BrS3 (MIM# 611875). The gene spans 640 kb at chromosome 12p13.33 and contains 50 exons [Soldatov, 1994]. Due to alternative splicing, 46–47 of these exons are transcribed in different splice forms [Takimoto et al., 1997]. *CACNA1C* is expressed in the heart, brain, lung, and smooth muscle in multiple splice forms [Takimoto et al., 1997]. The predominant splice form contains 2,138 amino acids. The $Ca_v1.2$ channel is activated upon depolarization of the cardiomyocyte, and is responsible for the depolarizing influx of Ca^{2+}, the L-type Ca^{2+} current ($I_{L,Ca}$), that inactivates so slowly that it is of major significance for maintaining the plateau phase of the AP. Furthermore, it represents a coupling between excitation and contraction by inducing release of Ca^{2+} from the sarcoplasmic reticulum. The $Ca_v1.2$ channel is the most important source of intracellular calcium and inhibition of the channel function, for example, pharmacologically induced, interferes negatively with contraction. The $I_{L,Ca}$ current is conducted by a complex composed of the pore-forming α_1-subunit, a β_2-subunit, an $\alpha_2\delta$ subunit and—at least in skeletal muscle—a γ-subunit [Singer et al., 1991]. The $Ca_v1.2$ channel α_1-subunit is composed of four homologous domains (DI–DIV), each composed of six transmembrane segments (S1–S6). The segments S5–S6 in each domain are connected via a P-segment. The four domains constitute the wall of the $Ca_v1.2$ ion channel with the P-segments lining the Ca^{2+} conducting pore [Bers, 2001]. The S4 segments are negatively charged and function as charge sensors. $Ca_v1.2$ interacts with the ryanodine receptor and calmodulin at the C-terminus, which also contains an EF-hand. $Ca_v\beta2$ interacts with $Ca_v1.2$ at the DI–DII linker and the ryanodine receptor at the DII–DIII linker, in addition to the C-terminus [Bers, 2004]. The interaction with calmodulin is responsible for the Ca^{2+} dependence of inactivation [Grandi et al., 2007].

Gain-of-function mutations in *CACNA1C* have previously been associated with Timothy syndrome (TS) [Splawski et al., 2005], a dominantly inherited genetic condition characterized by prolonged QT-interval, multiple malformations, and a very poor prognosis due to lethal cardiac arrhythmias. These mutations result in complete loss of voltage-dependent inactivation of $Ca_v1.2$, resulting in Ca^{2+} overload and delayed repolarization due to prolonged Ca^{2+} inward current during the plateau phase of the AP [Splawski et al., 2005].

The association between *CACNA1C* and BrS3 was established by the finding of two missense mutations in *CACNA1C* in 82 BrS

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patients [Antzelevitch et al., 2007] (see Table 2). The first index patient had a QTc of 346 msec, very little rate dependence of QT on heart rate, AF, and a brother who had died from cardiac arrest at the age of 45 years. The second patient had a QTc of 360 msec, a type I BrS pattern on the ECG, and a mother who had died suddenly at the age of 48 years, following repeated syncopal episodes. These two index patients were found to be heterozygous carriers of the mutations G490R and A39V in *CACNA1C*, respectively. When expressed with other $Ca_v1.2$ subunits in CHO cells, a clearly reduced $I_{L,Ca}$ was found in both cases, while confocal microscopy showed no signs of a trafficking defect [Antzelevitch et al., 2007]. The G490R mutation is located in the DI–DII linker (Fig. 3), and could hypothetically interfere with the binding of the $Ca_v\beta2$ subunit, which increases the $I_{L,Ca}$. The mechanism of BrS3 is thus independent of *SCN5A* and a result of decreased depolarization current in phase 1 of the AP. The A39V and G490R mutations are also associated with short QT syndrome (SQT4), and it has been suggested that BrS3/SQT4 constitutes a new clinical entity [Antzelevitch et al., 2007].

BrS4—Mutations in *CACNB2*

CACNB2 codes for the 660 amino acid β2-subunit ($Ca_v\beta2$) of $Ca_v1.2$ [Van et al., 2004], which modifies gating, increasing the $I_{L,Ca}$ current [Catterall et al., 2005], and is associated with BrS4 (MIM# 611876). *CACNB2* consists of 14 exons covering 421 kb at chromosome 10p12 [Allen and Mikala, 1998; Taviaux et al., 1997]. $Ca_v\beta2$ functions as a chaperone for the α-subunit of $Ca_v1.2$, ensuring its transport to the plasma membrane [Cornet et al., 2002]. It is the dominantly expressed $Ca_v1.2$ β-subunit in the heart. The phosphatidylinositol-3-kinase pathway is—via interaction with $Ca_v\beta2$—involved in ensuring a sufficient expression of $Ca_v1.2$ on the plasma membrane [Viard et al., 2004].

In one of 82 index patients with BrS subjected to comprehensive ion channel mutation screening, a missense mutation, S481L, in *CACNB2* was identified [Antzelevitch et al., 2007] (see Table 2). The patient had experienced an aborted sudden cardiac death and had a QT_c of 330 msec. The ECG was of a type 2 ST-segment elevation pattern. Of 10 family members six were phenotype-positive and the QTc time was short in all, range: 330 msec–370 msec; however, only the proband was symptomatic. When the mutation was expressed in CHO cells together with other components of the $Ca_v1.2$ channel, the $I_{L,Ca}$ current was markedly reduced but this was not due to a trafficking defect. The mutation is located in the C-terminal part of $Ca_v\beta2$ close to the $Ca_v1.2$ binding domain. As the mutation is located in close proximity to the DI–DII linker of $Ca_v1.2$, interference with the stimulatory role of $Ca_v\beta2$ on $I_{L,Ca}$ is a likely pathogenic mechanism for this mutation. The mechanism of BrS4 is related to that of BrS3, as the electrophysiological mechanism involves a reduction of the depolarizing $I_{L,Ca}$. The BrS4 family described above also exhibited SQT (SQT5) and—as for BrS3—the combination of BrS4 and SQT5 has been suggested to constitute a separate clinical entity, comprising the BrS3/SQT4 and BrS4/SQT5 combined phenotypes [Antzelevitch et al., 2007].

BrS5—Mutations in *SCN1B*

The gene *SCN1B* (MIM# 600235) encodes the β1-subunit of the cardiac sodium channel conducting the I_{Na} current. The gene spans 9.8 kb at chromosome 19q13.1 and is composed of six exons [Makita et al., 1994; Watanabe et al., 2008]. The *SCN1B* gene is translated into two isoforms: β1, which is a 218 amino acid, and β1b, which is a 268 amino acid protein. Both are composed of an N-terminal extracellular Ig-like domain, a transmembrane domain, and a C-terminal intracellular domain; however, their sequence identity is limited to the extracellular immunoglobulin domain [Qin et al., 2003]. The *SCN1B* gene is a member of the gene family *SCN1B*, *SCN2B*, *SCN3B*, and *SCN4B*, products of which encode small β-subunits, β1, β1b, β2, β3, and β4, of the Na_v complexes [Meadows and Isom, 2005]. The β-subunits have several functions, including interaction with ankyrin-B and -G. In the heart the biophysical function of the β1- and β1b-subunits is to modify the function of $Na_v1.5$, by increasing the I_{Na} 69 and 76%, respectively [Watanabe et al., 2008].

Mutations in *SCN1B* was recently found associated with BrS in three European kindreds (see Table 2). When the mutations were transiently expressed in CHO cells cotransfected with the $Na_v1.5$ ion channel, it was determined that the mutated forms of SCN1B were not able to increase I_{Na} [Watanabe et al., 2008].

BrS6—Mutations in *KCNE3*

BrS6 is caused by mutations in *KCNE3* (MIM# 604433). This gene encodes the 103 amino acid peptide MiRP2, one of five homologous ancillary β-subunits (KCNE peptides) of voltage-gated potassium ion channels [Abbott et al., 2001a,b; McCrossan et al., 2003]. The *KCNE3* gene consists of three exons distributed over 13 kb at chromosome 11q13–q14; only the last exon is translated [Abbott et al., 2001a]. The Kv:KCNE ion channel complexes are heterohexameric structures consisting of four α-subunits and two KCNE peptides [Morin and Kobertz, 2008]. The KCNE peptides modulate several potassium currents in the heart [Abbott and Goldstein, 2002; Abbott et al., 1999; Grunnet et al., 2003; Lewis et al., 2004; McCrossan et al., 2003; Yu et al., 2001; Zhang et al., 2001], including I_{Ks} [Bendahhou et al., 2005], I_{Kr} [Abbott and Goldstein, 2002], and possibly I_{to} [Delpon et al., 2008]. The minK peptide, encoded by *KCNE1*, has been shown convincingly to confer a slow activation to the KCNQ1 channel, characteristic of the I_{Ks} current, whereas the MiRP2:KCNQ1 complex is a constitutively active potassium channel [Schroeder et al., 2000]. MiRP2 is a type 1 transmembrane peptide [Abbott et al., 2001b], and the transmembrane domain is necessary and sufficient for the functional interaction between KCNQ1 and MiRP2; this interaction fixes the S4 segment of KCNQ1 in the active state [Nakajo and Kubo, 2007]. The cytoplasmic domain of MiRP2 has also been suggested to be involved in controlling gating, but probably to a lesser extent than the transmembrane domain [Melman et al., 2002].

Mutations in *KCNE3* have previously been suggested to cause hyper- and hypokalemic [Abbott et al., 2001a], as well as thyrotoxic, periodic paralysis [Dias da Silva et al., 2002], but later studies have not been able to confirm these findings [Jurkat-Rott and Lehmann-Horn, 2004; Sternberg et al., 2003]. Most likely, the association was based on the circumstantial association with a rare polymorphism in *KCNE3*.

The relation between mutations in *KCNE3* and BrS6 was established in a Danish family with four clinically affected BrS patients with type 1 Brugada pattern and normal QT-interval (Supp. Fig. S1), who were heterozygous carriers of a missense mutation, R99H, in *KCNE3* [Delpon et al., 2008] (see Table 2 and Fig. 4). When the mutated *KCNE3* was coexpressed in CHO cells with Kv4.3, the α-subunit of the ion channel conducting the repolarizing I_{to} current, an increase in the I_{to} current as well as an accelerated inactivation of the current was found [Delpon et al., 2008]. As the KCNE peptides are "promiscuous" [Abbott and Goldstein, 2002] in their choice of interacting α-subunit, it cannot

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be said for certain that this is the mechanism of the association between the R99H mutation and BrS; further studies are needed to ascertain the role of *KCNE3* in the aetiology of BrS.

BrS7—Mutations in *SCN3B*

The gene *SCN3B* (MIM# 600235) encodes the β3-subunit of the cardiac sodium channel conducting the I_{Na} current. The gene spans 25.6 kb at chromosome 11q23 and is composed of six exons [Morgan et al., 2000]. The *SCN3B* gene is translated into a 215 amino acid protein. The protein, $Na_v\beta3$, is composed of an N-terminal extracellular Ig-like domain, a transmembrane domain and a C-terminal intracellular domain. $Na_v\beta3$ is expressed in the brain, heart, lung, skeletal muscle, kidney, and pancreas [Stevens et al., 2001]. The *SCN3B* gene is a member of the gene family *SCNB* gene family [Meadows and Isom, 2005] described above under *SCN1B*. In the heart the function of the β3 subunit is to modify the function of $Na_v1.5$, by increasing the I_{Na}, as for the β1 subunit, albeit with another kinetics [Morgan et al., 2000].

A mutation in *SCN3B* was very recently found associated with BrS in a Caucasian male [Hu et al., 2009], see Table 2. When the mutation, L10P, was expressed in TSA201 cells together with *SCN5A* and *SCN1B*, the mutation was found to result in defective trafficking of Nav1.5 and reduced I_{Na} [Hu et al., 2009].

Genotype–Phenotype Correlation

In a comparison between the ECG morphology of *SCN5A* mutation carriers versus patients where mutations in *SCN5A* had been excluded with the mutation-screening techniques currently available [Smits et al., 2002], it was found that *SCN5A* mutation carriers had significantly longer PQ intervals on the ECG and prolonged His-to-ventricle time during electrical programmed stimulation. No significant differences were found in QT-time, QRS-duration, and the magnitude of ST-elevation [Smits et al., 2002]. No significant difference with respect to prognosis has been found between *SCN5A*-positive BrS patients and non-*SCN5A* carriers [Priori et al., 2003]. The BrS patients with mutations in *CACNA1C* and *CACNB2* express a phenotype that is essentially a combination of BrS and SQT [Antzelevitch et al., 2007], but it remains to be seen whether some mutations in these genes can express a "pure" BrS phenotype.

Clinical Significance of Mutation Identification

Presently, identification of BrS-causing mutations cannot be used for risk stratification, and it is still necessary to rely on clinical criteria. However, the identification of mutation carriers in BrS may be used to identify undiagnosed and/or asymptomatic patients in cascade screening. As these patients are at increased risk for arrhythmia, their identification is important, so that appropriate risk stratification analyses and ICD implantation in high risk cases can be facilitated [Antzelevitch et al., 2005]. This is relevant, particularly if ECG discloses spontaneous BrS type 1 pattern, but even in asymptomatic patients [Priori et al., 2002].

As mutations in *SCN5A* may result in sudden death, it is important to include genetic screening of *SCN5A* in "molecular autopsy" [Tester and Ackerman, 2006] of SIDS and SADS cases. This is particularly important if death occurs during the night or while resting, in order to offer cascade screening to family members. However, even more importantly, clinical assessment of relatives of the deceased is essential, as this may identify individuals with BrS and allow appropriate risk management in the survivors [Behr et al., 2008]. Screening for BrS-associated mutations may have a purpose when it is difficult to reach a diagnosis, for example, in cases with syncope, normal, or borderline abnormal ECG, no family members available for examination and/or the presence of conduction disease, DCM, or LQTS in the family. Such a use of genetic screening is, however, a specialist task and may still end inconclusively as the absence of a known mutation does not exclude BrS.

Conclusion

Presently, over 100 mutations have been associated with BrS. At the time of submission, BrS mutations have been identified in seven genes, involved in the correct execution of phase 1–2 of the AP. The presence of asymptomatic cases with an appreciable arrhythmia risk makes identification of the causative mutation followed by cascade screening important. The gene *SCN5A* is the most frequently involved. Mutations in *SCN5A* have been identified in 5–10% of sudden death cases in adulthood and childhood. Furthermore, the contribution of the other BrS-causing genes to sudden death is currently being examined. By analogy to other ion channel diseases, a comprehensive genetic examination of BrS cases should involve mutation screening of all involved genes.

References

Abbott GW, Butler MH, Bendahhou S, Dalakas MC, Ptacek LJ, Goldstein SA. 2001a. MiRP2 forms potassium channels in skeletal muscle with Kv3.4 and is associated with periodic paralysis. Cell 104:217–231.

Abbott GW, Goldstein SA, Sesti F. 2001b. Do all voltage-gated potassium channels use MiRPs? Circ Res 88:981–983.

Abbott GW, Goldstein SA. 2002. Disease-associated mutations in KCNE potassium channel subunits (MiRPs) reveal promiscuous disruption of multiple currents and conservation of mechanism. FASEB J 16:390–400.

Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA. 1999. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97:175–187.

Ackerman MJ, Splawski I, Makielski JC, Tester DJ, Will ML, Timothy KW, Keating MT, Jones G, Chadha M, Burrow CR, Stephens JC, Xu C, Judson R, Curran ME. 2004. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. Heart Rhythm 1:600–607.

Akai J, Makita N, Sakurada H, Shirai N, Ueda K, Kitabatake A, Nakazawa K, Kimura A, Hiraoka M. 2000. A novel SCN5A mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome. FEBS Lett 479:29–34.

Alings M, Wilde A. 1999. "Brugada" syndrome: clinical data and suggested pathophysiological mechanism. Circulation 99:666–673.

Allen TJ, Mikala G. 1998. Effects of temperature on human L-type cardiac Ca^{2+} channels expressed in *Xenopus* oocytes. Pflugers Arch 436:238–247.

Amin AS, Verkerk AO, Bhuiyan ZA, Wilde AA, Tan HL. 2005. Novel Brugada syndrome-causing mutation in ion-conducting pore of cardiac Na^+ channel does not affect ion selectivity properties. Acta Physiol Scand 185:291–301.

Antzelevitch C. 1998. The Brugada syndrome. J Cardiovasc Electrophysiol 9:513–516.

Antzelevitch C. 2001. The Brugada syndrome: ionic basis and arrhythmia mechanisms. J Cardiovasc Electrophysiol 12:268–272.

Antzelevitch C. 2005. Cardiac repolarization. The long and short of it. Europace 7(Suppl 2):3–9.

Antzelevitch C, Brugada R. 2002. Fever and Brugada syndrome. Pacing Clin Electrophysiol 25:1537–1539.

Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, Lemarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A. 2005. Brugada syndrome: report of the second consensus conference. Heart Rhythm 2:429–440.

Antzelevitch C, Brugada P, Brugada J, Brugada R, Shimizu W, Gussak I, Perez Riera AR. 2002. Brugada syndrome: a decade of progress. Circ Res 91:1114–1118.

Antzelevitch C, Brugada P, Brugada J, Brugada R, Towbin JA, Nademanee K. 2003. Brugada syndrome: 1992–2002: a historical perspective. J Am Coll Cardiol 41:1665–1671.

Antzelevitch C, Nof E. 2008. Brugada syndrome: recent advances and controversies. Curr Cardiol Rep 10:376–383.

Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros Jr EP, Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu LF, Haissaguerre M, Schimpf R, Borggrefe M, Wolpert C. 2007. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation 115:442–449.

Araki T, Konno T, Itoh H, Ino H, Shimizu M. 2003. Brugada syndrome with ventricular tachycardia and fibrillation related to hypokalemia. Circ J 67:93–95.

Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George Jr AL, Schwartz PJ. 2007. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. Circulation 115:361–367.

Balser JR. 2001. The cardiac sodium channel: gating function and molecular pharmacology. J Mol Cell Cardiol 33:599–613.

Behr ER, Dalageorgou C, Christiansen M, Syrris P, Hughes S, Tome Esteban MT, Rowland E, Jeffery S, McKenna WJ. 2008. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. Eur Heart J 29:1670–1680.

Belhassen B, Glick A, Viskin S. 2004. Efficacy of quinidine in high-risk patients with Brugada syndrome. Circulation 110:1731–1737.

Bendahhou S, Marionneau C, Haurogne K, Larroque MM, Derand R, Szuts V, Escande D, Demolombe S, Barhanin J. 2005. In vitro molecular interactions and distribution of KCNE family with KCNQ1 in the human heart. Cardiovasc Res 67:529–538.

Bers DM. 2001. Excitation-contraction coupling and cardiac contractile force. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Bers DM. 2004. Macromolecular complexes regulating cardiac ryanodine receptor function. J Mol Cell Cardiol 37:417–429.

Bezzina C, Veldkamp MW, van den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van der Hout AH, Mannens MM, Wilde AA. 1999. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. Circ Res 85:1206–1213.

Bezzina CR, Rook MB, Groenewegen WA, Herfst LJ, van der Wal AC, Lam J, Jongsma HJ, Wilde AA, Mannens MM. 2003. Compound heterozygosity for mutations (W156X and R225W) in SCN5A associated with severe cardiac conduction disturbances and degenerative changes in the conduction system. Circ Res 92:159–168.

Blangy H, Sadoul N, Coutelour JM, Rebmann JP, Joseph M, Scherrer C, de CC, Magnin-Poull I, Aliot E. 2005. [Prevalence of Brugada syndrome among 35,309 inhabitants of Lorraine screened at a preventive medicine centre]. Arch Mal Coeur Vaiss 98:175–180.

Bozkurt A, Yas D, Seydaoglu G, Acarturk E. 2006. Frequency of Brugada-type ECG pattern (Brugada sign) in Southern Turkey. Int Heart J 47:541–547.

Brugada J, Brugada R, Antzelevitch C, Towbin J, Nademanee K, Brugada P. 2002a. Long-term follow-up of individuals with the electrocardiographic pattern of right bundle-branch block and ST-segment elevation in precordial leads V1 to V3. Circulation 105:73–78.

Brugada J, Brugada R, Brugada P. 2000a. Pharmacological and device approach to therapy of inherited cardiac diseases associated with cardiac arrhythmias and sudden death. J Electrocardiol 33(Suppl):41–47.

Brugada J, Brugada R, Brugada P. 2003. Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of Brugada syndrome and no previous cardiac arrest. Circulation 108:3092–3096.

Brugada P, Brugada J. 1992. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol 20:1391–1396.

Brugada P, Brugada R, Brugada J. 2000b. The Brugada syndrome. Curr Cardiol Rep 2:507–514.

Brugada P, Brugada J, Brugada R. 2002b. The Brugada syndrome. Card Electrophysiol Rev 6:45–48.

Carlson MD, White RD, Trohman RG, Adler LP, Biblo LA, Merkatz KA, Waldo AL. 1994. Right ventricular outflow tract ventricular tachycardia: detection of previously unrecognized anatomic abnormalities using cine magnetic resonance imaging. J Am Coll Cardiol 24:720–727.

Casini S, Tan HL, Bhuiyan ZA, Bezzina CR, Barnett P, Cerbai E, Mugelli A, Wilde AA, Veldkamp MW. 2007. Characterization of a novel SCN5A mutation associated with Brugada syndrome reveals involvement of DIIIS4-S5 linker in slow inactivation. Cardiovasc Res 76:418–429.

Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. 2005. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev 57:411–425.

Cha A, Ruben PC, George Jr AL, Fujimoto E, Bezanilla F. 1999. Voltage sensors in domains III and IV, but not I and II, are immobilized by Na^{+} channel fast inactivation. Neuron 22:73–87.

Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, Ortiz-Lopez R, Wang Z, Antzelevitch C, O'Brien RE, Schulze-Bahr E, Keating MT, Towbin JA, Wang Q. 1998. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 392:293–296.

Chung SK, MacCormick JM, McCulley CH, Crawford J, Eddy CA, Mitchell EA, Shelling AN, French JK, Skinner JR, Rees MI. 2007. Long QT and Brugada syndrome gene mutations in New Zealand. Heart Rhythm 4:1306–1314.

Cordeiro JM, Barajas-Martinez H, Hong K, Burashnikov E, Pfeiffer R, Orsino AM, Wu YS, Hu D, Brugada J, Brugada P, Antzelevitch C, Dumaine R, Brugada R. 2006. Compound heterozygous mutations P336L and I1660V in the human cardiac sodium channel associated with the Brugada syndrome. Circulation 114:2026–2033.

Cordeiro JM, Marieb M, Pfeiffer R, Calloe K, Burashnikov E, Antzelevitch C. 2009. Accelerated inactivation of the L-type calcium current due to a mutation in CACNB2b underlies Brugada syndrome. J Mol Cell Cardiol 46:695–703.

Cornet V, Bichet D, Sandoz G, Marty I, Brocard J, Bourinet E, Mori Y, Villaz M, De WM. 2002. Multiple determinants in voltage-dependent P/Q calcium channels control their retention in the endoplasmic reticulum. Eur J Neurosci 16:883–895.

Darbar D, Kannankeril PJ, Donahue BS, Kucera G, Stubblefield T, Haines JL, George Jr AL, Roden DM. 2008. Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation. Circulation 117:1927–1935.

Delpon E, Cordeiro JM, Nunez L, Thomsen PE, Guerchicoff A, Pollevick GD, Wu Y, Kanters JK, Larsen CT, Burashnikov E, Christiansen M, Antzelevitch C. 2008. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. Circ Arrhythm Electrophysiol 1:209–218.

Deschenes I, Baroudi G, Berthet M, Barde I, Chalvidan T, Denjoy I, Guicheney P, Chahine M. 2000. Electrophysiological characterization of SCN5A mutations causing long QT (E1784K) and Brugada (R1512W and R1432G) syndromes. Cardiovasc Res 46:55–65.

Dias da Silva MR, Cerutti JM, Arnaldi LA, Maciel RM. 2002. A mutation in the KCNE3 potassium channel gene is associated with susceptibility to thyrotoxic hypokalemic periodic paralysis. J Clin Endocrinol Metab 87:4881–4884.

Di Diego JM, Cordeiro JM, Goodrow RJ, Fish JM, Zygmunt AC, Perez GJ, Scornik FS, Antzelevitch C. 2002. Circulation 106:2004–2011.

Dumaine R, Towbin JA, Brugada P, Vatta M, Nesterenko DV, Nesterenko VV, Brugada J, Brugada R, Antzelevitch C. 1999. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. Circ Res 85:803–809.

Eckardt L, Probst V, Smits JP, Bahr ES, Wolpert C, Schimpf R, Wichter T, Boisseau P, Heinecke A, Breithardt G, Borggrefe M, Lemarec H, Bocker D, Wilde AA. 2005. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. Circulation 111:257–263.

Eleftheriadis D, Panagiotis V, Eleftheriadis N. 2004. Recurrent syncopic episodes as a consequence of combined Brugada syndrome and paroxysmal atrial fibrillation. Which is the therapy of choice? Int J Cardiol 94:319–320.

Fish JM, Antzelevtich C. 2003. Cellular and ionic basis for the sex-related difference in the manifestation of the Brugada syndrome and progressive conduction disease phenotypes. J Electrocardiol 36(Suppl):173–179.

Fozzard HA, Hanck DA. 1996. Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. Physiol Rev 76:887–926.

Frustaci A, Priori SG, Pieroni M, Chimenti C, Napolitano C, Rivolta I, Sanna T, Bellocci F, Russo MA. 2005. Cardiac histological substrate in patients with clinical phenotype of Brugada syndrome. Circulation 112:3680–3687.

Gellens ME, George Jr AL, Chen LQ, Chahine M, Horn R, Barchi RL, Kallen RG. 1992. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proc Natl Acad Sci USA 89:554–558.

George Jr AL, Varkony TA, Drabkin HA, Han J, Knops JF, Finley WH, Brown GB, Ward DC, Haas M. 1995. Assignment of the human heart tetrodotoxin-resistant voltage-gated Na+ channel alpha-subunit gene (SCN5A) to band 3p21. Cytogenet Cell Genet 68:67–70.

Gervacio-Domingo G, Isidro J, Tirona J, Gabriel E, David G, Amarillo ML, Morales D, Dans A. 2008. The Brugada type 1 electrocardiographic pattern is common among Filipinos. J Clin Epidemiol 61:1067–1072.

Goldgran-Toledano D, Sideris G, Kevorkian JP. 2002. Overdose of cyclic antidepressants and the Brugada syndrome. N Engl J Med 346:1591–1592.

Grandi E, Puglisi JL, Wagner S, Maier LS, Severi S, Bers DM. 2007. Simulation of Ca-calmodulin-dependent protein kinase II on rabbit ventricular myocyte ion currents and action potentials. Biophys J 93:3835–3847.

Grunnet M, Rasmussen HB, Hay-Schmidt A, Rosenstierne M, Klaerke DA, Olesen SP, Jespersen T. 2003. KCNE4 is an inhibitory subunit to Kv1.1 and Kv1.3 potassium channels. Biophys J 85:1525–1537.

Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, McKenna WJ, Christiansen M. 2006. High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia. Clin Genet 69:504–511.

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Hong K, Berruezo-Sanchez A, Poungvarin N, Oliva A, Vatta M, Brugada J, Brugada P, Towbin JA, Dumaine R, Pinero-Galvez C, Antzelevitch C, Brugada R. 2004a. Phenotypic characterization of a large European family with Brugada syndrome displaying a sudden unexpected death syndrome mutation in *SCN5A*: female predominance in the signs and symptoms of the disease. J Cardiovasc Electrophysiol 15:64–69.

Hong K, Brugada J, Oliva A, Berruezo-Sanchez A, Potenza D, Pollevick GD, Guerchichoff A, Matsuo K, Burashnikov E, Dumaine R, Towbin JA, Nesterenko V, Brugada P, Antzelevitch C, Brugada R. 2004b. Value of electrocardiographic parameters and ajmaline test in the diagnosis of Brugada syndrome caused by SCN5A mutations. Circulation 110:3023–3027.

Hong K, Guerchicoff A, Pollevick GD, Oliva A, Dumaine R, De ZM, Burashnikov E, Wu YS, Brugada J, Brugada P, Brugada R. 2005. Cryptic 5′ splice site activation in SCN5A associated with Brugada syndrome. J Mol Cell Cardiol 38:555–560.

Hu D, Martinez HB, Burashnikov E, Springer M, Wu Y, Varro A, Pfeiffer R, Koopmann TT, Cordeiro JM, Guerchicoff A, Pollevick GD, Antzelevitch C. 2009. A mutation in the β3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. Circ Cardiovasc Genet (in press).

Itoh H, Shimizu M, Mabuchi H, Imoto K. 2005. Clinical and electrophysiological characteristics of Brugada syndrome caused by a missense mutation in the S5-pore site of SCN5A. J Cardiovasc Electrophysiol 16:378–383.

Ito H, Yano K, Chen R, He Q, Curb JD. 2006. The prevalence and prognosis of a Brugada-type electrocardiogram in a population of middle-aged Japanese–American men with follow-up of three decades. Am J Med Sci 331:25–29.

Jespersgaard C, Larsen LA, Baba S, Kukita Y, Tahira T, Christiansen M, Vuust J, Hayashi K, Andersen PS. 2006. Optimization of capillary array electrophoresis single-strand conformation polymorphism analysis for routine molecular diagnostics. Electrophoresis 27:3816–3822.

Jurkat-Rott K, Lehmann-Horn F. 2004. Periodic paralysis mutation MiRP2-R83H in controls: interpretations and general recommendation. Neurology 62:1012–1101.

Kawamura M, Ozawa T, Yao T, Ashihara T, Sugimoto Y, Yagi T, Itoh H, Ito M, Makiyama T, Horie M . 2009. Dynamic change in ST-segment and spontaneous occurrence of ventricular fibrillation in Brugada syndrome with a novel nonsense mutation in the SCN5A gene during long-term follow-up. Circ J 73:584–588.

Keller DI, Barrane FZ, Gouas L, Martin J, Pilote S, Suarez V, Osswald S, Brink M, Guicheney P, Schwick N, Chahine M. 2005a. A novel nonsense mutation in the SCN5A gene leads to Brugada syndrome and a silent gene mutation carrier state. Can J Cardiol 21:925–931.

Keller DI, Huang H, Zhao J, Frank R, Suarez V, Delacretaz E, Brink M, Osswald S, Schwick N, Chahine M. 2006. A novel SCN5A mutation, F1344S, identified in a patient with Brugada syndrome and fever-induced ventricular fibrillation. Cardiovasc Res 70:521–529.

Keller DI, Rougier JS, Kucera JP, Benammar N, Fressart V, Guicheney P, Madle A, Fromer M, Schlapfer J, Abriel H. 2005b. Brugada syndrome and fever: genetic and molecular characterization of patients carrying SCN5A mutations. Cardiovasc Res 67:510–519.

Kontis KJ, Goldin AL. 1997. Sodium channel inactivation is altered by substitution of voltage sensor positive charges. J Gen Physiol 110:403–413.

Kontis KJ, Rounaghi A, Goldin AL. 1997. Sodium channel activation gating is affected by substitutions of voltage sensor positive charges in all four domains. J Gen Physiol 110:391–401.

Kurita T, Shimizu W, Inagaki M, Suyama K, Taguchi A, Satomi K, Aihara N, Kamakura S, Kobayashi J, Kosakai Y. 2002. The electrophysiologic mechanism of ST-segment elevation in Brugada syndrome. J Am Coll Cardiol 40:330–334.

Kyndt F, Probst V, Potet F, Demolombe S, Chevallier JC, Baro I, Moisan JP, Boisseau P, Schott JJ, Escande D, Le MH. 2001. Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. Circulation 104:3081–3086.

Lai LP, Su YN, Hsieh FJ, Chiang FT, Juang JM, Liu YB, Ho YL, Chen WJ, Yeh SJ, Wang CC, Ko YL, Wu TJ, Ueng KC, Lei MH, Tsao HM, Chen SA, Lin TK, Wu MH, Lo HM, Huang SK, Lin JL. 2005. Denaturing high-performance liquid chromatography screening of the long QT syndrome-related cardiac sodium and potassium channel genes and identification of novel mutations and single nucleotide polymorphisms. J Hum Genet 50:490–496.

Laitinen-Forsblom PJ, Makynen P, Makynen H, Yli-Mayry S, Virtanen V, Kontula K, Aalto-Setala K. 2006. SCN5A mutation associated with cardiac conduction defect and atrial arrhythmias. J Cardiovasc Electrophysiol 17:480–485.

Levy-Nissenbaum E, Eldar M, Wang Q, Lahat H, Belhassen B, Ries L, Friedman E, Pras E. 2001. Genetic analysis of Brugada syndrome in Israel: two novel mutations and possible genetic heterogeneity. Genet Test 5:331–334.

Lewis A, McCrossan ZA, Abbott GW. 2004. MinK, MiRP1, and MiRP2 diversify Kv3.1 and Kv3.2 potassium channel gating. J Biol Chem 279:7884–7892.

Liang P, Liu WL, Hu DY, Li CL, Tao WH, Li L. 2006. [Novel SCN5A gene mutations associated with Brugada syndrome: V95I, A1649V and delF1617]. Zhonghua Xin Xue Guan Bing Za Zhi 34:616–619.

Littmann L, Monroe MH, Taylor III L, Brearley Jr WD. 2007a. The hyperkalemic Brugada sign. J Electrocardiol 40:53–59.

Littmann L, Taylor III L, Brearley Jr WD. 2007b. ST-segment elevation: a common finding in severe hypercalcemia. J Electrocardiol 40:60–62.

London B, Michalec M, Mehdi H, Zhu X, Kerchner L, Sanyal S, Viswanathan PC, Pfahnl AE, Shang LL, Madhusudanan M, Baty CJ, Lagana S, Aleong R, Gutmann R, Ackerman MJ, McNamara DM, Weiss R, Dudley Jr SC. 2007. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na^+ current and causes inherited arrhythmias. Circulation 116:2260–2268.

Lukas A, Antzelevitch C. 1996. Phase 2 reentry as a mechanism of initiation of circus movement reentry in canine epicardium exposed to simulated ischemia. Cardiovasc Res 32:593–603.

Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, Schulze-Bahr E, Fukuhara S, Mochizuki N, Makiyama T, Itoh H, Christiansen M, McKeown P, Miyamoto K, Kamakura S, Tsutsui H, Schwartz PJ, George Jr AL, Roden DM. 2008. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. J Clin Invest 118:2219–2229.

Makita N, Sasaki K, Groenewegen WA, Yokota T, Yokoshiki H, Murakami T, Tsutsui H. 2005. Congenital atrial standstill associated with coinheritance of a novel SCN5A mutation and connexin 40 polymorphisms. Heart Rhythm 2:1128–1134.

Makita N, Sloan-Brown K, Weghuis DO, Ropers HH, George Jr AL. 1994. Genomic organization and chromosomal assignment of the human voltage-gated Na(+) channel beta-1 subunit gene (SCN1B). Genomics 23:628–634.

Makita N, Sumitomo N, Watanabe I, Tsutsui H. 2007. Novel SCN5A mutation (Q55X) associated with age-dependent expression of Brugada syndrome presenting as neurally mediated syncope. Heart Rhythm 4:516–519.

Makiyama T, Akao M, Tsuji K, Doi T, Ohno S, Takenaka K, Kobori A, Ninomiya T, Yoshida H, Takano M, Makita N, Yanagisawa F, Higashi Y, Takeyama Y, Kita T, Horie M. 2005. High risk for bradyarrhythmic complications in patients with Brugada syndrome caused by SCN5A gene mutations. J Am Coll Cardiol 46:2100–2106.

Makiyama T, Akao M, Haruna Y, Tsuji K, Doi T, Ohno S, Nishio Y, Kita T, Horie M. 2008a. Mutation analysis of the glycerol-3 phosphate dehydrogenase-1 like (GPD1L) gene in Japanese patients with Brugada syndrome. Circ J 72:1705–1706.

Makiyama T, Akao M, Shizuta S, Doi T, Nishiyama K, Oka Y, Ohno S, Nishio Y, Tsuji K, Itoh H, Kimura T, Kita T, Horie M. 2008b. A novel SCN5A gain-of-function mutation M1875T associated with familial atrial fibrillation. J Am Coll Cardiol 52:1326–1334.

McCrossan ZA, Lewis A, Panaghie G, Jordan PN, Christini DJ, Lerner DJ, Abbott GW. 2003. MinK-related peptide 2 modulates Kv2.1 and Kv3.1 potassium channels in mammalian brain. J Neurosci 23:8077–8091.

McPhee JC, Ragsdale DS, Scheuer T, Catterall WA. 1998. A critical role for the S4–S5 intracellular loop in domain IV of the sodium channel alpha-subunit in fast inactivation. J Biol Chem 273:1121–1129.

Meadows LS, Isom LL. 2005. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. Cardiovasc Res 67: 448–458.

Melman YF, Krumerman A, McDonald TV. 2002. A single transmembrane site in the KCNE-encoded proteins controls the specificity of KvLQT1 channel gating. J Biol Chem 277:25187–25194.

Meregalli PG, Wilde AA, Tan HL. 2005. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? Cardiovasc Res 67:367–378.

Millat G, Chanavat V, Rodriguez-Lafrasse C, Rousson R. 2009. Rapid, sensitive and inexpensive detection of SCN5A genetic variations by high resolution melting analysis. Clin Biochem 42:491–499.

Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Aizawa Y, Ogawa S. 1996. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. J Am Coll Cardiol 27:1061–1070.

Mizumaki K, Fujiki A, Tsuneda T, Sakabe M, Nishida K, Sugao M, Inoue H. 2004. Vagal activity modulates spontaneous augmentation of ST elevation in the daily life of patients with Brugada syndrome. J Cardiovasc Electrophysiol 15:667–673.

Mizusawa Y, Sakurada H, Nishizaki M, Hiraoka M. 2006. Effects of low-dose quinidine on ventricular tachyarrhythmias in patients with Brugada syndrome: low-dose quinidine therapy as an adjunctive treatment. J Cardiovasc Pharmacol 47:359–364.

Morgan K, Stevens EB, Shah B, Cox PJ, Dixon AK, Lee K, Pinnock RD, Hughes J, Richardson PJ, Mizuguchi K, Jackson AP. 2000. Beta 3: an additional auxiliary subunit of the voltage-sensitive sodium channel that modulates channel gating with distinct kinetics. Proc Natl Acad Sci USA 97:2308–2313.

Morin TJ, Kobertz WR. 2008. Counting membrane-embedded KCNE beta-subunits in functioning K+ channel complexes. Proc Natl Acad Sci USA 105:1478–1482.

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Nademanee K. 1997. Sudden unexplained death syndrome in Southeast Asia. Am J Cardiol 79:10–11.

Nademanee K, Veerakul G, Mower M, Likittanasombat K, Krittayapong R, Bhuripanyo K, Sitthisook S, Chaothawee L, Lai MY, Azen SP. 2003. Defibrillator versus beta-blockers for unexplained death in Thailand (DEBUT): a randomized clinical trial. Circulation 107:2221–2226.

Nagase T, Seki N, Tanaka A, Ishikawa K, Nomura N. 1995. Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121–KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1. DNA Res 2:167–210.

Nakajima T, Kurachi Y, Ito H, Takikawa R, Sugimoto T. 1989. Anti-cholinergic effects of quinidine, disopyramide, and procainamide in isolated atrial myocytes: mediation by different molecular mechanisms. Circ Res 64: 297–303.

Nakajo K, Kubo Y. 2007. KCNE1 abd KCNE3 stabilize and/or slow voltage sensing S4 segment of KCNQ1 channel. J Gen Physiol 130:269–281.

Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S. 2005. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. JAMA 294:2975–2980.

Niimura H, Matsunaga A, Kumagai K, Ohwaki K, Ogawa M, Noguchi H, Yonemura K, Saku K. 2004. Genetic analysis of Brugada syndrome in Western Japan: two novel mutations. Circ J 68:740–746.

Ning L, Moss A, Zareba W, Robinson J, Rosero S, Ryan D, Qi M. 2003. Denaturing high-performance liquid chromatography quickly and reliably detects cardiac ion channel mutations in long QT syndrome. Genet Test 7:249–253.

Nuyens D, Stengl M, Dugarmaa S, Rossenbacker T, Compernolle V, Rudy Y, Smits JF, Flameng W, Clancy CE, Moons L, Vos MA, Dewerchin M, Benndorf K, Collen D, Carmeliet E, Carmeliet P. 2001. Abrupt rate accelerations or premature beats cause life-threatening arrhythmias in mice with long-QT3 syndrome. Nat Med 7:1021–1027.

Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, Horton SC, Rodeheffer RJ, Anderson JL. 2005. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. JAMA 293:447–454.

Ortega-Carnicer J, Bertos-Polo J, Gutierrez-Tirado C. 2001. Aborted sudden death, transient Brugada pattern, and wide QRS dysrrhythmias after massive cocaine ingestion. J Electrocardiol 34:345–349.

Papadatos GA, Wallerstein PM, Head CE, Ratcliff R, Brady PA, Benndorf K, Saumarez RC, Trezise AE, Huang CL, Vandenberg JI, Colledge WH, Grace AA. 2002. Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene Scn5a. Proc Natl Acad Sci USA 99:6210–6215.

Papavassiliu T, Wolpert C, Fluchter S, Schimpf R, Neff W, Haase KK, Duber C, Borggrefe M. 2004. Magnetic resonance imaging findings in patients with Brugada syndrome. J Cardiovasc Electrophysiol 15:1133–1138.

Pastor A, Nunez A, Cantale C, Cosio FG. 2001. Asymptomatic brugada syndrome case unmasked during dimenhydrinate infusion. J Cardiovasc Electrophysiol 12:1192–1194.

Petitprez S, Jespersen T, Pruvot E, Keller DI, Corbaz C, Schlapfer J, Abriel H, Kucera JP. 2008. Analyses of a novel SCN5A mutation (C1850S): conduction vs. repolarization disorder hypotheses in the Brugada syndrome. Cardiovasc Res 78:494–504.

Pfahnl AE, Viswanathan PC, Weiss R, Shang LL, Sanyal S, Shusterman V, Kornblit C, London B, Dudley Jr SC. 2007. A sodium channel pore mutation causing Brugada syndrome. Heart Rhythm 4:46–53.

Pilz B, Luft FC. 2003. Acquired Brugada syndrome. Am J Cardiol 92:771.

Priori SG, Napolitano C, Gasparini M, Pappone C, Bella PD, Brignole M, Giordano U, Giovannini T, Menozzi C, Bloise R, Crotti L, Terreni L, Schwartz PJ. 2000a. Clinical and Genetic Heterogeneity of right bundle branch block and ST-segment elevation syndrome. Circulation 102:2509–2515.

Priori SG, Napolitano C, Gasparini M, Pappone C, Bella PD, Giordano U, Bloise R, Giustetto C, De NR, Grillo M, Ronchetti E, Faggiano G, Nastoli J. 2002. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation 105:1342–1347.

Priori SG, Napolitano C, Vicentini A. 2003. Inherited arrhythmia syndromes: applying the molecular biology and genetic to the clinical management. J Interv Card Electrophysiol 9:93–101.

Probst V, Allouis M, Sacher F, Pattier S, Babuty D, Mabo P, Mansourati J, Victor J, Nguyen JM, Schott JJ, Boisseau P, Escande D, Le MH. 2006. Progressive cardiac conduction defect is the prevailing phenotype in carriers of a Brugada syndrome SCN5A mutation. J Cardiovasc Electrophysiol 17:270–275.

Probst V, Denjoy I, Meregalli PG, Amirault JC, Sacher F, Mansourati J, Babuty D, Villain E, Victor J, Schott JJ, Lupoglazoff JM, Mabo P, Veltmann C, Jesel L, Chevalier P, Clur SA, Haissaguerre M, Wolpert C, Le MH, Wilde AA. 2007. Clinical aspects and prognosis of Brugada syndrome in children. Circulation 115:2042–2048.

Qin N, D'Andrea MR, Lubin M-L, Shafaee N, Codd EE, Correa AM. 2003. Molecular cloning and functional expression of the human sodium channel β_{1B} subunit, a novel splicing variant of the β_1 subunit. Eur J Biochem 270:4762–4770.

Rivolta I, Abriel H, Tateyama M, Liu H, Memmi M, Vardas P, Napolitano C, Priori SG, Kass RS. 2001. Inherited Brugada and long QT-3 syndrome mutations of a single residue of the cardiac sodium channel confer distinct channel and clinical phenotypes. J Biol Chem 276:30623–30630.

Rossenbacker T, Carroll SJ, Liu H, Kuiperi C, de Ravel TJ, Devriendt K, Carmeliet P, Kass RS, Heidbuchel H. 2004. Novel pore mutation in SCN5A manifests as a spectrum of phenotypes ranging from atrial flutter, conduction disease, and Brugada syndrome to sudden cardiac death. Heart Rhythm 1:610–615.

Sacher F, Probst V, Iesaka Y, Jacon P, Laborderie J, Mizon-Gerard F, Mabo P, Reuter S, Lamaison D, Takahashi Y, O'Neill MD, Garrigue S, Pierre B, Jais P, Pasquie JL, Hocini M, Salvador-Mazenq M, Nogami A, Amiel A, Defaye P, Bordachar P, Boveda S, Maury P, Klug D, Babuty D, Haissaguerre M, Mansourati J, Clementy J, Le MH. 2006. Outcome after implantation of a cardioverter-defibrillator in patients with Brugada syndrome: a multicenter study. Circulation 114:2317–2324.

Saffitz JE. 2005. Structural heart disease, SCN5A gene mutations, and Brugada syndrome: a complex menage a trois. Circulation 112:3672–3674.

Schroeder BC, Waldegger S, Fehr S, Bleich M, Warth R, Greger R, Jentsch TJ. 2000. A constitutively open potassium channel formed by KCNQ1 and KCNE3. Nature 403:196–199.

Schulze-Bahr E, Eckardt L, Breithardt G, Seidl K, Wichter T, Wolpert C, Borggrefe M, Haverkamp W. 2003. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. Hum Mutat 21:651–652.

Shimizu W, Antzelevitch C, Suyama K, Kurita T, Taguchi A, Aihara N, Takaki H, Sunagawa K, Kamakura S. 2000. Effect of sodium channel blockers on ST segment, QRS duration, and corrected QT interval in patients with Brugada syndrome. J Cardiovasc Electrophysiol 11:1320–1329.

Shin DJ, Jang Y, Park HY, Lee JE, Yang K, Kim E, Bae Y, Kim J, Kim J, Kim SS, Lee MH, Chahine M, Yoon SK. 2004. Genetic analysis of the cardiac sodium channel gene SCN5A in Koreans with Brugada syndrome. J Hum Genet 49:573–578.

Shin DJ, Kim E, Park SB, Jang WC, Bae Y, Han J, Jang Y, Joung B, Lee MH, Kim SS, Huang H, Chahine M, Yoon SK. 2007. A novel mutation in the SCN5A gene is associated with Brugada syndrome. Life Sci 80:716–724.

Singer D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N. 1991. The roles of the subunits in the function of the calcium channel. Science 253:1553–1557.

Six I, Hermida JS, Huang H, Gouas L, Fressart V, Benammar N, Hainque B, Denjoy I, Chahine M, Guicheney P. 2008. The occurrence of Brugada syndrome and isolated cardiac conductive disease in the same family could be due to a single SCN5A mutation or to the accidental association of both diseases. Europace 10:79–85.

Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, Haverkamp W, Breithardt G, Escande D, Schulze-Bahr E, Lemarec H, Wilde AA. 2002. Genotype–phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. J Am Coll Cardiol 40:350–356.

Soldatov NM. 1994. Genomic structure of human L-type Ca^{2+} channel. Genomics 22:77–87.

Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, Sanguinetti MC, Keating MT. 2005. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. Proc Natl Acad Sci USA 102:8089–8096.

Sternberg D, Tabti N, Fournier E, Hainque B, Fontaine B. 2003. Lack of association of the potassium channel-associated peptide MiRP2-R83H variant with periodic paralysis. Neurology 61:857–859.

Stevens EB, Cox PJ, Shah BS, Dixon AK, Richardson PJ. 2001. Tissue distribution and functional expression of the human voltage gated sodium channel beta 3 subunit. Pflügers Arch 441:481–488.

Takahata T, Yasui-Furukori N, Sasaki S, Igarashi T, Okumura K, Munakata A, Tateishi T. 2003. Nucleotide changes in the translated region of SCN5A from Japanese patients with Brugada syndrome and control subjects. Life Sci 72:2391–2399.

Takehara N, Makita N, Kawabe J, Sato N, Kawamura Y, Kitabatake A, Kikuchi K. 2004. A cardiac sodium channel mutation identified in Brugada syndrome associated with atrial standstill. J Intern Med 255:137–142.

Takimoto K, Li D, Nerbonne JM, Levitan ES. 1997. Distribution, splicing and glucocorticoid-induced expression of cardiac alpha 1C and alpha 1D voltage-gated Ca^{2+} channel mRNAs. J Mol Cell Cardiol 29:3035–3042.

Tan HL, Bink-Boelkens MT, Bezzina CR, Viswanathan PC, Beaufort-Krol GC, van Tintelen PJ, van den Berg MP, Wilde AA, Balser JR. 2001. A sodium-channel mutation causes isolated cardiac conduction disease. Nature 409:1043–1047.

Taviaux S, Williams ME, Harpold MM, Nargeot J, Lory P. 1997. Assignment of human genes for beta 2 and beta 4 subunits of voltage-dependent Ca^{2+} channels to chromosomes 10p12 and 2q22–q23. Hum Genet 100:151–154.

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Tester DJ, Ackerman MJ. 2006. The role of molecular autopsy in unexplained sudden cardiac death. Curr Opin Cardiol 21:166–172.
Tester DJ, Will ML, Haglund CM, Ackerman MJ. 2005. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. Heart Rhythm 2:507–517.
Tfelt-Hansen J, Jespersen T, Hofman-Bang J, Rasmussen HB, Cedergreen P, Skovby F, Abriel H, Svendsen JH, Olesen SP, Christiansen M, Haunso S. 2009. Ventricular tachycardia in a Brugada syndrome patient caused by a novel deletion in SCN5A. Can J Cardiol 25:156–160.
Tian L, Zhu JF, Yang JG. 2007. [Gene (SCN5A) mutation analysis of a Chinese family with Brugada syndrome]. Zhonghua Xin Xue Guan Bing Za Zhi 35:1122–1125.
Van PF, Clark KA, Chatelain FC, Minor Jr DL. 2004. Structure of a complex between a voltage-gated calcium channel beta-subunit and an alpha-subunit domain. Nature 429:671–675.
Van Norstrand DW, Valdivia CR, Tester DJ, Ueda K, London B, Makielski JC, Ackerman MJ. 2007. Molecular and functional characterization of novel glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) mutations in sudden infant death syndrome. Circulation 116:2253–2259.
Vatta M, Dumaine R, Antzelevitch C, Brugada R, Li H, Bowles NE, Nademanee K, Brugada J, Brugada P, Towbin JA. 2002a. Novel mutations in domain I of SCN5A cause Brugada syndrome. Mol Genet Metab 75:317–324.
Vatta M, Dumaine R, Varghese G, Richard TA, Shimizu W, Aihara N, Nademanee K, Brugada R, Brugada J, Veerakul G, Li H, Bowles NE, Brugada P, Antzelevitch C, Towbin JA. 2002b. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. Hum Mol Genet 11:337–345.
Veltmann C, Schimpf R, Echternach C, Eckardt L, Kuschyk J, Streitner F, Spehl S, Borggrefe M, Wolpert C. 2006. A prospective study on spontaneous fluctuations between diagnostic and non-diagnostic ECGs in Brugada syndrome: implications for correct phenotyping and risk stratification. Eur Heart J 27:2544–2552.
Vernooy K, Sicouri S, Dumaine R, Hong K, Oliva A, Burashnikov E, Timmermans C, Delhaas T, Crijns HJGM, Antzelevitch C, Rodriguez L-M, Brugada R. 2006. Heart Rhythm 3:1074–1078.
Viard P, Butcher AJ, Halet G, Davies A, Nurnberg B, Heblich F, Dolphin AC. 2004. PI3K promotes voltage-dependent calcium channel trafficking to the plasma membrane. Nat Neurosci 7:939–946.
Viskin S, Wilde AA, Tan HL, Antzelevitch C, Shimizu W, Belhassen B. 2009. Empiric quinidine therapy for asymptomatic Brugada syndrome: time for a prospective registry. Heart Rhythm 6:401–404.
Wang DW, Desai RR, Crotti L, Arnestad M, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Rognum T, Schwartz PJ, George Jr AL. 2007. Cardiac sodium channel dysfunction in sudden infant death syndrome. Circulation 115:368–376.
Wang K, Yamauchi K, Li P, Kato H, Kobayashi M, Kato K, Shimizu Y. 2008. Cost-effectiveness of implantable cardioverter-defibrillators in Brugada syndrome treatment. J Med Syst 32:51–57.
Wang Q, Li Z, Shen J, Keating MT. 1996. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 34:9–16.
Wang Q, Shen J, Li Z, Timothy K, Vincent GM, Priori SG, Schwartz PJ, Keating MT. 1995. Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia. Hum Mol Genet 4:1603–1607.
Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott J-J, Demolombe S, Probst V, Anselme F, Escande D, Wiesfeld ACP, Pfeufer A, Kaab S, Wichmann H-E, Hasdemir C, Aizawa Y, Wilde AAM, Roden DM, Bezzina CR. 2008. Sodium channel β1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest 118:2260–2268.
Weiss R, Barmada MM, Nguyen T, Seibel JS, Cavlovich D, Kornblit CA, Angelilli A, Villanueva F, McNamara DM, London B. 2002. Clinical and molecular heterogeneity in the Brugada syndrome: a novel gene locus on chromosome 3. Circulation 105:707–713.
Wichter T, Matheja P, Eckardt L, Kies P, Schafers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schafers M. 2002. Cardiac autonomic dysfunction in Brugada syndrome. Circulation 105:702–706.
Yamakawa Y, Ishikawa T, Uchino K, Mochida Y, Ebina T, Sumita S, Kobayashi T, Matsushita K, Matsumoto K, Ohkusu Y, Nishizawa T, Takigiku K, Iwamoto M, Kimura K, Umemura S. 2004. Prevalence of right bundle-branch block and right precordial ST-segment elevation (Brugada-type electrocardiogram) in Japanese children. Circ J 68:275–279.
Yan GX, Antzelevitch C. 1999. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. Circulation 100:1660–1666.
Yang N, George Jr AL, Horn R. 1996. Molecular basis of charge movement in voltage-gated sodium channels. Neuron 16:113–122.
Yokoi H, Makita N, Sasaki K, Takagi Y, Okumura Y, Nishino T, Makiyama T, Kitabatake A, Horie M, Watanabe I, Tsutsui H. 2005. Double SCN5A mutation underlying asymptomatic Brugada syndrome. Heart Rhythm 2:285–292.
Yu H, Wu J, Potapova I, Wymore RT, Holmes B, Zuckerman J, Pan Z, Wang H, Shi W, Robinson RB, El-Maghrabi MR, Benjamin W, Dixon J, McKinnon D, Cohen IS, Wymore R. 2001. MinK-related peptide 1: a beta subunit for the HCN ion channel subunit family enhances expression and speeds activation. Circ Res 88:E84–E87.
Zhang M, Jiang M, Tseng GN. 2001. minK-related peptide 1 associates with Kv4.2 and modulates its gating function: potential role as beta subunit of cardiac transient outward channel? Circ Res 88:1012–1019.

1.5.4 EDITORIAL: LONG QT TESTING: IMPLICATIONS FOR COMPLEX DIAGNOSIS IN PERSONALISED MEDICINE

Michael Christiansen and Paula L. Hedley.

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Long QT testing: implications for complex diagnosis in personalized medicine

"...10% of long QT syndrome gene carriers are asymptomatic and at risk of sudden death."

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Long QT syndrome (LQTS) is a cardiac condition characterized by prolonged QT time on the ECG, representing delayed cardiac repolarization and a propensity for ventricular tachycardia. The disease has an estimated prevalence of 1 in 2500 and a clinical presentation that includes sudden death and syncope. Mutations in 12 genes have been demonstrated to cause the disease (LQTS1–12); all the genes code for either cardiac ion channels or proteins interacting with these channels [1].

Presently, LQTS genetic testing, performed by most laboratories, provides comprehensive open reading frame/splice site mutational analysis of the five most frequently affected LQTS-causing genes, that is, *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*, via high-throughput DNA sequencing [2,3]. A disease-causing mutation can be identified in 50–70% of symptomatic LQTS cases and cascade screening is routinely performed in affected families [2,3]. This is important as 10% of LQTS gene carriers are asymptomatic and at risk of sudden death [4]; LQTS has been estimated to be responsible for up to 10% of sudden infant death syndrome and 5% of sudden adult death syndrome cases [5]. There is no doubt that the real evidence-based value of LQTS testing at present relates to cascade screening in families of people with symptomatic disease, exemplified by sudden death cases with an identified presumed disease-causing mutation. However, it should be noted that the diagnosis of LQTS in most cases is a clinical one – genetic testing can not replace careful clinical examination as a negative mutation finding does not rule out the condition.

The genetics of LQTS is complicated, which is exemplified by the problems in ascertaining the clinical significance of novel mutations. These problems are not merely a consequence of allelic heterogeneity, but also due to the fact that most identified mutations are private, that is, specific for the examined family [1] and approximately 5% of LQTS cases are compound heterozygous [6,7]. The existence of many rare polymorphisms (and genetic modifiers) in the involved genes further complicates the genetic assessment of LQTS patients [8]. Many disease-causing mutations result in changes in the function of ion channels, for example, gating properties, and require highly specialized electrophysiological analysis for their characterization [1]. Such an analysis is not readily available and certainly not within a reasonable timeframe. Finally, small family sizes often preclude a certain assessment of disease segregation with the mutation in the pedigree. In reality, most of the mutations described in the literature as 'disease causing' have not been confirmed to be so *in sensu stricto*.

For the individual patient, several benefits can be noted if a disease-causing mutation can be found. First, prognostic information can occasionally be gained from knowing the affected gene [9] or the location of the mutation within a specific gene [10]. Second, if the person decides to have children they may be analyzed genetically at birth, or for that matter prenatally, and necessary prophylactic treatment can be instituted prior to occurrence of symptoms, reducing the need for extensive clinical investigations in early childhood. Third, knowledge of the gene involved may also aid in the identification of behavioral risk factors and enable a more precise recommendation of lifestyle changes. Mutations in some genes have been demonstrated to be associated with specific arrhythmia-precipitating factors, for example, physical activity in *KCNQ1* mutations and emotional distress in *KCNH2* mutations [1]. Thus, the identification of a disease-causing mutation may obviate the need for indiscriminate banning of physical activities. Finally, as the effect of treatment differs between affected genes and mutations in some genes – for example, *SCN5A* – exhibit complex phenotypes, genotyping may assist in defining optimal treatment or suggest further clinical testing [11]. The latter

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has recently been demonstrated to be the case for mutations in *SCN5A*, where some mutations present phenotypically as LQTS or atrial fibrillation, but upon flecainide stimulation reveal a Brugada phenotype that may require the insertion of an intracardiac defibrillator unit for optimal prophylaxis against sudden death [12].

> "There is no doubt that the real evidence-based value of long QT syndrome testing at present relates to cascade screening in families of people with symptomatic disease..."

Genetic testing for LQTS also raises problems for the individual. First, if genetic testing is carried out with individuals who have no symptoms or family history of LQTS symptoms, there is a considerable risk of identifying insignificant novel variants and misinterpreting them as being associated with LQTS in its 'forme fruste' presentation. Genetic testing should be reserved for people with clinical LQTS or relatives of patients with LQTS. Thus, screening for LQTS only through mutation screening in LQTS genes is not possible and is likely to be detrimental because it results in the introduction of non-evidence-based morbidity. Furthermore, most of the available information on prognosis of different mutations, treatment results and precipitating factors is based on a phenotyping of variable quality, performed on a limited subset of all genetic variants in particular genes, reducing the evidence base of the clinical inferences – particularly so in the individual case. Even in patients with clinical LQTS, the interpretation of finding a mutation has to be taken with caution, as the wide range of clinical severity of the same mutation, even within a family, makes it difficult to benefit from the mutation identification in the individual.

> "Genetic testing should be reserved for people with clinical long QT syndrome or relatives of patients with long QT syndrome."

It is the authors' opinion that several precautions can be taken to avoid a faulty interpretation of genetic testing for LQTS. The mutation screens should be expanded to incorporate all 12 genes, as this will reduce the risk of overlooking mutations in rarely affected genes as well as compound heterozygosity. Furthermore, the clinical inference of finding a mutation should be made with care, acknowledging the frequently weak knowledge base of statements on prognostic significance and phenotypical characteristics. However, these precautions should be weighed against the clinical situation of the affected family. This means that the significance accorded to a novel putative disease-causing mutation should be measured against the clinical severity of the disease in the family.

There is no doubt that the pros and cons of LQTS testing is very much in favor of the pros in cases where the person examined has LQTS or is at risk of having it owing to the family history. On the contrary, the cons are very important in cases where LQTS diagnosis has not been established.

> "Long QT syndrome illustrates the problems associated with the identification of disease-causing mutations in individual patients when a plethora of genes may be involved, occasionally simultaneously."

It is noteworthy that LQTS has turned out to be far more complicated than initially thought; the complexity, that is, the increasing number of genes to be analyzed when testing, is being overcome with the advances in sequencing technology. However, this does not solve the problems associated with the interpretation of the test results and the entailing clinical recommendations to the individual gene carrier. In addition, this simple autosomal dominant Mendelian disorder has turned into a polygenic disorder. Future research will certainly solve some of the problems associated with LQTS testing; the possibility of massive parallel sequencing of all exons in the genome [13] will increase the success rate of identification of putative disease-causing mutations but, at the same time, increase the need to refine the methods of eliminating insignificant rare variants and functional assessment of ion channel function. It remains an open question as to whether the dramatically increased sequence information will increase the diagnostic efficiency or increase the confusion with respect to the significance of genetic variants.

Long QT syndrome illustrates the problems associated with the identification of disease-causing mutations in individual patients when a plethora of genes may be involved, occasionally simultaneously. In such circumstances, it is important to limit testing to cases where it is clinically indicated and to interpret the results of testing with respect for the insufficient knowledge base of a too detailed clinical inference.

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Bibliography

1. Hedley PL, Jorgensen P, Schlamowitz S *et al.*: The genetic basis of long QT and short QT syndromes: a mutation update. *Hum. Mutat.* 30(11), 1486–1511 (2009).
2. Tester DJ, Will ML, Haglund CM, Ackerman MJ: Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm* 2(5), 507–517 (2005).
3. Napolitano C, Priori SG, Schwartz PJ *et al.*: Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *JAMA* 294(23), 2975–2980 (2005).
4. Schwartz PJ: The congenital long QT syndromes from genotype to phenotype: clinical implications. *J. Intern. Med.* 259(1), 39–47 (2006).
5. Ackerman MJ: Cardiac causes of sudden unexpected death in children and their relationship to seizures and syncope: genetic testing for cardiac electropathies. *Semin. Pediatr. Neurol.* 12(1), 52–58 (2005).
6. Berge KE, Haugaa KH, Fruh A *et al.*: Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers. *Scand. J. Clin. Lab. Invest.* 68(5), 362–368 (2008).
7. Larsen LA, Fosdal I, Andersen PS *et al.*: Recessive Romano–Ward syndrome associated with compound heterozygosity for two mutations in the *KVLQT1* gene. *Eur. J. Hum. Genet.* 7(6), 724–728 (1999).
8. Gouas L, Nicaud V, Berthet M *et al.*: Association of *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* polymorphisms with QTc interval length in a healthy population. *Eur. J. Hum. Genet.* 13(11), 1213–1222 (2005).
9. Priori SG, Schwartz PJ, Napolitano C *et al.*: Risk stratification in the long-QT syndrome. *N. Engl. J. Med.* 348(19), 1866–1874 (2003).
10. Moss AJ, Shimizu W, Wilde AA *et al.*: Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the *KCNQ1* gene. *Circulation* 115(19), 2481–2489 (2007).
11. Makita N, Behr E, Shimizu W *et al.*: The E1784K mutation in *SCN5A* is associated with mixed clinical phenotype of type 3 long QT syndrome. *J. Clin. Invest.* 118(6), 2219–2229 (2008).
12. Hedley PL, Jorgensen P, Schlamowitz S *et al.*: The genetic basis of Brugada syndrome: a mutation update. *Hum. Mutat.* 30(9), 1256–1266 (2009).
13. Ng SB, Turner EH, Robertson PD *et al.*: Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 461(7261), 272–276 (2009).

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HYPOTHESIS

The primary hypothesis is that there are molecular causes of LQTS in South African and Danish families, and that the disease-causing mutations may be harboured in the 13 genes that have been previously implicated in this disorder in international studies. The causative relationships between mutations and disease should be carefully established. Additionally, we hypothesise that some of the as yet unaccounted for genetic causes of LQTS may be found in genetic rearrangements of these genes. Alternatively, genes involved in transcript regulation of these genes may be harbouring potentially pathogenic variants. Finally, we hypothesise that the phenotypic spectrum of LQTS-associated genes may be very broad comprising other arrhythmias and structural heart diseases.

AIM

The first facet of this project is the identification of the spectrum of LQTS-causative mutations, in previously identified genes, in South African and Danish population groups. The second facet involves the assessment of the evidence-base of “LQTS-associated genes” and the identification of novel genes involved in LQTS aetiology. Finally, the role LQTS-causing genes play in other phenotypes will be determined.

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CHAPTER 2: THE GENETIC AETIOLOGY OF LONG QT SYNDROME IN SOUTH AFRICA

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PAPER 1: LONG QT SYNDROME IN SOUTH AFRICA: THE RESULTS OF COMPREHENSIVE GENETIC SCREENING

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Long QT syndrome in South Africa: the results of comprehensive genetic screening

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Abstract

Congenital long QT syndrome (cLQTS) is a genetic disorder predisposing to ventricular arrhythmia, syncope and sudden death. Over 700 different cLQTS-causing mutations in 13 genes are known. The genetic spectrum of LQTS in 44 South African cLQTS patients (23 known to carry the South African founder mutation p.A341V in *KCNQ1*) was established by screening for mutations in the coding regions of *KCNQ1, KCNH2, KCNE1, KCNE2* and *SCN5A*, the most frequently implicated cLQTS-causing genes (five-gene screening). Fourteen disease-causing mutations were identified, eight (including the founder mutation) in *KCNQ1*, five in *KCNH2* and one in *KCNE1*. Two mutations were novel. Two double heterozygotes were found among the 23 families (8.5%) carrying the founder mutation. In conclusion, cLQTS in South Africa reflects both a strong founder effect and a genetic spectrum similar to that seen in other populations. Consequently, five-gene screening should be offered as a standard screening option, as is the case internationally. This will disclose compound and double heterozygotes. Five-gene screening will most likely be even more informative in other South African sub-populations with a greater genetic diversity.

Keywords: LQTS, mutation, ion-channels, sudden death, arrhythmia

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The congenital long QT syndrome (cLQTS)[1] is an inherited disorder characterised by prolongation of the QT interval on a surface electrocardiogram (ECG) and an increased risk for life-threatening ventricular arrhythmias,[2] particularly ventricular tachycardia of the torsades de pointes type. cLQTS provides the archetypical monogenic disorder for studying the genetic basis of inherited arrhythmia syndromes in that it is relatively common (prevalence of 1:2 500 to 1:5 000),[3] and more than 700 disease-causing mutations have been identified in 13 genes encoding different cardiac ion channels or membrane adaptors.[4,5] The genetic and allelic heterogeneity results in subtly different clinical phenotypes.[6]

Several clinical syndromes have been described: Romano-Ward syndrome (RWS), an autosomal dominant form of cLQTS, which presents clinically with a pure cardiac phenotype; Jervell and Lange-Nielsen syndrome (JLNS), an autosomal recessive form of LQTS which is associated with congenital deafness;[2] Andersen-Tawil syndrome (ATS), which is an autosomal dominant multisystem disorder where cLQTS is variably present; and Timothy syndrome (TS), which is characterised by severe cLQTS as well as cardiac and somatic malformations.

cLQTS is associated with loss-of-function mutations in genes encoding repolarising K^+ ion channels, their subunits and certain interacting proteins, i.e. *KCNQ1* encoding Kv7.1 (LQT1),[6] *KCNH2* encoding Kv11.1 (LQT2),[7] *ANK2* encoding Ankyrin B (LQT4),[8] *KCNE1* encoding MinK (LQT5),[9,10] *KCNE2* encoding MiRP1 (LQT6),[11] *KCNJ2* encoding Kir2.1 (LQT7),[12] *CAV3* encoding M-Caveolin (LQT9),[13] *SCN4B* encoding Navβ3 (LQT10),[14] *AKAP9* encoding Yotiao (LQT11),[15] *SNTA1* encoding α1-Sytophin (LQT12)[16] and *KCNJ5* encoding Kir3.4 (LQT13).[17] Furthermore, gain-of-function mutations in genes encoding depolarising Na^+ and Ca^{2+} ion channels have also been associated with LQTS, i.e. *SCN5A* (LQT3)[18] and *CACNA1C* (LQT8).[19]

The ion channel defects result in either decreased K^+ efflux or increased Na^+ or Ca^{2+} influx over the cardiomyocyte plasma membrane, leading to reduced repolarisation and increased frequency of after-depolarisations (ADs) and prolonged refractory period. The latter predisposes to re-entrant ventricular arrhythmia.[4] Adrenergic stimulation, which increases the frequency of ADs, may precipitate fatal arrhythmia in asymptomatic mutation carriers.[1,4] Drugs, e.g. amiodarone, cisapride, sotalol and haloperidol, which reduce the repolarisation reserve, may cause drug-related LQTS (dLQTS) or, likewise, precipitate arrhythmia in mutation carriers.[1,4]

Previously, de Jager *et al.* and Brink *et al.* reported on the Kv7.1 (encoded by *KCNQ1*) founder mutation, p.A341V, identified in 23 Afrikaner families.[20,21] Little is known of the occurrence of other cLQTS-associated mutations in South Africa.

In order to describe the spectrum of mutations causing cLQTS in South Africa, we screened 44 apparently unrelated

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South African cLQTS probands, including the 23 probands of the families that are carriers of the founder p.A341V mutation, for mutations in the five most frequently implicated cLQTS genes, *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*, by multiplex capillary electrophoresis single-strand conformation polymorphism (multi-CE-SSCP). A sub-group was further analysed by the direct sequencing of these genes.

Methods

Forty-four LQTS probands (23 known founder mutation carriers and 21 unrelated patients shown not to carry the founder mutation) [77% female; mean (SD) age at first event 10 (6) years, were ascertained to have cLQTS, mean (SD) QTc 501 (59) ms]. See Table 1 for clinical and demographic information. The diagnosis was based on the 1993 standard diagnostic criteria[22] and included a physical examination, a standard 12-lead ECG and a personal interview. All probands were of the RWS type. All patients provided written informed consent (parental in the case of minors). The study was approved by the Health Research Ethics Committee of the University of Stellenbosch.

Molecular genetic procedures

DNA was isolated from peripheral blood using a commercially available procedure (Qiagen GmbH, Germany). Primers were designed to amplify all the exons of the five LQTS-causing genes under investigation, including the intron/exon boundaries. The primers were synthesised by the Synthetic DNA Laboratory (University of Cape Town, Cape Town, South Africa) and Applied Biosystems (Copenhagen, Denmark).

PCR amplification was performed by published protocol for *SCN5A*.[23] Primer sequences and PCR conditions for *KCNQ1, KCNH2, KCNE1* and *KCNE2* are available upon request (phy@ssi.dk). Primers used for generating PCR products for sequencing were tagged with M13 sequence. PCR products were qualitatively assessed on 2% agarose gels by standard procedures prior to multi-CE-SSCP and direct sequencing.

Multi-CE-SSCP

PCR primers were labelled at their 5′ end with one of the following fluorophors: 6FAM™, VIC®, NED™ or PET™ (Applied Biosystems, Foster City, California, USA). The labelled PCR amplicons were mixed and diluted according to published protocol[23] for *SCN5A*. Amplicons of *KCNQ1, KCNH2, KCNE1* and *KCNE2* were mixed and diluted in a similar manner; the specifics of these mixtures are available upon request.

Multi-CE-SSCP was performed on an ABI Prism™ 3100 Genetic Analyser (PE Applied Biosystems, Foster City, California, USA) with GeneScan polymer (Applied Biosystems, Foster City, California, USA) at 18°C and 30°C. PCR amplicons were sequenced to identify the variants responsible for alterations in the electrophoretic mobility detected by multi-CE-SSCP analysis.

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF 44 INDEX PATIENTS

Cohort	*% female*	*QTc mean (SD)*	*Age at first event mean (SD)*
Total	77	501 (59)	10 (6)
A341V carriers	81	513 (48)	8 (4)
Other mutation carriers	73	507 (69)	11 (5)
No mutation detected	75	452 (35)	17 (7)

Direct sequencing

A sub-group of 32 probands was additionally analysed by direct DNA sequencing. The probands of the founder families were included to assess the frequency of compound heterozygozity in our cohort. PCR products were purified by exonuclease 1 and shrimp alkaline phosphatase reaction.

Sequencing of both strands was performed using BigDye® Terminator v1.1 (Applied Biosystems, UK) and M13 primers (sense *5′-CAGTTCTCACAGGAGCCACA-3′*) and (antisense *5′-AGGTGAACTGGAACCACAGG-3′*) (Taq Copenhagen, Denmark). Sequences were analysed with the ABI 3730 DNA analyser (Applied Biosystem, UK).

Bio-informatics

Nucleotide sequences were aligned to GenBank (http://www.ncbi.nlm.nih.gov/Entrez) reference sequences *KCNQ1* (NM_000218.2/NP_000209.2), *KCNH2* (NM_000238.2/NP_000229.1), *SCN5A* (NM_000335.2/NP_000326.2), *KCNE1* (NM_000219.2/NP_000210.2) and *KCNE2* (NM_172201.1/NP_75195.1). Multiple sequence alignments of multiple species were performed with Clustal W (version 1.82) (http://www.ebi.ac.uk/clustalw/#). SNPs were compared to the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the NHLBI Exome Sequencing Project Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS/). These data were used as *in silico* control.

Criteria for disease association

A genetic variant was considered a disease-causing mutation if it resulted in an amino acid substitution, interfered with a splice site or had previously been shown to be associated with cLQTS and was not found in *in silico* controls and had not previously been described as a polymorphism. Sequence variants also found in controls were considered polymorphisms and are not reported here.

Results

We identified 14 different disease-causing mutations in 34 of the 44 probands tested (Table 2). Three mutations were identified among the 23 founder probands; all harboured the South African founder mutation KCNQ1:p.A341V, while two probands (8.5%) harboured an additional mutation in a second cLQTS-causing gene, which may contribute to disease. These are cases of double heterozygosity. Furthermore, 11 mutations were identified in 13 non-founder probands.

The multi-CE-SSCP results corroborated standard gel-based PCR-SSCP results (not shown), however, in 32 cases where the DNA concentration was sufficient, direct DNA sequencing led to the identification of four disease-causing mutations (KCNQ1:p.Y315C; KCNQ1:p.A344V; KCNH2:c.917-3T>C and KCNH2:p.R328C), which were missed by multi-CE-SSCP. In the case of KCNQ1:pA344V, two cases had been detected by SSCP, but an additional case was identified by direct sequencing.

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TABLE 2. DISEASE-CAUSING MUTATIONS IDENTIFIED IN THE SOUTH AFRICAN LQTS PROBANDS AS WELL AS AN INDICATION OF WHICH PLATFORM IDENTIFIED THE VARIANT

	Mutations		*Mutation detection technique*					
Gene/ protein	*Coding substitution*	*Protein consequence*	*SSCP*	*sequencing*	*total*	*Frequency in* in silico *controls*	*Av QTc/additional info*	*References*
KCNQ1/ Kv7.1	c.760G>A	p.V254M	1	ns	1	0	500 ms/ICD	4–6, 25–28, 33, 45, 55–58
	c.944A>G	p.Y315C	0	1	1	0	550 ms	4, 5, 25, 29, 33, 57, 59–62
	c.944A>C	p.Y315S	1	ns	1	0	512 ms	4, 26, 31, 57
	c.1022C>A	p.A341E	1	ns	1	0	502 ms/CA at 51 years	4, 5, 27, 32, 45, 57, 59, 63, 64
	c.1022C>T	p.A341V	23	23	23	0	513 ms/BB	4–6, 20, 21, 25–27, 45, 65–75
	c.1024C>T	p.L342F	1	1	1	0	527 ms	4, 5, 24, 26, 76, 77
	c.1031C>T	p.A344V	2	2	3[#]	0	557 ms	4, 5, 25, 26, 33, 34, 57, 68
	c.1760C>T	p.T587M	1	ns	1	0	400 ms	4, 5, 24, 35–37, 59, 78–80
KCNH2/ Kv11.1	c.208C>A	p.R100W	1	1	1	0	437 ms	5
	c.917-3T>C	defective splicing/ protein degradation	0	1	1	0	450 ms	This study
	c.982C>T*	p.R328C*	0	1	1	0	654 ms*	4, 5, 25, 30, 39, 40, 47, 48, 81–83
	c.1714G>A	p.G572S	1	1	1	0	402 ms/BB	4, 5, 24, 25, 39–44
	c.1882C>G	p.F627L	1	ns	1	0	462 ms	4, 45, 46
KCNE1/ MinK	c.273C>A*	p.D91E*	1	1	1	0 (EA); 0.0002 (AA)	533 ms*	This study

*Variants that co-occur with Kv7.1:p.A341V; [#]One of the probands in which p.A344V had been identified previously by SSCP was not available for sequencing. ns: not screened by sequencing; EA: 8 600 European-Americans screened by exome sequencing; AA: 4 406 African-Americans screened by exome sequencing; ICD: implantable cardioverter defibrillator; CA: cardiac arrest. BB: beta-blockers.

Discussion

We have identified 14 mutations in 34 of 44 (77 %) South African probands screened; two (14%) of the mutations identified are novel. The disease-causing mutations of 33 probands were identified by multi-CE-SSCP (Table 2), which represents a detection rate of 75%. Additionally, applying direct DNA sequencing to a smaller cohort resulted in the detection of the disease-causing mutation in 30 of the 32 probands screened, which represents a total detection rate of 94%.

These results are comparable to those reported in other screening studies.[22,24,25] Therefore, in comparison to just screening for the KCNQ1:p.A341V founder mutation in our cLQTS probands (which had a detection rate of 52%), the addition of other genes and the application of direct DNA sequencing for mutation screening resulted in manifest improvements in diagnostic efficiency.

Within the founder families, represented by 23 probands, two probands (8.5 %) were double heterozygotes. One case carried KCNQ1:p.A341V and KCNH2:p.R328C while the other carried KCNQ1:p.A341V and KCNE1:p.D91E. This is an important finding as one of the purposes of genetic screening in cLQTS probands is the identification of asymptomatic mutation carriers in family members (cascade screening) in order to clinically assess the need for prophylaxis.

In theory, half of the mutation carriers within a cLQTS family may be missed if only one of the mutations in a compound heterozygote or double heterozygote is identified through selective screening for only one mutation or the selective screening of only one of the involved genes. As the clinical presentation may be sudden death in cLQTS mutation carriers, this is a serious clinical problem. Our results demonstrate that the detection of compound and double heterozygotes is also an important consideration in clinical handling of cLQTS cases in South Africa.

KCNQ1 mutations

The common South African founder mutation (p.A341V) was identified in 23 probands.[20,21] p.V254M was first identified in a large LQT1-kindred[6] and later associated with sudden death before 40 years of age.[26] This mutation was found to exert a dominant negative effect on native IKs.[27] Assessment of beta-blocker therapy was performed on a cLQTS family carrying p.V254M; treatment was determined to be effective and safe.[28]

p.Y315C has been shown to be a dominant negative mutation, which, while the protein was normally trafficked to the cell surface, produced no measurable current.[29] This mutation has been shown to be associated with cisapride-induced QT prolongation.[30]

p.Y315S was first identified in a French LQTS family.[26] Jongbloed *et al.* (1999) identified this mutation as a *de novo* cause of cLQTS, which appeared to be triggered by both physical and emotional stress.[31] In both cases the mutation was associated with an onset of symptoms before 10 years of age.

p.L342F was previously reported by Donger *et al.* (1997) where it was identified in a single case; none of the other family members carrying the mutation presented with symptoms of LQTS.[26] However, the proband had experienced onset of symptoms before the age of 10 years.

p.A341E was identified in the first double heterozygote-carrying cLQTS family identified in a French cLQTS cohort,[32] where carriers of both mutations (KCNQ1:p.A341E and KCNH2:c.2592+1G>A) were reported to be severely symptomatic, with both stress- and rest-induced symptoms initiating in early childhood. The mutation was found to exert a dominant negative effect on native IKs.[27]

The p.A344V mutation was first identified as a *forme fruste* cause of LQTS.[26] Later, Choi *et al.* (2004) discovered this mutation in a cohort of patients, which had suffered swimming-triggered arrhythmias.[33] In addition to shifting the voltage

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dependence of the I_{Ks} channel activation, the p.A344V mutation increased the sensitivity of the channel for bupivacaine (a local anaesthetic).[34] KCNQ1:p.T587M was found to result in haplo-insufficiency as a consequence of being transport deficient.[35,36] Furushima *et al.* (2010) identified a case of foetal atrio-ventricular block and unmasked maternal QT prolongation in the postpartum period in a mother and baby carrying this mutation.[37]

KCNH2 mutations

KCNH2:c.917-3T>C has not been previously reported; however, splice-site analysis suggests that the tentative consequence of this variant is disruption the splice acceptor of exon 5. Such a splice-site disruption would be expected to cause aberrant mRNA splicing and result in either the synthesis of a truncated protein or, more likely, haplo-insufficiency. In both cases, the result is likely to be a reduced amount of Kv11.1 protein and therefore reduced repolarisation capacity.

KCNH2:p.R100W was identified in two of 2 500 patients reported by Kapplinger *et al.* in 2009, however, no specific clinical information is provided.[5] A mutation at residue 100 (p.R100G) has been reported in a 41-year-old French woman who had suffered an aborted cardiac arrest,[38] which may indicate that the R100 residue has a particular functional relevance.

The p.G572S mutation has been reported in LQTS cohorts around the world[4,5,23,25,39-44] but has not been identified in an exome sequencing project to date. Zhao *et al.* in 2009 determined that p.G572S causes a dominant negative trafficking defect.[44] KCNH2:p.F627L was first identified by Splawski *et al.* in 2000 as part of his LQTS screen of 262 probands[45] and later was reported to be the cause of LQTS with foetal onset of atrio-ventricular block and ventricular tachycardia.[46]

Double heterozygotes

In addition to the KCNQ1:p.A341V mutation, we have confirmed the presence of the KCNE1:p.D91E variant in a single proband. The clinical significance of this variant is unclear. It is a very rare variant, identified in a single African-American individual in an exome analysis of 4 406 African-American individuals (http://evs.gs.washington.edu/EVS/) (date accessed July 2012).

A more extensive analysis of the phenotypes in the family, where the clinical effect of individual mutations can be assessed as the *KCNQ1* mutation is located on chromosome 11 and the *KCNE1*-encoded MinK variant is located on chromosome 2,[14] is necessary to establish the clinical significance of the compound heterozygozity. Due to the rarity of the MinK variant and the known association between mutations in the N-terminus of MinK and adverse drug effects, we consider it a significant finding.

In a second proband, we identified both the KCNQ1:p.A341V mutation and KCNH2:p.R328C. The p.R328C variant was first reported by Chevalier *et al.* (2001) in an acquired LQTS cohort.[47] Grunnet *et al.* (2005) described a cLQTS patient harbouring double mutations (KCNQ1:pR591H; KCNH2:p.R328C). They determined that p.R328C did not produce a functional phenotype and that KCNQ1:p.R591H was sufficient to explain disease.[48]

Further functional assessment of KCNH2:p.R328C by Anderson *et al.* (2006) determined the Kv11.1 channels carrying p.R328C were normally trafficked to the membrane and conferred no functional phenotype.[39] However, a subsequent report by Chevalier *et al.* (2007) suggested that p.R328C had a dominant negative effect on I_{Kr}. Finally, Kapa *et al.* (2009) identified KCNH2:p.R328C in a control individual.[40] The p.R328C variant is, in all likelihood, a rare polymorphism; however, we cannot exclude that it has a functional effect in the presence of certain drugs or conditions, e.g. hypokalaemia.[49]

Apart from the variants described above and reported in Table 2, a number of synonymous (i.e. not having any effect on the amino acid sequence) variants as well as variants that have been found with a relatively high frequency (i.e. > 1%) in other populations were found during the screening of the 44 probands (data not shown). Such polymorphisms are not disease causing, but they may modify the phenotype.[4] These potential *forme fruste* mutations, i.e. mutations that do not appear to cause disease in isolation, should be noted.

Numerous non-synonymous polymorphisms have been reported to be associated with an effect in cardiac repolarisation currents.[50] KCNE1:p.D85N has been implicated in drug-induced LQTS[30] and KCNH2:p.R1047L has been reported to reduce I_{Kr} in a mammalian cell-based system.[51]

Occasionally, the presence of very rare variants in the normal population (e.g. the KCNE1: p.D91E mentioned above) that have not previously been associated with disease and have a population frequency less than 1:4 000 may raise questions about their possible role in disease causation. In such a case, family studies, which may not be possible in small families, and electrophysiological analysis, which is costly and time consuming, may be necessary to ascribe disease causation with certainty. Consequently, novel mutations where the disease association has not been established should be evaluated carefully and the uncertainty should be considered when deciding the appropriate management of the family, particularly as prophylactic treatment with beta-blockers or implantation of an ICD unit can have adverse effects.

To establish a genetic diagnosis in a cLQTS family is in most cases complicated, as described above, and is best done as a collaborative effort involving cardiologists, clinical geneticists and molecular geneticists. The findings reported here suggest that most South African cLQTS cases will be caused by mutations already described in other populations, as we only found two novel mutations. However, this probably reflects the fact that most of the patients included have a European ancestry and that to date no black South African LQTS patients have been encountered for enrolment in the study.

Furthermore, no other African-based research groups have reported the occurrence of LQTS patients within their black African populations.[52] It must be expected, due to the large genetic diversity of African populations, as is evident from the Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), that comprehensive genetic screening of at least five genes will be necessary to provide an adequate genetic diagnosis in these populations.

A limitation of this study was a failure to detect potential gene rearrangements, such as large duplications or deletions. Multi-CE-SSCP can only identify point mutations or small insertions and deletions in coding regions or at splice junctions. Koopman *et al.* detected a large duplication in the *KCNH2* gene, which they determined to be responsible for cLQTS in a Dutch family.[53] No mutation has so far been identified in approximately 10–20%

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of cLQTS cases globally; gene rearrangements could account for a portion of these.

Furthermore, we only screened five of the 13 known LQTS-causing genes. However, the small sample size limits the possibility of identifying variants in genes that are rarely associated with cLQTS. These genes typically exhibit a prevalence of <1% of the LQTS population.[4]

Conclusion

Genetic screening of five frequently implicated cLQTS causative genes in a predominantly white South African cLQTS cohort led to the identification of a disease-causing mutation in 77% of examined cases. The previously described founder mutation, p.A341V, is responsible for cLQTS in 52% of these probands, meaning that the extended screening increases the detection by 29% points. Furthermore, the frequency of double heterozygozity in South Africa is similar to the frequency seen in other populations.[54] Therefore, 8.5% of the founder mutation-carrying families had members that were double heterozygotes.

These findings emphasise the importance of performing a comprehensive genetic screening when doing genetic work up, even in a population with a large founder effect. Despite the impressive diagnostic rate found here, it should be remembered that, using current techniques, it is not yet possible to establish a genetic diagnosis in many (23%) cLQTS families. In these cases, and wherever the clinical picture is complex, it is of paramount importance to realise that LQTS is a clinical condition requiring clinical management, irrespective of the genetic aetiology.

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References

1. Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, *et al*. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS Registry. *Circulation* 1998; **97**(22): 2237–2244.
2. Priori SG, Barhanin J, Hauer RN, Haverkamp W, Jongsma HJ, Kleber AG, *et al*. Genetic and molecular basis of cardiac arrhythmias; impact on clinical management. Study group on molecular basis of arrhythmias of the working group on arrhythmias of the european society of cardiology. *Eur Heart J* 1999; **20**(3): 174–195.
3. Crotti L, Celano G, Dagradi F, Schwartz PJ. Congenital long QT syndrome. *Orphanet J Rare Dis* 2008; **3**: 18.
4. Hedley PL, Jorgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, et al. The genetic basis of long QT and short QT syndromes: a mutation update. *Hum Mutation* 2009; **30**(11): 1486–14511.
5. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, *et al*. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm* 2009; **6**(9): 1297–303.
6. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, *et al*. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nature Genet* 1996; **12**(1): 17–23.
7. Itoh T, Tanaka T, Nagai R, Kamiya T, Sawayama T, Nakayama T, *et al*. Genomic organization and mutational analysis of HERG, a gene responsible for familial long QT syndrome. *Hum Genet* 1998; **102**(4): 435–439.
8. Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, *et al*. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003; **421**(6923): 634–639.
9. Splawski I, Timothy KW, Vincent GM, Atkinson DL, Keating MT. Molecular basis of the long-QT syndrome associated with deafness. *New Engl J Med* 1997; **336**(22): 1562–1567.
10. Tyson J, Tranebjaerg L, Bellman S, Wren C, Taylor JF, Bathen J, *et al*. IsK and KvLQT1: mutation in either of the two subunits of the slow component of the delayed rectifier potassium channel can cause Jervell and Lange-Nielsen syndrome. *Hum Molec Genet* 1997; **6**(12): 2179–2185.
11. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, *et al*. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; **97**(2):175–187.
12. Plaster NM, Tawil R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, *et al*. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* 2001; **105**(4): 511–519.
13. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, *et al*. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation* 2006; **114**(20): 2104–2112.
14. Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, *et al*. SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. *Circulation* 2007; **116**(2): 134–142.
15. Chen L, Marquardt ML, Tester DJ, Sampson KJ, Ackerman MJ, Kass RS. Mutation of an A-kinase-anchoring protein causes long-QT syndrome. *Proc Natl Acad Sci USA* 2007; **104**(52): 20990–2995.
16. Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, *et al*. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. *Proc Natl Acad Sci USA* 2008; **105**(27): 9355–9360.
17. Yang Y, Yang Y, Liang B, Liu J, Li J, Grunnet M, *et al*. Identification of a Kir3.4 mutation in congenital long QT syndrome. *Am J Hum Genet* 2010; **86**(6): 872–880.
18. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, *et al*. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995; **80**(5): 805–811.
19. Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, *et al*. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci USA* 2005; **102**(23): 8089–8096; discussion 6–8.
20. Brink PA, Crotti L, Corfield V, Goosen A, Durrheim G, Hedley P, *et al*. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. *Circulation* 2005; **112**(17): 2602–2610.
21. De Jager T, Corbett CH, Badenhorst JC, Brink PA, Corfield VA. Evidence of a long QT founder gene with varying phenotypic expression in South African families. *J Med Genet* 1996; **33**(7): 567–573.
22. Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, *et al*. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *J Am Med Assoc* 2005; **294**(23): 2975–2980.
23. Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, *et al*. High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia. *Clin Genet* 2006; **69**(6): 504–511.
24. Berge KE, Haugaa KH, Fruh A, Anfinsen OG, Gjesdal K, Siem G, *et al*. Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers. *Scand J Clin Lab Invest* 2008; **68**(5): 362–368.
25. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm* 2005; **2**(5): 507–517.
26. Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, *et al*. KVLQT1 C-terminal missense mutation causes *a forme fruste* long-QT syndrome. *Circulation* 1997; **96**(9): 2778–2781.
27. Wang Z, Tristani-Firouzi M, Xu Q, Lin M, Keating MT, Sanguinetti

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MC. Functional effects of mutations in KvLQT1 that cause long QT syndrome. *J Cardiovasc Electrophysiol* 1999; **10**(6): 817–826.

28. Wedekind H, Schwarz M, Hauenschild S, Djonlagic H, Haverkamp W, Breithardt G, *et al*. Effective long-term control of cardiac events with beta-blockers in a family with a common LQT1 mutation. *Clin Genet* 2004; **65**(3): 233–241.
29. Bianchi L, Priori SG, Napolitano C, Surewicz KA, Dennis AT, Memmi M, *et al*. Mechanisms of I(Ks) suppression in LQT1 mutants. *Am J Physiol Heart Circ Physiol* 2000; **279**(6): H3003–11.
30. Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ, *et al*. Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. *J Molec Med* 2004; **82**(3): 182–188.
31. Jongbloed RJ, Wilde AA, Geelen JL, Doevendans P, Schaap C, Van Langen I, *et al*. Novel KCNQ1 and HERG missense mutations in Dutch long-QT families. *Hum Mutation* 1999; **13**(4): 301–310.
32. Berthet M, Denjoy I, Donger C, Demay L, Hammoude H, Klug D, *et al*. C-terminal HERG mutations: the role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. *Circulation* 1999; **99**(11): 1464–1470.
33. Choi G, Kopplin LJ, Tester DJ, Will ML, Haglund CM, Ackerman MJ. Spectrum and frequency of cardiac channel defects in swimming-triggered arrhythmia syndromes. *Circulation* 2004; **110**(15): 2119–2124.
34. Siebrands CC, Binder S, Eckhoff U, Schmitt N, Friederich P. Long QT 1 mutation KCNQ1A344V increases local anesthetic sensitivity of the slowly activating delayed rectifier potassium current. *Anesthesiology* 2006; **105**(3): 511–520.
35. Biliczki P, Girmatsion Z, Brandes RP, Harenkamp S, Pitard B, Charpentier F, *et al*. Trafficking-deficient long QT syndrome mutation KCNQ1-T587M confers severe clinical phenotype by impairment of KCNH2 membrane localization: evidence for clinically significant IKr-IKs alpha-subunit interaction. *Heart Rhythm* 2009; **6**(12): 1792–1801.
36. Yamashita F, Horie M, Kubota T, Yoshida H, Yumoto Y, Kobori A, *et al*. Characterization and subcellular localization of KCNQ1 with a heterozygous mutation in the C terminus. *J Molec Cell Cardiol* 2001; **33**(2):197–207.
37. Furushima H, Chinushi M, Sato A, Aizawa Y, Kikuchi A, Takakuwa K, *et al*. Fetal atrioventricular block and postpartum augmentative QT prolongation in a patient with long-QT syndrome with KCNQ1 mutation. *J Cardiovasc Electrophysiol* 2010; **21**(10): 1170–1173.
38. Millat G, Chevalier P, Restier-Miron L, Da Costa A, Bouvagnet P, Kugener B, *et al*. Spectrum of pathogenic mutations and associated polymorphisms in a cohort of 44 unrelated patients with long QT syndrome. *Clin Genet* 2006; **70**(3): 214–227.
39. Anderson CL, Delisle BP, Anson BD, Kilby JA, Will ML, Tester DJ, *et al*. Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation* 2006; **113**(3): 365–373.
40. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, *et al*. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation* 2009; **120**(18): 1752–1760.
41. Fodstad H, Swan H, Laitinen P, Piippo K, Paavonen K, Viitasalo M, *et al*. 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.
42. Liu WL, Hu DY, Li P, Li CL, Qin XG, Li YT, *et al*. [Novel mutations of potassium channel KCNQ1 S145L and KCNH2 Y475C genes in Chinese pedigrees of long QT syndrome]. *Zhonghua nei ke za zhi* [*Chinese J Int Med*] 2006; **45**(6): 463–466.
43. Chung SK, MacCormick JM, McCulley CH, Crawford J, Eddy CA, Mitchell EA, *et al*. Long QT and Brugada syndrome gene mutations in New Zealand. *Heart Rhythm* 2007; **4**(10): 1306–1314.
44. Zhao JT, Hill AP, Varghese A, Cooper AA, Swan H, Laitinen-Forsblom PJ, et *al*. Not all hERG pore domain mutations have a severe phenotype: G584S has an inactivation gating defect with mild phenotype compared to G572S, which has a dominant negative trafficking defect and a severe phenotype. *J Cardiovasc Electrophysiol* 2009; **20**(8): 923–930.
45. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, *et al*. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000; **102**(10): 1178–1185.
46. Lin MT, Wu MH, Chang CC, Chiu SN, Theriault O, Huang H, *et al*. *In utero* onset of long QT syndrome with atrioventricular block and spontaneous or lidocaine-induced ventricular tachycardia: compound effects of hERG pore region mutation and SCN5A N-terminus variant. *Heart Rhythm* 2008; **5**(11): 1567–1574.
47. Chevalier P, Rodriguez C, Bontemps L, Miquel M, Kirkorian G, Rousson R, *et al*. Non-invasive testing of acquired long QT syndrome: evidence for multiple arrhythmogenic substrates. *Cardiovasc Res* 2001; **50**(2): 386–398.
48. Grunnet M, Behr ER, Calloe K, Hofman-Bang J, Till J, Christiansen M, *et al*. Functional assessment of compound mutations in the KCNQ1 and KCNH2 genes associated with long QT syndrome. *Heart Rhythm* 2005; **2**(11): 1238–1249.
49. Christiansen M, Wettrell G, Larsen LA, Lundqvist L, Andersen PS, Kanters JK, Vuust J. Potassium must be considered in congenital long QT syndrome. *Heart Drug* 2005; **5**(1): 54–58.
50. Kaab S, Schulze-Bahr E. Susceptibility genes and modifiers for cardiac arrhythmias. *Cardiovasc Res* 2005; **67**(3): 397–413.
51. Laohakunakorn P, Benson DW, Yang P, Yang T, Roden DM, Kugler JD. Bidirectional ventricular tachycardia and channelopathy. *Am J Cardiol* 2003; **92**(8): 991–995.
52. Heradien MGA, Moolman-Smook JC, Brink PA. Race and gender representation of hypertrophic cardiomyopathy or long QT syndrome cases in a South African research setting : review article. *Cardiovasc J Afr* 2007; **18**(5): 308–311.
53. Koopmann TT, Alders M, Jongbloed RJ, Guerrero S, Mannens MM, Wilde AA, *et al*. Long QT syndrome caused by a large duplication in the KCNH2 (HERG) gene undetectable by current polymerase chain reaction-based exon-scanning methodologies. *Heart Rhythm* 2006; **3**(1): 52–55.
54. Zaklyazminskaya EV, Abriel H. Prevalence of significant genetic variants in congenital long QT syndrome is largely underestimated. *Frontiers Pharmacol* 2012; **3**: 72.
55. 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**(1): 57–61.
56. Struijk JJ, Kanters JK, Andersen MP, Hardahl T, Graff C, Christiansen M, *et al*. Classification of the long-QT syndrome based on discriminant analysis of T-wave morphology. *Med Biol Engineer Comput* 2006; **44**(7): 543–549.
57. Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, *et al*. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007; **115**(19): 2481–2489.
58. Barsheshet A, Goldenberg I, Ou J, Moss AJ, Jons C, Shimizu W, *et al*. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to beta-blocker therapy in type 1 long-QT syndrome. *Circulation* 2012; **125**(16): 1988–1996.
59. Chen S, Zhang L, Bryant RM, Vincent GM, Flippin M, Lee JC, *et al*. KCNQ1 mutations in patients with a family history of lethal cardiac arrhythmias and sudden death. *Clin Genet* 2003; **63**(4): 273–282.
60. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998; **51**(1): 86–97.
61. Napolitano C, Schwartz PJ, Brown AM, Ronchetti E, Bianchi L, Pinnavaia A, *et al*. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2000; **11**(6): 691–696.
62. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. *Circulation* 1999; **99**(4): 529–533.
63. Goldman AM, Glasscock E, Yoo J, Chen TT, Klassen TL, Noebels JL. Arrhythmia in heart and brain: KCNQ1 mutations link epilepsy and sudden unexplained death. *Sci Translat Med* 2009; **1**(2): 2ra6.

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64. Zareba W, Moss AJ, Sheu G, Kaufman ES, Priori S, Vincent GM, *et al*. Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. *J Cardiovasc Electrophysiol* 2003; **14**(11): 1149–1153.
65. Russell MW, Dick M, 2nd, Collins FS, Brody LC. KVLQT1 mutations in three families with familial or sporadic long QT syndrome. *Hum Molec Genet* 1996; **5**(9):1319–1324.
66. Kobori A, Sarai N, Shimizu W, Nakamura Y, Murakami Y, Makiyama T, *et al*. Additional gene variants reduce effectiveness of beta-blockers in the LQT1 form of long QT syndrome. *J Cardiovasc Electrophysiol* 2004; **15**(2): 190–199.
67. Jongbloed R, Marcelis C, Velter C, Doevendans P, Geraedts J, Smeets H. DHPLC analysis of potassium ion channel genes in congenital long QT syndrome. *Hum Mutation* 2002; **20**(5): 382–391.
68. Li H, Chen Q, Moss AJ, Robinson J, Goytia V, Perry JC, *et al*. New mutations in the KVLQT1 potassium channel that cause long-QT syndrome. *Circulation* 1998; **97**(13): 1264–1269.
69. Crotti L, Spazzolini C, Schwartz PJ, Shimizu W, Denjoy I, Schulze-Bahr E, *et al*. The common long-QT syndrome mutation KCNQ1/A341V causes unusually severe clinical manifestations in patients with different ethnic backgrounds: toward a mutation-specific risk stratification. *Circulation* 2007; **116**(21): 2366–2375.
70. Heradien MJ, Goosen A, Crotti L, Durrheim G, Corfield V, Brink PA, *et al*. Does pregnancy increase cardiac risk for LQT1 patients with the KCNQ1-A341V mutation? *J Am Coll Cardiol* 2006; **48**(7): 1410–1415.
71. Schwartz PJ, Vanoli E, Crotti L, Spazzolini C, Ferrandi C, Goosen A, *et al*. Neural control of heart rate is an arrhythmia risk modifier in long QT syndrome. *J Am Coll Cardiol* 2008; **51**(9): 920–929.
72. Heijman J, Spatjens RL, Seyen SR, Lentink V, Kuijpers HJ, Boulet IR, *et al*. Dominant-negative control of cAMP-dependent IKs upregulation in human long-QT syndrome type 1. *Circ Res* 2012; **110**(2): 211–219.
73. Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, *et al*. NOS1AP is a genetic modifier of the long-QT syndrome. *Circulation* 2009; **120**(17): 1657–1663.
74. Brink PA, Schwartz PJ. Of founder populations, long QT syndrome, and destiny. *Heart Rhythm* 2009; **6**(11 Suppl): S25–33.
75. Mikuni I, Torres CG, Bienengraeber MW, Kwok WM. Partial restoration of the long QT syndrome associated KCNQ1 A341V mutant by the KCNE1 beta-subunit. *Biochim Biophys Acta* 2011; **1810**(12): 1285–1293.
76. Chouabe C, Neyroud N, Guicheney P, Lazdunski M, Romey G, Barhanin J. Properties of KvLQT1 K+ channel mutations in Romano-Ward and Jervell and Lange-Nielsen inherited cardiac arrhythmias. *EMBO J* 1997; **16**(17): 5472–5479.
77. Chouabe C, Neyroud N, Richard P, Denjoy I, Hainque B, Romey G, *et al*. Novel mutations in KvLQT1 that affect Iks activation through interactions with Isk. *Cardiovasc Res* 2000; **45**(4): 971–980.
78. Itoh T, Tanaka T, Nagai R, Kikuchi K, Ogawa S, Okada S, *et al*. Genomic organization and mutational analysis of KVLQT1, a gene responsible for familial long QT syndrome. *Hum Genet* 1998; **103**(3): 290–294.
79. Neyroud N, Richard P, Vignier N, Donger C, Denjoy I, Demay L, *et al*. Genomic organization of the KCNQ1 K+ channel gene and identification of C-terminal mutations in the long-QT syndrome. *Circulation Res* 1999; **84**(3): 290–297.
80. Hayashi K, Shuai W, Sakamoto Y, Higashida H, Yamagishi M, Kupershmidt S. Trafficking-competent KCNQ1 variably influences the function of HERG long QT alleles. *Heart Rhythm* 2010; **7**(7): 973–980.
81. Hinterseer M, Irlbeck M, Ney L, Beckmann BM, Pfeufer A, Steinbeck G, *et al*. Acute respiratory distress syndrome with transiently impaired left ventricular function and torsades de pointes arrhythmia unmasking congenital long QT syndrome in a 25-yr-old woman. *Br J Anaesth* 2006; **97**(2): 150–153.
82. Millat G, Chevalier P, Restier-Miron L, Da Costa A, Bouvagnet P, Kugener B, *et al*. Spectrum of pathogenic mutations and associated polymorphisms in a cohort of 44 unrelated patients with long QT syndrome. *Clin Genet* 2006; **70**(3): 214–227.
83. Chevalier P, Bellocq C, Millat G, Piqueras E, Potet F, Schott JJ, *et al*. Torsades de pointes complicating atrioventricular block: evidence for a genetic predisposition. *Heart Rhythm* 2007; **4**(2): 170–174.

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CHAPTER 3: THE GENETIC AETIOLOGY OF LONG QT SYNDROME IN DENMARK

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PAPER 2: MUTATIONS IN DANISH PATIENTS WITH LONG QT SYNDROME AND IDENTIFICATION OF A LARGE FOUNDER FAMILY WITH P.F29L IN KCNH2

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Mutations in Danish patients with long QT syndrome and identification of a large founder family with p.F29L in *KCNH2*

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ABSTRACT:

Background: Long QT syndrome (LQTS) is a cardiac ion channelopathy which presents clinically with palpitations, syncope or sudden death. More than 700 LQTS-causing mutations have been identified in 13 genes, all of which encode proteins involved in the execution of the cardiac action potential. The most frequently affected genes, covering > 90% of cases, are *KCNQ1*, *KCNH2* and *SCN5A*.

Methods: We describe 64 different mutations in 70 unrelated Danish families using a routine five-gene screen, comprising *KCNQ1*, *KCNH2* and *SCN5A* as well as *KCNE1* and *KCNE2.*

Results: Twenty-two mutations were found in *KCNQ1*, 28 in *KCNH2,* 9 in *SCN5A,* 3 in *KCNE1* and 2 in KCNE2. Twenty-six of these have only been described in the Danish population and 24 of these are novel. One compound heterozygote (1.4 % of families) was found. A founder mutation, p.F29L in *KCNH2*, was identified in 5 unrelated families. Disease association, in 31.2 % of cases, was based on the type of mutation identified (nonsense, insertion/deletion, frameshift or splice-site). In 22.7 % of missense mutations functional analysis was available. Only one novel missense mutation was not found to be possibly causative using either Polyphen-2, SIFT or Mutation Assessor prediction servers. None of the mutations were found in 364 Danish alleles and only three, all functionally characterised, in the Exome Variation Server, albeit at a frequency of < 1:1000.

Conclusion: The genetic aetiology of LQTS in Denmark is similar to that found in other populations. A large founder family with p.F29L in *KCNH2* was identified. In 48.4 % of the mutations disease causation was based on mutation type or functional analysis.

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BACKGROUND

Long QT syndrome (LQTS) is a genetic disease of the cardiac electrical system which presents clinically with palpitations, syncope and sudden death [1, 2]. To date, more than

700 disease-causing mutations have been found in 13 genes [1-3] and the total number of mutations is probably even larger [4]. All these genes are directly or indirectly involved in the execution of the cardiac action potential (AP) [2]. LQTS is a consequence of a prolongation of the repolarisation phase of the AP, caused by decreased activity of the repolarising inward K^+-currents, I_{ks} and I_{kr}, or increased late activity of the outward depolarising Na^+-current, I_{Na}. The delayed repolarisation leads to the appearance of early after depolarisations (EADs), due to enhancement of the Na^+/Ca^{2+}-exchanger and the L- type Ca^{2+} channel [5]. These, together with increased refractoriness, may trigger malignant arrhythmias [2].

In Denmark, genetic diagnostics of LQTS has been performed since 1996. From 2006 the management of LQTS patients has followed national guidelines [6]. The genetic diagnostic work is centred in five University cardiology clinics and patients are offered a five-gene screen of the most frequently affected genes, *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*. Initially, the screen was performed using single strand conformation polymorphism analysis (SSCP) of the coding regions of the genes with intronic amplification primers [7-9]. In recent years, the mutation analysis has been performed by bi-directional Sanger sequencing of coding regions and all previous patients have been re-analysed. Here we report the disease-causing mutations identified in Danish LQTS families during the preceding 15 years. Furthermore, as the evidence base for considering mutations disease-causing is not always clear [10], and rare variants not associated with disease are found in controls [11], we report our reasoning for considering them disease- causing. Finally, we compare the distribution of mutations with that found in other population studies.

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MATERIALS AND METHODS

PATIENTS

The patients were 71 Danish LQTS probands from unrelated families where mutation screening in the five LQTS associated genes *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2* and *SCN5A* had led to identification of a disease-causing mutation. LQT diagnosis was based on the clinical examination of patients, which was performed according to guidelines issued by the Danish Cardiology Society [6] by specialists in cardiology from Danish cardiology departments at Rigshospitalet, Skejby Hospital, Aalborg Hospital, Gentofte Hospital, Haderslev Hospital and Odense Hospital. All patients had a QT_C interval > 440 ms for men and 450 ms for women. All patients were Caucasian. A clinical description of patients identified through a Danish nationwide survey comprising 59 families (all contributing to the current survey) has recently been published [12].

MUTATION IDENTIFICATION

Genomic DNA was extracted from EDTA-blood using the commercially available Maxwell^{TM} 16 Blood DNA purification kit on the Maxwell^{R} 16 System (Promega Biotech AB, Nacka, Sweden). Genetic screening was performed by bi-directional sequencing of PCR amplified exons with associated flanking intronic regions. Primers were M13 extended and sequences are given in supplementary tables S1 and S2. A minor proportion of mutations were identified at genetic departments at Skejby University Hospital, Rigshospitalet as well as in Norway and the Netherlands using other, but similar, technologies. All probands had the coding regions of the five genes sequenced. . All mutations were verified by sequencing a second amplified amplicon. A large proportion of patients were examined for large deletions in *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2* and a small part of *SCN5A* by multiplex-ligation-dependent amplification (MLPA) using the SALSA MLPA P114 kit (MRC-Holland, Amsterdam, The Netherlands).

MUTATION AND PROTEIN NOMENCLATURE

Mutation nomenclature uses numbering with the A of the initiation codon ATG as +1 (www.hgvs.org/mutnomen), based on the following RefSeqs: NM_000218.2 (*KCNQ1*), NM_000238.2 (*KCNH2*), NM_000335.4 (*SCN5A*), NM_000219.3 (*KCNE1*) and NM_172201.1 (*KCNE2*). All mutations were checked using Mutalyzer. The protein nomenclature was that used in the recent mutation update on LQTS [2].

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EVALUATION OF SEQUENCE CHANGES

Deletions, frameshift-, splice- and nonsense mutations were considered disease-causing if not found in controls. Concerning missense mutations, familial segregation was ascertained if possible, but nuclear family size was in all cases so small that it precluded a proper linkage analysis [12]. Instead it was ascertained that the family did not contain affected members that did not carry the family mutation. Conservation of residues across several species was examined- All genetic variants were evaluated in 182 randomly and anonymously collected blood donor controls (364 alleles). The frequency of identified variants was assessed using the Exome Variant Server v.0.0.21 (http://evs.gs.washington.edu). It was established whether genetic variants had previously been associated with LQTS and whether functional analysis had been performed. The potential functional effect of changes in amino acid composition was assessed in silico using the prediction servers Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) [13], SIFT (http://sift.bii.a-star.sg) [14], and MutationAssessor (http://www.ngrl.org.uk/Manchester/page/mutation-assessor) [15].

HAPLOTYPING KCNH2

Haplotyping was performed using the microsatellites D7S1824, D7S1826 up-stream of *KCNH2* and D7S636, D7S3070, D7S483 and D7S1803 downstream of *KCNH2* (Figure 1). CentiMorgan distances were obtained from the Map-O-Mat database for microsatellites (http://compgen.rutgers.edu/mapomat/). PCR amplicons were generated using fluorescently end-labelled primers (available at NCBI UniSTS) at 0.4µM per primer, per reaction. A loading mix of 0.5µl amplicon, 9µl HiDi formamide (Applied Biosciences) and 0.5µl 600LIZ size standard (Applied Biosystems) was prepared, and DNA products were electrophoresed on an ABI 3100 Genetic Analyser. Data were analysed using ABI GeneMapper software v4.0.

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RESULT

MUTATION SCREENING

The results of the mutation screening are summarized in Tables 1 - 3. In the 70 families we found 64 different mutations; 22 in *KCNQ1, 28* in *KCNH2*, 9 in *SCN5A*, 3 in *KCNE1* and 2 in *KCNE2*. All patients were heterozygous carriers of a single mutation, except one compound heterozygous proband (1.4% of families), with a p.R583G in *KCNQ1* and p.A93T in *KCNE1.* Twenty-six of the mutations have only been described in Danish LQTS patients (Table 1-3), 22 have not been reported previously. Two mutations in *KCNH2*, p.F29L and p.K101E were found in 5 and 2 unrelated families, respectively. The MLPA analysis, applied to 65 of the probands, resulted in the identification of a single three exon (7-9) deletion, IVS6_IVS10del, in *KCNQ1.* None of the mutations were found in 384 control alleles.

KCNQ1 MUTATIONS

The twenty-two mutations identified in *KCNQ1* seemed evenly spread out over the gene (Table 1). Only three of the mutations (IVS6_IVS10del, p.H363N and p.R583G) had not previously been described in other populations. The IVS6_IVS10del results, theoretically, either in the excision of a considerable part of the protein rendering it non-functional or in haploinsufficiency due to mRNA surveillance mechanisms [16], and is thus considered pathogenic. The remaining missense mutations all affect highly conserved residues and many missense mutations have been described in the S2-S3 and C-terminal regions of the protein, respectively [2]. The p.R583G affects a codon, where another missense mutation, p.R583C, has been shown to have an electrophysiological phenotype similar to that expected for a LQTS-associated mutation [17]. Among the 18 mutations previously seen in other populations, there were two frameshift mutations, two deletions, one splice-site mutation and one nonsense mutation. All of these must be expected to result in haploinsufficiency due to mRNA surveillance mechanisms. Of the remaining missense mutations, four had been electrophysiologically characterized *in vitro*, i.e. p.V254M [18], p.L273I [18-20], p.Y315C [21] and p.R583C [17]. Thus, only four out of 15 LQTS- associated missense mutations (27 %) had established electrophysiological characteristics at the molecular level.

KCNH2 MUTATIONS

Twenty-eight mutations were identified in *KCNH2*. Fourteen of these were located in the N-terminus, and four of these were located in the Per-Arnt-Sim domain. Five were located in the C-terminus, whereas only five were located in the pore-region (Table 2). This is surprising, as the majority of previously identified LQTS associated mutations in *KCNH2* have been located in the pore

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region [2]. Fifteen of the mutations have only been described in the Danish population. Nine of the Danish specific mutations were deletions, nonsense or frameshift mutations. The remaining eight Danish-specific mutations were missense mutations and all involved conserved amino acid residues. Among the mutations found in other populations, two were frameshift mutations and nine were missense mutations. Only one of these missense mutations, p. F29L, had been demonstrated to have an *in vitro* electrophysiological effect compatible with LQTS [21], so the remainder were considered disease-associated based on the evolutionary conservation of the involved amino acid residue and the absence of the mutation among 364 control alleles as well as a previously reported association with LQTS. The observation of the association of these mutations with LQTS in the Danish population corroborates their role in the causation of LQTS. Finally, a large number of mutations in the regions affected by the mutations described here have been associated with LQTS [2].

All the remaining missense mutations in *KCNH2,* except p.A913V, were conserved to the level of zebrafish. The p.A913V mutation changed an alanine into a valine, where the valine was found at the same codon in rodents. However, the mutation had previously been associated with LQTS [22] and in the absence of deviations from familial disease segregation, and the absence of other mutations in the five genes as well as of the mutation in 364 control alleles, it was considered disease-causing.

SCN5A MUTATIONS

Nine mutations were identified in *SCN5A* (Table 3), six of which have only been described in the Danish population. Four mutations have not been reported before. Two mutations were intronic splice-site mutations and the remaining mutations were missense mutations located in the DI, DIV and C-terminal regions. This distribution is compatible with the one found when accumulating all known *SCN5A* LQTS-associated mutations [2]. The splice- site mutations were considered disease-causing because they are believed to result in aberrant mRNA splicing resulting in the synthesis of *SCN5A* ion channels with prolonged depolarisation contributing to delayed repolarisation. The splice mutations most likely do not result in haploinsufficiency as this would most likely give a Brugada syndrome phenotype [23]. One of the missense mutations, p.P2005A, has previously been associated with sudden infant death syndrome and shown to result in late persistent I_{Na} current, compatible with LQTS [24]. An *in vitro* electrophysiological analysis of the functional consequence of the p.L1785Q mutation has shown that it results in increased late persistent I_{Na} current, but also in a reduction of the total I_{Na} (Kanters et al., submitted), suggesting that the electrophysiological

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phenotype may be a combination of Brugada syndrome and LQTS, as previously described for *SCN5A* mutations [25], e.g. the E1784K mutation [26]. The remaining missense mutations interfere with conserved residues and have not been found in controls; further the mutations p.F1596I and p.V1597M, are located in the C-terminal part of *SCN5A*, where mutations causing LQTS are particularly frequent [2]. The rare polymorphism p.D1819N, known to be associated with increased QT-interval in normal individuals [27], was found in a single p.F29L family and not considered pathogenic, despite being previously reported as associated with LQTS [28] The C-terminally located mutations are likely to interfere with fast inactivation of the I_{Na} current [29]. Likewise, the remaining missense mutations, p.G319S, p.R340W and p.V411M, are located in the DI-S5- S6 region, a region with a high frequency of LQTS-associated mutations [2].

KCNE1 MUTATIONS

Three mutations were found in *KCNE1*, two of which, p.G60D and p.A93T, have only been found in the Danish population and have not been reported before. The p.A93T was found associated with p.R583G in *KCNQ1* in an isolated proband, where family data were not attainable. The p.G60D and p.D76N interfered with amino acid residues conserved in human, rat, mouse, cow and frog, whereas the alanine at residue 93 was only conserved in mouse, rat, cow and human. However, the N-terminus of frog minK is not conserved at all from amino acid residue 85 – 105 in the human sequence (corresponding to residue 80 – 95 in the frog minK sequence). However, the missense mutations p. Y81C, p.W87R, p.R98W, and p.P127T – as well as p.D76N, located in the cytoplasmic C-terminus of minK, have previously been associated with LQTS [2].

KCNE2 MUTATIONS

Two mutations were found, p.I57T and p.V65M, both previously described in other populations as associated with LQTS [28, 30] and shown [31] to interfere with Kv7.1 function in a way compatible with an association with LQTS. Both interfered with highly conserved residues and were not found in 384 control alleles.

DISEASE CAUSATION

The association between a mutation and disease is of paramount importance when the mutation findings are used for cascade screening and clinical management as is the case in Denmark [12]. This is a particular problem when family data are not sufficient to establish linkage. As none of the mutations are present in the 364 control alleles and not in the Exome Variant Server, except for p.P2005A in *SCN5A*, p.D76N in *KCNE1* and p.I57T in *KCNE2,* that were present, but with frequencies

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< 1:1000, it is unlikely that they are polymorphisms. Looking at the mutations given in table 1 – 3 it is seen that 31.2 % are frame-shift, splice-site- nonsense- or indel mutations. Such mutations have a direct effect on the integrity of the polypeptide chain and are considered explanatory for LQTS. However, the remaining 68.8 % are missense mutations that need to be differentiated from the naturally occurring functionally insignificant non-synonymous variants in the same proteins. A classical method of supporting disease causality in LQTS is electrophysiological examination of mutated channel proteins to disclose a reduction in repolarising K^+- current (*KCNQ1, KCNH2, KCNE1, KCNE2*) or late persistence of depolarising Na^+ - current (*SCN5A*) [2]. Such information was, however, only available for 22.7 % of the missense mutations, table 1-3.

We used the prediction servers Polyphen-2, SIFT and Mutation Assessor to assess the significance of all the missense mutations for interference with protein function. The results are given in table 4. It is seen that the majority of mutations where an electrophysiological assessment was available (p.V254M, p.L273F, p.Y315C in *KCNQ1*, p.F29L in *KCNH2*, p.L1785Q in *SCN5A*, p.D76N in *KCNE1*, p.I57T and p.V65M in *KCNE2*) were expected to have an effect on protein function. Two mutations, p.R583C in *KCNQ1* and p.P2005A in *SCN5A* were not found to be disruptive of protein function, despite functional significance in vitro. Thus, there seems to be a reasonable sensitivity for the prediction servers. The remaining, not functionally characterised, missense mutations, except p.P73T in *KCNQ1,* and the remaining mutations in codon 583 of *KCNQ1* as well as p.A913V in *KCNH2*, were all found to be at least possibly disruptive of protein function.

THE FOUNDER MUTATIONS IN KCNH2

Two of the missense mutations, p.F29L [21] and p.K101E [32], were found in five and two "unrelated" families, respectively. Haplotype analysis, using six polymorphic microsatellite markers, spanning 24.6cM, flanking the *KCNH2* gene at distances ranging from 10.1Mb 3' to 2.9Mb 5' as shown in Figure 1, demonstrated that both mutations were founder mutations (Table 5).

POPULATION DISTRIBUTION OF MUTATIONS

The distribution of Danish LQTS mutations is compared with four other large mutation surveys comprising verified LQTS patients in Table 6. The proportion of *KCNQ1* mutations seems to be lower in Danish patients, 34.3 %, than in other populations, where the proportion of *KCNQ1* mutations ranged from 39.4 % - 48.6 %. This trend was not significant, however, using a Chi-Square test. The proportion of *KCNH2* mutations in Denmark is within the range seen in the other populations, whereas the proportion of patients with mutations in the three rarely affected genes, *SCN5A, KCNE1* and *KCNE2* is considerably higher, 21.9 %, than seen in the other populations (range:

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10.7 % - 16.2 %). The distribution of mutation types does not seem to differ substantially between the populations. Likewise, the frequency of compound heterozygosity was very similar in the populations where it was established. In a Norwegian study no compound heterozygotes were convincingly demonstrated, but 18 cases of Jervell and Lange-Nielsen syndrome had been found [33].

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DISCUSSION

We have identified 64 different mutations in 70 Danish LQTS families referred for five- gene screening. This is by far the highest number of different mutations identified per capita in any country and it amounts to approximately 1: 72.000 inhabitants. The signature of the genetics of LQTS in Denmark is that of many "private" mutations (Table 1-3). With respect to this considerable inter-allelic and inter-genic variation, the Danish mutation spectrum is similar to that found in other populations (Table 6). However, there was a trend, albeit not significant, that the proportion of mutations in *KCNQ1* is reduced and the proportion of mutations in *SCN5A*, *KCNE1* and *KCNE2* correspondingly increased in the Danish LQTS patients. The same tendency is registered when comparing the Danish mutation spectrum to a collection of mutations identified in the five genes in persons referred for LQT testing, but where no knowledge on the clinical phenotype was available [4]. The relatively high frequency of mutations in the more rarely affected genes stresses the need of performing a five-gene screen when establishing the aetiology of individual Danish LQTS families.

The patients described here were studied and collected over a long span of years, from 1996– 2010. In this period the clinical picture of LQTS was better defined, the possibility of referral for genetic analysis increased and the indication for genetic analysis in LQTS cases was established in 2006 [6]. Consequently, it is very difficult to establish a success rate for the genetic screening across this period. But it is probably comparable to the 70% reported from Norway in a much smaller collection of mutations [33]. Likewise, we cannot really state anything about the cost-effectiveness of including more genes in the basic screen or the suitability or cost-effectiveness of MLPA analysis of the five genes. A large proportion of cases, 65/70 had MLPA analysis performed in order to detect larger insertion/deletion mutations and a single case with a three exon deletion in *KCNQ1* was identified. However, considering the relatively low cost of MLPA analysis and the possibility to detect deletions that would have escaped classical Sanger sequencing, our data suggests that MLPA analysis – or more extensive methods for detection of minor structural abnormalities - should be part of the five gene screen as the frequency of deletions (1.4%) is comparable to that of KCNE mutations.

In general, disease causation was based on the identification of a mutation that; either resulted in a deletion or a frameshift, introduced a stop codon or disrupted correct splicing. In the case of missense mutations, the mutation should involve the exchange of a conserved amino acid and not be present in > 100 control alleles. If the mutation had previously been associated with LQTS this

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strengthened the argument for disease-causation. Functional assessment was not available for novel mutations. However, a large proportion, 48.4 %, of mutations was well classified following the application of the mutation type principle and functional in vitro assessment. None of the mutations were present in Danish controls and three were found with very low frequencies in the EVS server. The remaining missense mutations all, with two exceptions, were positive for disrupting protein function in one or several prediction servers. As the servers had a reasonable sensitivity, 8/10 functionally characterised mutations were correctly classified, they seem useful, but detailed analyses on larger datasets are necessary to establish specificity. Despite the promising performance of protein function interference prediction servers it will still be of great significance to be able to perform a functional analysis of identified novel variants.

Interestingly, this study and a subsequent survey of the cardiology clinics revealed that Andersen syndrome, despite being originally described in Denmark [34], as well as Jervell and Lange-Nielsen syndrome and Timothy syndrome patients, are not found in Danish cardiology clinics (Kanters, pers.com.).

The identification of a large *KCNH2* p.F29L founder family, comprising 7.1 % of Danish LQTS families, is interesting, and the location of the family in the Northern part of Jutland, where it constitutes ca. 50% of affected families makes it easier to genotype patients from this part of Denmark. The p.F29L mutation has previously been found in North America [21] in a family of Northern European origin (Splawski, pers. com.). The mutation has been found to have an electrophysiological effect *in vitro* compatible with LQTS [21].

Each LQTS family has to be carefully examined as there is a risk of compound heterozygosity or digenic inheritance. Our data suggest that this risk is similar to that reported in other countries (Table 6). The clarification of the individual significance of either mutation in a family with a compound heterozygous index patient requires cascade screening to be performed and identification of carriers of the single mutations. In our experience this is rarely possible due to the small size of families [12].

The translation of molecular findings in LQTS patients into patient-specific clinical management decisions is difficult due to the low level of strict evidence, the complexity of the genetics, including the existence of genetic modifiers of phenotype [10]. Some of these problems may be alleviated if the use of patient-specific pluripotent stem cells turns out to give relevant information [35]. However, until such new approaches become routine, it is, with reference to the large role played by the use of previously reported information on disease-causation, important that all mutation

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findings and clinical as well as molecular follow-up of mutations are made available to the larger scientific community.

CONCLUSIONS

The Danish spectrum of LQTS causing mutations is very similar to that of the rest of the world, even though the frequency of *KCNQ1* mutations seems relatively reduced and the proportion of mutations in rarely affected genes increased. A considerable proportion of novel mutations were identified, but they were distributed on the genes largely as seen elsewhere. Despite a shortage of functional information and a long collection period nearly all mutations identified were reasonably classified as causative. The identification of a large founder family with p.F29L in *KCNH2* may become of importance for local patient management as well as studies into the prevention of sudden cardiac death in LQT2. Sharing of genotype and phenotype data as well as development of improved *in silico* predictions of functional consequences of mutations will improve the management of LQTS.

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REFERENCES

1. Crotti L, Celano G, Dagradi F, Schwartz PJ: **Congenital long QT syndrome**. *Orphanet journal of rare diseases* 2008, **3**:18.

2. Hedley PL, Jorgensen 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**(11):1486-1511.

3. Yang Y, Liang B, Liu J, Li J, Grunnet M, Olesen SP, Rasmussen HB, Ellinor PT, Gao L, Lin X *et al*: **Identification of a Kir3.4 Mutation in Congenital Long QT Syndrome**. *Am J Hum Genet* 2010.

4. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AA, Ackerman MJ: **Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test**. *Heart rhythm* 2009, **6**(9):1297-1303.

5. Antzelevitch C: **Role of spatial dispersion of repolarization in inherited and acquired sudden cardiac death syndromes**. *American journal of physiology Heart and circulatory physiology* 2007, **293**(4):H2024-2038.

6. DCS: **Arvelige hjertesydomme**, vol. 1, 1 edn. Copenhagen: KLS Grafisk Hus A/S; 2006.

7. Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, McKenna WJ, Christiansen M: **High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia**. *Clin Genet* 2006, **69**(6):504-511.

8. Larsen LA, Andersen PS, Kanters J, Svendsen IH, Jacobsen JR, Vuust J, Wettrell G, Tranebjaerg L, Bathen J, Christiansen M: **Screening for mutations and polymorphisms in the genes KCNH2 and KCNE2 encoding the cardiac HERG/MiRP1 ion channel: implications for acquired and congenital long Q-T syndrome**. *Clin Chem* 2001, **47**(8):1390-1395.

9. Larsen LA, Andersen PS, Kanters JK, Jacobsen JR, Vuust J, Christiansen M: **A single strand conformation polymorphism/heteroduplex (SSCP/HD) method for detection of mutations in 15 exons of the KVLQT1 gene, associated with long QT syndrome**. *Clin Chim Acta* 1999, **280**(1-2):113-125.

Stellenbosch University http://scholar.sun.ac.za

10. Moller DV, Pecini R, Gustafsson F, Hassager C, Hedley P, Jespersgaard C, Torp-Pedersen C, Christiansen M, Kober LV: **Hereditary hemochromatosis (HFE) genotypes in heart failure: relation to etiology and prognosis**. *BMC Med Genet* 2010, **11**:117.

11. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, Wilde AA, Ackerman MJ: **Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants**. *Circulation* 2009, **120**(18):1752-1760.

12. Theilade J, Kanters J, Henriksen FL, Gilsa-Hansen M, Svendsen JH, Eschen O, Toft E, Reimers JI, Tybjaerg-Hansen A, Christiansen M *et al*: **Cascade Screening in Families with Inherited Cardiac Diseases Driven by Cardiologists: Feasibility and Nationwide Outcome in Long QT Syndrome**. *Cardiology* 2013, **126**(2):131-137.

13. Adzhubei I, Jordan DM, Sunyaev SR: **Predicting functional effect of human missense mutations using PolyPhen-2**. *Current protocols in human genetics / editorial board, Jonathan L Haines [et al]* 2013, **Chapter 7**:Unit7 20.

14. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC: **SIFT web server: predicting effects of amino acid substitutions on proteins**. *Nucleic acids research* 2012, **40**(Web Server issue):W452-457.

15. Reva B, Antipin Y, Sander C: **Predicting the functional impact of protein mutations: application to cancer genomics**. *Nucleic acids research* 2011, **39**(17):e118.

16. Akimitsu N: **Messenger RNA surveillance systems monitoring proper translation termination**. *J Biochem* 2008, **143**(1):1-8.

17. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M *et al*: **Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes**. *Circulation* 2002, **105**(16):1943-1948.

18. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T *et al*: **Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias**. *Nature genetics* 1996, **12**(1):17-23.

19. Shalaby FY, Levesque PC, Yang WP, Little WA, Conder ML, Jenkins-West T, Blanar MA: **Dominant-negative KvLQT1 mutations underlie the LQT1 form of long QT syndrome**. *Circulation* 1997, **96**(6):1733-1736.

Stellenbosch University http://scholar.sun.ac.za

20. Wilson AJ, Quinn KV, Graves FM, Bitner-Glindzicz M, Tinker A: **Abnormal KCNQ1 trafficking influences disease pathogenesis in hereditary long QT syndromes (LQT1)**. *Cardiovascular research* 2005, **67**(3):476-486.

21. Chen J, Zou A, Splawski I, Keating MT, Sanguinetti MC: **Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation**. *The Journal of biological chemistry* 1999, **274**(15):10113-10118.

22. Tester DJ, Will ML, Haglund CM, Ackerman MJ: **Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing**. *Heart rhythm* 2005, **2**(5):507-517.

23. Hedley P, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters J, Corfield V, Christiansen M: **The Genetic Basis of Brugada Syndrome: A Mutation Update**. *Human mutation* 2009, **30**(9):1256-1266.

24. Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George AL, Jr. *et al*: **Prevalence of long-QT syndrome gene variants in sudden infant death syndrome**. *Circulation* 2007, **115**(3):361-367.

25. Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA, Christiansen M: **The genetic basis of Brugada syndrome: a mutation update**. *Hum Mutat* 2009, **30**(9):1256-1266.

26. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, Schulze-Bahr E, Fukuhara S, Mochizuki N, Makiyama T *et al*: **The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome**. *The Journal of clinical investigation* 2008, **118**(6):2219-2229.

27. Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B, Guicheney P: **Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population**. *European journal of human genetics : EJHG* 2005, **13**(11):1213-1222.

28. Millat G, Chevalier P, Restier-Miron L, Da Costa A, Bouvagnet P, Kugener B, Fayol L, Gonzalez Armengod C, Oddou B, Chanavat V *et al*: **Spectrum of pathogenic mutations and associated polymorphisms in a cohort of 44 unrelated patients with long QT syndrome**. *Clinical genetics* 2006, **70**(3):214-227.

Stellenbosch University http://scholar.sun.ac.za

29. Wang DW, Yazawa K, George AL, Jr., Bennett PB: **Characterization of human cardiac Na+ channel mutations in the congenital long QT syndrome**. *Proceedings of the National Academy of Sciences of the United States of America* 1996, **93**(23):13200-13205.

30. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA: **MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia**. *Cell* 1999, **97**(2):175-187.

31. Isbrandt D, Friederich P, Solth A, Haverkamp W, Ebneth A, Borggrefe M, Funke H, Sauter K, Breithardt G, Pongs O *et al*: **Identification and functional characterization of a novel KCNE2 (MiRP1) mutation that alters HERG channel kinetics**. *Journal of molecular medicine* 2002, **80**(8):524-532.

32. Christiansen M, Tonder N, Larsen LA, Andersen PS, Simonsen H, Oyen N, Kanters JK, Jacobsen JR, Fosdal I, Wettrell G *et al*: **Mutations in the HERG K+-ion channel: a novel link between long QT syndrome and sudden infant death syndrome**. *Am J Cardiol* 2005, **95**(3):433-434.

33. Berge KE, Haugaa KH, Fruh A, Anfinsen OG, Gjesdal K, Siem G, Oyen N, Greve G, Carlsson A, Rognum TO *et al*: **Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers**. *Scandinavian journal of clinical and laboratory investigation* 2008, **68**(5):362-368.

34. Andersen ED, Krasilnikoff PA, Overvad H: **Intermittent muscular weakness, extrasystoles, and multiple developmental anomalies. A new syndrome?** *Acta Paediatr Scand* 1971, **60**(5):559-564.

35. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, Dorn T, Goedel A, Hohnke C, Hofmann F *et al*: **Patient-specific induced pluripotent stem-cell models for long-QT syndrome**. *The New England journal of medicine* 2010, **363**(15):1397-1409.

36. Larsen LA, Christiansen M, Vuust J, Andersen PS: **High-throughput single-strand conformation polymorphism analysis by automated capillary electrophoresis: robust multiplex analysis and pattern-based identification of allelic variants**. *Hum Mutat* 1999, **13**(4):318-327.

37. Liu W, Yang J, Hu D, Kang C, Li C, Zhang S, Li P, Chen Z, Qin X, Ying K *et al*: **KCNQ1 and KCNH2 mutations associated with long QT syndrome in a Chinese population**. *Human mutation* 2002, **20**(6):475-476.

Stellenbosch University http://scholar.sun.ac.za

38. Priori SG, Napolitano C, Schwartz PJ: **Low penetrance in the long-QT syndrome: clinical impact**. *Circulation* 1999, **99**(4):529-533.

39. Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES *et al*: **Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene**. *Circulation* 2007, **115**(19):2481-2489.

40. Choi G, Kopplin LJ, Tester DJ, Will ML, Haglund CM, Ackerman MJ: **Spectrum and frequency of cardiac channel defects in swimming-triggered arrhythmia syndromes**. *Circulation* 2004, **110**(15):2119-2124.

41. Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, Chivoret G, Schwartz K, Coumel P, Guicheney P: **KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome**. *Circulation* 1997, **96**(9):2778-2781.

42. 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**. *Genetic testing* 2003, **7**(1):57-61.

43. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM *et al*: **Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2**. *Circulation* 2000, **102**(10):1178-1185.

44. Struijk JJ, Kanters JK, Andersen MP, Hardahl T, Graff C, Christiansen M, Toft E: **Classification of the long-QT syndrome based on discriminant analysis of T-wave morphology**. *Med Biol Eng Comput* 2006, **44**(7):543-549.

45. Chen S, Zhang L, Bryant RM, Vincent GM, Flippin M, Lee JC, Brown E, Zimmerman F, Rozich R, Szafranski P *et al*: **KCNQ1 mutations in patients with a family history of lethal cardiac arrhythmias and sudden death**. *Clinical genetics* 2003, **63**(4):273-282.

46. Napolitano C, Schwartz PJ, Brown AM, Ronchetti E, Bianchi L, Pinnavaia A, Acquaro G, Priori SG: **Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias**. *J Cardiovasc Electrophysiol* 2000, **11**(6):691-696.

47. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT: **Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1**. *Genomics* 1998, **51**(1):86-97.

Stellenbosch University http://scholar.sun.ac.za

48. Ackerman MJ, Schroeder JJ, Berry R, Schaid DJ, Porter CJ, Michels VV, Thibodeau SN: **A novel mutation in KVLQT1 is the molecular basis of inherited long QT syndrome in a near-drowning patient's family**. *Pediatric research* 1998, **44**(2):148-153.

49. Ackerman MJ, Porter CJ: **Identification of a family with inherited long QT syndrome after a pediatric near-drowning**. *Pediatrics* 1998, **101**(2):306-308.

50. Itoh T, Tanaka T, Nagai R, Kikuchi K, Ogawa S, Okada S, Yamagata S, Yano K, Yazaki Y, Nakamura Y: **Genomic organization and mutational analysis of KVLQT1, a gene responsible for familial long QT syndrome**. *Hum Genet* 1998, **103**(3):290-294.

51. Kanters JK, Larsen LA, Orholm M, Agner E, Andersen PS, Vuust J, Christiansen M: **Novel donor splice site mutation in the KVLQT1 gene is associated with long QT syndrome**. *J Cardiovasc Electrophysiol* 1998, **9**(6):620-624.

52. Li H, Chen Q, Moss AJ, Robinson J, Goytia V, Perry JC, Vincent GM, Priori SG, Lehmann MH, Denfield SW *et al*: **New mutations in the KVLQT1 potassium channel that cause long-QT syndrome**. *Circulation* 1998, **97**(13):1264-1269.

53. Murray A, Donger C, Fenske C, Spillman I, Richard P, Dong YB, Neyroud N, Chevalier P, Denjoy I, Carter N *et al*: **Splicing mutations in KCNQ1: a mutation hot spot at codon 344 that produces in frame transcripts**. *Circulation* 1999, **100**(10):1077-1084.

54. Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S: **Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice**. *JAMA : the journal of the American Medical Association* 2005, **294**(23):2975-2980.

55. Sherman J, Tester DJ, Ackerman MJ: **Targeted mutational analysis of ankyrin-B in 541 consecutive, unrelated patients referred for long QT syndrome genetic testing and 200 healthy subjects**. *Heart rhythm : the official journal of the Heart Rhythm Society* 2005, **2**(11):1218-1223.

56. Huang L, Bitner-Glindzicz M, Tranebjaerg L, Tinker A: **A spectrum of functional effects for disease causing mutations in the Jervell and Lange-Nielsen syndrome**. *Cardiovascular research* 2001, **51**(4):670-680.

57. Tyson J, Tranebjaerg L, McEntagart M, Larsen LA, Christiansen M, Whiteford ML, Bathen J, Aslaksen B, Sorland SJ, Lund O *et al*: **Mutational spectrum in the cardioauditory syndrome of Jervell and Lange-Nielsen**. *Hum Genet* 2000, **107**(5):499-503.

Stellenbosch University http://scholar.sun.ac.za

58. Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L: **Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects**. *Journal of the American College of Cardiology* 1999, **34**(3):823-829.

59. 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**(6):603-608.

60. Tanaka T, Nagai R, Tomoike H, Takata S, Yano K, Yabuta K, Haneda N, Nakano O, Shibata A, Sawayama T *et al*: **Four novel KVLQT1 and four novel HERG mutations in familial long-QT syndrome**. *Circulation* 1997, **95**(3):565-567.

61. Larsen LA, Svendsen IH, Jensen AM, Kanters JK, Andersen PS, Moller M, Sorensen SA, Sandoe E, Jacobsen JR, Vuust J *et al*: **Long QT syndrome with a high mortality rate caused by a novel G572R missense mutation in KCNH2**. *Clin Genet* 2000, **57**(2):125-130.

62. Lupoglazoff JM, Denjoy I, Berthet M, Neyroud N, Demay L, Richard P, Hainque B, Vaksmann G, Klug D, Leenhardt A *et al*: **Notched T waves on Holter recordings enhance detection of patients with LQt2 (HERG) mutations**. *Circulation* 2001, **103**(8):1095-1101.

63. Lupoglazoff JM, Denjoy I, Villain E, Fressart V, Simon F, Bozio A, Berthet M, Benammar N, Hainque B, Guicheney P: **Long QT syndrome in neonates: conduction disorders associated with HERG mutations and sinus bradycardia with KCNQ1 mutations**. *Journal of the American College of Cardiology* 2004, **43**(5):826-830.

64. Satler CA, Vesely MR, Duggal P, Ginsburg GS, Beggs AH: **Multiple different missense mutations in the pore region of HERG in patients with long QT syndrome**. *Hum Genet* 1998, **102**(3):265-272.

65. Lai LP, Su YN, Hsieh FJ, Chiang FT, Juang JM, Liu YB, Ho YL, Chen WJ, Yeh SJ, Wang CC *et al*: **Denaturing high-performance liquid chromatography screening of the long QT syndrome-related cardiac sodium and potassium channel genes and identification of novel mutations and single nucleotide polymorphisms**. *Journal of human genetics* 2005, **50**(9):490-496.

66. Bianchi L, Shen Z, Dennis AT, Priori SG, Napolitano C, Ronchetti E, Bryskin R, Schwartz PJ, Brown AM: **Cellular dysfunction of LQT5-minK mutants: abnormalities of IKs, IKr and trafficking in long QT syndrome**. *Human molecular genetics* 1999, **8**(8):1499-1507.

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67. Duggal P, Vesely MR, Wattanasirichaigoon D, Villafane J, Kaushik V, Beggs AH: **Mutation of the gene for IsK associated with both Jervell and Lange-Nielsen and Romano-Ward forms of Long-QT syndrome**. *Circulation* 1998, **97**(2):142-146.

68. Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, Keating MT: **Mutations in the hminK gene cause long QT syndrome and suppress IKs function**. *Nature genetics* 1997, **17**(3):338-340.

69. Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ, Schulze-Bahr E, Haverkamp W, Breithardt G, Cohen N *et al*: **Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients**. *Journal of molecular medicine* 2004, **82**(3):182-188.

70. 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**. *Proceedings of the National Academy of Sciences of the United States of America* 2000, **97**(19):10613-10618.

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LEGENDS TO FIGURES

FIGURE 1. Location of microsatellite markers used in haplotyping the p.F29L and p.k101E mutations in *KCNH2.*

Table 1. Mutations identified in KCNQ1.

Gene	cDNA	Protein	Mutation	Genomic	Protein	Phenotype	References
KCNQ1	**NM_000218**	**NP_000209**	Type	Region	Region		
	c.217C>A	p.P73T	Missense	Exon_01	N-term	RWS	[4, 22]
DK	c.470T>G	p.F157C	Missense	Exon_02	S2	RWS	[36]
	c.572-576del	p.L191fs	Frameshift	Exon_03	S2-S3	RWS	[4, 37]
	c.592A>G	p.I198V	Missense	Exon_03	S2-S3	RWS	[4]
	c.674C>T	p.S225L	Missense	Exon_04	S3-S4	RWS	[4, 38]
	c.667C>T	p.A226V	Missense	Exon_04	S3-S4	RWS	[4, 39]
	c.760G>A*	p.V254M	Missense	Exon_05	S4-S5	RWS	[4, 18, 39-44]
	c.817C>T*	p.L273F	Missense	Exon_06	S5	RWS	[4, 18-20, 39, 43, 44]
	c.905C>T	p.A302V	Missense	Exon_06	Pore	RWS	[4, 40]
	c.944A>G*	p.Y315C	Missense	Exon_07	Pore	RWS	[4, 38-40, 45-47]
	c.1015-1017del	p.F339del	Deletion	Exon_07	S6	RWS	[48]
DK	IVS6_IVS10del	Unknown	Deletion	Exon_07-Exon_9	Unknown	RWS	This study
	c.1017-1019del	p.F340del	Deletion	Exon_07	S6	RWS	[4, 39, 43, 49]
	c.1032G>A	p.A344sp	Splice-site	IVS_07	C-term	RWS	[4, 40, 43, 44, 50-53]
	c.1048G>A	p.G350R	Missense	Exon_08	C-term	RWS	[4, 54]
DK	c.1087C>A	p.H363N	Missense	Exon_08	C-term	RWS	[2, 44]
	c.1096C>T	p.R366W	Missense	Exon_08	C-term	RWS	[4, 9, 40, 44, 47]
	c.1202insC	p.P400fs	Frameshift	Exon_09	C-term	RWS	[4, 39, 55]
	c.1588C>T*	p.Q530X	Nonsense	Exon_12	C-term	RWS	[4, 20, 39, 43, 56, 57]
	c.1747C>T*	p.R583C	Missense	Exon_15	C-term	RWS	[17, 43]
DK	c.1747C>G	p.R583G	Missense	Exon_15	C-term	RWS	This study
	c.1748G>A	p.R583H	Missense	Exon_15	C-term	RWS	[54]

DK: Only seen in patients of Danish origin. *In vitro functional characterisation performed. RWS: Romano-Ward syndrome

Table 2. Mutations identified in KCNH2.

Gene ***KCNH2***	cDNA **NM_000238**	Protein **NP_000229**	Mutation Type	Genomic region	Protein Region	Phenotype	References
DK	c.65T>C	p.F22S	Missense	Exon_01	N-term	RWS	This study
	c.87C>A*	p.F29L	Missense	Exon_01	N-term	RWS	[4, 21, 44]
DK	c.88-90del	p.I30del	Deletion	Exon_01	N-term	RWS	This study
	c.221C>T	p.T74M	Missense	Exon_02	PAS	RWS	[4, 54]
DK	c.234_241dupTGCCGCGC	p.A83fs	Frameshift	Exon_02	PAS	RWS	This study
DK	c.287T>C	p.I96T	Missense	Exon_02	PAS	RWS	[8]
DK	c.301A>G	p.K101E	Missense	Exon_02	PAS	RWS	[8, 32]
DK	c.326T>C	p.L109P	Missense	Exon_03	PAS	RWS	This study
DK	c.446insC	p.R148fs	Frameshift	Exon_03	N-term	RWS	This study
	c.453delC	p.P151fs	Frameshift	Exon_03	N-term	RWS	[58]
	c.526C>T	p.R176W	Missense	Exon_04	N-term	RWS	[58]
DK	c.552-560del	p.G184-G188del	Deletion	Exon_04	N-term	RWS	This study
	c.1096C>T	p.R366X	Nonsense	Exon_05	N-term	RWS	[4, 8]
DK	c.1199T>A	p.I400N	Missense	Exon_06	N-term	RWS	[8]
	c.1283C>T	p.S428L	Missense	Exon_06	S1-S2	RWS	[54]
DK	c.1286delC	p.S428fs	Frameshift	Exon_06	S1-S2	RWS	[59]
DK	c.1591-1671del	p.R531-L539del	Deletion	Exon_07	S4-S5	RWS	This study
	c.1682C>T	p.A561V	Missense	Exon_07	S5	RWS	[4, 38, 43, 60]
DK	c.1714G>C	p.G572R	Missense	Exon_07	S5-pore	RWS	[61]
	c.1750G>A	p.G584S	Missense	Exon_07	Pore	RWS	[43, 58]
	c.1862G>A	p.S621N	Missense	Exon_07	Pore	RWS	[8, 62, 63]
	c.1886A>G	p.N629S	Missense	Exon_07	Pore	RWS	[4, 64]
	c.1898A>G	p.N633S	Missense	Exon_07	Pore-S6	RWS	[64]
DK	c.2111_2114dup	p.W705fs	Frameshift	Exon_08	C-term	RWS	This study
DK	c.2573T>C	p.I858T	Missense	Exon_10	C-term	RWS	This study
	c.2738C>T	p.A913V	Missense	Exon_12	C-term	RWS	[4, 22]
	c.2768delC	p.P923fs	Frameshift	Exon_12	C-term	RWS	[65]
DK	c.3090-3102del	p.S1029fs	Frameshift	Exon_13	C-term	RWS	This study

DK: Only seen in patients of Danish origin. *In vitro functional characterization performed. RWS: Romano-Ward syndrome.

Table 3. Mutations identified in SCN5A, KCNE1 or KCNE2.

Gene	cDNA	Protein	Mutation Type	Genomic Region	Protein Region	Phenotype	References
SCN5A	**NM_000335**	**NP_000326**					
DK	c.611+G>A	Intronic splice	Splice-site	IVS5	D1-S3	RWS	This study
DK	c.955C>A	p.G319S	Missense	Exon_08	D1-S5-S6	RWS	[7]
	c.1018C>T	p.R340W	Missense	Exon_09	D1-S5-S6	RWS	[4]
DK	c.1141-3C>A	Intronic splice	Splice-site	IVS9	D1-S6	RWS	This study
	c.1231G>A	p.V411M	Missense	Exon_10	D1-S6	RWS	[4, 22]
	c.4783T>A	p.F1595I	Missense	Exon_27	DIV-S3	RWS	[4]
DK	c.4786G>A	p.V1596M	Missense	Exon_27	DIV-S3	RWS	This study
DK	c.5354T>A*	p.L1785Q	Missense	Exon_28	C-term	RWS	This study
	c.6013C>G*	p.P2005A	Missense	Exon_28	C-term	RWS	[24]
KCNE1	**NM_000219**	**NP_000210**					
DK	c.179G>A	p.G60D	Missense	Exon_03	TM	RWS	This study
	c.226G>A*	p.D76N	Missense	Exon_03	Cyto	RWS	[4, 43, 65-68]
DK	c.277G>A	p.A93T	Missense	Exon_03	Cyto	RWS	This study
KCNE2	**NM_172201**	**NP_751951**					
	c.170T>C*	p.I57T	Missense	Exon_02	TM	RWS	[4, 28, 30, 31, 69, 70]
	c.193G>A*	p.V65M	Missense	Exon_02	TM	RWS	[31]

DK: Only seen in patients of Danish origin. *In vitro functional characterization performed. RWS: Romano-Ward syndrome.

Table 4. In silico functional analysis of missense variants.

Gene	Protein	Polyphen-2	SIFT	MutationAssessor
KCNQ1	p.P73T	0	0	0
KCNQ1	p.F157C	0	1	2
KCNQ1	p.I198V	1	1	1
KCNQ1	p.S225L	1	1	1
KCNQ1	p.A226V	2	1	2
KCNQ1	p.V254M	2	1	2
KCNQ1	p.L273F	2	1	2
KXNQ1	p.A302V	2	1	2
KCNQ1	p.Y315C	2	1	3
KCNQ1	p.G350R	2	1	2
KXNQ1	p.H363N	1	1	2
KCNQ1	p.R366W	2	1	2
KCNQ1	p.R583C	1	0	1
KXNQ1	p.R583G	0	0	1
KCNQ1	p.R583H	0	0	1
KCNH2	p.F22S	2	0	2
KCNH2	p.F29L	0	1	2
KCNH2	p.T74M	2	1	2
KCNH2	p.I96T	1	1	2
KCNH2	p.K101E	0	1	3
KCNH2	p.L109P	1	0	2
KCNH2	p.R176W	2	1	0
KCNH2	p.I400N	2	1	2
KCNH2	p.S428L	0	0	2
KCNH2	p.A561V	2	1	2
KCNH2	p.G572R	2	1	2
KCNH2	p.G584S	1	0	0
KCNH2	p.S621N	1	1	3
KCNH2	p.N629S	2	1	2
KCNH2	p.N633S	1	0	1
KCNH2	p.I858T	1	1	2
KCNH2	p.A913V	0	0	0
SCN5A	p.G319S	0	0	2
SCN5A	p.R340W	1	1	0
SCN5A	p.V411M	2	1	3
SCN5A	p.F1595I	0	0	2
SCN5A	p.V1596M	1	1	2
SCN5A	p.L1785Q	2	1	3
SCN5A	p.P2005A	0	0	0
KCNE1	p.G60D	2	1	2
KCNE1	p.D76N	1	0	2
KCNE1	p.A93T	0	0	1
KCNE2	p.I57T	2	1	1
KCNE2	p.V65M	2	1	1
Polyphen-2 scores: 0: benign, 1 possibly damaging for function; 2: Probably damaging for function. SIFT scores: =: Tolerated and 1: Not tolerated, Mutation				

Table 5. Haplotyping of the p.K101E and p.F29L families. The ancestral alleles are indicated blue or red text with regard to each mutation. Alleles are represented by approximate number of repeats.

	ped 33	ped 135	ped 39	Ped 89	Ped 523	ped 779	ped 248_641_795
D7S1824	0307	0104	08	06	**02**	**02**	**02**
D7S1826	**07**	**07**	**07**	**07**	**07**	**07**	**07**
KCNH2	**101E**	**101E**	**29L**	**29L**	**29L**	**29L**	**29L**
D7S636	**07**	**07**	**04**	**04**	**04**	**04**	**04**
D7S3070	**08**	**08**	**06**	**06**	**08**	**06**	**06**
D7S483	**06**	**06**	**08**	**08**	**07**	**08**	**08**
D7S1807	0108	06	**02**	**02**	**04**	**02**	**02**

Table 6. Distribution of mutations and mutation types in this study and four other large studies

	This study	Berge et al (2008) [33]	Napolitano et al (2005) [54]	Tester et al (2005) [22]	Splawski et al (2000) [43]
Number of mutations	64	37	233	211	177
KCNQ1 (%)	34.3	42.6	48.6	41.7	39.4
KCNH2 (%)	43.8	46.3	38.8	42.2	51.5
SCN5A (%)	14.1	9.3	10.1	15.2	6.1
KCNE1 (%)	4.7	1.9	1.7	0.5	2.3
KCNE2 (%)	3.1	0.0	0.7	0.5	2.3
Mutation type					
Missense (%)	68.8	64.9	72.0	75.0	72.3
Nonsense (%)	3.1	13.5	5.1	5.7	6.2
Deletion (%)	9.4	2.7	14.1	2.5	5.0
Frameshift (%)	14.1	13.5	6.1	11.4	9.6
Splice site (%)	4.7	5.4	2.7	4.3	6.7
Compound heterozygotes (%)*	1.4	0	3.9	5.4	n.a.

*Percentage of families.

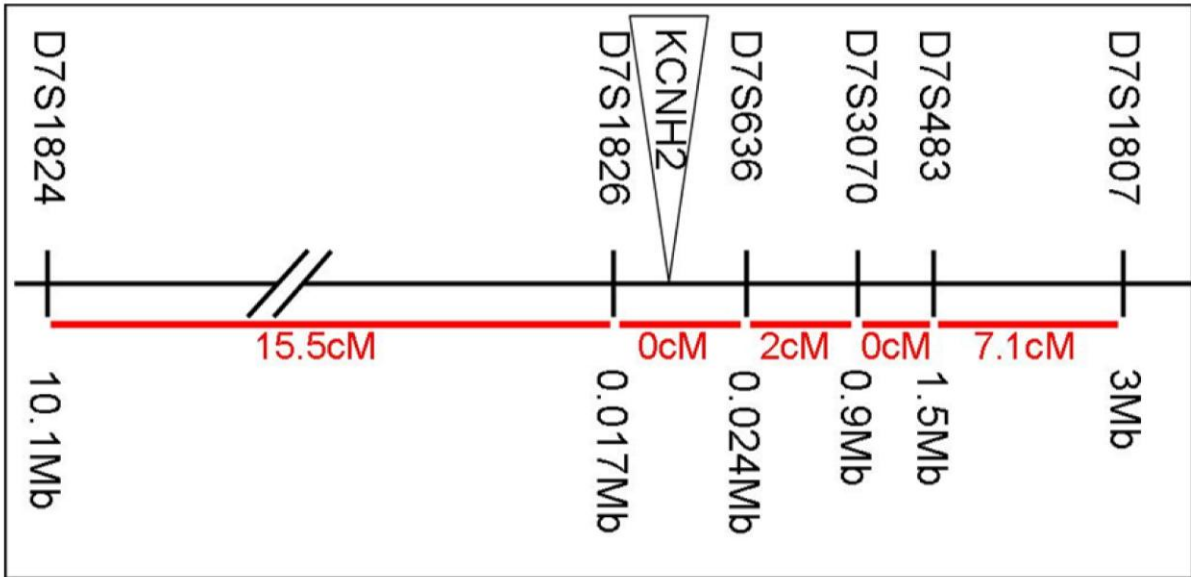

FIGURE 1

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CHAPTER 4: AN EXAMINATION OF PUTATIVE NOVEL GENETIC CAUSES OF ARRHYTHMIC SYNDROMES

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PAPER 3: THE ROLE OF CAV3 IN LONG QT SYNDROME: CLINICAL AND FUNCTIONAL ASSESSMENT OF A CAVEOLIN-3/KV11.1 DOUBLE HETEROZYGOTE VERSUS CAVEOLIN-3 SINGLE HETEROZYGOTE

Paula L. Hedley, Jørgen K. Kanters, Maja Dembic, Thomas Jespersen, Lasse Skibsbye, Frederik H Aidt, Ole Eschen, Claus Graff, Elijah R. Behr, Sarah Schlamowitz, Valerie Corfield, William J McKenna and Michael Christiansen.

Circulation: Cardiovascular Genetics **2013; 6:452-461**.

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Original Article

The Role of *CAV3* in Long–QT Syndrome

Clinical and Functional Assessment of a Caveolin-3/Kv11.1 Double Heterozygote Versus Caveolin-3 Single Heterozygote

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Background—Mutations in *CAV3*, coding for caveolin-3, the major constituent scaffolding protein of cardiac caveolae, have been associated with skeletal muscle disease, cardiomyopathy, and most recently long–QT syndrome (LQTS) and sudden infant death syndrome. We examined the occurrence of *CAV3* mutations in a large cohort of patients with LQTS.

Methods and Results—Probands with LQTS (n=167) were screened for mutations in *CAV3* using direct DNA sequencing. A single proband (0.6%) was found to be a heterozygous carrier of a previously described missense mutation, caveolin-3:p.T78M. The proband was also a heterozygous carrier of the trafficking-deficient Kv11.1:p.I400N mutation. The caveolin-3:p.T78M mutation was found isolated in 3 family members, none of whom had a prolonged QT_c interval. Coimmunoprecipitations of caveolin-3 and the voltage-gated potassium channel subunit (Kv11.1) were performed, and the electrophysiological classification of the Kv11.1 mutant was carried out by patch-clamp technique in human embryonic kidney 293 cells. Furthermore, the T-wave morphology was assessed in mutation carriers, double mutation carriers, and nonmutation carriers by applying a morphology combination score. The morphology combination score was normal for isolated caveolin-3:p.T78M carriers and of LQT2 type in double heterozygotes.

Conclusions—Mutations in *CAV3* are rare in LQTS. Furthermore, caveolin-3:p.T78M did not exhibit a LQTS phenotype. Because no association has ever been found between LQTS and isolated *CAV3* mutations, we suggest that LQTS9 is considered a provisional entity. (*Circ Cardiovasc Genet*. **2013;6:452-461.**)

Key Words: arrhythmias, cardiac ■ genetics ■ ion channel ■ long–QT syndrome ■ molecular biology

Caveolae are small (50–100 nm in size) plasma invaginations with a cup-like shape[1] and a composition of cholesterol and other lipids that classify them as lipid rafts. These rafts are enriched in a large number of protein complexes involved in signaling and vesicular trafficking.[2]

Editorial see p 439
Clinical Perspective on p 461

Mutations in the *CAV3* gene, coding for caveolin-3, are associated with a variety of muscle diseases (ie, rippling muscle disease,[3] limb-girdle-muscle dystrophy,[4] muscular dystrophy,[5,6] and cardiomyopathy).[7,8] Most recently, mutations in *CAV3* have been found to be associated with long–QT syndrome-9 (LQTS9; Figure 1).[9–11]

Caveolae are structurally composed of caveolins, a family of proteins comprising caveolin-1, caveolin-2, and caveolin-3 in humans. They exhibit a ubiquitous tissue distribution; however, caveolin-3 is selectively expressed in the caveolae of heart and skeletal muscle.[2,12,13]

Caveolin-3 comprises 151 amino acids and is divided into 4 domains; the N-terminal domain contains the FEDVIAEP caveolin signature domain (Figure 1). The transmembrane domain loops through the membrane, and both the N and C termini are cytoplasmic. After homo-oligomerization, which occurs in the endoplasmic reticulum, caveolin-3 forms caveolar complexes that subsequently fuse with the plasma membrane to form the caveolae.[13] The scaffolding domain is essential for the homo-oligomerization of caveolin-3.

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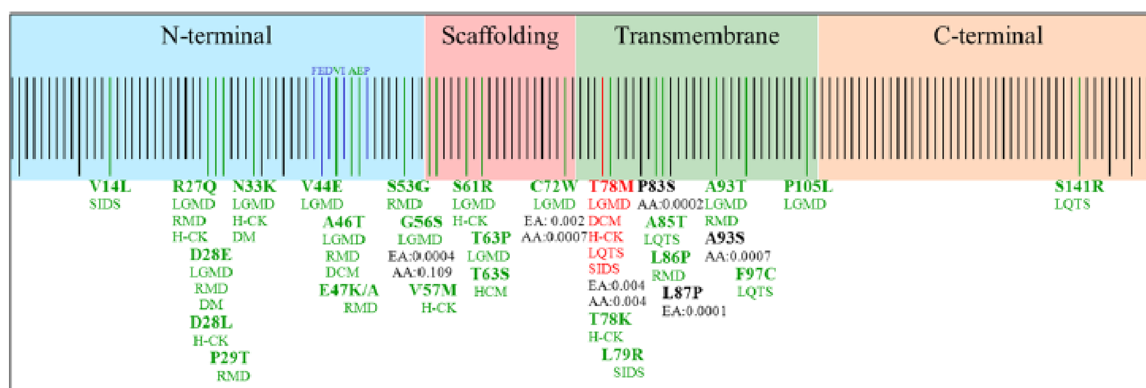

Figure 1. Caveolin-3 domain organization and mutation map. The primary structure of caveolin-3 in which each amino acid is represented as a stripe, the 4 domains are demarcated by colored blocks and are labeled in the figure (N-terminal [amino acid {aa} 1–53]—blue, scaffolding domain [aa 54–73]—pink, transmembrane domain [aa 74–106]—green, and C-terminal domain [aa 107–151]—orange). The FEDVIAEP caveolin signature domain is indicated within the N terminal. Previously reported mutations have been indicated as green stripes along with the diseases reported to be associated with them. Caveolin-3:p.T78M is represented by a red stripe. Nonsynonymous variants represented in the exome variant server are indicated in black, and the frequencies of these variants are shown for European Americans (EA) and African Americans (AA). DCM indicates dilated cardiomyopathy; DM, distal myopathy; H-CK, hyperCKemia; HCM, hypertrophic cardiomyopathy; LGMD, limb-girdle muscular dystrophy; LQTS, long–QT syndrome; RMD, rippling muscular dystrophy; and SIDS, sudden infant death syndrome.

The physical orientation and localization of ion channels[14] and signaling molecules, for example, β_2-adrenoreceptor, in caveolae are of functional significance.[2,15–17] Caveolin-3 is associated with the α-subunit of the Nav1.5 ion channel conducting the depolarizing I_{Na} current,[9] and interference with this association has been suggested as the mechanism of *CAV3*-associated LQTS or sudden infant death syndrome.[9,11] Another tentative mechanism may be the altered interaction between caveolin-3 and the α-subunit of the Kv11.1 (encoded by *KCNH2*) conducting the I_{Kr} current also involved in LQTS. An association with Kv11.1 has been documented for both caveolin-1, in stably transfected human embryonic kidney 293 cells (human ether-a-go-go-related gene [hERG]:HEK),[18,19] and caveolin-3, in neonatal rat ventricular myocytes.[19] These associations result in the sensitivity of Kv11.1 to extracellular K^+.[19]

We examined the occurrence of *CAV3* mutations in 167 LQTS probands by sequencing the coding regions of the gene. Furthermore, we describe a family with mutations in *CAV3* and a novel *KCNH2* mutation, where the functional characterization indicates that the *KCNH2* mutation is disease causing. Finally, we use our own findings, together with published reports on *CAV3* mutations, to question whether described mutations in this gene are in fact LQTS causative.

Materials and Methods

Patients

Unrelated probands, referred from specialist cardiology centers in Denmark and the United Kingdom to Statens Serum Institut for genetic investigation of LQTS (n=167; 61% women), were included in the study. The probands had been screened by capillary-electrophoresis single-strand conformation polymorphism for mutations in *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, and *SCN5A*, as described elsewhere.[20–22] Fifty-eight (35%) patients had been found to carry a probably damaging variant in any one of these genes. A control panel comprising 189 Danish blood donors was used to assess population frequencies of the identified variants. Furthermore, data from the 1000 genomes project[23] (retrieved from National Center for Biotechnology Information single-nucleotide polymorphism [SNP] database) and the Exome Variant Server data were used as an in silico control of allele frequencies (Exome Variant Server; NHLBI Exome Sequencing Project, Seattle, WA [http://evs.gs.washington.edu/EVS/, accessed June 2012]).

CAV3 Screening

DNA was extracted from frozen EDTA-blood using a Qiagen kit (Qiagen; QmbH, Hilden, Germany). Screening for mutations in *CAV3* was performed by direct sequencing using primers that flanked the coding region (primer sequences and polymerase chain reaction conditions are available on request). Polymerase chain reaction products were quantified on agarose gel electrophoresis, purified using ExoSap (Affymetrix, Santa Clara, CA), and sequenced using the Big Dye dideoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and the 3100 ABI-PRISM automated sequencer (Applied Biosystems). Sequence analysis was performed using the Sequencher 4.8 software (Gene Codes Corporation, Ann Arbor, MI).

Definition of Disease Causation or Association

Genetic variants were considered probably damaging if (1) the nucleotide variation was deduced to result in a missense mutation, frameshift, and abnormal splicing; (2) relevant, the variation affected a conserved amino acid; (3) the variation cosegregated with the disease in affected family members; and (4) the variation was not identified among 189 Danish blood donors. In the absence of available family members for cosegregation studies, disease association was presumed if criteria 1, 2, and 4 were fulfilled. If the mutation had been associated with disease previously—in accordance with the criteria mentioned here and relevant functional studies—disease causation was presumed when just criteria 1 and 4 were met.

Cell Cultures, Transfections, and DNA Constructs

Human embryonic kidney 293 cells were cultured in Eagle minimal essential medium (MEM) with Glutamax supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/mL; 100 mg/mL) at 37°C and 5% CO_2. For Western blot and immunoprecipitation analysis, cells were transfected transiently with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to manufacturer instruction. Cells were plated to be 90% confluent at transfection day and transfected with 4 µg of total DNA in 6-well plates for Western blot.

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For electrophysiology recordings, cells were cotransfected in T25 flasks with 2 µg of total amount of DNA together with 0.2 µg of green fluorescent protein for detection of successfully transfected cells.

The human *KCNH2* and the *CAV3* cDNA constructs were purchased from OriGene Technologies, Inc, (Rockville, MD). The caveolin-3:p.T78M and the Kv11.1:p.I400N mutants were obtained by inserting point mutations in the *CAV3* and *KCNH2* constructs, respectively. The constructs were confirmed by direct sequencing, and the proteins were verified with Western blot analysis.

Coimmunoprecipitation

Protein samples were prepared 48 hours after cell transfection by 30-minute solubilization in ice-cold buffer containing Triton 1%, NP40 1%, 50 mmol/L Tris–HCl pH 7.4, 150 mmol/L NaCl, 2 mmol/L EDTA, 10% glycerol, 1 mmol/L PMSF, 1 mmol/L Na_3VO_4, 5 mmol/L NaF supplemented with fresh protease inhibitor cocktail EDTA free (La Roche, Indianapolis, IN). A total of 400 µg of protein extract was precleared by incubation with 60 µL of 1:1 slurry of protein G-Agarose beads for 2 hours at 4°C. The clarified fractions were then incubated overnight at 4°C with goat polyclonal anti-hERG antibody (sc-15968; Santa Cruz Biotechnology Inc, Santa Cruz, CA) or polyclonal IgG isolated from normal goat serum for negative control. Immune complexes were precipitated finally with protein G-Agarose for 2 hours at 4°C, collected by centrifugation, washed 5× in washing buffer (Triton 0.1%, NP40 0.1%, 50 mmol/L Tris–HCl pH 7.4, 150 mmol/L NaCl, 2 mmol/L EDTA, and 10% glycerol), and disrupted by incubation in denaturing Laemmli sample buffer. The complexes were separated on 4% to 15% gel and transferred to polyvinylidene difluoride membrane. After blocking, membranes were probed with anti-hERG (1:1000; sc-15968; Santa Cruz) or anti-Cav3 antibody (1:500; sc-5310; Santa Cruz), and the appropriate secondary antibody was conjugated with horseradish peroxidase. A total of 20 µg of each protein extract was also loaded on the gel to check the efficiency of transfection. Signals were detected using an ECL west-pico detection kit (Thermo Scientific, Rockford, IL).

Cellular Electrophysiology

Two days after transfection, cells were harvested and laid on 0.5-cm coverslips for 1 hour. Electrophysiology recordings were performed in the whole-cell patch-clamp configuration at room temperature using an EPC-9 patch-clamp amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany). Data acquisition was performed with the Pulse software (HEKA Elektronik). Resistances obtained ranged from 1.5 to 2.5 MΩ when pipettes were filled with a solution containing 5.17 mmol/L $CaCl_2$, 1.42 mmol/L $MgCl_2$, 31/10 mmol/L KOH/EGTA, 110 mmol/L KCl, 4 mmol/L K_2ATP, and 10 mmol/L Hepes (pH 7.4 adjusted with KOH). The cells were in superfusion with solution containing: 140 mmol/L NaCl, 4 mmol/L KCl, 2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, and 10 mmol/L Hepes (pH 7.4 adjusted with NaOH). The series resistances recorded in the whole-cell configuration ranged from 1.5 to 8 mΩ. Voltage protocols used for channel activation recordings were depolarizing steps ranging from −80 to +60 mV preceded by a short sweep at −100 mV to ensure that all channels had recovered from inactivation. A step protocol ranging between −120 and −40 mV for 1 second was used for channel deactivation. For all protocols, the holding potential was 80 mV. Exact protocols are indicated in the corresponding figures.

Data Analysis

Data analysis was performed using Igor Pro Software (WaveMetrics, Lake Oswego, OR). No leak subtraction was used during current recordings, and current densities were normalized to cell capacitance. Deactivation kinetic analysis was performed by calculating the time constants of double exponential functions fitted on the currents measured. Data were fit with the Boltzmann function. The significance of differences between the recordings was calculated using the *t* test.

Analysis of Trafficking

Forty-eight hours after transfection, cells were lysed in Triton 1%, 50 mmol/L Tris–HCl pH 7.4, 150 mmol/L NaCl, 2 mmol/L EDTA, 10% glycerol, 1 mmol/L PMSF, 1 mmol/L Na_3VO_4, and 5 mmol/L NaF buffer supplemented with fresh protease inhibitor cocktail EDTA free (La Roche). Lysates were cleared at 12 000 rpm for 15 minutes, and protein concentration was determined by Bradford assay. Equal amounts of protein (20 µg) were heated for 10 minutes at 60°C and subjected to SDS-PAGE on a 7.5% gel followed by transfer on polyvinylidene difluoride membranes. After blocking, membranes were incubated with primary antibodies against hERG C-terminal (1:1000; sc-15968; Santa Cruz) or rabbit antiactin (Sigma) and appropriate secondary horseradish peroxidase–conjugated antibody.

Bioinformatics

Caveolin-3 reference sequences NM_001234.3 and NP_001225.1 were used for variant annotation. All variants were checked with Mutalyser Name Checker,[24] and SNPCheck v2.1 (https://ngrl.manchester.ac.uk/SNPCheckV2/snpcheck.htm) was used to assess primer sequences for potential SNPs, as well as to collect known SNP data for the amplified regions. National Center for Biotechnology Information SNP database and the Exome Variant Server (http://evs.gs.washington.edu/EVS/) were used to collect frequency data on known SNPs. Clustal W2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/)[25] was used to align caveolin-3 sequences across species (*Homo sapiens* [humans], *Gorilla gorilla* [gorilla], *Macaca mulatta* [macaque], *Pan troglodytes* [chimpanzee], *Bos taurus* [cow], *Rattus norvegicus* [rat], *Gallus gallus* [chicken], *Melegris gallapavo* [turkey], *Anolis carolinensis* [anole lizard], *Xenopus tropicalis* [Western clawed frog], *Danio rerio* [zebrafish], *Otolemur garnettii* [bushbaby], *Procavia capensis* [hyrax], *Oryzias latipes* [medaka], *Monodelphis domestica* [opposum]). Human Splicing Finder v2.4.1 (www.umd.be/HSF)[26] was used to assess the effect the identified variants might have on splicing. Deviation from Hardy–Weinberg Equilibrium was tested using GenePop (http://genepop.curtin.edu.au/genepop_op1.html).[27,28]

Electrocardiography

ECGs of selected family members were recorded in the supine position at rest on digital ECG recorders (GE Medical Systems, Milwaukee, WI), with a standard 12-lead system and 10-second duration at a sampling rate of 500 Hz. Data from a control group were acquired from the GE-Marquette research database of healthy volunteers.[29] QT intervals were preferentially measured manually in lead V5. If T-waves were immeasurable, lead II or lead V2 were chosen. T-wave morphology features, including flatness, asymmetry, and notches, were measured as morphology combination score (MCS) as described by Graff et al[30] to quantitate differences in T-wave morphology between carriers of caveolin-3:p.T78M, Kv11.1:p.I400N, double heterozygote carriers, and healthy subjects. A mean (SD) MCS value of 1.80 (0.60) has been reported in 30 patients with LQTS2 as opposed to 0.71 (0.24) in 917 healthy controls. An MCS value of 1.1 defines the cutoff between normal controls and patient with significant I_{Kr} reduction because of 160 mg of sotalol or an LQTS2 phenotype.[30]

Ethics

All patients had given informed consent of genetic screening according to the law and clinical practices of the respective countries.

Results

One LQTS proband (0.6%) was identified as a heterozygous carrier of the missense mutation *CAV3* c.233C>T: p.T78M that has been previously associated with LQTS,[9] as well as sudden infant death syndrome.[11] This threonine residue is highly conserved in mammals.

A further 15 non-disease–causing genetic variants, 3 in the 5-prime untranslated region part of the gene, 5 from the intronic regions around the intron/exon boundaries, and 7 synonymous variants from the coding region, were identified as detailed in Table 1. Ten of the variants described here,

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including caveolin-3:p.T78M, had been registered in the SNP database. Some of the variants had a very low prevalence. The frequent genotypes were in Hardy–Weinberg Equilibrium, although the frequency of the rare variants was too low for a reliable Hardy–Weinberg Equilibrium estimation.

Of the variants not previously described in an SNP database, 2 were 5-prime untranslated region SNPs (c.-34C>T and c.-15C>A), 2 were intronic variants (c.114+32A>G and c.115-32delT), and 2 were synonymous variants (c.12A>G:p.E4= and c.240G>A:p.L80=). All these variants were extremely rare and occurred only in the LQTS cohort. There was no appreciable effect on splicing by these variants as assessed by in silico analysis.

The caveolin-3:p.T78M carrying proband was also a heterozygous carrier of the Kv11.1:p.I400N mutation. The pedigree and clinical synopsis are shown in Figure 2. Three family members (II-2, II-4, and III-7) carry the caveolin-3:p.T78M mutation in isolation. All 3 reported syncopal episodes: II-2, an 80-year-old woman (8 years after diagnosis at 72 years), had 2 syncopes and a severely prolonged PQ interval of 0.52 ms, AV block was assumed and a DDD pacemaker was implanted, and the patient has been symptom free since; II-4 had a syncopal episode once during painful delivery; and III-7 fainted once during an accident where her hand was lacerated in a machine. The 3 isolated caveolin-3:p.T78M carriers (II-2, II-4, and III-7) had heart rates of 56, 75, and 75 bpm, respectively. All Kv11.1:p.I400N carriers had a Schwartz score ≥4 except IV-3 with a score of 3. Family members only carrying the caveolin-3:p.T78M mutation had a Schwartz score of 2, on the basis of normal QTc interval, nonexertional syncope, and positive family history. Twelve-lead ECG traces of the caveolin-3:p.T78M carriers are shown in Figure I of the online-only Data Supplement. Furthermore, individuals carrying both caveolin-3:p.T78M and Kv11.1:p.I400N do not have a worse clinical phenotype (median QTc=500 ms; range, 470–550 ms) than individuals carrying Kv11.1:p.I400N in isolation (median QTc=530 ms; range, 430–570 ms). The application of T-wave morphology analysis[30] to 8 family members, where digital ECGs were available, established that the mean MCS (SD) of double heterozygotes was 1.79 (0.01) (n=2), Table 2, indistinguishable from the mean MCS (SD) of 1.73 (0.42) in 4 family members solely carrying Kv11.1:p.I400N. The mean MCS (SD) of caveolin-3:p.T78M carriers was 0.57 (0.04), Table 2, indistinguishable from that of healthy subjects.[30] Furthermore, no SCN5A morphology with prolonged isoelectric ST segments and narrow T-waves was apparent in these cases (Figure I in the online-only Data Supplement).

Western blot analysis indicated that cells expressing wild-type (WT) Kv11.1 channel proteins exhibited both a 135-kDa and a mature 155-kDa protein band, whereas cells expressing the Kv11.1:p.I400N substitution exhibited only the 135-kDa protein band (Figure 3A). Quantification of the amount of mature channel at the plasma membrane confirmed that, compared with WT Kv11.1, less Kv11.1:p.I400N channel was present at the plasma membrane (n=8; P=0.001; Figure 3B). Electrophysiological characterization of the Kv11.1:p.I400N mutant is shown in Figure 4. The Kv11.1:p.I400N mutant displayed considerably lower peak currents. The mean currents measured at the end of a 2-second depolarizing pulse at 0 mV were 0.030±0.007 pA/pF for WT compared with 0.014±0.003 pA/pF for Kv11.1:p.I400N (P=0.046). Mean tail currents recorded at 60 mV were 0.114±0.022 pA/pF for WT compared with 0.023±0.006 pA/pF for Kv11.1:p.I400N (P<0.0001). Normalized tail currents for progressive increase of voltage

Table 1. Genetic Variants in *CAV3* and Their Frequency in a White Population and LQTS

NCBI SNP identifier	NM_001234.3	NP_001225.1	NCBI SNP: MAF	EVS EA: MAF	DK control MAF (n=189)	LQTS (n=167)
rs116840771	c.-37G>A	…	…	A=0.005	A=0.003	0.006
…	c.-34C>T	…	…	…	…	0.003
…	c.-15C>A	…	…	…	…	0.003
…	c.12A>G	p.Glu4=	…	…	…	0.003
rs1974763	c.27C>T	p.Leu9=	T=0.059	T=0.125	T=0.124	0.159
rs1008642	c.99C>T	p.Asn33=	T=0.371	T=0.239	T=0.265	0.254
rs11922879	c.114+26G>A	…	A=0.048	A=0.049	A=0.063	0.073
…	c.114+32A>G	…	…	…	…	0.003
rs139242554	c.115-47_115-31del	…	…	…	del=0.091	0.114
…	c.115-32delT	…	…	…	…	0.008
rs57159780	c.115-23G>C	…	C=0.060	C=0.089	C=0.130	0.079
rs13087941	c.123T>C	p.Phe41=	C=0.167	C=0.241	C=0.250	0.239
rs61147808	c.171G>A	p.Val57=	A=0.014	A=0.0001	…	0.003
rs72546668	c.233C>T*	p.Thr78Met*	T=0.001	T=0.004	T=0.006	0.003
…	c.240G>A	p.Leu80=	…	…	…	0.003
rs139985460	c.336C>T	p.Ile112=	…	T=0.002	T=0.013	0.006

NCBI SNP: MAF are generated from 1000 genomes data. EVS EA data are generated from the exome sequencing of 8600 individuals. DK indicates Denmark; EA, European American; EVS, Exome Variant Server; LQTS, long–QT syndrome; MAF, minor allele frequency; NCBI, National Center for Biotechnology Information; and SNP, single-nucleotide polymorphism.

*Only variant previously reported in the literature.[3,9–11]

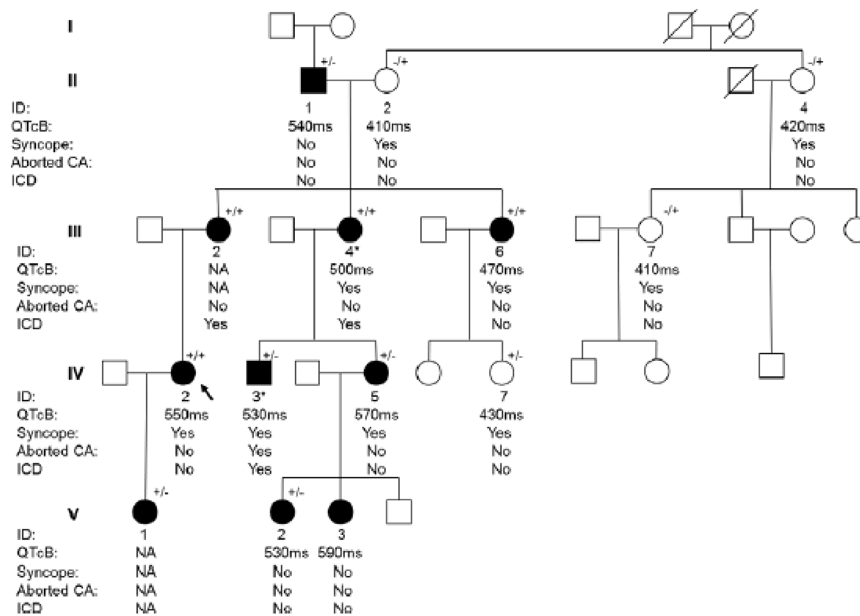

Figure 2. Pedigree representation of the family carrying both caveolin-3:p.T78M and Kv11.1:p.I400N. The pedigree indicates the transmission of both the caveolin-3:p.T78M mutation and the Kv11.1:p.I400N mutation. The proband is indicated with an arrow. □ Male members of the pedigree and ○ female members. Filled symbols represent long–QT syndrome affected individuals. QTcB=corrected QT interval using Bazett formula (QTcB=QT/√ RR); +/− Kv11.1:p.I400N carriers; −/+ caveolin-3:p.T78M carriers; +/+ double heterozygote carriers of Kv11.1:p.I400N and caveolin-3:p.T78M; *ICD units, no appropriate shocks have been registered. CA indicates cardiac arrest; and ICD, implantable cardioverter defibrillator.

were lower in Kv11.1:p.I400N channels when compared with WT (Figure 4D). No difference was observed between WT and Kv11.1:p.I400N in the deactivation rates.

Interaction between Kv11.1 and caveolin-3 was confirmed by coimmunoprecipitation analysis (Figure 5A). Furthermore, the interaction between caveolin-3 and Kv11.1 is not compromised by either caveolin-3:p.T78M or Kv11.1:p.I400N mutations (Figure 5B).

Discussion

Clinical Genetics

We have identified 1 *CAV3* variant (c.233C>T; p.T78M) in 167 LQTS cases. This variant falls within the central hydrophobic transmembrane domain (amino acids 75–106) of caveolin-3 and has previously been reported to be disease causing. Of the 16 *CAV3* variants identified in the LQTS population, 10 of them occurred at a frequency of <1% in this population. Three of these rare variants were identified in the Danish control group, and 2 occurred with a frequency of <1% in this group. Only 1 of these, caveolin-3:p.T78M, was also identified in the 1000 genomes cohort at a frequency of 0.1%. The significance of these rare variants is unclear. In silico analyses of these variants suggest that they all have the possibility of disrupting exon splicing silencer or enhancer sites and thereby affecting splicing. However, without further experimental evidence, it is difficult to ascribe pathophysiological significance to these variants.

Double heterozygotes [caveolin-3:p.T78M][Kv11.1:p.I400N] but not single caveolin-3:p.T78M carriers exhibited QTc prolongation and abnormal T-wave morphology.

Table 2. Clinical Characteristics of Caveolin-3:p.T78M Carriers, An Aggregation of Data From Vatta et al[9] and the Present Study

Case	Sex	Age at Diagnosis, y	Nucleotide Change	Caveolin-3 Variant	Other LQTS Mutations	Presenting Symptom	QTc, ms	MCS	Other ECG Abnormalities	References
1	F	14	c.233 C>T	p.T78M	p.A913V-KCNH2	Nonexertional syncope	405	n.a.	U waves, sinus bradycardia	9
2	M	8	c.233 C>T	p.T78M	…	Nonexertional syncope	433	n.a.	Marked sinus bradycardia	9
3	M	40	c.233 C>T	p.T78M	…	None	456	n.a.	…	9
II-2	F	72	c.233 C>T	p.T78M	…	AV block	410	n.a.	1° AV block	This study
II-4	F	64	c.233 C>T	p.T78M	…	Syncope during birth delivery	420	0.53	…	This study
III-7	F	38	c.233 C>T	p.T78M	…	Syncope during emotional stress	410	0.60	…	This study
III-4	F	48	c.233 C>T	p.T78M	p.I400N-KCNH2	Nonexertional syncope	500	1.78	Atrial pacing, notches	This study
III-6	F	44	c.233 C>T	p.T78M	p.I400N-KCNH2	None	470	1.80	Notches, abnormal U-waves	This study
IV-2	F	28	c.233 C>T	p.T78M	p.I400N-KCNH2	Syncope	550	n.a.	Notches	This study

AV, atrioventricular; F, female; LQTS, long–QT syndrome; M, male; and MCS, morphology combination score.

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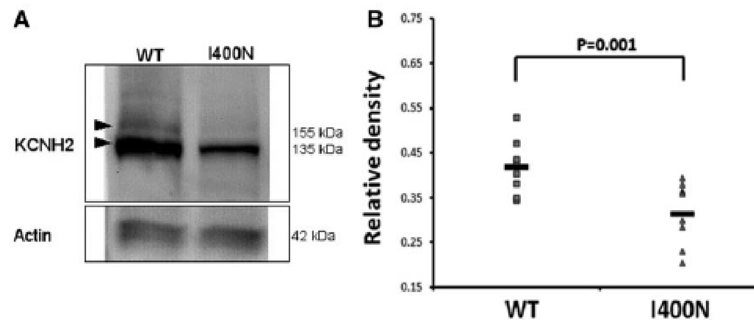

Figure 3. Western blot of Kv11.1 wild-type (WT) and Kv11.1:p.I400N mutant (**A**). The densities of the 155-kDa bands of the WT and the Kv11.1:I400N mutant are normalized to their respective 135-kDa band and are plotted in **B** as relative values to the amount of Kv11.1 channel present in the endoplasmic reticulum (n=8; *P*=0.001). The actin bands in **A** show that equal amounts of total protein were loaded on the gel.

Furthermore, caveolin-3:p.T78M carriers do not manifest an *SCN5A* ST-T wave pattern. Although all the 3 single caveolin-3:p.T78M carriers had experienced syncope, these could be attributed to AV block or a neurocardiogenic mechanism.

CAV3 Mutations and Disease Association

The caveolin-3:p.T78M mutation was reported originally in 3 unrelated patients with LQTS, 1 of whom also carried Kv11.1:p.A913V. On the basis of clinical presentation of 2 of the patients, the authors concluded that caveolin-3:p.T78M may modulate the I_{Na} and may reduce the sinus rate producing clinical features characteristic of LQT3.[9] However, of the 3 patients carrying only the caveolin-3:p.T78M mutant, 2—a 14-year-old girl and an 8-year-old boy—had normal QTc intervals of 405 and 433 ms, respectively. The last, a 40-year-old man, had a QTc interval of 456 ms that could be considered borderline prolonged[9]; however, as reported by Drew et al,[31] QTc intervals of 470 ms in postpubertal men and 480 ms in postpubertal women are considered the upper limits of normal. Data aggregated from Vatta et al[9] and the present study (Table 2) indicate a paucity of evidence supporting *CAV3* as an LQTS-causing gene. A subsequent study by Cronk et al[11] identified caveolin-3:p.T78M in a sudden infant death syndrome case. The authors concluded that, as caveolin-3 colocalizes with β_2-adrenoreceptors in ventricular and sinoatrial myocytes,[32–34] disruption of caveolae by mutations in caveolin-3 might affect the β-adrenergic responsiveness and the excitation–contraction coupling of cardiac myocytes. The caveolin-3 p.T78M mutation has also been found in a subject with idiopathic hyperCKemia, although in this case immunohistochemical analysis of caveolin-3 in a muscle biopsy was normal.[35] Traverso et al[3] described a case where a homozygous caveolin-3:p.T78M carrier developed severe limb-girdle muscular dystrophy with dilated cardiomyopathy, whereas a heterozygous offspring was asymptomatic, suggesting an autosomal recessive mode of inheritance for this trait. In vitro studies showed that caveolin-3:p.T78M impairs the ability of the caveolin-3 hydrophobic domain to assemble caveolin-3 homo-oligomers units for the formation of caveolae in muscle cells.[36–38] Furthermore, the first case of a heterozygous caveolin-3:p.T78M mutation associated with rippling muscle disease and proximal weakness was reported by Ricci et al[39] in which immunohistochemical analysis of skeletal muscle confirmed reduced caveolin-3 immunolabeling. The patient was also affected by mild facioscapulohumeral muscular dystrophy that was explained by a D4Z4 partial deletion. However, weakness of the pelvic girdle muscles, a complaint not usually present in facioscapulohumeral muscular dystrophy,[40] and the rippling phenomenon, a specific sign of caveolinopathy, has never been associated with D4Z4-reduced allele expression. Subsequently, Spadafora et al[41] questioned the association of caveolin-3:p.T78M with disease. They presented a case in which caveolin-3:p.T78M was found in a woman presumed to suffer from distal myopathy. A final diagnosis of mild frontotemporal dementia was determined, and as the proband's father was suspected to have died with parkinsonism, it was surprising that the proband had inherited the caveolin-3:p.T78M variant from her clinically nonaffected mother. The authors also point out that caveolin-3:p.T78M has a frequency of 1.5% in Italy and hypothesize that either the association of this variant with disease is incidental or specific population factors are required to modify the effect of this variant.[41] Collectively, these data do not support the hypothesis that the caveolin-3:p.T78M mutation is pathogenic in the heterozygous state. It may be that caveolin-3:p.T78M exerts an effect in association with other genetic factors. Genotype–phenotype correlation studies in caveolinopathies suggest that genetic modifiers may contribute to the phenotype.[3,13,42,43]

As seen in Table 2, the caveolin-3:p.T78M is not independently associated with a prolonged QT or with previous syncope, despite its previously described electrophysiology effect (a 4-fold increase in the late I_{Na}).[11] Several cardiac ion channels have been reported to be localized within caveolae: HCN4, Nav1.5, Kv1.5, Cav1.2, and Kv11.1.[44,45] The complex regulation of these ion channels within macromolecular complexes in caveolae[19,45] suggests that the effect *CAV3* mutations might exert on individual channels would produce a cumulative effect on the currents that make up the action potential. The ion channels listed above play critical roles in different phases of the cardiac action potential. Therefore, it is meaningless to assess the effects that *CAV3* mutations have on a single ion channel without being able to assess the effect on the action potential as a whole. The findings that 2 of the caveolin-3:p.T78M carriers had reported a previous syncope and 1 had a slight 1° AV block could, however, indicate that *CAV3* mutations may increase the propensity for numerous types of arrhythmia depending on the involved interacting ion channel.

An interesting possibility is that CAV3 mutations affect the I_{Kr} current predominantly under hypokalemic

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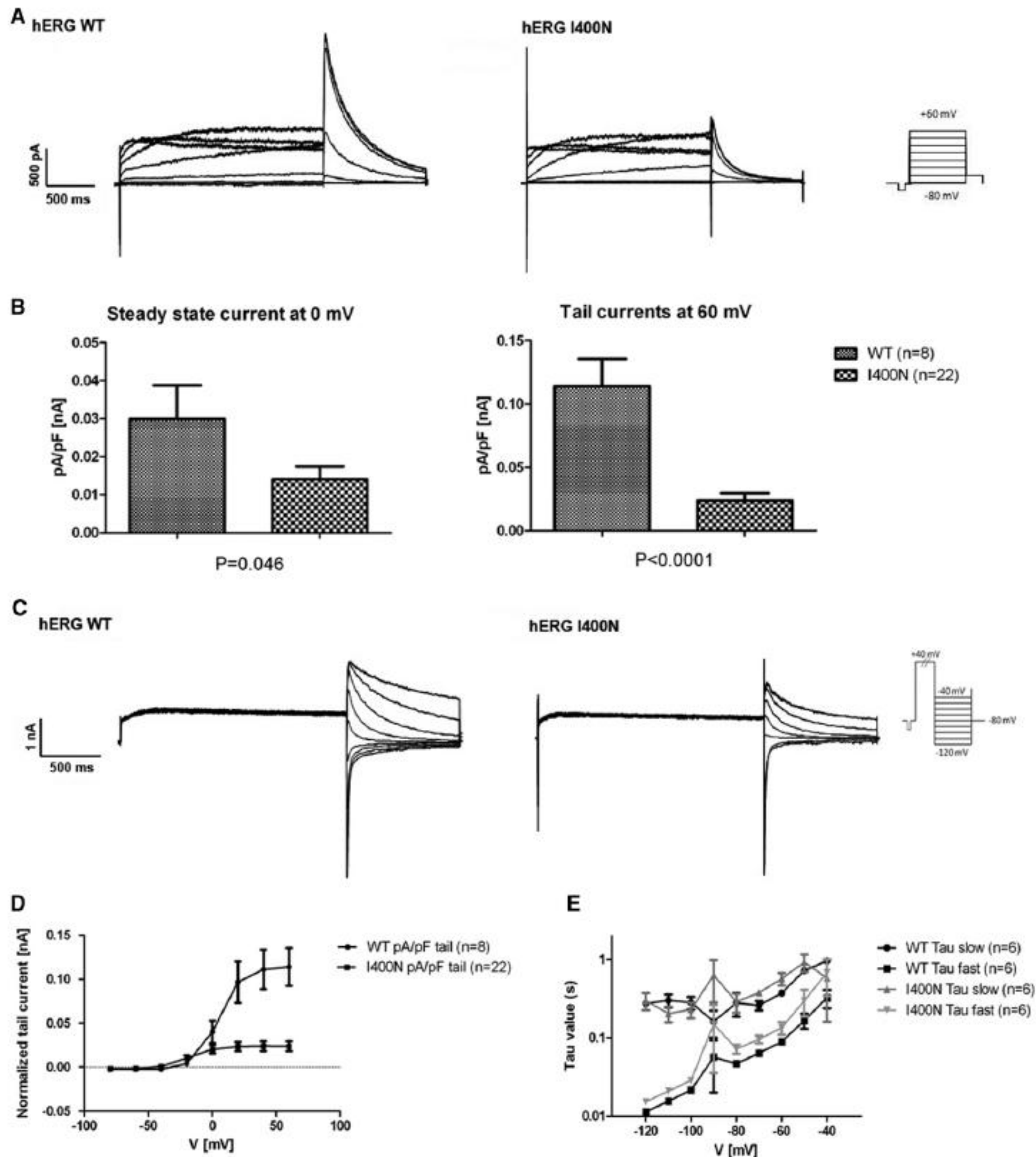

Figure 4. Electrophysiological readings. **A**, Representative Kv11.1 wild-type (WT) and Kv11.1:p.I400N traces. The voltage clamp protocol is shown in inset. **B**, Steady state peak currents measured at 0 mV and peak tail currents measured at 60 mV for WT and Kv11.1:p.I400N. **C**, Deactivation; representative current traces after a deactivation protocol (in inset). **D**, Maximum tail currents measured in WT and Kv11.1:p.I400N. **E**, Tau values after double exponential fitting to the decaying phase of the traces obtained for WT and Kv11.1:p.I400N. All deviations of mean averages are given as SEM. hERG indicates human ether-a-go-go-related gene.

conditions, where *CAV3* is known to play a role in the availability of Kv11.1 at the cell membrane.[19] This would be in accordance with the known sensitivity of the I_{Kr} current to external hypo- and hyperkalemia.[46] This interdependence between hypokalemia and *CAV3* function may explain the highly variable phenotypic expression of *CAV3* mutations.

However, in the absence of an LQTS phenotype in any of the isolated caveolin-3:p/T78M carriers, despite that this mutation has the signature in vitro electrophysiological effect of LQTS9, it would seem reasonable to consider LQTS9 a provisional entity until an association between the clinical LQTS phenotype and *CAV3* mutations has been established.

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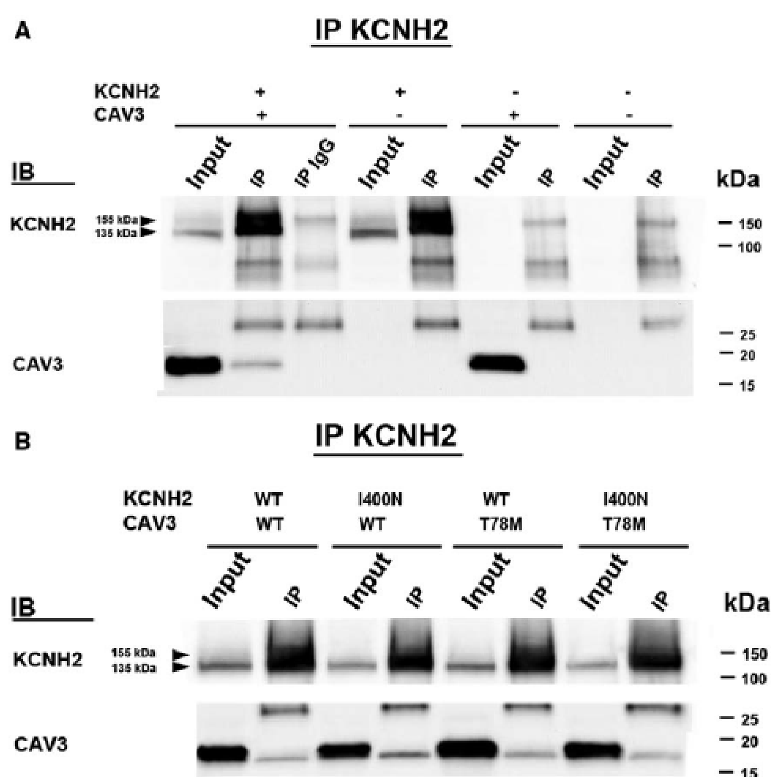

Figure 5. **A**, Western blot analysis of immunoprecipitated human embryonic kidney 293 protein extracts. Double-transfected HEK293 cells with KCNH2 and CAV3 were immunoprecipitated with anti-Kv11.1 antibodies and blotted for the presence of caveolin-3. Lane 2 shows that caveolin-3 coimmunoprecipitates with Kv11.1. Cells transfected with only 1 of the constructs (CAV3 or KCNH2) and cells transfected with empty plasmids were used as controls (lanes 5, 7, and 9). No caveolin-3 is detected in any of the control lanes, including the sample, where immunoprecipitation was carried out with normal goat serum polyclonal IgG (lane 3) instead of anti-Kv11.1 antibodies, thus confirming the specificity of the Kv11.1–caveolin-3 binding. **B**, Western blot analysis of immunoprecipitated Kv11.1 wild-type (WT) and mutant protein. The caveolin-3:p.T78M and the Kv11.1:p.I400N mutants were investigated in coimmunoprecipitation experiments and compared with WT. The interaction between caveolin-3 and Kv11.1 mutants is not disrupted in any combination as shown by the presence of the caveolin-3 band in all the immunoprecipitation lanes.

Cellular Electrophysiology

Cellular protein synthesis is a tightly controlled process where incorrectly processed proteins may be retained until they are correctly processed, retrogradely transported to an earlier compartment, and targeted for degradation. The processing of Kv11.1 channel proteins includes 2 glycosylation steps. Immature Kv11.1 proteins undergo N-linked core glycosylation in the endoplasmic reticulum (135 kDa), whereas complex glycosylation occurs in the Golgi apparatus (155 kDa); this mature form of Kv11.1 is inserted into the cell membrane.[47,48] Using this defective glycosylation as a representation of defective trafficking, Anderson et al[49] reported that most Kv11.1 mutations were trafficking deficient. Our results suggest that Kv11.1:p. I400N results in a trafficking deficiency of the Kv11.1 channel subunit.

Electrophysiological assessment of the Kv11.1:p.I400N mutation indicated that there is no effect on the gating of the mutant and confirmed the Western blot analysis that the mutation results in a reduced number of total functional channels present on the membrane.

Limitations

This study may be limited with respect to the method used for mutation screening. Direct sequencing of the coding regions of *CAV3* cannot identify large deletions or duplications that would be more suitably identified by MLPA; it is also limited in identifying intronic variants as only the intronic regions close to the intron/exon boundaries are interrogated by this method. Consequently, we cannot comment on the role of *CAV3* structural rearrangements or the genetic variation *CAV3* regulatory regions may play in LQTS.

Conclusions

CAV3 mutations are present in LQTS, but they are rare and may not have any clinical consequence, despite an electrophysiology in vitro phenotype. There is no evidence indicating that the caveolin-3:p.T78M mutation is disease causing. As caveolin-3:p.T78M has been shown to generate the signature electrophysiological effect of LQTS9, and we have demonstrated that this effect is not associated with LQTS, it is most reasonable to consider LQTS9 a provisional entity until an association with clinical LQTS can be established. In this study, all symptomatic patients are heterozygote carriers of

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the Kv11.1:p.I400N mutation, which has shown to be associated with trafficking deficiency and reduced peak current. This functional effect is the clinical pathogenic mechanism in LQTS2.[50,51]

CAV3 analysis need not be performed routinely in the genetic workup of patients with LQTS, and the findings of rare variants in *CAV3* should be handled with caution, particularly with respect to their clinical significance.

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Disclosures

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References

1. Schlörmann W, Steiniger F, Richter W, Kaufmann R, Hause G, Lemke C, et al. The shape of caveolae is omega-like after glutaraldehyde fixation and cup-like after cryofixation. *Histochem Cell Biol*. 2010;133:223–228.
2. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev*. 2004;84:1341–1379.
3. Traverso M, Bruno C, Broccolini A, Sotgia F, Donati MA, Assereto S, et al. Truncation of Caveolin-3 causes autosomal-recessive Rippling Muscle Disease. *J Neurol Neurosurg Psychiatry*. 2008;79:735–737.
4. Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, et al. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. *Nat Genet*. 1998;18:365–368.
5. McNally EM, de Sá Moreira E, Duggan DJ, Bönnemann CG, Lisanti MP, Lidov HG, et al. Caveolin-3 in muscular dystrophy. *Hum Mol Genet*. 1998;7:871–877.
6. Galbiati F, Razani B, Lisanti MP. Caveolae and caveolin-3 in muscular dystrophy. *Trends Mol Med*. 2001;7:435–441.
7. Carbone I, Bruno C, Sotgia F, Bado M, Broda P, Masetti E, et al. Mutation in the CAV3 gene causes partial caveolin-3 deficiency and hyperCKemia. *Neurology*. 2000;54:1373–1376.
8. Hayashi T, Arimura T, Ueda K, Shibata H, Hohda S, Takahashi M, et al. Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun*. 2004;313:178–184.
9. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation*. 2006;114:2104–2112.
10. Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, et al. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. *Circulation*. 2007;115:361–367.
11. Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, et al. Novel mechanism for sudden infant death syndrome: persistent late sodium current secondary to mutations in caveolin-3. *Heart Rhythm*. 2007;4:161–166.
12. Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, et al. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J Biol Chem*. 1996;271:2255–2261.
13. Gazzerro E, Sotgia F, Bruno C, Lisanti MP, Minetti C. Caveolinopathies: from the biology of caveolin-3 to human diseases. *Eur J Hum Genet*. 2010;18:137–145.
14. Feron O, Kelly RA. Gaining respectability: membrane-delimited, caveolar-restricted activation of ion channels. *Circ Res*. 2002;90:369–370.
15. Couet J, Li S, Okamoto T, Ikezu T, Lisanti MP. Identification of peptide and protein ligands for the caveolin-scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. *J Biol Chem*. 1997;272:6525–6533.
16. Cohen AW, Combs TP, Scherer PE, Lisanti MP. Role of caveolin and caveolae in insulin signaling and diabetes. *Am J Physiol Endocrinol Metab*. 2003;285:E1151–E1160.
17. Ackerman MJ, Mohler PJ. Defining a new paradigm for human arrhythmia syndromes. *Circ Res*. 2010;107:457–465.
18. Cherubini A, Hofmann G, Pillozzi S, Guasti L, Crociani O, Cilia E, et al. Human ether-a-go-go-related gene 1 channels are physically linked to beta1 integrins and modulate adhesion-dependent signaling. *Mol Biol Cell*. 2005;16:2972–2983.
19. Massaeli H, Sun T, Li X, Shallow H, Wu J, Xu J, et al. Involvement of caveolin in low K+-induced endocytic degradation of cell-surface human ether-a-go-go-related gene (hERG) channels. *J Biol Chem*. 2010;285:27259–27264.
20. Larsen LA, Andersen PS, Kanters JK, Jacobsen JR, Vuust J, Christiansen M. A single strand conformation polymorphism/heteroduplex (SSCP/HD) method for detection of mutations in 15 exons of the KVLQT1 gene, associated with long QT syndrome. *Clin Chim Acta*. 1999;280:113–125.
21. Larsen LA, Christiansen M, Vuust J, Andersen PS. High-throughput single-strand conformation polymorphism analysis by automated capillary electrophoresis: robust multiplex analysis and pattern-based identification of allelic variants. *Hum Mutat*. 1999;13:318–327.
22. Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsøe S, et al. High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia. *Clin Genet*. 2006;69:504–511.
23. Consortium TGP. A map of human genome variation from population-scale sequencing. *Nature*. 2010;467:1061–1073.
24. Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PE. Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. *Hum Mutat*. 2008;29:6–13.
25. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007;23:2947–2948.
26. Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res*. 2009;37:e67.
27. Raymond M, Rousset F. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *J Heredity*. 1995;86:248–249.
28. Rousset F. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour*. 2008;8:103–106.
29. Haarmark C, Hansen PR, Vedel-Larsen E, Pedersen SH, Graff C, Andersen MP, et al. The prognostic value of the Tpeak-Tend interval in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. *J Electrocardiol*. 2009;42:555–560.
30. Graff C, Andersen MP, Xue JQ, Hardahl TB, Kanters JK, Toft E, et al. Identifying drug-induced repolarization abnormalities from distinct ECG patterns in congenital long QT syndrome: a study of sotalol effects on T-wave morphology. *Drug Saf*. 2009;32:599–611.
31. Drew BJ, Ackerman MJ, Funk M, Gibler WB, Kligfield P, Menon V, et al. Prevention of torsade de pointes in hospital settings: a scientific statement from the American Heart Association and the American College of Cardiology Foundation. *Circulation*. 2010;121:1047–1060.
32. Balijepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ. Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation. *Proc Natl Acad Sci USA*. 2006;103:7500–7505.
33. Calaghan S, White E. Caveolae modulate excitation-contraction coupling and beta2-adrenergic signalling in adult rat ventricular myocytes. *Cardiovasc Res*. 2006;69:816–824.
34. Barbuti A, Terragni B, Brioschi C, DiFrancesco D. Localization of f-channels to caveolae mediates specific beta2-adrenergic receptor modulation of rate in sinoatrial myocytes. *J Mol Cell Cardiol*. 2007;42:71–78.
35. Reijneveld JC, Ginjaar IB, Frankhuizen WS, Notermans NC. CAV3 gene mutation analysis in patients with idiopathic hyper-CK-emia. *Muscle Nerve*. 2006;34:656–658.
36. Parton RG. Caveolae and caveolins. *Curr Opin Cell Biol*. 1996;8:542–548.
37. Das K, Lewis RY, Scherer PE, Lisanti MP. The membrane-spanning domains of caveolins-1 and -2 mediate the formation of caveolin hetero-oligomers. Implications for the assembly of caveolae membranes in vivo. *J Biol Chem*. 1999;274:18721–18728.
38. Schlegel A, Lisanti MP. A molecular dissection of caveolin-1 membrane attachment and oligomerization: two separate regions of the caveolin-1 C-terminal domain mediate membrane binding and oligomer/oligomer interactions in vivo. *J Biol Chem*. 2000;275:21605–21617.
39. Ricci G, Scionti I, Alì G, Volpi L, Zampa V, Fanin M, et al. Rippling muscle disease and facioscapulohumeral dystrophy-like phenotype in a patient carrying a heterozygous CAV3 T78M mutation and a D4Z4 partial

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deletion: further evidence for "double trouble" overlapping syndromes. *Neuromuscul Disord*. 2012;22:534–540.
40. Padberg GW, Lunt PW, Koch M, Fardeau M. Diagnostic criteria for facioscapulohumeral muscular dystrophy. *Neuromuscul Disord*. 1991;1:231–234.
41. Spadafora P, Liguori M, Andreoli V, Quattrone A, Gambardella A. Cav3 t78m mutation as polymorphic variant in South Italy. *Neuromuscul Disord*. 2012;22:669–670.
42. Fee DB, So YT, Barraza C, Figueroa KP, Pulst SM. Phenotypic variability associated with Arg26Gln mutation in caveolin3. *Muscle Nerve*. 2004;30:375–378.
43. Fulizio L, Nascimbeni AC, Fanin M, Piluso G, Politano L, Nigro V, et al. Molecular and muscle pathology in a series of caveolinopathy patients. *Hum Mutat*. 2005;25:82–89.
44. Maguy A, Hebert TE, Nattel S. Involvement of lipid rafts and caveolae in cardiac ion channel function. *Cardiovasc Res*. 2006;69:798–807.
45. Balijepalli RC, Kamp TJ. Caveolae, ion channels and cardiac arrhythmias. *Prog Biophys Mol Biol*. 2008;98:149–160.
46. Christiansen M, Wettrell G, Larsen LA, Lundqvist L, Andersen PS, Kanters JK, Vuust J. Potassium must be considered in congenital long qt syndrome. *Heart Drug*. 2005;5:54–58.
47. Petrecca K, Atanasiu R, Akhavan A, Shrier A. N-linked glycosylation sites determine HERG channel surface membrane expression. *J Physiol*. 1999;515(Pt 1):41–48.
48. Gong Q, Anderson CL, January CT, Zhou Z. Role of glycosylation in cell surface expression and stability of HERG potassium channels. *Am J Physiol Heart Circ Physiol*. 2002;283:H77–H84.
49. Anderson CL, Delisle BP, Anson BD, Kilby JA, Will ML, Tester DJ, et al. Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation*. 2006;113:365–373.
50. Hedley PL, Jørgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, et al. The genetic basis of long QT and short QT syndromes: a mutation update. *Hum Mutat*. 2009;30:1486–1511.
51. Christiansen M, Hedley PL. Long qt testing: implications for complex diagnosis in personalized medicine. *Pers Med*. 2010;7:125–127.

CLINICAL PERSPECTIVE

We examined the occurrence of *CAV3* mutations in 167 long-QT syndrome (LQTS) probands by sequencing the coding regions of the gene. We describe a family with double heterozygosity, harboring mutations in *CAV3* and *KCNH2*; however, caveolin-3:p.T78M did not exhibit a LQTS phenotype, and functional characterization indicates that the *KCNH2* mutation is disease causing. Our findings in aggregate with published reports on *CAV3* mutations suggest that, despite an in vitro electrophysiology phenotype, *CAV3* mutations may not have any clinical consequence. There is no evidence indicating that the caveolin-3:p.T78M mutation is disease causing, and as caveolin-3:p.T78M has been shown to generate the signature LQTS9 electrophysiological effect, it is reasonable to consider LQTS9 a provisional entity until an association with clinical LQTS can be established, and the findings of rare variants in *CAV3* should be handled with caution, particularly with respect to their clinical significance.

SUPPLEMENTAL MATERIAL

Legend to Figure 1 in Supplemental Material

Figure 1. ECG traces of family members solely carrying the caveolin 3:p.T78M mutations. **A** II2, **B** II4 and **C** III7.

Figure 1A

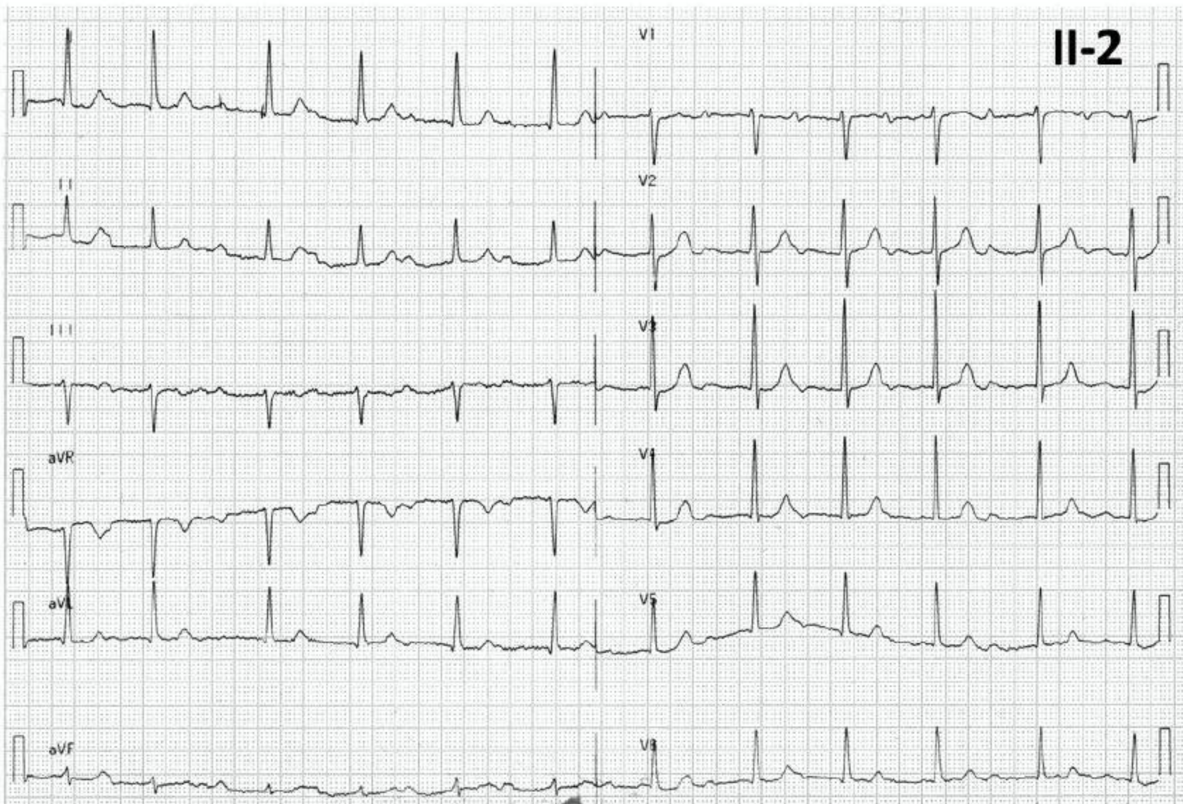


Figure 1B

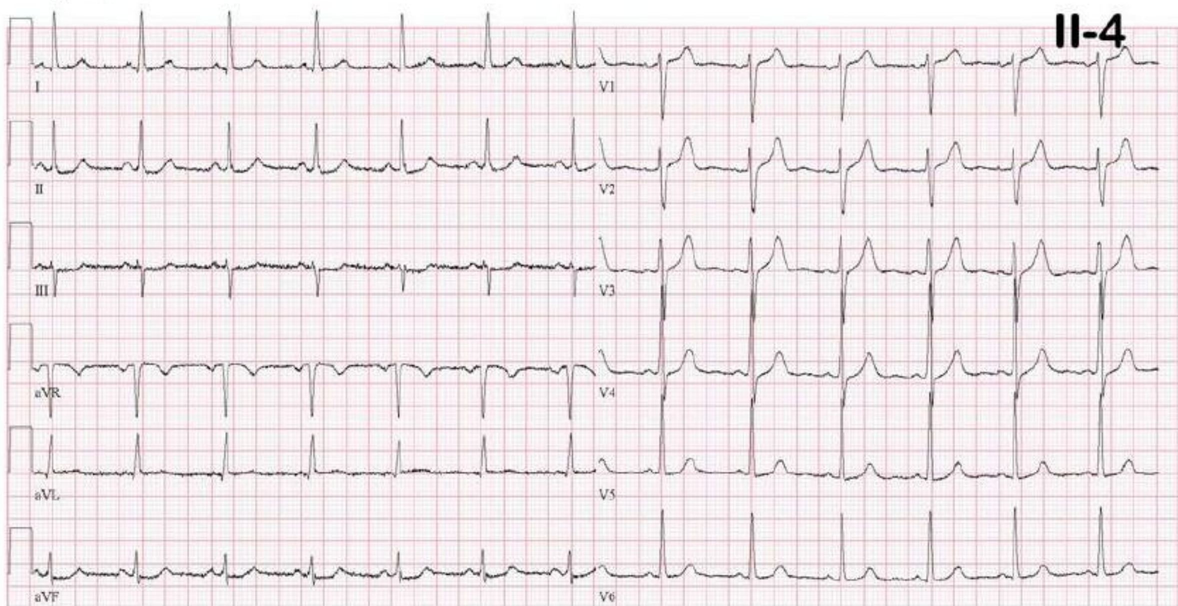
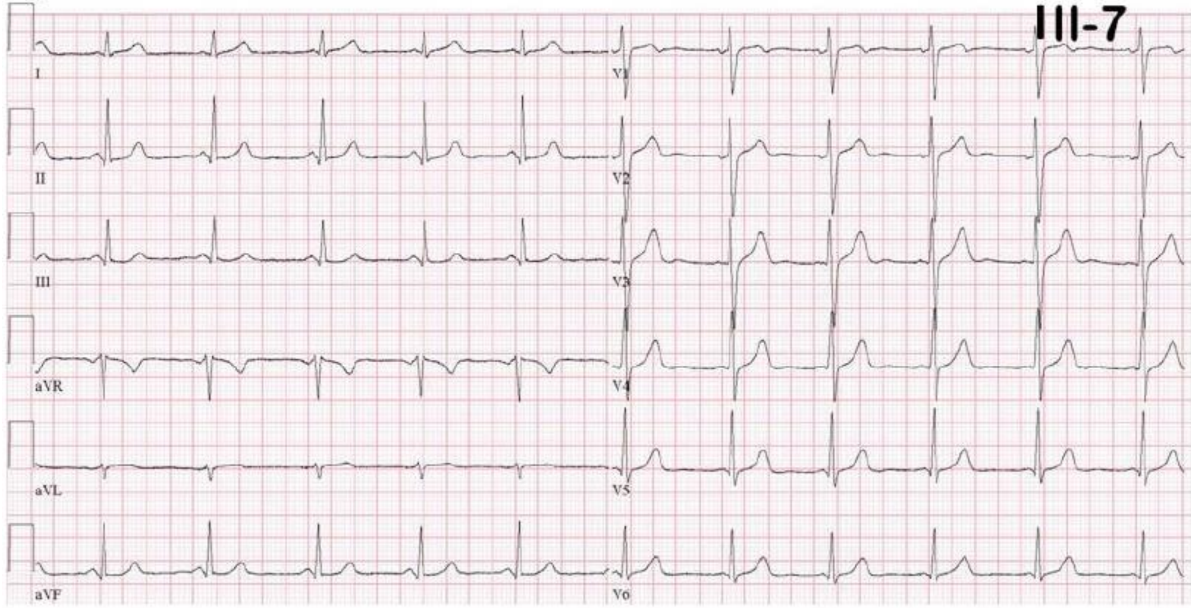


Figure 1C



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PAPER 4: MICRORNAS IN CARDIAC ARRHYTHMIA: THE SEQUENCE VARIATION OF MIR-1 AND MIR-133A IN LONG QT SYNDROME

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MicroRNAs in cardiac arrhythmia: DNA sequence variation of MiR-1 and MiR-133A in long QT syndrome.

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Abstract

Background

Long QT syndrome (LQTS) is a genetic cardiac condition associated with prolonged ventricular repolarisation, primarily a result of perturbations in cardiac ion channels, which predisposes individuals to life-threatening arrhythmias. Using DNA screening and sequencing methods, over 700 different LQTS-causing mutations have been identified in 13 genes worldwide. Despite this, the genetic cause of 30-50% of LQTS is presently unknown.

MicroRNAs (miRNAs) are small (~22 nucleotides) noncoding RNAs which post-transcriptionally regulate gene expression by binding complementary sequences within messenger RNAs (mRNAs). The human genome encodes over 1800 miRNAs, which target about 60% of human genes. Consequently, miRNAs are likely to regulate many complex processes in the body, indeed aberrant expression of various miRNA species has been implicated in numerous disease states, including cardiovascular diseases. MiR-1 and MiR-133A are the most abundant miRNAs in the heart and have both been reported to regulate cardiac ion channels.

We hypothesised that, as a consequence of their role in regulating cardiac ion channels, genetic variation in the genes which encode MiR-1 and MiR-133A might explain some cases of LQTS.

Results

Four miRNA genes (*miR-1-1*, *miR-1-2*, *miR-133a-1* & *miR-133a-2*), which encode MiR-1 and MiR-133A, were sequenced in 125 LQTS probands. No genetic variants were identified in *miR-1-1* or *miR-133a-1*; but in miR-1-2 we identified a single substitution (*n.100G>A*) and in *miR-133a-2* we identified two substitutions (*n.-19G>A* and *n.98C>T*). None of the variants affect the mature miRNA products.

Conclusion

2

Our findings indicate that sequence variants of *miR-1-1*, *miR-1-2*, *miR-133a-1* and *miR-133a-2* are not a cause of LQTS.

Keywords: long-QT syndrome, miRNA, genetics

Background

Long QT syndrome (LQTS) is a genetic condition characterised by prolongation of the QT interval, syncopal attacks, T-wave abnormalities, ventricular tachycardia of the torsades de pointes (TdP) type and an increased risk of sudden death [1].

The population prevalence of LQTS is estimated to be between 1:2000 and 1:5000 [1, 2] and the disease phenotype is associated with highly variable expressivity [3] and incomplete penetrance [4]. Presently, using genetic screening and DNA sequencing techniques, over 700 LQTS-causing mutations have been identified in 13 genes [5, 6]. These genes are involved in the correct execution of the cardiac action potential. Genetic screening of the five most frequently affected genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*) results in the identification of a disease-causing mutation in 50-70% of symptomatic LQTS cases [7]; the additional eight genes, which complete the list of LQTS-causative genes known presently, each account for very few LQTS cases [5, 6]. A number of LQTS cases, with as-yet-unknown genetic aetiology, might be attributable to mutations in genes which regulate the expression of these LQTS-causing genes.

Initially characterised by Lee et al [8] in 1993, microRNAs (miRNA) are first transcribed as long pri-miRNA transcripts containing a stem loop secondary structure, which, when excised by splicing or cleaved by the nuclear RNA cleaving enzyme, Drosher, is called pre-miRNA. Removal of the terminal loop from the pre-miRNA by the cytoplasmic RNA cleaving enzyme, Dicer, produces the small (~22 nucleotides) mature miRNA duplex [9]. Usually, one strand is preferentially selected for entry into the RNA-induced silencing complex (RISC), while the other strand, known as the miRNA* strand, is degraded. RISC is a multi-protein complex which uses the single stranded miRNA as a template for recognizing complementary messenger RNAs (mRNAs). The targeted transcripts are post-transcriptionally regulated through the binding these complementary sequences and subsequent repression of gene expression through the regulation of mRNA

translation or degradation [8, 10-12]. The miRNAs of animals achieve this regulation through an imperfect association with mRNA target regions [13-15]. Furthermore, this incomplete complementarity with targets provides an opportunity for animal miRNA's to bind multiple different mRNA targets, similarly a given target might bind multiple miRNAs [16, 17].

The human genome may encode over 1800 miRNAs, [18, 19] which target about 60% of human genes [13, 20]. Consequently, miRNAs are likely to be involved in most biological processes [14, 21]. Indeed, miRNAs have been reported to regulate many complex processes in the body and aberrant expression of various miRNA species has been implicated in numerous disease states including cancer [22], diabetes [23, 24], systemic lupus erythematosus [25] and cardiovascular disease e.g. heart failure, hypertrophy, conduction disturbances and arrhythmogenesis [26-35].

MiR-1 and MiR-133A are muscle-specific miRNAs, expressed predominantly in the heart and skeletal muscle [38], they are the most abundant miRNAs expressed in the heart [30, 39]. The bicistronic miRNA clusters encoding *miR-133a-1/miR-1-2* (located on chromosome 18) and *miR-133a-2/miR-1*-1 (located on chromosome 20) are regulated cooperatively by the myocyte enhancer factor-2 (MEF2) transcription factor and the serum response factor (SRF) which are essential regulators of muscle development [36, 37]. The sequences of the mature *miR-1-1* and *miR-1-2* are identical (MiR-1), as are those of *miR-133a-1* and *miR-133a-2* (MiR-133A). Targets of MiR-1 and MiR-133A include those mRNA transcripts encoding proteins involved in cardiovascular development [29], hypertrophy [26, 27] and ion channel function [30]. Ion channels, such as *RYR2*, *KCNQ1* and *KCND2*, have been shown to be regulated, at least in part, by MiR-1 or MiR-133A [34, 35, 40].

MiR-1 was shown to play a role in cardiac morphogenesis and conduction in a genetic knockout mouse model [35]. Mice lacking miR-1-2 show an approximate 50% lethality between late embryogenesis and

shortly after birth, as a consequence of ventricular septal defects. Postnatal survivors displayed a range of cardiac phenotypes, however while most displayed no structural dysfunction many suffered sudden death. Electrocardiography of mutant mice showed that many mutant carriers displayed prolonged ventricular depolarisation [35]. Mice lacking either MiR-133A gene did not exhibit cardiac defects nor was their life span reduced compared to control animals [41]. However, increased levels of MiR-133A prolong the action potential in isolated ventricular myocytes and cause QT prolongation in mice [31]. Furthermore, using a guinea pig model, Shan et al could show that increasing MiR-1 and MiR-133A induced QT prolongation, while antisense silencing of MiR-1 and MiR-133A abolished Arsenic trioxyde-induced QT prolongation [42]. MiR-133A was shown to bind to the 3'UTR of KCNQ1 mRNA which encodes Kv7.1 [40]. Kv7.1 is the pore-forming subunit of the voltage-gated potassium channel which is responsible for the I_{Ks} cardiac potassium current. This current is essential for the repolarisation phase of the cardiac action potential, and mutations in *KCNQ1* are known to cause a loss-of-function phenotype, which manifests clinically as LQTS [5].

We hypothesised that genetic variation in MiR-1 or MiR-133A might explain some cases of LQTS as the phenotype is a result of loss-of-function and gain-of-function perturbations in cardiac ion channels.

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Results and Discussion

No genetic variants were identified in *miR-1-1*; but in *miR-1-2* we identified a single substitution (*n.100G>A*) in all probands screened (116 homozygote carriers and 9 heterozygote carriers). The allele frequency of the A-allele is 0.964 in this cohort (Figure 1). This polymorphism has been reported in dbSNP (rs9989532) with a reported allele frequency, in the CEU population, of the A allele of 0.988. Multi-species sequence comparison of DNA sequences similar to the miR-1-2 gene region is represented in figure 2; the *n.100G* nucleotide is highly conserved among all mammals queried as well as zebrafish in a region which is highly conserved among apes.

No sequence variants were detected in *miR-133a-1*, but in *miR-133a-2* we identified two substitutions (*n.-19G>A* and *n.98C>T*) (Figure 1). The *n.-19G>A* polymorphism occurred in 64 probands (8 homozygote carriers and 56 heterozygote carriers) corresponding to a minor allele frequency (MAF) of 0.288, while the *n.98C>T* variant occurred in a single individual and thereby had a MAF of 0.004 in this cohort. Similar allele frequencies are reported in dbSNP for these variants; rs13040413, *n.19G>A* has a reported MAF of 0.2824 in the CEU population and rs200375711, *n.98C>T* has a reported MAF of 0.002 in the ClinSeq population, a cohort of primarily Caucasian, atherosclerotic heart patients [43], this variant was not identified in the 1000 genomes populations. Multi-species sequence comparison of DNA sequences similar to the *miR-133a-2* gene region is represented in figure 3; the *n.-19G* nucleotide is not conserved, however the *n.98C* nucleotide is highly conserved among all mammals queried as well as chicken and zebrafish; the region surrounding *n.98C* is highly conserved among apes.

Presently, mutations in 13 genes are described to cause LQTS; taken together mutations in these genes explain 50-70% of LQTS cases. This means that causality in 30-50% of LQTS cases is as yet unknown. Animal models suggest that miRNAs might be involved in the regulation of cardiac action potential [35].

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A number of associations between SNPs in predicted miRNA binding sites on target mRNAs and phenotypic traits have been reported [22, 44-46]. A limitation of this study is that we have not assessed the MiR-1 and MiR-133A binding sites of putative target transcripts; instead we focused on identifying genetic variation within the miRNA genes themselves.

SNPs are rarely seen in the seed regions (short 3-8 nucleotide regions important for target specificity) of miRNAs; which suggests a strong selective constraint on the seed regions of mature miRNA. However, Mencia et al provided the first example of human inherited condition associated with miRNA mutations; when they discovered that mutations in the seed region of MiR-96 were responsible for non-syndromic progressive hearing loss in two families [47]. Variants outside of the seed region of mature miRNA have also been associated with clinical phenotypes; Dorn et al demonstrated that a rare variant in mature MiR-499 protected against cardiomyopathy in a transgenic mouse model [48] and Ohanian et al identified a genetic variant in *mir-133a-2* which altered strand abundance resulting in an accumulation of the miRNA* strand in an atrial fibrillation patient [49]. Furthermore, several SNPs in pri-miRNA genes have been reported to affect processing and expression levels of mature miRNA [46].

Conclusion

To our knowledge, this is the first study to report miRNA genetic variation in LQTS patients. We found three allelic variations none of which affect the mature miRNA products, although the *miR-133a-2:n.98C>T* variant is present in the excised pre-miRNA molecule (figure 1) and may affect subsequent processing to mature MiR133A. Our findings indicate that sequence variation of *miR1-1*, *miR1-2*, *miR133a-1* and *miR133a-2* are not a cause of LQTS.

Methods

Patient samples

Unrelated probands, referred from specialist cardiology centres in Denmark and the UK to Statens Serum Institut for genetic investigation of LQTS (n=125; 70% female), were included in this retrospective study. The probands had been screened for mutations in *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2* by capillary-electrophoresis single strand conformation polymorphism (CE-SSCP) followed by DNA sequencing of aberrant conformers as described elsewhere [50-52]. Screening for mutations in *CAV3* was previously performed by direct sequencing [53]. Forty-eight (38%) had been found to carry a probably damaging variant in any one of these genes (Christiansen et al submitted 2012).

Genetic screening

DNA extraction

Genomic DNA (gDNA) was extracted from frozen EDTA-blood using a Qiagen kit (Qiagen, QmbH, Hilden, Germany).

Polymerase chain reaction

Four miRNA genes (*mir-1-1*, *mir-1-2*, *mir-133a-1* & *mir-133a-2*) were PCR amplified from 125 gDNA samples (Table 1). Primers were designed using NCBI/Primer-BLAST program [54] (Table1); all primers were modified with M13 tails (F: TGTAAAACGACGGCCAGT; R: CAGGAAACAGCTATGACC).

A 25 µl PCR mix incorporated 1 U of TEMPase Hot Start DNA Polymerase (Ampliqon ApS), 1x Tempase buffer I (Ampliqon ApS), 2mM dNTPs, 0.4 um of the forward and reverse primers, and 2.5 ng gDNA template.

PCR amplifications were performed in GeneAmp® PCR System 2700 (Applied Biosystems, Foster City, CA, USA) using the following amplification protocol: initial heat activation step at 95°C for 15 minutes, 38 cycles of 30 s at denaturing temperature 94°C, 45 s at annealing temperature 58°C, and 40 s at extension temperature 72°C, and a final indefinite hold step of 4°C.

PCR products were quantified by 2% agarose gel electrophoresis and product size was confirmed through a visual comparison with Sigma Direct load Wide Range DNA marker (Sigma-Aldrich Denmark A/S).

Direct sequencing and data analysis

PCR products were purified using ExoSap (Affymetrix, Santa Clara, CA, USA) and sequenced using the Big Dye dideoxy terminator cycle sequencing kit (Applied Biosystems) and the 3730 DNA Analyzer (Applied Biosystems). Sequence analysis was carried out using the Sequencher 4.9 software (Gene Codes Corporation, Ann Arbor, MI, USA)

Multiple sequence alignment and nucleotide conservation across species

BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analyses of the two miRNA genes which harboured nucleotide variants (*miR-1-2* – NR_029662.1 and *miR-133a-2* – NR_029676.1) identified several similar sequences in a number of species. These sequences were aligned in Bioedit using ClustalW in order to identify conserved regions [55].

Prediction of RNA secondary structure

RNAfold Webserver (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) was used to compute the minimum free energy (MFE), the partition function, the matrix of base pairing probabilities, and the centroid structure [56].

List if abbreviations

CE-SSCP	capillary-electrophoresis single strand conformation polymorphism
g DNA	genomic DNA
LQTS	long QT syndrome
MAF	minor allele frequency
MEF2	myocyte enhancer factor-2
MFE	minimum free energy
miRNA	microRNA
mRNA	messenger RNA
pre-miRNA	preliminary microRNA
pri-miRNA	primary microRNA
RISC	RNA-induced silencing complex
SRF	serum response factor
TdP	torsade de pointes

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PLH participated in the design of the study, interpreted the data and drafted the manuscript. ALC participated in the design and coordination of the study and critical revision of the manuscript. KMC made substantial contributions to the conception of the study, acquired data and interpreted data. JKK provided data and critically revised the manuscript. ERB provided data. VAC critically revised the manuscript. MC conceived of the study, participated in its design and critically revised the manuscript. All authors read and approved the final manuscript.

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References

1. Crotti L, Celano G, Dagradi F, Schwartz PJ: **Congenital long QT syndrome.** *Orphanet J Rare Dis* 2008, **3**:18.

2. Goldenberg I, Zareba W, Moss AJ: **Long QT Syndrome.** *Curr Probl Cardiol* 2008, **33:**629-694.

3. 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:**846-852.

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

5. Hedley PL, Jorgensen 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:**1486-1511.

6. Yang Y, Yang Y, Liang B, Liu J, Li J, Grunnet M, Olesen S-P, Rasmussen HB, Ellinor PT, Gao L, et al: **Identification of a Kir3.4 Mutation in Congenital Long QT Syndrome.** *The American Journal of Human Genetics* 2010, **86:**872-880.

7. Christiansen M, Hedley PL: **Long QT testing: implications for complex diagnosis in personalized medicine.** *Personalized Medicine* 2010, **7:**125-127.

8. Lee RC, Feinbaum RL, Ambros V: **The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14.** *Cell* 1993, **75:**843-854.

9. Carthew RW, Sontheimer EJ: **Origins and Mechanisms of miRNAs and siRNAs.** *Cell* 2009, **136:**642-655.

10. Bartel DP: **MicroRNAs: genomics, biogenesis, mechanism, and function.** *Cell* 2004, **116:**281-297.

11. Filipowicz W, Bhattacharyya SN, Sonenberg N: **Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?** *Nat Rev Genet* 2008, **9:**102-114.

12. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T: **Identification of novel genes coding for small expressed RNAs.** *Science* 2001, **294:**853-858.

13. Lewis BP, Burge CB, Bartel DP: **Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets.** *Cell* 2005, **120:**15-20.

14. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB: **Prediction of mammalian microRNA targets.** *Cell* 2003, **115:**787-798.

15. Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R: **Control of translation and mRNA degradation by miRNAs and siRNAs.** *Genes Dev* 2006, **20:**515-524.

16. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, Rajewsky N: **Combinatorial microRNA target predictions.** *Nat Genet* 2005, **37:**495-500.

17. Rajewsky N: **microRNA target predictions in animals.** *Nat Genet* 2006, **38 Suppl:**S8-13.

18. **miRBase** [http://www.mirbase.org/cgi-bin/mirna_summary.pl?org=hsa]

19. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, et al: **Identification of hundreds of conserved and nonconserved human microRNAs.** *Nat Genet* 2005, **37:**766-770.

20. Friedman RC, Farh KK, Burge CB, Bartel DP: **Most mammalian mRNAs are conserved targets of microRNAs.** *Genome Res* 2009, **19:**92-105.

21. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM: **Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs.** *Nature* 2005, **433:**769-773.

22. Ryan BM, Robles AI, Harris CC: **Genetic variation in microRNA networks: the implications for cancer research.** *Nat Rev Cancer* 2010, **10:**389-402.

23. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M: **A pancreatic islet-specific microRNA regulates insulin secretion.** *Nature* 2004, **432:**226-230.

24. Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, Zavolan M, Stoffel M: **miR-375 maintains normal pancreatic alpha- and beta-cell mass.** *Proc Natl Acad Sci U S A* 2009, **106:**5813-5818.

25. Carlsen AL, Schetter AJ, Nielsen CT, Lood C, Knudsen S, Voss A, Harris CC, Hellmark T, Segelmark M, Jacobsen S, et al: **Circulating microRNA expression profiles associated with systemic lupus erythematosus.** *Arthritis Rheum* 2013, **65:**1324-1334.

26. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, et al: **MicroRNA-133 controls cardiac hypertrophy.** *Nat Med* 2007, **13:**613-618.

27. Ikeda S, He A, Kong SW, Lu J, Bejar R, Bodyak N, Lee KH, Ma Q, Kang PM, Golub TR, Pu WT: **MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes.** *Mol Cell Biol* 2009, **29:**2193-2204.

28. Kim GH: **MicroRNA regulation of cardiac conduction and arrhythmias.** *Transl Res* 2013, **161:**381-392.

29. Liu N, Olson EN: **MicroRNA regulatory networks in cardiovascular development.** *Dev Cell* 2010, **18:**510-525.

30. Luo X, Zhang H, Xiao J, Wang Z: **Regulation of human cardiac ion channel genes by microRNAs: theoretical perspective and pathophysiological implications.** *Cell Physiol Biochem* 2010, **25:**571-586.

31. Matkovich SJ, Wang W, Tu Y, Eschenbacher WH, Dorn LE, Condorelli G, Diwan A, Nerbonne JM, Dorn GW, 2nd: **MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts.** *Circ Res* 2010, **106:**166-175.

32. Nair N, Kumar S, Gongora E, Gupta S: **Circulating miRNA as novel markers for diastolic dysfunction.** *Mol Cell Biochem* 2013, **376:**33-40.

33. Small EM, Olson EN: **Pervasive roles of microRNAs in cardiovascular biology.** *Nature* 2011, **469:**336-342.

34. Terentyev D, Belevych AE, Terentyeva R, Martin MM, Malana GE, Kuhn DE, Abdellatif M, Feldman DS, Elton TS, Gyorke S: **miR-1 overexpression enhances Ca(2+) release and promotes cardiac arrhythmogenesis by targeting PP2A regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2.** *Circ Res* 2009, **104:**514-521.

35. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D: **Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2.** *Cell* 2007, **129:**303-317.

36. Liu N, Williams AH, Kim Y, McAnally J, Bezprozvannaya S, Sutherland LB, Richardson JA, Bassel-Duby R, Olson EN: **An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133.** *Proc Natl Acad Sci U S A* 2007, **104:**20844-20849.

37. Zhao Y, Samal E, Srivastava D: **Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis.** *Nature* 2005, **436:**214-220.

38. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T: **Identification of tissue-specific microRNAs from mouse.** *Curr Biol* 2002, **12:**735-739.

39. Liang Y, Ridzon D, Wong L, Chen C: **Characterization of microRNA expression profiles in normal human tissues.** *BMC Genomics* 2007, **8:**166.

40. Xiao L, Xiao J, Luo X, Lin H, Wang Z, Nattel S: **Feedback remodeling of cardiac potassium current expression: a novel potential mechanism for control of repolarization reserve.** *Circulation* 2008, **118:**983-992.

41. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, Olson EN: **microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart.** *Genes Dev* 2008, **22:**3242-3254.

42. Shan H, Zhang Y, Lu Y, Zhang Y, Pan Z, Cai B, Wang N, Li X, Feng T, Hong Y, Yang B: **Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines.** *Cardiovasc Res* 2009, **83:**465-472.

43. Biesecker LG: **Opportunities and challenges for the integration of massively parallel genomic sequencing into clinical practice: lessons from the ClinSeq project.** *Genet Med* 2012, **14:**393-398.

44. Borel C, Antonarakis SE: **Functional genetic variation of human miRNAs and phenotypic consequences.** *Mamm Genome* 2008, **19:**503-509.

45. Kim J, Bartel DP: **Allelic imbalance sequencing reveals that single-nucleotide polymorphisms frequently alter microRNA-directed repression.** *Nat Biotechnol* 2009, **27:**472-477.

46. Saunders MA, Liang H, Li WH: **Human polymorphism at microRNAs and microRNA target sites.** *Proc Natl Acad Sci U S A* 2007, **104:**3300-3305.

47. Mencia A, Modamio-Hoybjor S, Redshaw N, Morin M, Mayo-Merino F, Olavarrieta L, Aguirre LA, del Castillo I, Steel KP, Dalmay T, et al: **Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss.** *Nat Genet* 2009, **41:**609-613.

48. Dorn GW, 2nd, Matkovich SJ, Eschenbacher WH, Zhang Y: **A human 3' miR-499 mutation alters cardiac mRNA targeting and function.** *Circ Res* 2012, **110:**958-967.

49. Ohanian M, Humphreys DT, Anderson E, Preiss T, Fatkin D: **A heterozygous variant in the human cardiac miR-133 gene, MIR133A2, alters miRNA duplex processing and strand abundance.** *BMC Genet* 2013, **14:**18.

50. Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, McKenna WJ, Christiansen M: **High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia.** *Clin Genet* 2006, **69:**504-511.

51. Larsen LA, Andersen PS, Kanters JK, Jacobsen JR, Vuust J, Christiansen M: **A single strand conformation polymorphism/heteroduplex (SSCP/HD) method for detection of mutations in 15 exons of the KVLQT1 gene, associated with long QT syndrome.** *Clin Chim Acta* 1999, **280:**113-125.

52. Larsen LA, Christiansen M, Vuust J, Andersen PS: **High-throughput single-strand conformation polymorphism analysis by automated capillary electrophoresis: robust multiplex analysis and pattern-based identification of allelic variants.** *Hum Mutat* 1999, **13:**318-327.

53. Hedley PL, Kanters JK, Dembic M, Jespersen T, Skibsbye L, Aidt FH, Eschen O, Graff C, Behr ER, Schlamowitz S, et al: **The role of CAV3 in long QT syndrome: clinical and functional assessment of a caveolin-3/Kv11.1 double heterozygote versus caveolin-3 single heterozygote.** *Circulation: Cardiovascular Genetics* 2013, **Accepted**.

54. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL: **Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction.** *BMC Bioinformatics* 2012, **13:**134.

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55. Hall TA: **BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.** *Nucleic Acids Symposium Series* 1999, **41:**95-98.

56. Gruber AR, Lorenz R, Bernhart SH, Neubock R, Hofacker IL: **The Vienna RNA websuite.** *Nucleic Acids Res* 2008, **36:**W70-74.

57. Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA: **An integrated map of genetic variation from 1,092 human genomes.** *Nature* 2012, **491:**56-65.

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Figure Legends

Figure 1. A) Genomic structure of the miR-1 and miR-133 clusters. B) Genomic structure of *mir-1-2* and *mir-133a-2*, variants identified in this study are represented by a red line. The minor allele frequencies (MAF) indicated here are representative of the CEU population as reported in 1000 genomes [57], except rs200375711 (*mir-133a-2:n.98C>T*) which was not identified in 1000 genomes but was identified in one of 493 atherosclerosis patients of European descent from the ClinSeq whole-exome sequencing project [43].

Figure 2. A) Pri-miR-1-2 multiple species sequence alignment. Pre-miR-1-2 is indicated in a blue box, mature MiR-1 is indicated in a red box, the seed region is highlighted, rs9989532 is indicated by a black arrow. B) Pre-miR-1-2 secondary structure, mature MiR-1 is indicated in Red. Secondary structure was predicted using RNAfold Web Server [56]

Figure 3. A) Pri-miR-133a-2 multiple species sequence alignment. Pre-miR-133a-2 is indicated in a blue box, mature MiR-133A is indicated in a red box, the seed region is highlighted, rs13040413 and rs200375711 are indicated by black arrows. B) Pre-miR133a-2 secondary structure, mature MiR-133A is indicated in Red, n.98C is indicated in blue. Secondary structure was predicted using RNAfold Web Server [56].

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Table 1. Primers for *mir-1-1*, *mir-1-2*, *mir-133a-1* and *mir-133a-2* amplification

NCBI REF SEQ	MIRNA GENE	FORWARD PRIMER	REVERSE PRIMER	AMPLICON
NR_029780.1	mir-1-1 (71 bp)	ACACAGAGAGGGCTCCGGCA	ACACGACCGTCCACCAACGC	342 bp
NR_029662.1	mir-1-2 (85 bp)	TTGCCAAAGGTCATCTGTTCATGACT	TGGAACCATTAATGCCATGCTTCAGG	365 bp
NR_029675.1	mir-133a-1 (88 bp)	AGCGCAGGAAAACAGTAGGA	TTTGAAATCCTTAAGTCATCCATACA	444 bp
NR_029676.1	mir-133a-2 (102 bp)	ATCTCCATCGGGACTGCTT	GGGCTTCACTTACTTGGAGCT	264 bp

Figure 1

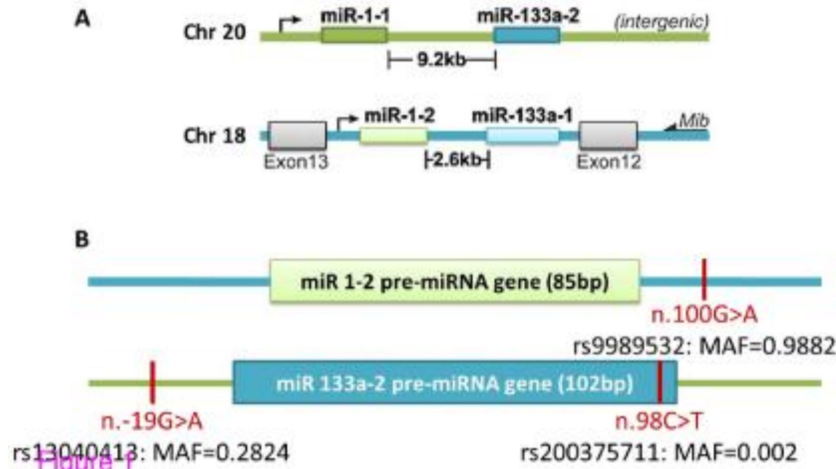


Figure 2

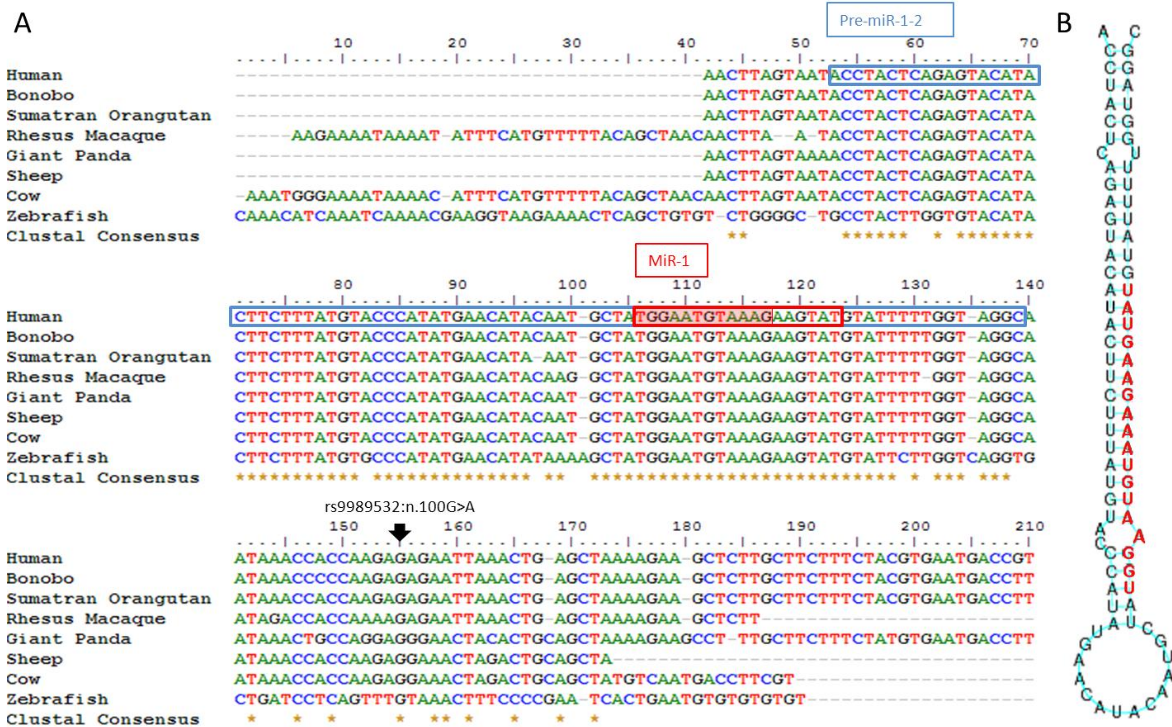
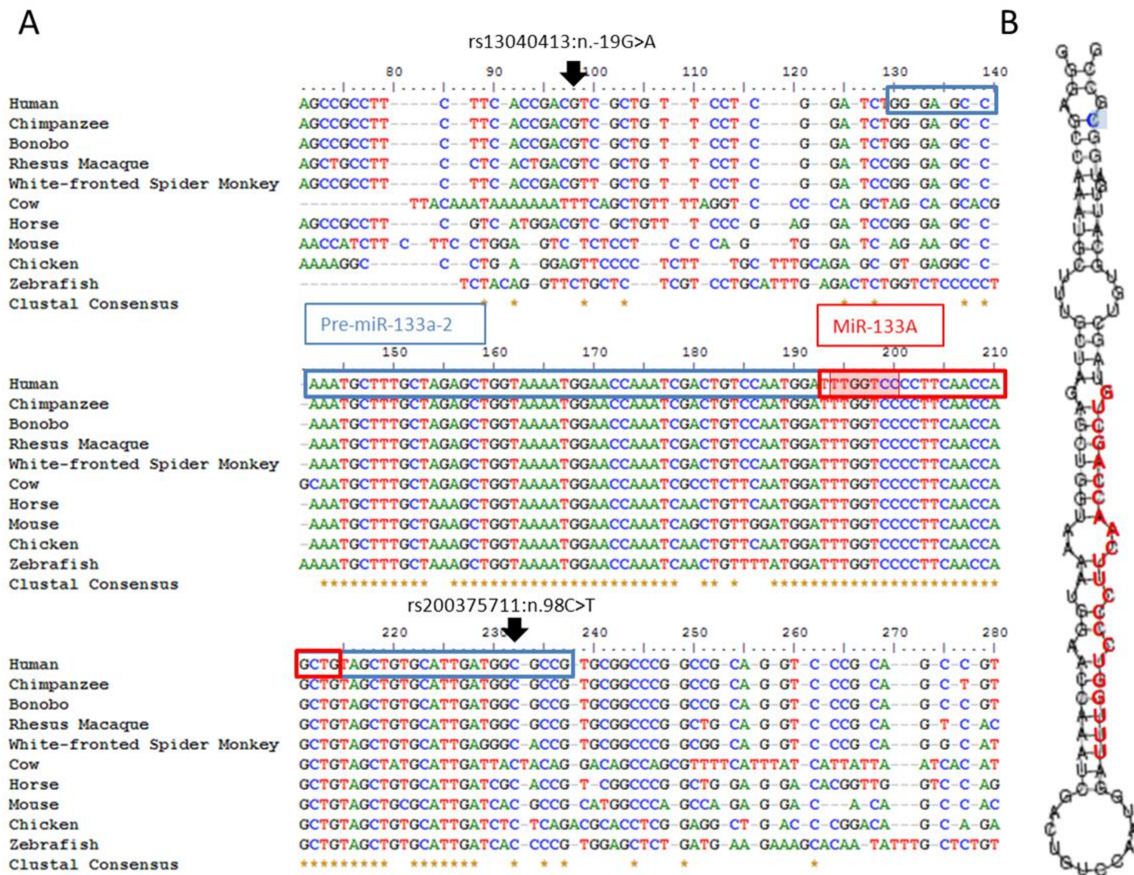


Figure 3



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CHAPTER 5: SODIUM CHANNEL DISEASE

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PAPER 5: FLECAINIDE PROVOCATION REVEALS CONCEALED BRUGADA SYNDROME IN A LONG QT SYNDROME FAMILY WITH A NOVEL L1786Q MUTATION IN SCN5A

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Flecainide provocation reveals concealed Brugada syndrome in a Long QT Syndrome family with a novel L1786Q mutation in SCN5A

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Short title: Brugada Syndrome with SCNA5A L1786Q mutation

5166 words

Keywords: Sodium channel blockade – repolarization – T-wave morphology – Ion Channel Kinetics

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great care should be taken in defining the phenotype. It may be considered to let LQTS patients with *SCN5A* mutations undergo a sodium blocker provocation test.

For Review Only

ABSTRACT

Background: Mutations in *SCN5A*, encoding the cardiac sodium channel Nav1.5, can result in both long QT type 3 (LQT3) and Brugada syndrome (BrS). However, a few mutations have shown an overlapping phenotype in single patients as well as between family members. Long QT syndrome is characterized by a prolonged QT interval and a prerequisite for a BrS diagnosis is an ST elevation in the right precordial leads of the electrocardiogram (ECG). These ST elevations are dynamic and may only appear after sodium channel blockage. Hence, diagnosing BrS patients can be difficult.

Results: In a Danish family suffering from long QT syndrome, a novel missense mutation in *SCN5A*, changing a leucine residue into a glutamine residue at position 1786 (L1786Q) was found to be present in heterozygous form co-segregating with prolonged QT-interval. The proband presented with an aborted cardiac arrest, and his mother died suddenly and unexpectedly at the age of 65. Flecainide treatment revealed coved ST elevation in all mutation carriers. Electrophysiological investigations of the mutant in HEK293 cells revealed a reduced peak current, a negative shift in inactivation properties and a positive shift in activation properties, compatible with BrS. Furthermore, the sustained ($I_{Na,late}$) TTX sensitive sodium current was found drastically increased, explaining the association between the mutation and LQT syndrome.

Conclusion: The L1786Q mutation is associated with a combined LQT3 and concealed BrS phenotype explained by gating characteristics of the mutated ion channel protein. Hence, sodium channel blockade should be considered in clinical evaluation of apparent LQT3 patients.

Keywords: LQTS, BrS, SCN5A, electrophysiology, comorbidity

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INTRODUCTION

Mutations in the *SCN5A* gene, encoding the voltage gated cardiac sodium channel, can lead to a multitude of diseases[1] including long QT syndrome (LQTS), Brugada syndrome (BrS)[2], progressive cardiac conduction defect disease (CCD)[3], sinus node disease[4], and dilated cardiomyopathy[5]. Many of the mutations in *SCN5A* are only linked to one of these diseases, although combined phenotypes like BrS + LQTS[6] or BrS + CCD[3] have been described. In the latter family it was noted that CCD and BrS ran in separate branches of the family despite having the same SCN5A mutation (G1406R)[3], suggesting that other genetic factors may play a role. These additional genetic factors may be polymorphisms in other genes which have been shown to influence the QT interval in control populations[7], as well as congenital [8-10] and acquired LQTS cohorts[10]. There is a large overlap in ECG phenotypes between healthy and affected subjects complicating the clinical diagnosis of both LQTS and BrS. Approximately 10 % of LQTS mutation carriers have a normal QT interval[11]. Although the prognosis of LQTS mutation carriers with normal QT intervals is generally better than in carriers with a prolonged QT interval there is still an increased risk of sudden cardiac death, warranting treatment with beta blockers and/or implantable cardioverter defibrillator (ICD)[12]. The ECG phenotype in BrS is even more complicated by intraindividual variability, with the occasional normalization of ST segment elevation occurring in many carriers. Furthermore, it is well known from BrS[13] that it is not uncommon for healthy subjects in screening studies to show either an ST segment elevation in the right precordial leads or a shortened QT interval. Unless coexisting with symptoms or a family history these ECG parameters may be normal variants without association with sudden cardiac death. LQTS in patients with a *SCN5A* mutation is due to gain-of-function of the late sodium current, categorized as LQT3, leading to an increased persisting depolarizing late sodium current prolonging the cardiac action potential. In contrast, BrS caused by *SCN5A* mutations is due to a loss-of-function of the sodium channel. This can be caused either by reduced I_{Na} peak amplitudes, negative voltage shift in steady-state inactivation, positive shift in activation, reduced release from inactivation or any combinations of these changes in gating. As

the reduction in the transient sodium current in *SCN5A* BrS patients occurs in the initial part of the action potential (phase 1), whereas the persistent late sodium current in *SCN5A* LQTS patients are active throughout the whole action potential, coexistence of the two apparent opposing sodium channel phenotypes is possible.

Here we describe a family with a novel *SCN5A* mutation (L1786Q) which presented with a clinical LQTS phenotype. Due to subtle early repolarization, an atypical arrhythmia initiation mechanism and the location of the *SCN5A* mutation near the position of other mutations underlying mixed phenotypes, we performed a Flecainide test in both the proband and the other *SCN5A* L1786Q gene carriers revealing a clear type 1 ST segment elevation pattern indicating BrS.

METHODS

FLECAINIDE TEST

Flecainide 2 mg/kg bodyweight (maximal 150 mg) was injected over 10 min into a cubital vein with simultaneous ECG recordings during flecainide administration and 30 min afterwards.

CONSERVATION SCORE

The conservation score was calculated as described by Jons et al.[14] with the ten sodium channels (SCN1A-SCN11A) in figure 1 as reference values. A score of 1 indicates maximal conservation and 0 no conservation.

GENETIC TESTING

Mutation screening of *SCN5A* was performed with capillary array electrophoresis (CAE) single stranded conformation polymorphism analysis (SSCP)[15, 16] on genomic DNA isolated from blood, followed by direct DNA sequencing of aberrant conformers. The primers used are described previously in detail[16]. The proband was also screened for mutations in *KCNQ1, KCNH2* (HERG), *KCNE1* (MinK), and *KCNE2* (MiRP1). Primer sequences and conditions are available upon request (mic@ssi.dk). The mutation was denoted based on the full-length 2,016 amino acid splice variant (Genebank NM_198056.2 / NP_932173.1).

DNA CONSTRUCTS

Human SCN5A (hH1) in pcDNA3 was a gift from Dr. H. Abriel (Lausanne University). The L1786Q mutation was introduced into pcDNA3-hSCN5A by the use of overlapping oligonucleotides followed by full plasmid PCR. The integrity of the sub-cloned construct was verified by sequencing.

PATCH-CLAMPING

For electrophysiological studies, HEK293 cells were transiently co-transfected with 0.3 µg Wild-Type (WT) or L1786Q Nav1.5 encoding constructs together with 0.2 µg of pcDNA3-EGFP as a reporter gene, using Lipofectamine (Invitrogen, USA) according to the manufacturer instructions. Measurements were performed 1–3 days after transfection. Whole-cell currents

were measured at 37±1 °C, except for the measurements of the sustained current, which were done at room temperature. The internal pipette solution (mM) was: CsCl 60, Cesium aspartate 70, $CaCl_2$ 1, $MgCl_2$ 1, Hepes 10, EGTA 11, Na_2ATP 5, pH 7.2, with CsOH; external solution (mM): NaCl 130, CsCl 5, $CaCl_2$ 2, $MgCl_2$ 1.2, Hepes 10, glucose 5, pH 7.4, with CsOH. Measurements were controlled with Pulse software (HEKA Elektronik, Lambrecht, Germany) and using an EPC-9 amplifier (HEKA Elektronik, Lambrecht, Germany). Borosilicate glass pipettes were pulled on a DPZ-Universal puller (Zeit Instrumente, Munich, Germany). The pipettes had a resistance of 1.5–2.5 MΩ when filled with intracellular solution. The series resistances recorded in the whole-cell configuration were 2–4 MΩ and were compensated (80 %). No leak subtraction was performed. The seal resistance was in all experiments above 1.5 GΩ, and for the sustained current measurements above 2 GΩ. I_{Na} was blocked with 50 μM tetrodotoxin (TTX) (Alomone labs, Jerusalem, Israel). The sustained current data shown in Figure 2 and 3 is an average value of the last 50 ms (450-500 ms) of the voltage steps. Series resistance was < 10 MΩ during the entire experiment. Update was performed between each sweep.

Data analysis: Peak current densities were measured during an activation protocol and I_{Na} densities (pA/pF) were obtained by dividing the peak I_{Na} by the cell capacitance. For the activation and steady-state inactivation curves, data from individual cells were fitted with a Boltzmann equation, $y(V_m) = 1/[1 + \exp[(Vm-V_{1/2})/K]]$, in which y is the normalized current or conductance, V_m the membrane potential, $V_{1/2}$ the voltage at which half of the channels are activated or inactivated, and K the slope factor. Electrophysiological data were analyzed using Excel (Microsoft), Igor Pro (Wavemetrics, Lake Oswego, OR, USA), GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA).

STATISTICAL ANALYSIS

Data are represented as mean values ± SEM. Two-tailed Student's t-test was used to compare means; $p < 0.05$ was considered as statistically significant.

RESULTS

GENETICS

Genetic analysis was performed in all family members with the exception of the proband's mother (Figure 4: I-2) where the carrier status was assumed from the fact that she died suddenly and unexpectedly at age 65 and that her husband did not carry the mutation, making her an obligate carrier. All clinically affected subjects (black circles/squares) were heterozygous carriers of a novel missense mutation in *SCN5A*, L1786Q. The mutated residue is highly conserved in voltage-gated Na^{+}-channels with a conservation score of 1.0[14] as the original leucine is present in all known human SCN-proteins and, additionally, cross-species alignment of Nav1.5 reveals this leucine to be conserved beyond the mammalian kingdom (Figure 4). All clinically unaffected family members were found to have the wild-type with lysine at codon 1786.

CLINICAL PRESENTATION

The proband (Figure 4: II-1) was resuscitated after a cardiac arrest at the age of 48 without any neurological deficits. The ECG revealed a clearly prolonged corrected QT interval of 0.48 sec, but without signs of an ST segment elevation pattern in the resting ECGs (Figure 5A). The proband was not inducible during an electrophysiology (EP) study using a standard protocol with up to three extra stimuli (minimum of 200 ms) and two basic drive cycle lengths (600 and 400 ms) from the right ventricular apex and outflow tract. Due to the cardiac arrest an ICD was implanted. Afterwards the proband received three appropriate shocks. During flecainide testing a coved type 1 ST segment elevation pattern was induced in lead V1. The brother (Figure 4: II-3) and a niece of the proband (Figure 4: III-3) also had clear QT prolongation without significant BrS pattern, but after flecainide provocation a clear BrS type 1 pattern were seen (Figure 5 E+F). The brother was asymptomatic but his daughter had experienced a single syncopal event at home following a tooth extraction with infection. Both were treated with a prophylactic ICD.

The brother's daughter did not experience any shocks during follow-up, whereas the brother has received two inappropriate shocks due to episodes of rapidly conducted atrial flutter.

Figure 5C and D shows the recorded ICD electrogram (EGM) strips with two induction modes of arrhythmia: LQTS mode with short long short coupling sequence (Figure 5D) and BrS mode with a sudden premature beat eliciting the ventricular tachycardia (Figure 5C).

WHOLE-CELL PATCH CLAMPING

In order to investigate whether altered biophysical properties of the L1786Q Nav1.5 mutation can explain the observed patient phenotype voltage-clamp patch clamping was performed. Transient expression in HEK293 cells, followed by whole-cell patch clamping of the cells superfused with 37±1 °C in an extracellular solution containing 130 mM sodium, revealed fast activating and inactivating sodium currents for both WT and L1786Q $Na_v1.5$ channels (Figure 2, Table 1). The applied voltage steps were preceded by a -120 mV step to insure full release from inactivation. The current peaks after 0.2-0.3 ms and is almost completely inactivated after 5-10 ms. However, although the L1786Q mutant produced measurable current, the peak current was reduced approximately 3.5-fold as compared to WT $Na_v1.5$ (Figure 2A,B,C). Analysis of steady-state activation and inactivation properties were performed to investigate whether these parameters could contributes to a reduced transient current, which could explain the BrS phenotype. The half steady-state activation potential was shifted from -28.5 mV to -13.3 mV (Figure 2E and Table 1), while the half steady-state inactivation potential was shifted from -67.0 mV to -88.3 mV for WT and L1786Q, respectively (Figure 2C & Table 1). A positive shift in activation potential and a negative shift in inactivation potential will lead to a reduced availability of the channels, which will be consistent with a loss-of-function phenotype as observed in BrS.

A biophysical explanation for the observed LQTS phenotype was addressed by analysing the late sodium current before and after addition of the sodium channel blocker TTX (Figure 3A). 50 µM TTX enforces an 80-90 % block of both WT and L1786Q $Na_v1.5$ conducted peak Na^+ current.

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These experiments were conducted at 20±1 ºC to maintain a stable recording over the time course of TTX application. The TTX block revealed a three-fold increase in the sustained ($I_{Na, late}$) L1786Q current as compared to WT current when holding the potential at -20 mV (Figure 3B) in spite of the fact that L1786Q peak current was reduced drastically (Figure 3C). Further, as the membrane potential in a cardiomyocyte changes throughout the action potential the voltage dependence of the sustained current was investigated at different voltages (Figure 2F). The sustained current was recorded in sodium channel expressing cells at 37±1 ºC without TTX block. Mock transfected HEK293 cells do only show a very low sustained current (data not shown). Both wt and L1786Q channels have voltage dependent sustained currents with the highest current at the most negative values. The L1786Q sustained current (normalised to cell size) is significantly larger at -15 mV to -30 mV. Thus, the data reveal an increased sustained current for the L1786Q mutant channel which would be compatible with the observed LQTS phenotype.

In summary, the electrophysiological investigations of the L1786Q $Na_v1.5$ sodium channel provide evidence for the observed mixed phenotype of LQT and BrS.

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DISCUSSION

The main finding of the study is that the *SCN5A* L1786Q mutation leads to an overlap syndrome with combined LQTS and BrS, without any signs of ST elevation in the resting ECG.

Today almost 100 mutations in *SCN5A* leading to BrS have been identified[2], some of them with overlap syndromes between LQTS, CCD and sick sinus node syndromes[17]. As shown in Table 2 only six other mutations have been clinically documented with comorbid BrS and LQTS. These six mutations span from the DII-III linker to the C-terminus.

The SCN5A L1786Q mutation is located in the C-terminal end of *SCN5A*, in proximity to the E1784K and 1795insD mutations[6, 18]. The C-terminus is thought to have a role in stabilizing the inactivated channel, where it interacts with the intracellular linker between the DIII and DIV domain of SCN5A[19]. As demonstrated in Figure 2, the L1786Q mutation leads to a reduced peak sodium current, a leftwards shift in the inactivation curve, and a rightward shift in the activation curve, which would result in a reduced availability of functional sodium channels and thereby attribute to a loss of function phenotype. Whereas most wild type channels are released from inactivation at the resting membrane potential, the leftward shift in voltage dependent inactivation makes a large fraction of L1786Q channels inactivated at the resting potential, hence, not available for activation during the depolarizing process. The rightward shift in activation kinetics causes the sodium channels to activate at more positive potentials, which would also result in reduced peak sodium current.

For the overlap mutations, encompassing a dual BrS and LQTS phenotype (Table 2), where electrophysiological analyses have been performed; a ubiquitous increase in late sodium current causes the LQTS phenotype, and a reduction of early sodium current causes the BrS phenotype. All but delK1500 had reduced peak sodium current, but the characterization of delK1500[20] was performed at a resting potential of -100 mV instead of at a more physiological value of -85 mV, masking the real effect on the peak sodium current difficult to quantify.

However, the large negative shift in the steady-state inactivation of delK1500 would lead to a net reduction of peak sodium current compatible with BrS.

L1786Q provides a significant increase in the late sodium current. Interestingly, our analyses show a voltage dependency of this late current. The L1786Q late current is more pronounced at more negative voltages. These data thereby indicate that the sustained current has the largest impact in the late part of the phase II of the action potential, which gradually shows a declining potential, and may as well have an impact on phase III repolarisation. Such a relative increase in a depolarising current in the later part of the action potential will lead to a prolongation of the action potential duration and thereby prolonged QT interval.

The clinical history of the proband indicates a malignant mutation with a course of aborted cardiac arrest and several occurrences of appropriate ICD shocks. According to the proposed diagnostic criteria, coved type ST segment elevation should be present in two right precordial leads (>2mm)[21, 22] which was not fulfilled in the proband. However recently it has been shown that patients with only single lead coved ST elevation in lead V1 or V2 has similar prognosis as the classical pattern with elevation in two leads[23].

From the ICD print-outs (Figure 5 C+D), it is clear that there are two modes of initiating events. In Figure 5D a premature ventricular beat is followed by a post ectopic pause giving rise to the short-long-short sequence classical for LQTS, but in Figure 5C an early premature ventricular beat with a short coupling interval suggests a phase 2 re-entry arrhythmia[24], a classical pattern for BrS.

Treatment of overlap syndrome patients with combined LQTS+BrS impose a clinical problem. Beta blockers are effective in LQTS, but are known to increase ST elevation in BrS[25]. Sodium blocker may worsen BrS, although quinidine has been suggested to be useful in BrS due to the beneficial I_{to} blocking effect, but its I_{Kr} blocking abilities could induce further QT prolongation and torsades de pointes. Mexilitine has been shown not to unmask BrS in the overlap mutation SCN5A E1784K, but there are no reports of its continued use in these overlap syndrome

patients. A new alternative could be ranolazine, a late sodium current blocker, which may shorten the QT interval without affecting the peak current, hence should be beneficial for the LQTS without harm for the BrS part. In the present family we decided to implant ICDs in all affected subjects even the asymptomatic subject because of the possible need for beta blocker treatment for the LQTS which may be harmful in BrS. Due to the limited evidence in overlap syndromes, it is unknown whether this will be justified in the future or reflect an overtreatment. The baseline T-wave morphology in the three patients is similar to late onset of a normal T-wave pattern as described by Zhang et al[26]. This pattern is seen in less than 10% of all LQTS patients with *SCN5A* mutations, where late onset peaked/biphasic (53%) and asymmetrical peaked T-waves (12%) are more commonly seen[26]. Interestingly, the similar late onset of a normal T-wave pattern was also seen in two of the other combined BrS/LQTS mutations (E1784K[18], 1795insD[6]), whereas in the other mutations published the baseline full 12 lead ECGs without flecainide were not revealed for evaluation. It is also noticeable that these three mutations with late onset normal pattern were C-terminal mutations in *SCN5A*.

CONCLUSION

We have described a case of combined BrS and LQTS in a patient with *SCN5A* L1786Q mutation where the typical BrS type 1 ST segment elevation was unmasked by flecainide provocation. Our results thereby confirm that a proportion of LQTS patients with a mutation in SCN5A may harbour a type 1 ECG Brugada ECG pattern when exposed to a class 1C antiarrhythmic. As the pharmacological treatment of BrS is different from that of LQTS, great care should be taken in defining the phenotype. It may be considered to let LQTS patients with *SCN5A* mutations undergo a sodium blocker provocation test.

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DISCLOSURES:

None

FIGURE LEGENDS

Figure 1. A) The homology alignment of the SCN5A sequences across eight species indicating the conservation of the mutated L1786 residue. Published LQTS and BrS mutations in this region are also indicated. B) This residue is also conserved in all types of human SCN alpha channels.

Figure 2. Biophysical properties of wt vs. L1786Q $Na_v1.5$ channels A) Voltage clamping of either WT or mutant (L1786Q) *SCN5A* transfected HEK293 cells at 37 °C. Representative traces. Increasing depolarizing voltages increasingly activates the Nav1.5 channels. However, as the reversal potential of sodium is approximately 50 mV, the peak current decreases at potentials higher than approximately – 20 mV. B) Current voltage (I-V) relationship. C) Normalized peak current at the maximum current recorded (wt; -20 mV, L1786Q; -5 mV). D) Steady-state inactivation as a function of voltage. E) Steady-state activation as a function of voltage. The Boltzmann curves were obtained as described under "Experimental Procedures". The applied voltage protocols are shown in inserts. Arrows indicate were the current values are recorded. The left-ward shift in inactivation and the right-ward shift in activation of L1786Q compared to WT are both parameters that will reduce peak sodium current. F) Sustained current at different potentials recorded from wt and L1786Q expressing cells at 37 °C, normalised for cell size. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure 3. A) The sustained current was addressed by depolarising the $Na_v1.5$ transfected cells to -20 mV for 500 ms. Following the initial opening and inactivation of the sodium channel a small sustained current was observed (black traces). The part of this current conducted through the $Na_v1.5$ channels was established by adding 50 μM tetrodotoxin (TTX) (red traces). B) Normalised TTX sensitive peak current. C) Normalised TTX sensitive sustained current. $^{**}p < 0.01$. [37]

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Figure 4. Family pedigree, empty symbols (circles indicate females, and squares, males) depict unaffected members, filled symbols depict carriers of the SCN5A L1786Q mutation. The corrected QT interval (QTc) is shown above the gender symbol. The arrow indicates the proband (II-1).

Figure 5. ECG and EGM traces: A) ECG trace from the proband with QT prolongation but without any signs of ST elevation. B) ECG trace from the proband during flecainide test with coved pattern. C) BrS pattern with ST elevation and a premature beat with a short coupling interval eliciting the arrhythmia. D) LQTS pattern of induction without ST elevation in EGM and a short-long-short coupling sequence. E) ECG trace from the proband's brother (Figure 1: II-3) during flecainide testing. F) ECG trace from the proband's niece (Figure 1: III-3) during flecainide testing.

Table 1. Biophysical properties of Ina recorded in HEK293 cells expressing WT Nav1.5 and L1786Q Nav1.5. *p<0.005, **p<0.005, ***p<0.001.

	Wild type	**L1786Q**
Peak current (pA/pF) (pre-pulse: -120 mV)	-604 ± 113 (n = 7, at -20 mV)	-190 ± 55(**) (n = 8, at -5 mV)
Steady-state activation $V_{½}$ (mV) slope, k value	 -28.5 ± 1.9 (n = 7) 5.0 ± 0,4 mV/e-fold	 -13.3 ± 1.1 (n = 8) (***) 9.3 ± 0,9 mV/e-fold(**)
Steady-state inactivation, $V_{½}$ (mV) slope, k value	-67.0 ± 1.4 mV (n = 11) 4.7 ± 0,2 mV/e-fold	-88.3 ± 2,2 mV (n = 8) (***) 4.7 ± 0.3 mV/e-fold

Table 2. Electrophysiological characteristics of mutations associated with a mixed LQTS and BrS phenotype.

Mutation	Late Sodium current	Activation shift	Inactivation shift	Peak Sodium current	Clinical Phenotype
D1114N	NA	NA	NA	NA	BrS[27]+LQTS[28]
W1191X	NA	NA	NA	NA	BrS+LQTS[29]
delK1500	+[20]	+[20]	-[20]	-[20]	BrS+LQTS[23]
Del KPQ	+[28,29]	0[29]	-[29]	NA	BrS+LQTS[27], LQTS[30]
Del F1617	+/- *)[31]	0[31]	-[31]	0 [31]	BrS[31],LQTS[28]
E1784K	+[24]	+	-[24]	-[24]	LQTS+BrS[21], 25
L1786Q	+	+	-	-	Present paper
1795 insD	+[33]	+[5]	-[5]	-[5]	LQTS+BrS[6]

*) Late sodium current reduced at negative voltage potentials and increased at positive voltage potentials. NA = Not available

Reference List

1. Tfelt-Hansen J, Winkel BG, Grunnet M, Jespersen T. Inherited Cardiac Diseases Caused by Mutations in the Nav1.5 Sodium Channel. J Cardiovasc Electrophysiol 2009;21:107-115.

2. Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA *et al.* The genetic basis of Brugada syndrome: A mutation update. Hum Mutat 2009;30:1256-1266.

3. Kyndt F, Probst V, Potet F, Demolombe S, Chevallier JC, Baro I *et al.* Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. Circulation 2001;104:3081-3086.

4. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ *et al.* Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). J Clin Invest 2003;112:1019-1028.

5. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ *et al.* Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. JAMA 2005;293:447-454.

For Review Only

Stellenbosch University http://scholar.sun.ac.za

6. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma *et al.* A single Na(+) channel mutation causing both long-QT and Brugada syndromes. Circ Res 1999;85:1206-1213.

7. Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B *et al.* Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population. Eur J Hum Genet 2005;13:1213-1222.

8. Hedley PL, Jorgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA *et al.* The genetic basis of long QT and short QT syndromes: a mutation update. Hum Mutat 2009;30:1486-1511.

9. Mank-Seymour AR, Richmond JL, Wood LS, Reynolds JM, Fan YT, Warnes GR *et al.* Association of torsades de pointes with novel and known single nucleotide polymorphisms in long QT syndrome genes. Am Heart J 2006;152:1116-1122.

10. Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ *et al.* Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. J Mol Med 2004;82:182-188.

11. Sauer AJ, Moss AJ, McNitt S, Peterson DR, Zareba W, Robinson JL *et al.* Long QT syndrome in adults. J Am Coll Cardiol 2007;49:329-337.

For Review Only

Stellenbosch University http://scholar.sun.ac.za

12. Schwartz PJ. The congenital long QT syndromes from genotype to phenotype: clinical implications. J Intern Med 2006;259:39-47.

13. Sakabe M, Fujiki A, Tani M, Nishida K, Mizumaki K, Inoue H. Proportion and prognosis of healthy people with coved or saddle-back type ST segment elevation in the right precordial leads during 10 years follow-up. Eur Heart J 2003;24:1488-1493.

14. Jons C, Moss AJ, Lopez CM, McNitt S, Zareba W, Goldenberg I *et al.* Mutations in Conserved Amino Acids in the KCNQ1 Channel and Risk of Cardiac Events in Type-1 Long-QT Syndrome. J Cardiovasc Electrophysiol 2009;20:859-865.

15. Larsen LA, Christiansen M, Vuust J, Andersen PS. High-throughput single-strand conformation polymorphism analysis by automated capillary electrophoresis: robust multiplex analysis and pattern-based identification of allelic variants. Hum Mutat 1999;13:318-327.

16. Hofman-Bang J, Behr E, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S *et al.* High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: a rapid genetic approach to cardiac arrhythmia. Clin Genet 2006;69:504-511.

17. Remme CA, Wilde AA, Bezzina CR. Cardiac sodium channel overlap syndromes: different faces of SCN5A mutations. Trends Cardiovasc Med 2008;18:78-87.

For Review Only

18. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L *et al.* The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. J Clin Invest 2008;118:2219-2229.

19. Motoike HK, Liu H, Glaaser IW, Yang AS, Tateyama M, Kass RS. The Na+ channel inactivation gate is a molecular complex: a novel role of the COOH-terminal domain. J Gen Physiol 2004;123:155-165.

20. Grant AO, Carboni MP, Neplioueva V, Starmer CF, Memmi M, Napolitano C *et al.* Long QT syndrome, Brugada syndrome, and conduction system disease are linked to a single sodium channel mutation. J Clin Invest 2002;110:1201-1209.

21. Antzelevitch C, Brugada P, Brugada J, Brugada R. Brugada syndrome: from cell to bedside. Curr Probl Cardiol 2005;30:9-54.

22. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P *et al.* Proposed diagnostic criteria for the Brugada syndrome: consensus report. Circulation 2002;106.

23. Richter S, Sarkozy A, Paparella G, Henkens S, Boussy T, Chierchia GB *et al.* Number of electrocardiogram leads displaying the diagnostic coved-type pattern in Brugada syndrome: a diagnostic consensus criterion to be revised. Eur Heart J 2010;31:1357-1364.

24. Bloch Thomsen PE, Joergensen RM, Kanters JK, Jensen TJ, Haarbo J, Hagemann A *et al.* Phase 2 reentry in man. Heart Rhythm 2005;2:797-803.

For Review Only

Stellenbosch University http://scholar.sun.ac.za

25. Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Aizawa Y, Ogawa S. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. J Am Coll Cardiol 1996;27:1061-1070.

26. Zhang L, Timothy KW, Vincent GM, Lehmann MH, Fox J, Giuli LC *et al.* Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. Circulation 2000;102:2849-2855.

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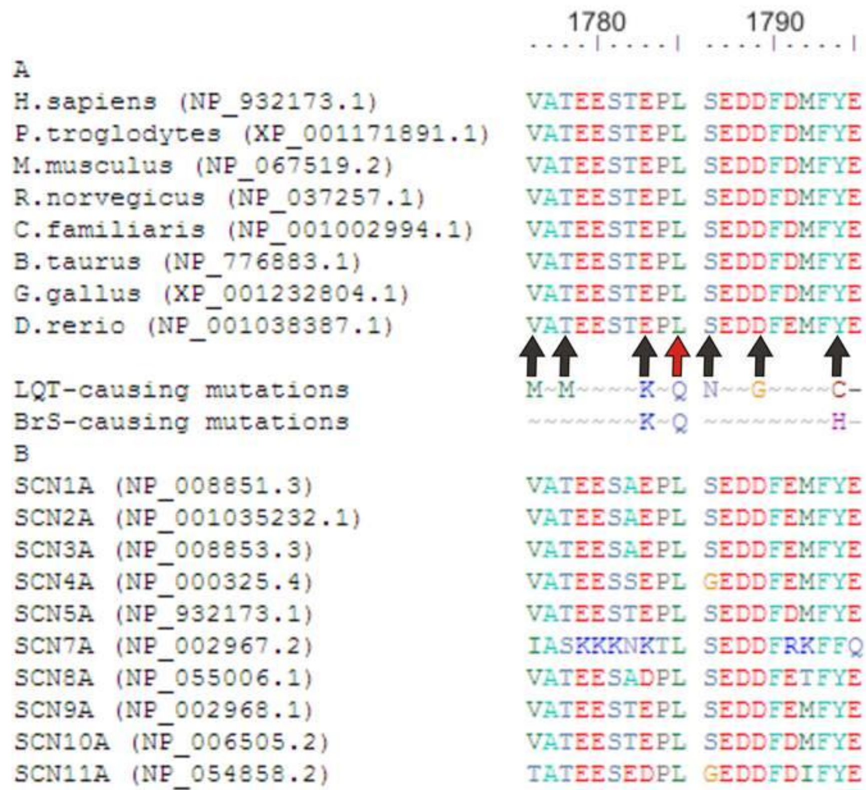
Figure 1.



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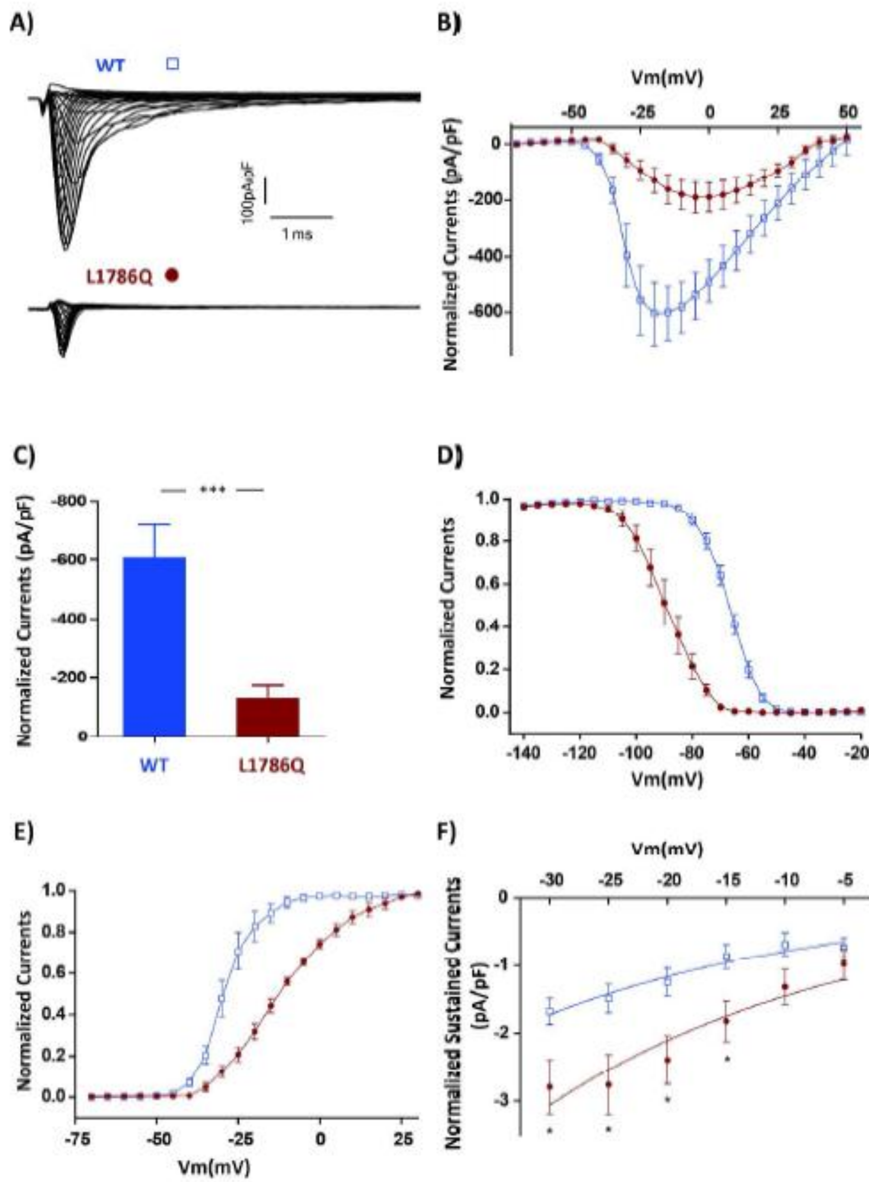

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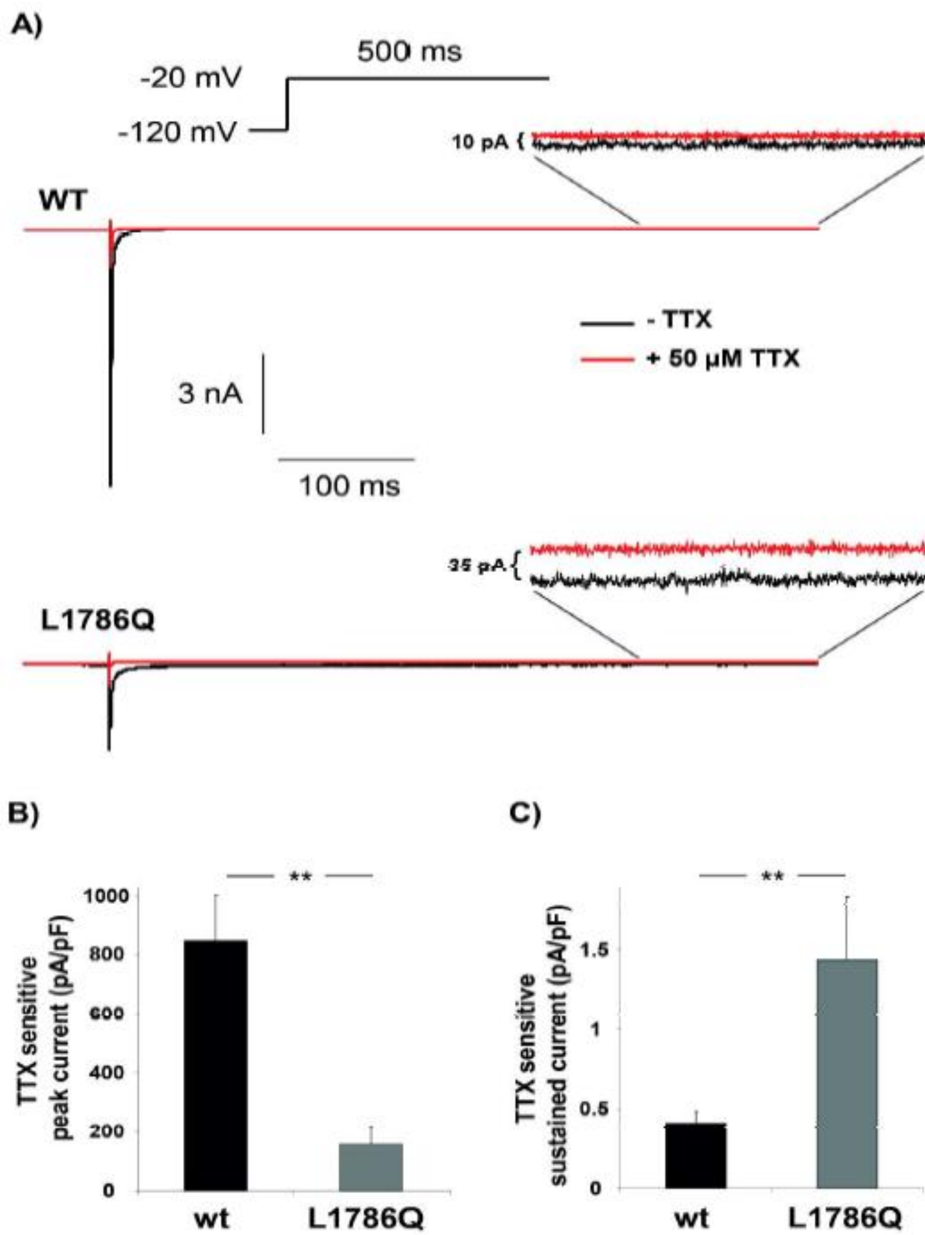

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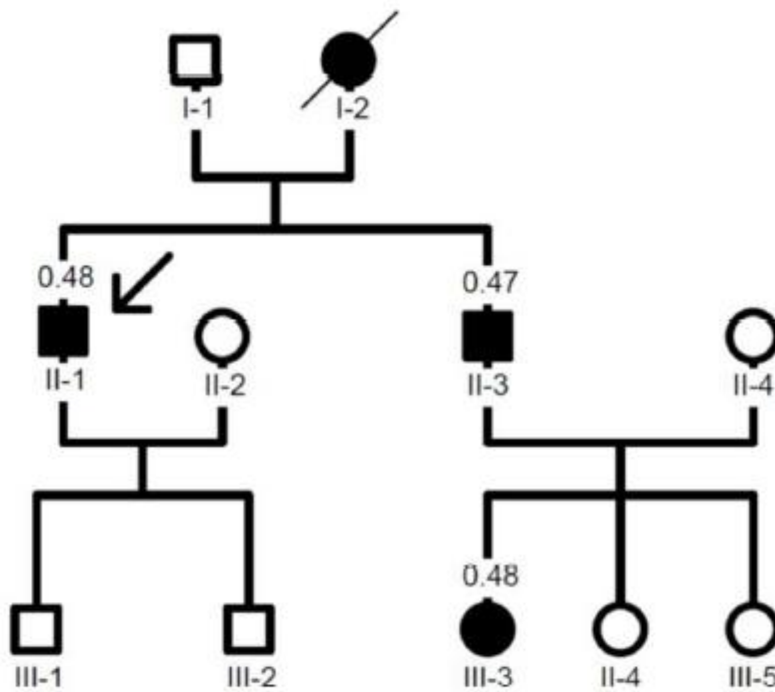
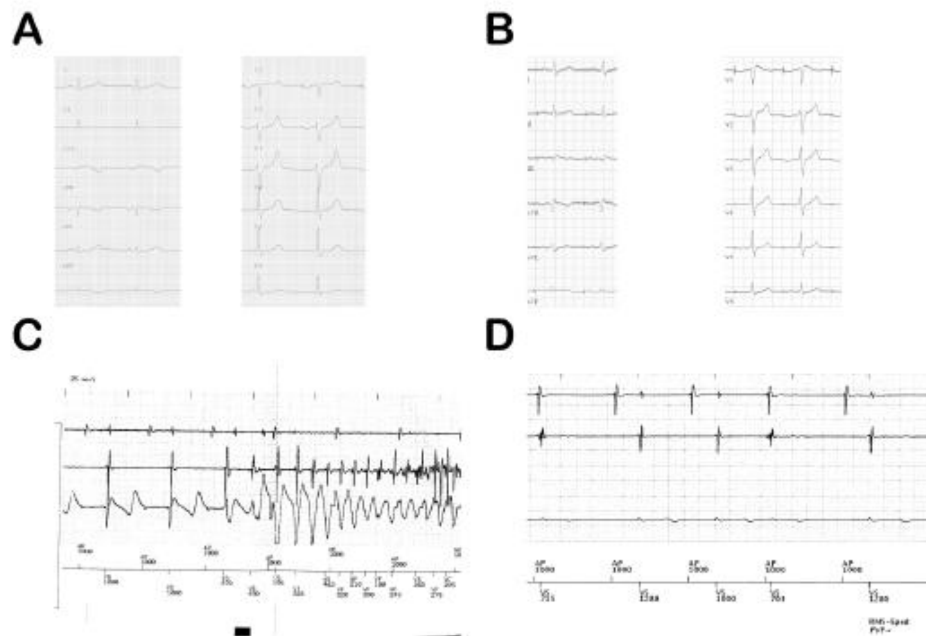


Figure 5.



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PAPER 6: HIGH PREVALENCE OF LONG QT SYNDROME–ASSOCIATED SCN5A VARIANTS IN PATIENTS WITH EARLY-ONSET LONE ATRIAL FIBRILLATION

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High Prevalence of Long QT Syndrome–Associated *SCN5A* Variants in Patients With Early-Onset Lone Atrial Fibrillation

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Background—Atrial fibrillation (AF) is the most common cardiac arrhythmia. The cardiac sodium channel, $Na_V1.5$, plays a pivotal role in setting the conduction velocity and the initial depolarization of the cardiac myocytes. We hypothesized that early-onset lone AF was associated with genetic variation in *SCN5A*.

Methods and Results—The coding sequence of *SCN5A* was sequenced in 192 patients with early-onset lone AF. Eight nonsynonymous mutations (T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W, and V1951M) and 2 rare variants (S216L in 2 patients and F2004L) were identified. Of 11 genopositive probands, 6 (3.2% of the total population) had a variant previously associated with long QT syndrome type 3 (LQTS3). The prevalence of LQTS3-associated variants in the patients with lone AF was much higher than expected, compared with the prevalence in recent exome data (minor allele frequency, 1.6% versus 0.3%; P=0.003), mainly representing the general population. The functional effects of the mutations were analyzed by whole cell patch clamp in HEK293 cells; for 5 of the mutations previously associated with LQTS3, patch-clamp experiments showed an increased sustained sodium current, suggesting a mechanistic overlap between LQTS3 and early-onset lone AF. In 9 of 10 identified mutations and rare variants, we observed compromised biophysical properties affecting the transient peak current.

Conclusions—In a cohort of patients with early-onset lone AF, we identified a high prevalence of *SCN5A* mutations previously associated with LQTS3. Functional investigations of the mutations revealed both compromised transient peak current and increased sustained current. **(*Circ Cardiovasc Genet.* 2012;5:450-459.)**

Key Words: atrial fibrillation ■ genes ■ long-QT syndrome ■ QT interval electrocardiography ■ *SCN5A*

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia, affecting almost 7 million patients in the European Union and United States combined.[1–4] In most cases, AF arises secondary to hypertension, ischemic, and/or structural heart disease relatively late in life.[1,5] However, 10% to 20% of patients experiencing AF are younger than 60 years and free of traditional predisposing conditions. These patients are said to have lone AF. The mechanisms underlying lone AF are not fully understood, but interplay between multiple substrates and triggers may constitute the etiology of AF.[6] As such, early-onset lone AF may be a primary electric disease caused by disturbances in ion channel function.[7]

Clinical Perspective on p 459

Familial predisposition for AF has recently been recognized. Fox et al[8] showed that the development of AF in offspring is associated with parental AF. The importance of common genetic variants in the development of AF has been revealed in recent genome-wide association studies.[9] Rare mutations in genes encoding potassium channels (*KCNQ1*, *KCNH2*, *KCNA5*, *KCNJ2,5*, and *KCNE1,2,3,5*), sodium channels (*SCN5A* and *SCN1-3B*), a peptide hormone (*ANP*), a gap junction protein (*GJA5*), and a nuclear membrane protein (*LMNA*) have been linked to AF.[10–14]

Mutations in *SCN5A*, the gene encoding the α-subunit underlying the dominant cardiac sodium current, is composed

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Table 1. Clinical Characteristics of the Lone AF Population (n=192)

Characteristics	Value
Age of onset, median (IQR), y	31.5 (26–36)
Male sex, %	82
Height, cm	183±9
Weight, kg	89±17
BMI, kg/m^2	26.7±4.6
Blood pressure, mm Hg	
Systolic	131±13
Diastolic	78±9
AF type	
Paroxysmal, %	55.9
Persistent, %	35.9
Permanent, %	8.2
Family history of AF	
First-degree relatives with AF, %	31

Data are reported as mean±SD, unless otherwise noted. AF indicates atrial fibrillation; IQR, interquartile range; BMI, body mass index.

of a central pore-forming α-subunit, Na$_V$1.5,[15,16] and 2 β-subunits of the Na$_V$β type. In addition to its implication in Brugada syndrome (BrS), long QT syndrome type 3 (LQTS3), and conduction defects, Na$_V$1.5 has recently a role in AF.[17] However, functional characterizations of *SCN5A* mutations associated with AF have been sparse. Two studies reported mutations that increased the transient peak current but showed no effect on the sustained current.[18,19] Another study described a mutation with decreased transient peak current.[20] In a third study of a family affected by an LQTS3 mutation, resulting in increased sustained sodium current, some members also had AF, suggesting that an increased sustained current could also play a role in AF.[21] A recent study of a human LQTS3 mutation expressed in a knock-in mouse model also suggested that the sustained sodium current could play a role in lone AF.[22]

We hypothesized that patients with early-onset lone AF would carry a high prevalence of *SCN5A* mutations, because such individuals are particularly likely to have a primary genetic defect as a substrate for AF. Also, we aimed to characterize possible mutations electrophysiologically by a patch-clamp approach to elucidate the functional impact of these mutations because such experiments have not previously been performed systematically.

Methods

Study Subjects

Patients were recruited from cardiology departments in 8 hospitals in the Copenhagen region of Denmark. Patient records from all inpatient and outpatient activity in the past 10 years with the diagnose code *International Statistical Classification of Diseases, 10th Revision (ICD-10)* I48.9 (AF and atrial flutter) were collected. Only patients with onset of lone AF before the age of 40 years (ie, absence of clinical or echocardiograph findings of other cardiovascular diseases, hypertension, or metabolic or pulmonary diseases) were included (Table 1). All patients were interviewed about family history of arrhythmia. Patients that carried mutations had a second interview specifically about family history of arrhythmia, sudden death, dilated cardiomyopathy, and other *SCN5A* associated disease. All mutation carriers were offered a flecainide provocation test (2 mg/kg flecainide intravenously) to exclude BrS.

To distinguish between common genetic polymorphisms, rare variants, and mutations, a group of ECG-documented healthy controls without cardiac symptoms were collected.

The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee (reference no. KF 01313322). All included patients gave written informed consent.

Mutation Screening

Genomic DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN; Hilden, Germany). The entire coding sequence and splice junctions of *SCN5A* (NM_198056.2) (primers and polymerase chain reaction conditions are available on request) were amplified and analyzed using a high-resolution melting curve analysis, as previously described[23] (Light Scanner, Idaho Technology; Salt Lake City, UT). Fragments with melting curves differing from the curves of wild-type DNA were purified and directly sequenced using Big Dye chemistry (DNA analyzer 3730, Applied Biosystems; Carlsbad, CA).

All identified nonsynonymous variants were validated by resequencing in an independent polymerase chain reaction. DNA from 22 of the patients was sequenced directly using Big Dye chemistry. The group of healthy controls was screened using high-resolution melting curve analysis, with probands included as positive controls. In probands with nonsynonymous variants, bidirectional sequencing of *SCN1–3B* (NM_001037.4, NM_004588, and NM_018400.3), *KCNQ1* (NM_000218.2), *KCNH2* (NM_000238), *KCNN3* (NM_002249.5), *KCNA5* (NM_002234.2), *KCNE1/2/3/5* (NM_001127668, NM_172201, NM_005472.4, and NM_012282.2), *KCNJ2,5* (NM_000891.2 and NM_000890.3), *KCNN3* (NM_002249.4),[24] *ANP* (NM_006172.3), and *LMNA* (NM_005572) was performed.

Bioinformatics

We performed species alignment (Figure 1) and Polyphen-2 prediction analyses of variants.[25] The Single Nucleotide Polymorphism Database (http://www.ncbi.nlm.nih.gov/projects/SNP) was searched for identified mutations and variants.

The National Heart, Lung, and Blood Institute GO Exome Sequencing Project (ESP) holds information on exome data from next-generation sequencing of all protein-coding regions in 3510 persons of European American ancestry.[26] We compared the proportion of mutations or rare variants in *SCN5A* with minor allele frequency (MAF) <0.1% in ESP with those variants in the lone AF population also with an MAF <0.1% in ESP, assuming genetic homogenicity. We also compared the 2 populations regarding the proportion of previously published LQTS-associated variants, as reported in a recent comprehensive review by Hedley et al[27] and in data from the Human Gene Mutation Database.[28]

In Vitro Electrophysiological Data

Mutations were introduced into Na$_V$1.5 cDNA cloned in pcDNA3.1 (Invitrogen; Nærum, Denmark), using standard mutated oligonucleotide extension polymerase chain reaction. All constructs were verified by DNA sequencing. For patch-clamp studies, HEK293 cells were transiently cotransfected with 0.3 µg pcDNA3-hNa$_V$1.5 (wild-type or mutants) and 0.2 µg of pcDNA3-eGFP as a reporter gene, using Lipofectamine and Plus reagent (Invitrogen), according to the manufacturer's instructions. Patch-clamp experiments were performed at room temperature (20°C–22°C) 2 to 3 days after transfection. Patch-clamp recordings were conducted using an internal solution containing the following (mmol/L): CsCl 60; CsAspartate 70; EGTA 11; $MgCl_2$ 1; $CaCl_2$ 1; HEPES 10; and Na_2-ATP 5, pH 7.2, with CsOH; external solution NaCl 130; $CaCl_2$ 2; $MgCl_2$ 1.2; CsCl 5; HEPES 10; and glucose 5, pH 7.4, with CsOH. Data analyses were performed as previously described.[29]

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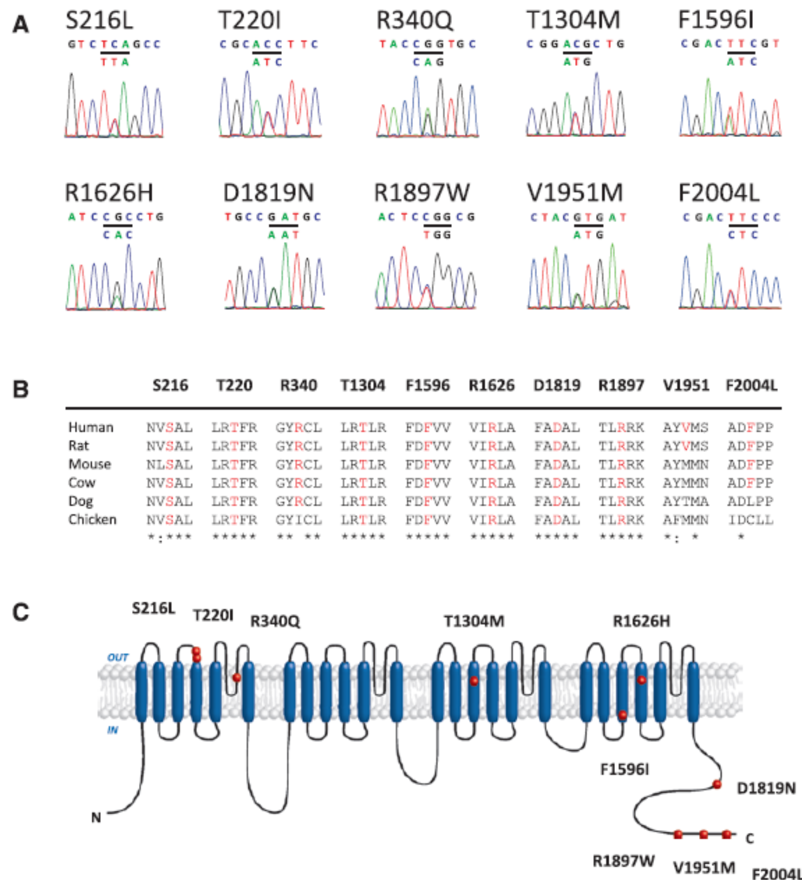

Figure 1. **A**, DNA sequencing traces (chromatograms) for variants identified in *SCN5A*. **B**, Evolutionary conservation between species. The location of mutated amino acid is marked in red. **C**, The position of the mutations indicated in schematic of protein topology.

A potential increase in sustained current was investigated by applying 30 μmol/L tetrodotoxin for the mutations R1626H and D1819N.

Data Analysis of Electrophysiological Experiments

Peak current densities were measured during an activation protocol, and sodium current I_{Na} densities (pA/pF) were obtained by dividing peak I_{Na} by cell capacitance. For activation and steady-state inactivation curves, data from individual cells were fitted with a Boltzmann equation, $y(V_m)=1/\{1+\exp[(Vm-V_{1/2})/K]\}$, in which y is the normalized current or conductance; V_m, the membrane potential; $V_{1/2}$, the voltage at which half of the channels are activated or inactivated; and K, the slope factor. The decay characteristics of the fast transient current was fitted best with time constants using the following equation: $I=A_0+A_1 \exp(-t/T)$, where t is the time from the beginning of the test pulse and T is the time constant of current decay. Recovery curves from inactivation were obtained by giving a 50-ms, −20-mV depolarizing pulse, followed by clamping to 4 different prepotentials. Recovery was fitted with monoexponential function: $Itest/Ipre=Y_0+A\exp^{-T/T}$, where Y_0 is the offset, A is amplitude, and T is the time constant. Data are presented as mean±SEM unless otherwise noted. A Student unpaired t test, 1-way ANOVA, or Fisher exact tests were used to test for significant differences. Normal distribution of the data set was tested by Shapiro-Wilk normality test using GraphPadPrism 5.0 software. $P<0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity.

Results

Study Cohort

The study population consisted of 192 patients with onset of AF ranging from 16 to 39 years without any concomitant disease. All included individuals were of Danish/white ethnicity. Clinical data are shown in Table 1.[30]

Mutation Screening

Screening of *SCN5A* in the 192 patients with lone AF revealed 10 nonsynonymous mutations (S216L, T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W, V1951M, and F2004L; Table 2), 5 that had not been functionally characterized before (see later). S216L was found in 2 patients with lone AF. S216L and F2004L were previously described in healthy controls (both with an MAF of 0.09%) and were, therefore, confided as rare variants.[34] In addition, T220I, T1304M, and R1897W were identified with a low frequency in the ESP; however, the disease status of these individuals was unknown.

None of the mutations were present in our control population (n=216) and have not previously been reported in another large group of healthy controls (n=1100).[34] All patients were heterozygous for the mutations.

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Table 2. Summary of Na$_v$1.5 Sodium Channel Mutations and Rare Variants in *SCN5A*

Amino Acid Change	Nucleotide Change	Frequency in 102 Patients	Conserv.*	Exon	Location	Polypen –2 Score	Reported Studies	Function
S216L	c.647C>T	2	HC	6	Transmembrane	Benign **0.000**	LQTS[27], SIDS[31]	Positive voltage shift in steady-state inactivation; increase in fast inactivation; negative voltage shift in recovery from inactivation; 8-fold increased sustained sodium current[31]
T220I	c.659C>T	1	HC	6	Transmembrane, helical, voltage sensor	Probably damaging **0.97**	AF[32], SSS[33]	Decreased peak current; negative voltage shift in steady-state inactivation; slower time-dependent recovery from inactivation[33]
R340Q	c.1018C>G	1	CM	8	Extracellular	Possibly damaging **0.93**	LQTS[27], SIDS[31]	Negative voltage shift in steady-state activation; negative voltage shift in steady-state inactivation; increased fast inactivation
T1304M	c.3911C>T	1	HC	22	Transmembrane, voltage sensor	Probably damaging **1.00**	LQTS[27], SIDS[31]	Positive voltage shift in steady-state activation; positive voltage shift in steady-state inactivation; increased fast inactivation; faster time-dependent recovery from inactivation; 7-fold increased sustained sodium current[31]
F1596I	c.4786T>A	1	HC	26	Transmembrane, helical	Probably damaging: **0.96**	AF[7]	Normal peak current parameter, sustained current not investigated[7]
R1626H	c.4877G>A	1	HC	28	Transmembrane, helical, voltage sensor	Probably damaging **1.00**	Novel	Positive voltage shift in steady-state activation; negative voltage shift in steady-state inactivation; decreased fast inactivation; 2- to 3-fold increased sustained sodium current
D1819N	c.5455G>A	1	HC	28	Intracellular	Probably damaging **0.99**	LQTS[27]	Negative voltage shift in steady-state inactivation; decreased fast inactivation; 6- to 10-fold increased sustained sodium current
R1897W	c.5689C>T	1	HC	28	Intracellular	Probably damaging **1.00**	Novel	Negative voltage shift in steady-state inactivation
V1951M	c.5851G>A	1	NC	28	Intracellular	Benign **0.000**	AF[17]	Decreased fast inactivation; positive voltage shift in recovery from inactivation
L2004F	c.5851G>A	1	NC	28	Intracellular	Benign **0.000**	SIDS[31]	Positive voltage shift in steady-state inactivation; decreases fast inactivation; faster recovery from inactivation; 4-fold increased sustained sodium current[31]

AF indicates atrial fibrillation; LQTS, long QT syndrome; SIDS, sudden infant death syndrome.
*Conserv. is the degree of conservation for the mutated site among multiple species: CM, conserved among large mammals; HC, highly conserved; NC, not conserved.

All patients carrying nonsynonymous mutations were subsequently screened for mutations in the whole coding region of the genes already known to be associated with AF: *SCN1–3B*, *KCNQ1*, *KCNH2*, *KCNA5*, *KCNJ2-3*, *KCNJ5*, and *KCNN3*[24]; *KCNE1,2,3,5*, *ANP*, and *LMNA*, but no additional mutations, were found.

Bioinformatics

All mutations were highly conserved across species, except for V1951M and F2004L, which are not conserved, and R340Q, which was conserved only in eutherian mammals (Figure 1). A PolyPhen2 prediction indicated that 7 of the 10 mutations and rare variants in *SCN5A* were predicted to be probably or at least possibly damaging (Table 2). We identified a significantly higher frequency of rare *SCN5A* variants (MAF <0.1%) in the patients with lone AF when compared with the frequencies reported in ESP (MAF, 2.9% versus 1.1%; P=0.013). This was also the case regarding *SCN5A* variants previously associated with LQTS3 (MAF, 1.6% versus 0.3%; P=0.003).

Family History

All patients carrying a mutation in *SCN5A* were interviewed specifically about family history of arrhythmias and *SCN5A*-related diseases, and 5 of the probands had a family history of arrhythmia. The proband carrying the mutation F1596I had a mother and a sister who were both affected by AF, but because they were both deceased, genetic screening was not possible. The proband carrying D1819N also had a family history of AF, but without cosegregation of the mutation. The proband carrying R1897W had a mother diagnosed as having postoperative AF at an old age, but she did not carry the mutation. The patient carrying the mutation V1951M had a father diagnosed as having nonsustained ventricular tachycardia (VT), although he was not a mutation carrier either. The proband

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carrying F2004L had a father with AF, but he was unavailable for genetic testing. For the other patients, there was no family history of AF and, therefore, no genetic testing of relatives was performed.

In Vitro Electrophysiological Data

SCN5A mutations not previously characterized electrophysiologically (R340Q, R1626H, D1819N, R1897W, and V1951M) were investigated for a potential functional impact. We expressed wild-type or mutant channels in mammalian HEK293 cells and addressed electrophysiological parameters by whole cell patch-clamp experiments (Figure 2 and Table 3). No significant difference in peak current density was observed in any of the mutants compared with control. However, differences were observed in steady-state activation and in several different inactivation parameters summarized in Table 3. In brief, R340Q showed a negative voltage shift of both steady-state activation and inactivation, together with a reduced time constant for onset (decay) of fast inactivation (Figure 2D, E, and B, respectively). R1626H gave a positive voltage shift of steady-state activation and a negative voltage shift of steady-state inactivation, together with a decreased onset of fast inactivation (Figure 2D, E, and B, respectively). D1819N revealed a minor change in the onset of fast inactivation parameters with an increase of the decaying time constant at depolarizing potentials (Figure 2B). R1897W showed a drastic negative voltage shift of the steady-state inactivation potential (Figure 2E), and V1951M gave a decrease of the time-dependent inactivation at different potentials and a decrease of onset of inactivation time constant (Figure 2B and F, respectively).

The R1626H mutant had a moderately increased sustained current component, whereas D1819N produced pronounced sustained sodium currents (Figure 3B–D).

Electrocardiographic Data

Flecainide provocation tests did not induce Brugada ECG patterns in any of the tested probands (Table 4). Of 10 of the identified mutations and rare variants, 5 (S216L, R340Q, T1304M, D1819N, and V1951M) have previously been associated with LQT3 syndrome.[27] Of the 2 patients carrying S216L, 1 had a borderline prolonged QT_c interval of 469 ms, whereas the other proband, who was also a carrier of H558R, a variant previously able to rescue other mutations functionally,[35] had a QT_c within the normal range of 438 ms.

At baseline, the patient harboring the R1626H mutation had a 443-ms QT_c interval, but interestingly, during flecainide testing, the QT_c interval increased to 495 ms. The patient carrying D1819N had a borderline prolonged QT_c interval of 467 ms. This patient also had a relatively large 43-ms increase in QT_c interval during the flecainide test. The V1951M proband had a QT_c of 425 ms at baseline, but this patient also displayed a relatively large increase in QT_c of 41 ms during flecainide testing. Both patients carrying R340Q or T1304M had normal QT_c intervals (Table 4).

Discussion

To our knowledge, this study is the first to comprehensively attempt to associate early-onset lone AF with mutations in *SCN5A*. In a cohort of 192 patients with onset of lone AF before the age of 40 years, we identified 8 mutations (T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W, and V1951M,) in *SCN5A*. The high degree of conservation across species indicates that these residues are important for channel function. We also identified 2 rare *SCN5A* variants (S216L in 2 probands and F2004 in 1 proband). Of 11 *SCN5A*-positive probands, 6 (3.2% of the total population) carried a mutation or rare variant previously associated with LQT3 syndrome.

Genetic screening of the patients with lone AF revealed a much higher prevalence of mutations or rare variants in *SCN5A*, as expected from the prevalences in ESP (MAF, 2.9% versus 1.1%; P=0.013), representing the general population. Also, mutations or rare variants previously associated with LQTS3 were present with a higher prevalence in the patients with lone AF patients compared with ESP (MAF, 1.6% versus 0.3%; P=0.003). Despite some limitations in comparing MAF in the 2 populations (different screening techniques, no possibility of matching on age and sex, and different geographic regions), this quantitative approach strongly supports the hypothesis that the present *SCN5A* mutations or rare variants identified in the patients with lone AF might be involved in the pathogenesis of AF.

All mutation carriers had a QT_c interval within the normal range (<470 ms); however, 2 probands had a borderline prolonged QT_c interval of 467 and 469 ms, respectively. Recently, individuals carrying an LQTS-associated mutation with a QT_c interval within the normal range (<440 ms) also had an increased risk for life-threatening cardiac events.[36] Hence, we speculate that the patients with lone AF in our cohort carrying mutations previously associated with LQTS3 may have an increased risk of life-threatening arrhythmias. If patients with lone AF in general carry a high prevalence of LQTS3-associated variants, then as a group, they might have an increased risk of life-threatening arrhythmias. This novel finding could have potentially clinical implications for future risk stratification in patients with lone AF. However, further investigations are warranted to address a potential benefit of genetic screening of patients with lone AF in a clinical setting. Interestingly, in the present group of *SCN5A* genotype-positive patients, those patients carrying an LQTS3-associated variant also presented with the longest QT_c intervals. The 2 patients with the shortest QT_c intervals were the only 2 *SCN5A*-positive probands who carried the variant R558H (Table 4), which has rescued several other *SCN5A* variants (Table 2).[35, 37]

Our results from the present cohort of patients with early-onset lone AF indicate that *SCN5A* mutations only in rare cases produce highly penetrant monogenic forms of AF (Table 2). This is in contrast to a study by Darbar et al,[17] who reported several *SCN5A* mutations that cosegregated with familial AF. We envision several explanations for this discrepancy. First, the 2 cohorts differed in sex, age, and size of the families. Second, because of the relative age of the probands' parents, they may not have developed AF; however, they were predisposed for this. Third, a cohort selection bias may exist, in that patients with familial aggregation potentially more often could have been referred to the cohort described by Darbar et al.[17] The proband carrying R1897W had a mother diagnosed as having postoperative AF at an old age and she did not carry the mutation; AF after surgery is common. The

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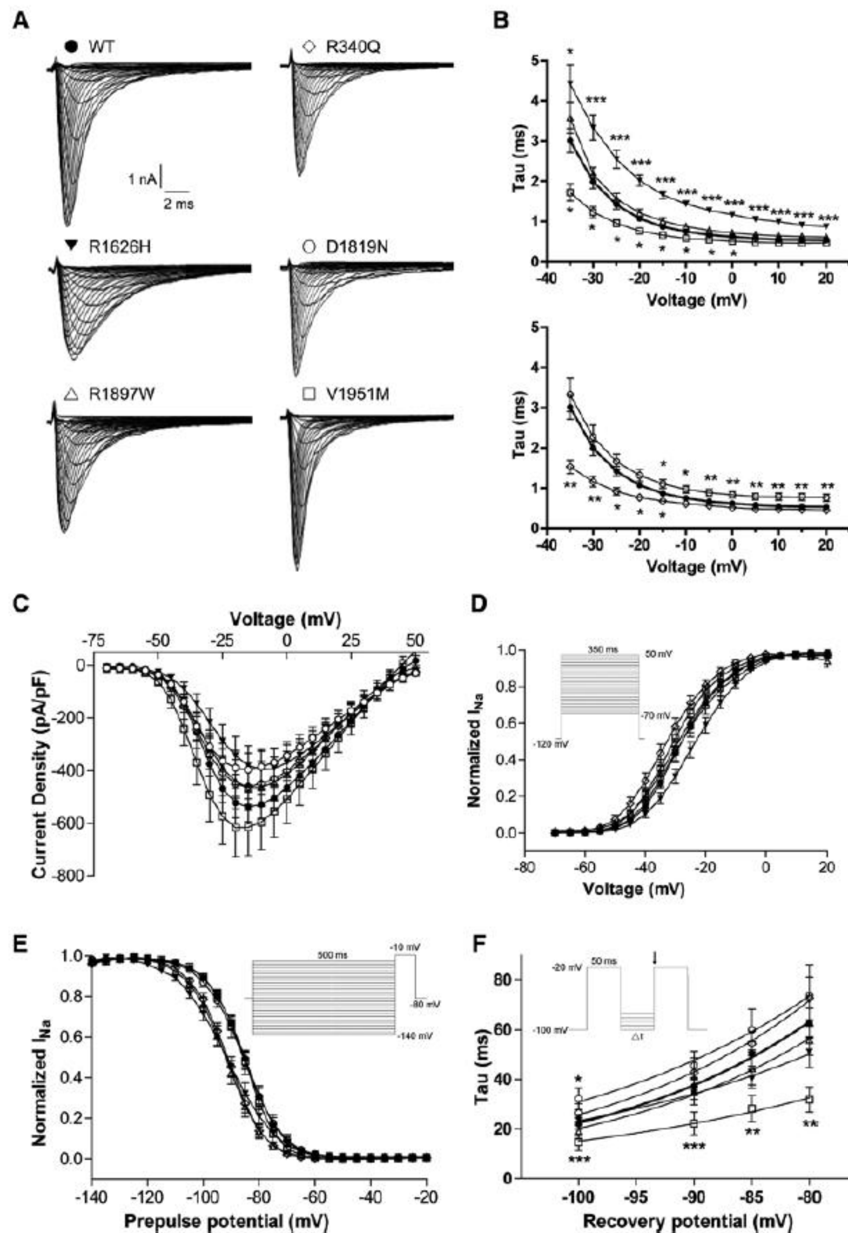

Figure 2. Electrophysiological characterization of *SCN5A* mutants. **A**, Representative current traces obtained with a current/voltage protocol (inset in **D**) for wild-type (WT) and the 5 Na$_v$1.5 mutations. **B**, Onset of fast inactivation. Single exponential fit to the decaying phase of the current traces (as shown in **A**). **C**, Current/voltage relationship of WT and Na$_v$1.5 mutants. **D**, Steady-state activation curves. Activation properties were determined from I/V relationships by normalizing peak I_{Na} to driving force and maximal I_{Na}, and plotting normalized conductance vs mV. **E**, Steady-state inactivation curves. Boltzmann curves were fitted to both steady-state activation and inactivation data. **F**, Time- and voltage-dependent recovery from inactivation. The time-dependent recovery from inactivation at different voltage potentials (inset) was fitted with a monoexponential relationship, and the τ values were plotted. **A** and **C-E**, Averaged values and the numbers of cells measured are presented in Table 3. **B** and **F**, n=10 for each group. *P<0.05, **P<0,01, and ***P<0,001. ●WT, ◊ R340Q, ▼ R1626H, Δ R1897W, ○ D1819N, □ V1951M. Error bars represent the mean±SEM. In some figures, the SEM bars are smaller than the data symbols.

patient carrying the mutation V1951M had a father diagnosed as having nonsustained VT, although he was not a mutation carrier either. A recent article provided important input to the discussion about reduced penetrance. In LQTS type 1 patients, variants in the 3′UTR-region of the *KCNQ1* gene modify disease severity in an allele-specific manner,[38] and this mechanism may also be important in other LQTS genes, such as *SCN5A*. The lack of familial cosegregation, in combination with the fact that both rare *SCN5A* variants and previously LQTS3-associated variants are highly

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Table 3. Clinical Characteristics of Probands With *SCN5A* Variants

Patient No.	Genotype	H558R Genotype	Sex	Phenotype	Onset, y	ECG at Inclusion	ECG During Flecainide Testing	Flecainide Testing	Family History of AF
1	S216L	HR	M	Persistent	36	Normal (QT_c 438 ms)	...	Not done	None
2	S216L	HH	M	Persistent	39	Normal (QT_c 469 ms)	...	Not done	None
3	T220I	HH	M	Paroxystic	35	Normal (QT_c 422 ms)	...	Not done	None
4	R340Q	HH	M	Paroxystic	26	Normal (QT_c 422 ms)	Not available	Negative	None
5	T1304M	HH	M	Paroxystic	37	r'-Wave in V1/V2 (QT_c 439 ms)	QRS increase 28 ms QT_c increase 30 ms	Negative	None
6	F1596I	HH	M	Persistent	39	Low voltage (QT_c 428)	QRS increase 15 ms QT_c increase 19 ms	Negative	Mother and mother's sister with AF, both decreased
7	R1626H	HH	M	Paroxystic	37	Normal (QT_c 443 ms)	QRS increase 26 ms QT_c increase 52 ms	Negative	None
8	D1819N	HH	F	Paroxystic	25	Short PR of 106 ms (QT_c 467 ms)	QRS increase 4 ms QT_c increase 48 ms	Negative	Family history of AF, but no mutation cosegregation
9	R1897W	HH	M	Paroxystic	38	r'-Wave and J-point elevation in V1/V2 (QT_c 397)	QRS increase 20 ms QT_c increase 16 ms	Negative	Mother with postoperative AF, no mutation
10	V1951M	HR	M	Persistent	22	J-wave in II, III and aVF (QT_c 425 ms)	QRS increase 18 ms QT_c increase 41 ms	Negative	Father diagnosed as having VT, no mutation
11	F2004L	HR	M	Chronic	38	AF	...	Not done	Father AF, not available for genetic testing

AF indicates atrial fibrillation; HH, H558H; HR, H558R.

overrepresented in the patients with lone AF, compared with the general population, points in the direction that these variants might be important disease modifiers rather than monogenic causes of lone AF.

Compromised Peak Sodium Current

To investigate whether the mutations reported herein are disease causing, whole cell patch-clamp electrophysiological investigations were performed. We analyzed 5 *SCN5A* mutations that have not been functionally investigated previously (namely, R340Q, R1626H, D1819N, R1897W, and V1951M). None of the mutations had a significant effect on peak current density. Steady-state activation was altered for 2 mutations. Channels harboring the R340Q mutation showed a negative potential shift, which means that these channels open at more negative membrane potentials, increasing the availability of the channels (Figure 2 and Table 2). R1626H channels showed a positive voltage shift of activation, which is expected to reduce channel availability. Because $Na_V1.5$ channels are inactivated at potentials close to the resting membrane potential of cardiomyocytes, small changes in steady-state inactivation are expected to give large impact on sodium channel availability. Indeed, for the R340Q, R1626H, and R1897W mutations, we observed a >5-mV negative shift in steady-state inactivation (Figure 2 and Table 3). We also investigated the onset (or decay) of inactivation, which is a measure of the width of the sodium peak. If the onset of inactivation time constant is decreased, the channel will close faster, resulting in a decreased depolarizing power of the mutated channel. Decreased time constants were found for R340Q and V1951M. A third inactivation property is the time-dependent recovery from inactivation. The V1951M mutation had a shorter recovery time at all tested potentials. A faster recovery could be speculated to be proarrhythmic because the sodium channel complexes are released from inactivation earlier and can thereby contribute to wavelength shortening.[17] In conclusion, all 5 mutations displayed altered phenotypes compared with wild-type $Na_V1.5$. Most of the observed changes indicated a decreased transient peak current. Of those mutations previously studied by others, T220I[33] displayed decreased peak current, whereas S216L,[31] T1304M,[31] and L2004F[31] showed electrophysiological parameters, resulting in increased availability of the sodium channels in the early (transient) part of the action potential (Table 2). F1596I has not affected any of the peak current parameters, and its role in AF is questionable (Table 2).[7] Our results, together with other available data, support the notion that both an increase[18,19] and a decrease in the transient sodium peak current predispose for AF.[33]

Pappone et al[39] recently reported that, in 6% of a lone AF population, a BrS type 1 ECG pattern could be induced by flecainide testing. Flecainide testing of our *SCN5A*-positive probands did not reveal any BrS type 1 ECG pattern, indicating that these subjects are unlikely to have a concealed BrS.

Increased Sustained Sodium Current

Flecainide has, apart from blocking I_{Na}, blocked the $K_V11.1$ (hERG1) potassium channel, which is responsible for the fast delayed rectifier current and can, therefore, potentially unmask increased sustained sodium current.[40] The 6 probands carrying an *SCN5A* mutation previously associated with LQTS3 may be predisposed for AF through increased sustained sodium current, a mechanism thought to at least partly underlie LQTS3. Indeed, for 3 (S216L, T1304M, and L2004F) of the 6 mutations, increased sustained sodium current has been reported (8-, 7-, and 4-fold, respectively).[31] In addition, 3 of 7 patients carrying a mutation previously associated with LQTS3 had either a borderline prolonged QT_c interval at baseline or a higher increase in QT_c interval during flecainide testing

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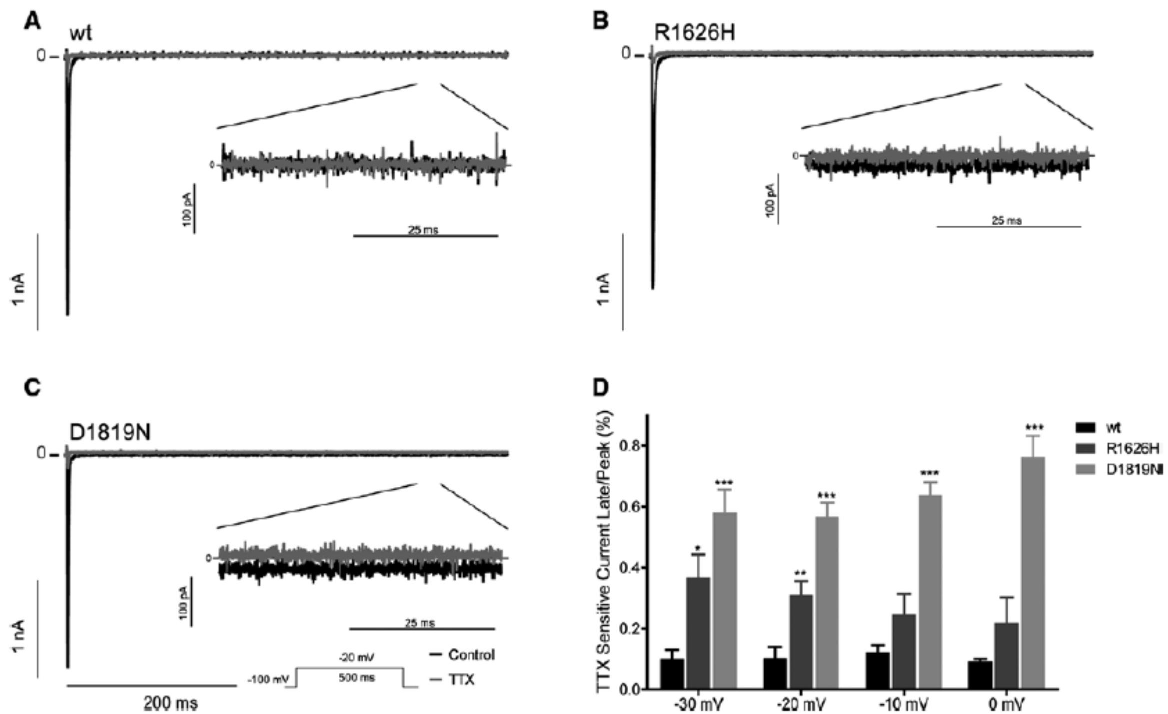

Figure 3. The sustained sodium current (I_{NaL}) of R1626H and D1819N is increased at different voltages. Representative recordings in the absence or presence of tetrodotoxin (TTX) for wild-type (WT) (*n*=6) (**A**), R1626H (*n*=6) (**B**), and D1819N (*n*=6) (**C**). Currents were activated by a 500-ms step to –20 mV from a holding potential of –100 mV. For comparison, the peak and late current are shown at different scales. The sustained currents were normalized to the peak current observed in each trace. **D**, Summarized data of I_{NaL} at different voltages. Currents were activated by a 500-ms step from –30 to 0 mV in 10-mV increments from a holding potential of –100 mV. Currents in the presence of TTX were subtracted from currents recorded in the absence of TTX to determine the TTX-sensitive current. I_{NaL} was measured as the mean current between 450 and 500 ms, and the ratio between the TTX-sensitive peak and the late current was calculated for WT, R1626H, and D1819N. At each condition, the difference in I_{NaL} between R1626H or D1819N and WT was significant. *$P<0.05$, **$P<0.01$, and ***$P<0.001$.

than the expected value of 21±17 ms for healthy individuals (Table 4).[41]

In our cohort, the patient carrying the novel R1626H mutation had an unexpectedly large increase in QT_c (52 ms) during flecainide testing and the patient carrying D1819N had a borderline prolonged QT_c interval of 467 ms at baseline. Hence, we investigated the sustained sodium current for R1626H and D1819N. Patch-clamp experiments revealed a 2- to 3-fold increase in sustained current for the R1626H mutation, whereas the D1819N conducts a dramatically 6- to 10-fold increased sustained current. Hence, the in vitro investigations confirmed the effect of flecainide on the QT_c interval observed in the 2 probands, indicating that the sustained component of the sodium current might play a role in the pathogenesis of AF. This is in line with a study using isolated atrial myocytes from patients with AF, which showed increased sustained sodium current.[42] Furthermore, Lemoine et al[22] have recently showed atrial action potential prolongation, atrial early after depolarizations, and triggered activity in a genetically modified animal model of human LQTS3. Treatment with ranolazine,

Table 4. Electrophysiological Properties of Wild-Type and Mutant Na$_v$1.5 Channels

Variable	Peak Current at –15 mV, pA/pF	No. of Experiments	Steady-State Activation $V_{1/2}$, mV	Slope k Value	*n*	Steady-State Inactivation $V_{1/2}$, mV	Slope k Value	No. of Experiments
WT	–539± 55	23	–27.9±1.3	6.6± 0.3	22	–85.6±0.9	5.5±0.1	25
R340Q	–462± 90	14	–34.1±1.5*	6.3± 0.5	14	–91.1±1.3*	5.7±0.2	14
R1626H	–380± 74	15	–23.5±1.8**	7.6±0.4**	14	–91.2±1.9*	5.6±0.2	13
D1819N	–385± 59	15	–27.77±1.8	7.5±0.6	14	–85.4±0.5	5.9±0.2**	14
R1897W	–465± 65	15	–29.5±1.6	7.2±0.5	15	–91.8±1.5*	5.5±0.2	14
V1951M	–616± 108	10	–31.5±1.8	6.9±0.4	10	–85.6±1.0	5.6±0.2	10

*$P<0.01$, **$P<0.05$, significantly different from Na$_v$1.5-WT.

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a blocker of the sustained sodium currents, normalized the number of early after depolarizations in this model.[22] Patients treated with ranolazine for angina pectoris had a lower incidence of supraventricular tachycardias.[43]

Our study on human AF, together with the mice experiments by Lemoine et al,[22] for the first time, to our knowledge, indicate a possible overlap between the mechanisms underlying LQTS3 and lone AF with action potential prolongation as a substrate and early after depolarizations as triggers for arrhythmia. However, both decreased and increased transient peak sodium current have also been suggested as possible mechanisms for AF,[18,19,20] and further studies are needed to reveal the electrophysiological mechanisms behind AF.

Limitations

We only analyzed the coding regions of *SCN5A*, and mutations occurring in gene regions other than coding regions cannot be excluded. We used a conventional heterologous expression system, which differs from native cardiomyocytes. Furthermore, for the electrophysiological parameters investigated, several changes of several parameters were observed. Although these data provide strong support for discussing whether a given mutation is preferentially a loss- or gain-of-function mutation, they cannot be regarded as conclusive.

Conclusions

We identified 8 mutations and 2 rare variants in *SCN5A* in 192 patients with early-onset lone AF. Many of these patients with lone AF carried a mutation or rare variant previously associated with LQTS3, compared with the expected frequency in the general population (MAF, 1.6% versus 0.3%; P=0.003). All identified variants have been investigated electrophysiologically, and in 9 of them, compromised peak sodium current was found, whereas 5 variants showed increased sustained sodium current. Our results thereby indicate that both gain- and loss-of-function alterations in the electrophysiological properties of the cardiac sodium current may lead to the development of AF in young adults.

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Disclosures

None.

References

1. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation*. 1998;98:946–952.
2. Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, et al. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation--executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation). *Rev Port Cardiol*. 2007;26:383–446.
3. Stewart S, Hart CL, Hole DJ, McMurray JJ. Population prevalence, incidence, and predictors of atrial fibrillation in the Renfrew/Paisley study. *Heart*. 2001;86:516–521.
4. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, et al. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA*. 2001;285: 2370–2375.
5. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, et al. Incidence of and risk factors for atrial fibrillation in older adults. *Circulation*. 1997;96:2455–2461.
6. Sinner MF, Ellinor PT, Meitinger T, Benjamin EJ, Kääb S. Genome-wide association studies of atrial fibrillation: past, present, and future. *Cardiovasc Res*. 2011;89:701–709.
7. Olesen MS, Jespersen T, Nielsen JB, Liang B, Møller DV, Hedley P, et al. Mutations in sodium channel β-subunit SCN3B are associated with early-onset lone atrial fibrillation. *Cardiovasc Res*. 2011;89:786–793.
8. Fox CS, Parise H, D'Agostino RB, Lloyd-Jones DM, Vasan RS, Wang TJ, et al. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. *JAMA*. 2004;291:2851–2855.
9. Gudbjartsson DF, Arnar DO, Helgadottir A, Gretarsdottir S, Holm H, Sigurdsson A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature*. 2007;448:353–357.
10. Campuzano O, Brugada R. Genetics of familial atrial fibrillation. *Europace*. 2009;11:1267–1271.
11. Mahida S, Lubitz SA, Rienstra M, Milan DJ, Ellinor PT. Monogenic atrial fibrillation as pathophysiological paradigms. *Cardiovasc Res*. 2011;89:692–700.
12. Kozlowski D, Budrejko S, Lip GYH, Rysz J, Mikhailidis DP, Raczak G, et al. Lone atrial fibrillation: what do we know? *Heart*. 2010;96:498–503.
13. Olesen MS, Holst AG, Haunsø S, Tfelt-Hansen J. SCN1Bb R214Q found in 3 patients: 1 with Brugada syndrome and 2 with lone atrial fibrillation. *Heart Rhythm*. Cited January 2, 2012. Available at: http://www.sciencedirect.com/science/article/pii/S1547527111014603.
14. Olesen MS, Bentzen BH, Nielsen JB, Steffensen AB, David J-P, Jabbari J, et al. Mutations in the potassium channel subunit KCNE1 are associated with early-onset familial atrial fibrillation. *BMC Med Genet*. 2012;13:24.
15. Tan HL, Bezzina CR, Smits JP, Verkerk AO, Wilde AA. Genetic control of sodium channel function. *Cardiovasc Res*. 2003;57:961–973.
16. Rook MB, Evers MM, Vos MA, Bierhuizen MFA. Biology of cardiac sodium channel Nav1.5 expression. *Cardiovasc Res*. 2012;93:12–23.
17. Darbar D, Kannankeril PJ, Donahue BS, Kucera G, Stubblefield T, Haines JL, et al. Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation. *Circulation*. 2008;117:1927–1935.
18. Makiyama T, Akao M, Shizuta S, Doi T, Nishiyama K, Oka Y, et al. A novel SCN5A gain-of-function mutation M1875T associated with familial atrial fibrillation. *J Am Coll Cardiol*. 2008;52:1326–1334.
19. Li Q, Huang H, Liu G, Lam K, Rutberg J, Green MS, et al. Gain-of-function mutation of Nav1.5 in atrial fibrillation enhances cellular excitability and lowers the threshold for action potential firing. *Biochem Biophys Res Commun*. 2009;380:132–137.
20. Ellinor PT, Nam EG, Shea MA, Milan DJ, Ruskin JN, MacRae CA. Cardiac sodium channel mutation in atrial fibrillation. *Heart Rhythm*. 2008;5:99–105.
21. Benito B, Brugada R, Perich RM, Lizotte E, Cinca J, Mont L, et al. A mutation in the sodium channel is responsible for the association of long QT syndrome and familial atrial fibrillation. *Heart Rhythm*. 2008;5:1434–1440.
22. Lemoine MD, Duverger JE, Naud P, Chartier D, Qi XY, Comtois P, et al.Arrhythmogenic left atrial cellular electrophysiology in a murine genetic long QT syndrome model. *Cardiovasc Res*. 2011;92:67–74.
23. Olesen MS, Jensen NF, Holst AG, Nielsen JB, Tfelt-Hansen J, Jespersen T, et al. A novel nonsense variant in Nav1.5 cofactor MOG1 eliminates its sodium current increasing effect and may increase the risk of arrhythmias. *Can J Cardiol*. 2011;27:523.e17–523.e23.
24. Olesen MS, Jabbari J, Holst AG, Nielsen JB, Steinbrüchel DA, Jespersen T, et al. Screening of KCNN3 in patients with early-onset lone atrial fibrillation. *Europace*. 2011;13:963–967.
25. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249.

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26. Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA. Available at: http://snp.gs.washington.edu/poggenSNP. Accessed July 10, 2011.
27. Hedley PL, Jørgensen P, Schlamowitz S, Wangari R, Moolman Smook J, Brink PA, et al. The genetic basis of long QT and short QT syndromes: a mutation update. *Hum Mutat*. 2009;30:1486–1511.
28. The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff. Cited April 3, 2012. Available at: http://www.hgmd.org. Accessed April 4, 2012.
29. Petitprez S, Jespersen T, Pruvot E, Keller DI, Corbaz C, Schlapfer J, et al. Analyses of a novel SCN5A mutation (C1850S): conduction vs. repolarization disorder hypotheses in the Brugada syndrome. *Cardiovasc Res*. 2008;78:494–504.
30. Watanabe H, Darbar D, Kaiser DW, Jiramongkolchai K, Chopra S, Donahue BS, et al. Mutations in sodium channel beta1- and beta2-subunits associated with atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2009;2:268–275.
31. Wang DW, Desai RR, Crotti L, Arnestad M, Insolia R, Pedrazzini M, et al. Cardiac sodium channel dysfunction in sudden infant death syndrome. *Circulation*. 2007;115:368–376.
32. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, et al. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. *JAMA*. 2005;293:447–454.
33. 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;21:564–573.
34. Ackerman MJ, Splawski I, Makielski JC, Tester DJ, Will ML, Timothy KW, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm*. 2004;1:600–607.
35. Poelzing S, Forleo C, Samodell M, Dudash L, Sorrentino S, Anaclerio M, et al. SCN5A polymorphism restores trafficking of a Brugada syndrome mutation on a separate gene. *Circulation*. 2006;114:368–376.
36. Goldenberg I, Horr S, Moss AJ, Lopes CM, Barsheshet A, McNitt S, et al. Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. *J Am Coll Cardiol*. 2011;57:51–59.
37. Shinlapawittayatorn K, Dudash LA, Du XX, Heller L, Poelzing S, Ficker E, et al. A novel strategy using cardiac sodium channel polymorphic fragments to rescue trafficking-deficient SCN5A mutations. *Circ Cardiovasc Genet*. 2011;4:500–509.
38. Amin AS, Giudicessi JR, Tijsen AJ, Spanjaart AM, Reckman YJ, Klemens CA, et al. Variants in the 3′ untranslated region of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. *Eur Heart J*. 2012;33:714–723.
39. Pappone C, Radinovic A, Manguso F, Vicedomini G, Sala S, Sacco FM, et al. New-onset atrial fibrillation as first clinical manifestation of latent Brugada syndrome: prevalence and clinical significance. *Eur Heart J*. 2009;30:2985–2992.
40. Du CY, El Harchi A, Zhang YH, Orchard CH, Hancox JC. Pharmacological inhibition of the hERG potassium channel is modulated by extracellular but not intracellular acidosis. *J Cardiovasc Electrophysiol*. 2011;22:1163–1170.
41. Kawata H, Noda T, Yamada Y, Okamura H, Satomi K, Aiba T, et al. Effect of sodium-channel blockade on early repolarization in inferior/lateral leads in patients with idiopathic ventricular fibrillation and Brugada syndrome. *Heart Rhythm*. Cited December 22, 2011. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21855521.
42. Sossalla S, Kallmeyer B, Wagner S, Mazur M, Maurer U, Toischer K, et al. Altered Na+ currents in atrial fibrillation: effects of ranolazine on arrhythmias and contractility in human atrial myocardium. *J Am Coll Cardiol*. 2010;55:2330–2342.
43. Scirica BM, Morrow DA, Hod H, Murphy SA, Belardinelli L, Hedgepeth CM, et al. Effect of ranolazine, an antianginal agent with novel electrophysiological properties, on the incidence of arrhythmias in patients with non–ST-segment–elevation acute coronary syndrome. *Circulation*. 2007;116:1647–1652.

CLINICAL PERSPECTIVE

The cardiac sodium channel is responsible for both the fast depolarization upstroke of the cardiac action potential (peak component) and the late phase of the action potential (sustained component). Mutations in the gene SCN5A encoding the human cardiac sodium channel have been associated with inherited susceptibility to a plethora of diseases, such as long QT syndrome (LQTS), Brugada syndrome, sudden infant death syndrome, progressive cardiac conduction disorders, and atrial fibrillation (AF). To our knowledge, this study is the first to investigate the prevalence of SCN5A variants in patients with early-onset lone AF. In 192 subjects with early-onset lone AF, we identified 8 mutations and 2 rare variants in SCN5A. Intriguingly, 6 of these have previously been associated with LQTS, and LQTS variants exceeded the expected frequency in the general population (minor allele frequency, 1.6% versus 0.3%; P=0.003). All identified variants have been investigated electrophysiologically, and in 9 of them, a compromised peak sodium current was found, whereas 5 variants showed increased sustained sodium current. Our results thereby indicate that both gain- and loss-of-function alterations in the electrophysiological properties of the cardiac sodium current may lead to the development of AF, and that the same genetic variants that are involved in the LQTS syndrome are also involved in lone atrial fibrillation. To our knowledge, this study is the first to investigate the sustained sodium current in this context of AF and may indicate a role for the specific blocker of the sustained sodium current ranolazine in clinical practice.

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CHAPTER 6: ION CHANNEL GENES IMPLICATED IN STRUCTURAL HEART DISEASE

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PAPER 7: THE KCNE GENES IN HYPERTROPHIC CARDIOMYOPATHY: A CANDIDATE GENE STUDY

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RESEARCH Open Access

The KCNE genes in hypertrophic cardiomyopathy: a candidate gene study

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Abstract

Background: The gene family *KCNE1-5*, which encode modulating β-subunits of several repolarising K^+-ion channels, has been associated with genetic cardiac diseases such as long QT syndrome, atrial fibrillation and Brugada syndrome. The minK peptide, encoded by *KCNE1*, is attached to the Z-disc of the sarcomere as well as the T-tubules of the sarcolemma. It has been suggested that minK forms part of an "electro-mechanical feed-back" which links cardiomyocyte stretching to changes in ion channel function. We examined whether mutations in KCNE genes were associated with hypertrophic cardiomyopathy (HCM), a genetic disease associated with an improper hypertrophic response.

Results: The coding regions of *KCNE1*, *KCNE2*, *KCNE3*, *KCNE4*, and *KCNE5* were examined, by direct DNA sequencing, in a cohort of 93 unrelated HCM probands and 188 blood donor controls.
Fifteen genetic variants, four previously unknown, were identified in the HCM probands. Eight variants were non-synonymous and one was located in the 3'UTR-region of *KCNE4*. No disease-causing mutations were found and no significant difference in the frequency of genetic variants was found between HCM probands and controls. Two variants of likely functional significance were found in controls only.

Conclusions: Mutations in KCNE genes are not a common cause of HCM and polymorphisms in these genes do not seem to be associated with a propensity to develop arrhythmia

Background

Hypertrophic cardiomyopathy (HCM) is a condition characterised by increased wall (predominantly septal) thickness, diastolic dysfunction, and an increased risk of heart failure, stroke and cardiac arrhythmia [1]. The disease has a prevalence of 1:500 in young adults [2], and is considered a hereditary disease caused by mutations in more than 12 genes [3], most of which encode proteins of the sarcomere. The disease exhibits considerable intra-allelic as well as phenotypic heterogeneity. Presently, a genetic aetiology can be identified in 70% of familial cases and 30% of non-familial cases [3].

Recently, mutations in genes coding for ion channels have been shown to cause cardiomyopathy. Mutations in *SCN5A*, coding for the α-subunit of the ion channel conducting the depolarising I_{Na} -current [4,5], and in *ABCC9* [6], coding for the cardiac specific SUR2A subunit of the K_{ATP} potassium channel, have been associated with dilated cardiomyopathy (DCM). The DCM caused by mutations in both *SCN5A* and *ABCC9* is accompanied by cardiac arrhythmia.

The *KCNE*-gene family (*KCNE1-5*) encodes five small single transmembrane peptides (minK and MiRP1-4, respectively) that function as β-subunits to potassium and pacemaker ion channels [7,8]. The KCNE peptides confer distinctive characteristics to a variety of currents [9-11]. For example, the slow increase and high conductance characteristic of $I_{Ks}$is conferred by minK (encoded by *KCNE1*) to the α-subunit (encoded by *KCNQ1*) [12]. The KCNE peptides are also involved in correct trafficking of α-subunits [13]. Mutations in *KCNE* genes have been associated with a number of diseases, i.e. cardiac arrhythmia by mutations in *KCNE1* (long QT syndrome and Jervell Lange Nielsen Syndrome) [14-17], *KCNE2* (long QT syndrome, atrial fibrillation, drug induced ventricular fibrillation) [18-20], *KCNE3* (Brugada syndrome)

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[21] and *KCNE5* (atrial fibrillation) [22]; mutations in *KCNE3* have also been associated with periodic paralysis and hypo-and hyperkalemic disorders [23]. Furthermore, *kcne2* null mice develop rhythm disturbances [24] and *kcne2* null pups to *kcne2* null dams develop hypertrophy among other abnormalities as a consequence of hypothyroidism [25]. This suggests that in addition to the development of arrhythmias, mutations in *KCNE2* could give rise to cardiac hypertrophy through the dysregulation of thyroid hormones. Likewise, other investigations using *kcne2* null mice have revealed an association with gastric pathology [26]. These finding suggest that the KCNE genes may influence phenotypic presentation of HCM in multiple ways.

All *KCNE* genes are expressed in the heart but to a varying extent [27]. The minK and MirP peptides exhibit considerable functional promiscuity, consequently, they may substitute for each other with different α-subunits [28] and the relative levels of peptides in different parts of the heart influence the regional variation of ion channel function [27].

Yeast-two-hybrid (Y2H) experiments have shown that minK is linked to the z-disc of the sarcomere via interaction with titin-cap (telethonin) [29]. The link between the T-tubule, where minK is attached and the Z-disc, has been suggested to constitute a "mechano-electrical feed-back system", linking the function of repolarising ion channels to stretch of the cardiomyocytes [29].

The Z-disc proteins are involved in the control of cardiac hypertrophy as mutations in the protein constituents of the Z-disc, T-cap, titin, muscle LIM protein, actinin and cypher/ZASP, have been shown to cause both HCM and DCM [30,31]. The electrical remodelling seen in heart failure is characterised by a marked increase in the expression of KCNE1 [32] in the heart.

We hypothesised that variants in *KCNE* genes, might result in changes in mechano-electrical feed-back, and could be responsible for a maladaptation of the stretch-response of the heart. This could explain an exaggerated hypertrophic response and thus HCM development in patients with mutations in Z-disc proteins. Alternatively, an increased occurrence of electrophysiologically significant *KCNE* variants might explain the increased propensity of arrhythmia in HCM.

We screened the genes *KCNE1*, *KCNE2*, *KCNE3*, *KCNE4*, and *KCNE5* for genetic variants in 93 unrelated probands with HCM and related the findings to occurrence of disease or propensity to a particular phenotype.

Results

No putative disease causing mutations were found in HCM index patients in any of the five *KCNE* genes. Fifteen genetic variants were identified; four of which were previously unknown. Fourteen of the genetic variants were located in the coding regions of the genes. The variants are detailed in Table 1. All variants were in Hardy-Weinberg equilibrium, when the variants were so frequent that this could be assessed.

Two variants, p.M1T in *KCNE3* and p.E141A in *KCNE4*, were found in single controls. The p.M1T variant abolishes the translation initiation codon and most likely results in haplo-insufficiency. The p.E141A variant, affects an amino acid which is conserved in seven species, and represents a charge change and may well modify the functional properties.

Some of the identified variants have previously been associated with arrhythmia, i.e. p.S38G in *KCNE1* and p.P33S in *KCNE5*, that are known polymorphisms associated with increased risk of atrial fibrillation. There was no significant difference in the frequency of any of the polymorphisms between HCM and the normal population. Two variants, i.e. p.D85N in *KCNE1* and p.T8A in *KCNE2* have previously been associated with increased risk for drug-induced ventricular fibrillation. For both variants, the frequency was lower in HCM, for p.D85N rare allele frequency 0.5% vs 1.2% in controls, and for p.T8A a rare allele frequency of 0.5% vs. 4.3% in controls. For both variants the allele frequency was so low, however, that the difference is not significant when compensating for multiple comparisons.

The p.R83H variant in KCNE3 has previously been associated with hypo- and hyper-kalemia and paralysis [7], and here it was found in two cases. In one family the mutation was co-inherited with a mutation in troponin T and in another the comprehensive sarcomeric gene screening had not revealed other mutations. There were no special clinical characteristics of the carriers of the p.R83H variant. However, the p.R83H has, following the association with hypo- and hyper-kalemic paralysis, been described as a polymorphism in several populations [33].

None of the identified variants had any significant effect on splicing, i.e. did not interfere *in silico* with ESEs or SSEs.

Discussion

The *KCNE* genes do not, despite the association with electromechanical feedback, seem to cause HCM, even though the number of probands examined does not preclude an involvement at the level of less than 1%. However, except in special cases, there does not seem to be any reason for including *KCNE* gene screening in the screening of genes in the genetic work-up of HCM.

The frequency of arrhythmia associated genetic variants was so low that it did not convincingly differ from that of controls and it cannot explain the increased occurrence of arrhythmia in HCM [34]. However, the previously arrhythmia-associated variants p.D85N [18,35,36] and p.T8A [7] both occurred more frequently

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Table 1 Genetic variants within the *KCNE* genes identified in a Danish HCM cohort

nucleotide	peptide	rs#	Pop rare allele frequency	HCM rare allele frequency	Disease association	Reference
KCNE1: [NM_000219.2/NP_000210.2]						
c.24 G > A	p.A8A		0.000	0.005		
c.112G > A	p.G38S	rs1784617 9	0.494	0.376	AF	[40]
c.253G > A	p.D85N	rs1805128	0.012	0.005	IVF, drug induced	[18,35,36]
KCNE2: [NM_172201.1/NP_751951.1]						
c.22A > G	p.T8A	rs2234916	0.043	0.005	IVF, drug-induced	
KCNE3: [NM_005472.4/NP_005463.1]						
c.2T > C	p.M1T		0.003	0.000		
c.198T > C	p.F66F	rs2270676	0.104	0.080		
c.248G > A	p.R83H	rs17215437	0.003	0.011	Hypokalemia	[7,41]
KCNE4: [NM_080671.2/NP_542402.2]						
c.69C > T	p.S23S	rs12720447	0.011	0.006		
c.81C > T	p.G27G	rs3795886	0.730	0.717		
c.264T > C	p.P88P	rs10201907	0.949	0.933		
c.422A > C	p.E141A		0.003	0.000		
c.435T > G	p.D145E	rs12621643	0.712	0.724		
c.471G > A	p.E157E		0.042	0.023		
c.*19G > C	3'UTR	rs10189762	0.059	0.046		
KCNE5: [NM_012282.2/NP_036414.1]						
c.97C > T	p.P33S	rs17003955	0.206*	0.150*	AF	[22,42]

*** gender corrected value**

in controls than in HCM patients. We cannot exclude, however, that a small minority of HCM patients with arrhythmia associated variants in *KCNE* genes have an increased propensity for arrhythmia.

The finding of very rare genetic variants with likely functional significance, i.e. p.M1T in *KCNE3* and p. E141A in *KCNE4*, in controls is interesting and suggests that such variants may contribute to the arrhythmia risk in various conditions in the general population.

Conclusions

Our findings suggest that neither *KCNE1*, despite its physical association with the Z-disc [29], nor the other *KCNE* genes are common causes of HCM.

Methods

Patients

Ninety-three unrelated consecutively diagnosed HCM patients identified at, or referred to, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark were included in the study. All patients were of Northern European descent. Patients were subjected to a full clinical evaluation including family history, physical examination, echocardiography and ECG. All fulfilled classical diagnostic criteria for HCM [37,38]. The mean age of index patients was 49 years, 62% were male, and 48% were familial. Ninety-two % had septal hypertrophy, 6% apical hypertrophy and 2% mid-ventricular hypertrophy. All patients had been screened for mutations in the coding regions of *MYH7, MYBPC3, TTNT2, TPM1, TNNI3, MYL3, MYL2, ACTC, TCAP, CSRP3*, and exons 3,7,14,18, and 49 of *TTN*, as detailed in a previous study [3]. All index patients were also screened for mutations in *GLA*. In 32 index patients this screening had identified presumably disease-causing mutations, i.e. 12 in *MYH7*, 8 in *MYBPC3*, 2 in each of *TNNT2, TNNI3* and *GLA*, 1 in each of *ACTC, TPM1, MYL3* and *MYL2*. Two patients were carriers of mutations in both *MYL2* and *MYH7*. A control panel of 188 (50% men) anonymous blood donors obtained from Rigshospitalet, Copenhagen, were used.

Molecular genetic studies

Genomic DNA was isolated from whole blood samples (Qiagen, Hilden, Germany). The genomic sequences of *KCNE1, KCNE2, KCNE3, KCNE4*, and *KCNE5* were used for designing intronic primers covering the coding region of the genes. Primers and conditions are given in Table 2. DNA sequencing was performed using Big Dye technology. Variant numbering was verified using the Mutalyzer program [39].

Disease-causation and association

Genetic variants were considered disease-causing if 1) the nucleotide variation was deduced to result in a

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Table 2 Primer sequences, amplicon size and melting temperatures for the *KCNE* gene mutation screening

Name	Sequence	amplicon size (bp)	Tm (°C)
KCNE1.1F	5' GCA GCA GTG GAA CCT TAA TG 3'	225	58
KCNE1.1R	5' CGG ATG TAG CTC AGC ATG AT 3'		
KCNE1.2F	5' CTT CGG CTT CTT CAC CCT G 3'	250	58
KCNE1.2R	5' TTA GCC AGT GGT GGG GTT C 3'		
KCNE2.1F	5' TCC GTT TTC CTA ACC TTG TTC 3'	250	58
KCNE2.1R	5' GCC ACG ATG ATG AAA GAG AAC 3'		
KCNE2.2F	5' GAT GCT GAG AAC TTC TAC TAT G 3'	300	58
KCNE2.2R	5' GTC TGG ACG TCA GAT GTT AG 3'		
KCNE3F	5' GCT AAG ATT TTA CCT GGG ATC TGA 3'	626	65
KCNE3R	5' TAT GCA CAA GGC TTC GGT CTA C 3'		
KCNE4F	5' CTC TTG TCA GCT GTT TGG CGA ACC 3'	886	65
KCNE4R	5' CAC AGG CAC CTC CCG GAC TC 3'		
KCNE5F	5' CCG CCG TGT CAC TCC CCG AAA 3'	493	62
KCNE5R	5' AGA TGA GGA GGG CGC GAA CCA 3'		

missense mutation, frameshift and/or abnormal splicing; 2) if relevant, the variation affected a conserved amino acid; 3) the variation co-segregated with the disease in affected family members and; 4) if the variation was not identified among 188 ethnically controlled samples. In the absence of available family members for co-segregation studies, disease association was presumed if criteria 1, 2 and 4 were fulfilled. If the mutation had previously, in accordance with the criteria mentioned here and/or relevant functional studies, been associated with disease, disease causation was presumed when just the criteria 1 and 4 were met. The association between gene variants and disease was assessed by comparing the distribution of variants in disease group and controls. χ^2-testing was used to examine for significant association using a level of significance of 0.05, with correction for multiple comparisons, if such were made.

Bioinformatics

ESE/SSE-*in silico* assessment was performed using the online web-servers: FAS-ESS [34], RESCUE-ESE [35], HMMgene [36], GENSCANW [37] and ESEFinder v.3.0 [38]. Multiple species alignments were performed using ClustalW2 [39].

Ethics

Informed consent was obtained from study participants. The study was approved by the Local Science Ethics Committees, Copenhagen and Frederiksberg, protocol no. KF V92213.

List of abbreviations

DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; Y2H: yeast-two-hybrid.

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Authors' contributions

PLH, PSA, JMS and MC Participated in the study design, PLH carried out the molecular genetic studies, PLH and FA participated in the sequence alignment and bioinformatics assessment of variants, PLH and MC drafted the manuscript, OH, MJ and HB Performed clinical characterisation of the patients, MC: Conceived the study.
All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Behr ER, McKenna WJ: **Hypertrophic Cardiomyopathy.** *Curr Treat Options Cardiovasc Med* 2002, **4(6)**:443-453.
2. Maron BJ: **Hypertrophic cardiomyopathy: a systematic review.** *JAMA* 2002, **287(10)**:1308-1320.
3. Andersen PS, Havndrup O, Hougs L, Sorensen KM, Jensen M, Larsen LA, Hedley P, Thomsen AR, Moolman-Smook J, Christiansen M, *et al*: **Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives.** *Hum Mutat* 2009, **30(3)**:363-370.
4. McNair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, Mestroni L: **SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia.** *Circulation* 2004, **110(15)**:2163-2167.
5. Hesse M, Kondo CS, Clark RB, Su L, Allen FL, Geary-Joo CT, Kunnathu S, Severson DL, Nygren A, Giles WR, *et al*: **Dilated cardiomyopathy is associated with reduced expression of the cardiac sodium channel Scn5a.** *Cardiovasc Res* 2007, **75(3)**:498-509.
6. Bienengraeber M, Olson TM, Selivanov VA, Kathmann EC, O'Cochlain F, Gao F, Karger AB, Ballew JD, Hodgson DM, Zingman LV, *et al*: **ABCC9**

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mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. *Nat Genet* 2004, 36(4):382-387.
7. Abbott GW, Goldstein SA: Potassium channel subunits encoded by the KCNE gene family: physiology and pathophysiology of the MinK-related peptides (MiRPs). *Mol Interv* 2001, 1(2):95-107.
8. Angelo K, Jespersen T, Grunnet M, Nielsen MS, Klaerke DA, Olesen SP: KCNE5 induces time- and voltage-dependent modulation of the KCNQ1 current. *Biophys J* 2002, 83(4):1997-2006.
9. Grunnet M, Jespersen T, Rasmussen HB, Ljungstrom T, Jorgensen NK, Olesen SP, Klaerke DA: KCNE4 is an inhibitory subunit to the KCNQ1 channel. *J Physiol* 2002, 542(Pt 1):119-130.
10. Grunnet M, Rasmussen HB, Hay-Schmidt A, Rosenstierne M, Klaerke DA, Olesen SP, Jespersen T: KCNE4 is an inhibitory subunit to Kv1.1 and Kv1.3 potassium channels. *Biophys J* 2003, 85(3):1525-1537.
11. Lundby A, Olesen SP: KCNE3 is an inhibitory subunit of the Kv4.3 potassium channel. *Biochem Biophys Res Commun* 2006, 346(3):958-967.
12. Melman YF, Um SY, Krumerman A, Kagan A, McDonald TV: KCNE1 binds to the KCNQ1 pore to regulate potassium channel activity. *Neuron* 2004, 42(6):927-937.
13. Xu X, Kanda VA, Choi E, Panaghie G, Roepke TK, Gaeta SA, Christini DJ, Lerner DJ, Abbott GW: MinK-dependent internalization of the IKs potassium channel. *Cardiovasc Res* 2009, 82(3):430-438.
14. Bianchi L, Shen Z, Dennis AT, Priori SG, Napolitano C, Ronchetti E, Bryskin R, Schwartz PJ, Brown AM: Cellular dysfunction of LQT5-minK mutants: abnormalities of IKs, IKr and trafficking in long QT syndrome. *Hum Mol Genet* 1999, 8(8):1499-1507.
15. Duggal P, Vesely MR, Wattanasirichaigoon D, Villafane J, Kaushik V, Beggs AH: Mutation of the gene for IsK associated with both Jervell and Lange-Nielsen and Romano-Ward forms of Long-QT syndrome. *Circulation* 1998, 97(2):142-146.
16. Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA, Christiansen M: The genetic basis of Brugada syndrome: a mutation update. *Hum Mutat* 2009, 30(9):1256-1266.
17. Hedley PL, Jorgensen 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(11):1486-1511.
18. Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ, Schulze-Bahr E, Haverkamp W, Breithardt G, Cohen N, *et al*: Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. *J Mol Med* 2004, 82(3):182-188.
19. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, *et al*: Spectrum of mutations in long-QT syndrome genes. *KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation* 2000, 102(10):1178-1185.
20. Yang Y, Xia M, Jin Q, Bendahhou S, Shi J, Chen Y, Liang B, Lin J, Liu Y, Liu B, *et al*: Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. *Am J Hum Genet* 2004, 75(5):899-905.
21. Delpon E, Cordeiro JM, Nunez L, Thomsen PE, Guerchicoff A, Pollevick GD, Wu Y, Kanters JK, Larsen CT, Burashnikov E, *et al*: Functional Effects of KCNE3 Mutation and its Role in the Development of Brugada Syndrome. *Circ Arrhythm Electrophysiol* 2008, 1(3):209-218.
22. Ravn LS, Aizawa Y, Pollevick GD, Hofman-Bang J, Cordeiro JM, Dixen U, Jensen G, Wu Y, Burashnikov E, Haunso S, *et al*: Gain of function in IKs secondary to a mutation in KCNE5 associated with atrial fibrillation. *Heart Rhythm* 2008, 5(3):427-435.
23. Abbott GW, Butler MH, Bendahhou S, Dalakas MC, Ptacek LJ, Goldstein SA: MiRP2 forms potassium channels in skeletal muscle with Kv3.4 and is associated with periodic paralysis. *Cell* 2001, 104(2):217-231.
24. Roepke TK, Kontogeorgis A, Ovanez C, Xu X, Young JB, Purtell K, Goldstein PA, Christini DJ, Peters NS, Akar FG, *et al*: Targeted deletion of kcne2 impairs ventricular repolarization via disruption of I(K,slow1) and I (to,f). *FASEB J* 2008, 22(10):3648-3660.
25. Roepke TK, King EC, Reyna-Neyra A, Paroder M, Purtell K, Koba W, Fine E, Lerner DJ, Carrasco N, Abbott GW: Kcne2 deletion uncovers its crucial role in thyroid hormone biosynthesis. *Nat Med* 2009, 15(10):1186-1194.
26. Roepke TK, Purtell K, King EC, La Perle KM, Lerner DJ, Abbott GW: Targeted deletion of Kcne2 causes gastritis cystica profunda and gastric neoplasia. *PLoS One* 2010, 5(7):e11451.
27. Lundquist AL, Manderfield LJ, Vanoye CG, Rogers CS, Donahue BS, Chang PA, Drinkwater DC, Murray KT, George AL Jr: Expression of multiple KCNE genes in human heart may enable variable modulation of I(Ks). *J Mol Cell Cardiol* 2005, 38(2):277-287.
28. Abbott GW, Goldstein SA: Disease-associated mutations in KCNE potassium channel subunits (MiRPs) reveal promiscuous disruption of multiple currents and conservation of mechanism. *FASEB J* 2002, 16(3):390-400.
29. Furukawa T, Ono Y, Tsuchiya H, Katayama Y, Bang ML, Labeit D, Labeit S, Inagaki N, Gregorio CC: Specific interaction of the potassium channel beta-subunit minK with the sarcomeric protein T-cap suggests a T-tubule-myofibril linking system. *J Mol Biol* 2001, 313(4):775-784.
30. Bos JM, Poley RN, Ny M, Tester DJ, Xu X, Vatta M, Towbin JA, Gersh BJ, Ommen SR, Ackerman MJ: Genotype-phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle LIM protein, and telethonin. *Mol Genet Metab* 2006, 88(1):78-85.
31. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, *et al*: The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* 2002, 111(7):943-955.
32. Radicke S, Cotella D, Graf EM, Banse U, Jost N, Varro A, Tseng GN, Ravens U, Wettwer E: Functional modulation of the transient outward current Ito by KCNE beta-subunits and regional distribution in human non-failing and failing hearts. *Cardiovasc Res* 2006, 71(4):695-703.
33. Jurkat-Rott K, Lehmann-Horn F: Periodic paralysis mutation MiRP2-R83H in controls: Interpretations and general recommendation. *Neurology* 2004, 62(6):1012-1015.
34. McKenna WJ, England D, Doi YL, Deanfield JE, Oakley C, Goodwin JF: Arrhythmia in hypertrophic cardiomyopathy. *I: Influence on prognosis Br Heart J* 1981, 46(2):168-172.
35. Van Laer L, Carlsson PI, Ottschytsch N, Bondeson ML, Konings A, Vandevelde A, Dieltjens N, Fransen E, Snyders D, Borg E, *et al*: The contribution of genes involved in potassium-recycling in the inner ear to noise-induced hearing loss. *Hum Mutat* 2006, 27(8):786-795.
36. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC: Compound mutations: a common cause of severe long-QT syndrome. *Circulation* 2004, 109(15):1834-1841.
37. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfas I, *et al*: Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 1996, 93(5):841-842.
38. ten Cate FJ, Hugenholtz PG, van Dorp WG, Roelandt J: Prevalence of dignostic abnormalities in patients with genetically transmitted asymmetric septal hypertrophy. *Am J Cardiol* 1979, 43(4):731-737.
39. Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PE: Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. *Hum Mutat* 2008, 29(1):6-13.
40. Ehrlich JR, Zicha S, Coutu P, Hebert TE, Nattel S: Atrial fibrillation-associated minK38G/S polymorphism modulates delayed rectifier current and membrane localization. *Cardiovasc Res* 2005, 67(3):520-528.
41. Dias Da Silva MR, Cerutti JM, Arnaldi LA, Maciel RM: A mutation in the KCNE3 potassium channel gene is associated with susceptibility to thyrotoxic hypokalemic periodic paralysis. *J Clin Endocrinol Metab* 2002, 87(11):4881-4884.
42. Hofman-Bang J, Jespersen T, Grunnet M, Larsen LA, Andersen PS, Kanters JK, Kjeldsen K, Christiansen M: Does KCNE5 play a role in long QT syndrome? *Clin Chim Acta* 2004, 345(1-2):49-53.

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CHAPTER 7: DISCUSSION

The societal significance of LQTS is emphasised by the prevalence of SCD among LQTS patients [38]. An appreciable portion of unexplained SCD in the young (individuals under 40-years-of-age at the time of their death) and sudden infant death (SIDS) (the unexplained death of an infant under one-year-of-age) may be attributed to mutations in LQTS-causing genes [39-43].

Genetic testing for LQTS is a useful clinical tool in cases where family history is ambiguous or clinical data is missing (absent clinical data in SCD cases and absent or inconclusive QT interval prolongation on a single clinical test). Genetic testing in such cases may be useful in excluding one cause of sudden arrhythmia death syndrome (SADS) over another [44, 45]. Genetic testing is also useful in cases where the LQTS diagnosis is clear-cut and genetic diagnosis is used to inform patient management decisions [46-48]. Furthermore, cascade screening in family members can identify those relatives who are asymptomatic but are at increased risk of experiencing an arrhythmic event [44, 45, 49, 50]. It should be highlighted that genetic diagnosis of LQTS is complicated by variable penetrance, even among related individuals with the same genotype [51], and the possibility of compound and multiple mutations [1, 4, 6, 52-56].

Genetic screening of the five major LQTS-causing genes identified putative pathogenic mutations in approximately 75 to 80 % of cases [4, 57]. The missing mutations may be present in the unscreened LQTS-causing genes or in as yet unknown disease-causing genes. Furthermore, relevant mutations may be missed in the genes currently screened as a consequence of limitations of the sequencing methodologies applied i.e. intronic mutations which lie outside tested splice sites [58], and large deletion and duplication mutations [59, 60].

7.1 GENETIC AETIOLOGY OF LONG QT SYNDROME

To establish a genetic diagnosis in a LQTS family is a complicated process [61] which often requires the collaboration of cardiologists, clinical geneticists and molecular geneticists. The studies reported here have limited genetic screening to the five most frequently implicated of the 13 known LQTS-causing genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*). The relatively small sample size of these studies limits the possibility of identifying variants in genes that are rarely associated with LQTS [1]. However, these limited genetic screens have led to the identification of putative disease-causing mutations in 77 % and 82 % within the South African and Danish cohorts, respectively [4, 57]. This is comparable to those reported in other screening studies [54, 55, 62].

LQTS is characteristically caused by "private" mutations (distinct genetic mutations which are observed in a single family) and screening of the two cohorts, presented here, identified many such mutations. However, the South African cohort included 23 founder families [63, 64] and screening of the Danish cohort identified several probands who carried identical mutations (five carriers of KCNH2:p.F29L and two carriers of KCNH2:p.K101E), these mutations were shown to be identical-by-descent in these probands and are thus the first Danish LQTS founder mutations to be described. Despite these founder mutations, the mutation spectrum in Denmark is similar to that found in other populations [1]. Whereas, the spectrum of mutations in the South African cohort is strongly skewed by the South African founder mutation (KCNQ1:p.A341V), which is carried by 52 % of the South African probands; in fact, even when accounting for the frequency of this mutation in the cohort, the mutation spectrum in South Africa is still very much skewed with respect to the number of *KCNQ1* mutations. Furthermore, no *SCN5A* mutations were identified in this cohort. The South African LQTS cohort is strongly biased to individuals of European descent (~9 % of the total population of South Africa) and is not representative of all population groups in South Africa; this is despite extensive efforts to identify LQTS patients in the Black population. The "missed" LQTS mutations in the South African population may be present in a population group not well represented in the South African LQTS cohort. The Danish LQTS cohort, on the other hand, is representative of the Danish population. This difference in the representativity of the two cohorts might account for the differences in the mutation distribution observed within the two groups.

We also determined that 4.5 % and 1.4 % of the South African and Danish cohorts were double or compound heterozygotes, respectively [4, 5]; this too is comparable to the frequency of double and compound heterozygotes seen in other populations [65]. The identification of double heterozygotes within the South African founder cohort emphasises the importance of performing comprehensive genetic screens on all LQTS probands, irrespective of the frequency of a founder mutation within the population; this is clinically relevant with respect to cascade screening within these families. Prior to preparing "Paper 2: Mutations in Danish Patients with Long QT Syndrome and Identification of a Large Founder Family with p.F29L in KCNH2" Grunnet *et* al. reported identifying a putative double heterozygote in this cohort (KCNQ1:p.R591H – KCNH2:p.R328C) [52]. Furthermore, analysis of the *CAV3* gene in this cohort also identified another putative double heterozygote (KCNH2:p.I400N – CAV3:p.T78M) [6]. Functional assessment of these mutations and additional clinical investigations, particularly in family members who carry the mutations singularly, lead to the conclusion that the KCNQ1:p.R591H and the CAV3:p.T78M mutations were not contributing to disease in these families [6, 52]. This highlights the need for greater clinical and functional characterisation of novel mutations, particularly in cases of double and compound heterozygotes, as carriers of

multiple disease-causing mutations have been previously reported to exhibit more severe clinical phenotypes and a consequent increased need for clinical attention [66].

LQTS is characterised by broad clinical heterogeneity and incomplete penetrance which point to the existence of additional factors (genetic or environmental) that affect the QT interval and clinical presentation [63, 67, 68]. Several synonymous genetic variants, as well as frequently occurring non-synonymous genetic variants, were also identified in the South African cohort. Such polymorphisms are not disease causing, but they may modify the phenotype [1]. These potential *forme fruste* mutations, i.e. mutations that do not appear to cause disease in isolation, may affect cardiac repolarisation and thereby play a role in disease. A number of non-synonymous polymorphisms have been reported to be associated with an effect in cardiac repolarisation currents [69]. KCNE1:p.D85N has been implicated in drug-induced LQTS [70] and KCNH2:p.R1047L has been reported to reduce I_{Kr} in a mammalian cell-based system [71]. Additionally, several polymorphisms have been reported to modify the functional effects of the LQTS-causing mutation which affects the South African founder group (*KCNQ1*:p.A341V) e.g. *KCNH2*:p.K897T [72] and several polymorphisms in *NOS1AP* [73].

While the pathophysiological effect of such genetic modifiers is not yet fully understood, it is hoped that, with the increasing application of next generation sequencing technologies in human genetics, it will become possible to identify novel causes and modifiers of diseases.

7.2 DETERMINING THE PATHOGENICITY OF VARIANTS IN LQTS-CAUSING GENES

Determining pathogenicity of genetic variants has changed dramatically during the course of these studies. The past decade has seen remarkable technical developments in the field of genetics along with an unprecedented ability to generate, store and evaluate large amounts of genetic data. There are currently over 6500 exomes available in the Exome Variant Server [74] and this growing number of well-documented exomes represents thousands of *in silico* control chromosomes, which subsequently increases the power to detect causal alleles, even when the number of available cases is limited as is frequently the case in LQTS. An assessment of SIDS causing mutations within the exome data collected in various population studies found that many of these variants occurred at a high frequency within these populations, thus bringing in to question the role that these variants may play in SIDS [75].

The sheer volume of publically available genetic data generated in both disease and normal populations necessitates that we, as medical geneticists, reassess genetic variants associated with our disease of interest. Variants of unknown significance (VUS), as well as variants which

have been reported to be pathogenic, may be reclassified as genetic data is generated in other populations (a great deal of genetic data is Eurocentric) or clinical and functional data provide additional evidence supporting or opposing the initial characterisation of the variant. For instance, KCNH2:p.R176W was initially identified as a Finnish founder mutation [76] and a number of studies supported the pathogenicity of this variant [77-79], however, recently this variant has been determined to be a benign polymorphism [80, 81]. Another interesting example of how a variant might be reclassified as new data is generated is the KCNE1:p.D85N variant which poses an interesting challenge for medical geneticist working with LQTS. The KCNE1:p.D85N variant affects the I_{Ks} current, influences the QTc interval length [82] and is associated with drug-induced LQTS [70]. Furthermore, in 2009 a Japanese group reported that KCNE1:p.D85N was a disease-causing variant in Japan [83], a subsequent publication by this group reported that in a family carrying both KCNE1:p.D85N and KCNH2:p.E58K; individuals who were double heterozygotes experienced syncope while single mutation carriers were asymptomatic [84]. KCNE1:p.D85N occurs at MAF of 1.2 % in a population of European descent and a MAF of 0.2 % in a population of African descent [74]. While it is without doubt that this variant influences the QTc interval, it is unclear to what extent this variant is clinically relevant, but it is currently considered to be a low-penetrant causal variant or a modifying variant which is insufficient to cause disease in isolation.

We should consider that the criteria that we traditionally apply when characterising genetic variants do not address questions of functional impairment or of clinical relevance directly (Figure 9).

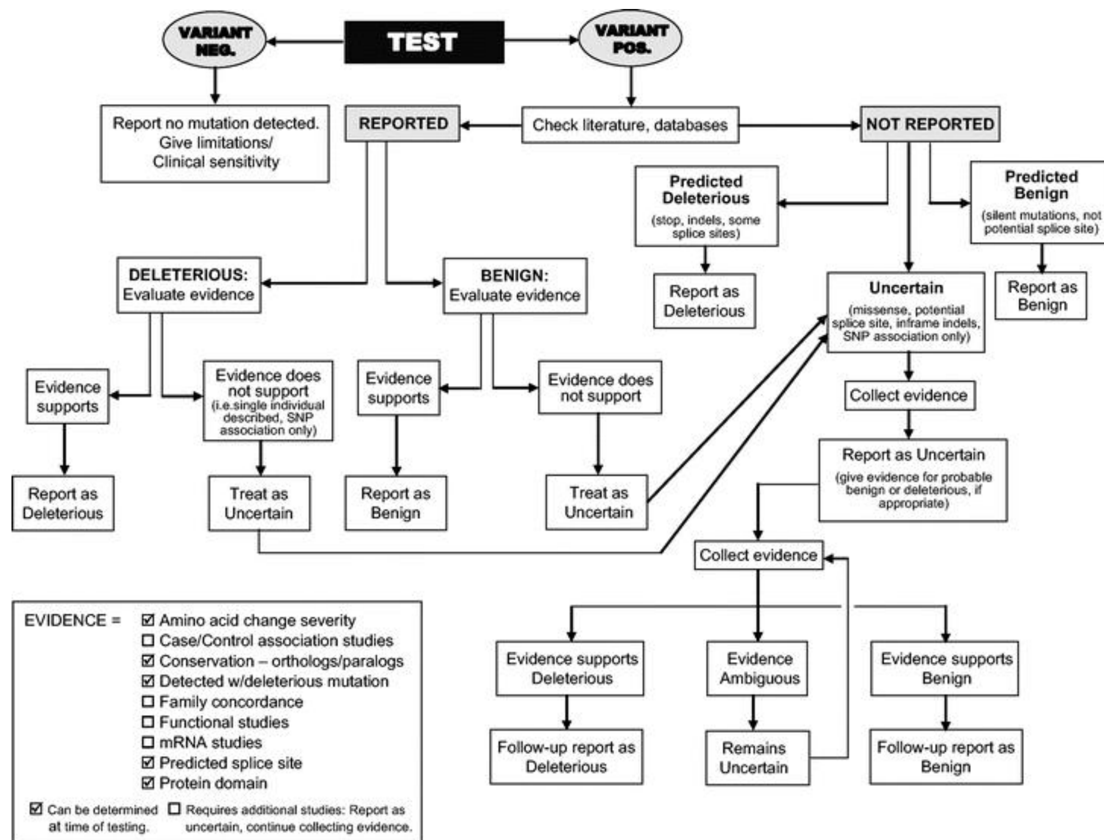

Figure 9: A decision tree for the clinical interpretation of genetic variants. Types of evidence are shown in the box at the bottom left of the figure [85].

Generally, a genetic variant is predicted to be deleterious and thereby "likely-pathogenic" if it has an apparent functional effect i.e. it results in a frameshift, introduces a stop codon or disrupts correct splicing. Furthermore, a missense mutation is considered "probably-pathogenic" if there is evidence that it has a deleterious effect on protein function. Such evidence includes a prediction of the severity of the amino acid substitution, the level of conservation of the affected amino acid residue and its segregation with disease in an affected kindred and/or its absence in > 100 control alleles.

The argument for disease-causation is strengthened if the likely- or probably-pathogenic variant has been previously associated with disease. But the question of the clinical relevance of a putative mutation is difficult to address in diseases, like LQTS, which are characterised by "private" mutations, often found in small families. Functional assessments of putative disease-causing mutations are rarely available and the clinical evidence for disease-causation is not readily available in the literature. Consequently, the classification of a genetic variant as disease-causing is based on the inference of a few functional studies performed under *in vitro* conditions. Several prediction programs (e.g. SIFT, PANTHER, PolyPhen-2 and MutPred) have been developed to predict the likelihood that a given variant is pathogenic and so provide a means of distinguishing pathogenic mutations from rare, benign variants. These programs

typically apply algorithms which take into account evolutionary conservation of the primary structure of the protein as well as some measure of the effect a variant may have on the secondary structure. While these prediction programs require further validation and cannot, currently, substitute for a detailed understanding of the disease-mechanism several groups have determined that using the consensus predictions of several programs significantly improved the overall predictive value [86].

7.3 IDENTIFYING AND EVALUATING NEW GENETIC CAUSES OF LONG QT SYNDROME

The two population-based genetic screening studies reported here [4, 5] were limited to screening the five most frequently implicated LQTS-causing genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*). Using this limited approach, ~20% of LQTS cases are currently mutation-negative. However, including more LQTS-causing genes in this standard screen may not be a cost effective approach to identifying the cause of disease in these cases, as the contribution of these additional genes is, in most cases, <1 % [1, 3].

7.3.1 COPY NUMBER VARIANTS

While, unknown LQTS-causing genes could account for some of the mutation-negative LQTS-cases observed here, a number of these cases might be attributable to exonic rearrangements in known LQTS-causing genes which are not detectable using standard PCR-based methods, which form the basis of most mutation screening methodologies.

Copy number variants (CNVs) are defined as > 1Kb segments of DNA that differ in copy number between genomes. They encompass more DNA than single nucleotide polymorphisms (SNPs) within the genome and are a major source of genetic variation in humans [87]. CNVs can be limited to a single exon in a gene or include a contiguous set of genes [88]. Additionally, *de novo* locus-specific mutation rates for CNVs are between 2-4 orders of magnitude greater than those for SNPs [89].

As with other forms of genetic variation, CNVs may be of no significance to the phenotype or may be associated with clinical disease. Dosage sensitive genes may be susceptible to haploinsufficiency as a result of a CNV encompassing a whole gene, alternatively the deletion or duplication of an exon within a gene could result in the haploinsufficiency as a result of nonsense-mediated decay [90]. CNVs may exert their influence on phenotypic variability and disease susceptibility by modifying the expression of affected genes or by modifying the expression of genes close to the rearranged region [91]. Purifying selection has been reported to

act on several CNV loci, suggesting that rare variants potentially play a role in disease [92]. Furthermore, several whole genome assessments of structural variation have discovered CNVs in the genomic regions containing LQTS genes [93-100].

Using multiplex ligation-dependent probe amplification (MLPA) to assess a large portion of the Danish LQTS probands (91 %) lead to the identification of a large deletion affecting three exons in *KCNQ1* [5]. Indicating that CNVs within *KCNQ1* account for ~1.5 % of LQTS cases, greater than the occurrence of disease-causing mutations in *KCNE1* or *KCNE2* which are currently part of the standard LQTS screen [5]. Swedish and French studies identified a similar proportion of CNVs in their LQTS-cohorts [101, 102]. The significance of confirming these results using a second method cannot be overstated. An unpublished, study of 205 Danish LQTS probands identified CNV's within the five LQTS genes at a similar frequency, however these findings could not be confirmed by long range PCR or Genome-Wide Human SNP Array 6.0 (Affymetrix) (Hedley *et al*. unpublished data) proving the MLPA discoveries to be false and highlighting the necessity of confirming MLPA results using a second method.

7.3.2 ASSESSING THE EVIDENCE

Screening of the *CAV3* gene in Danish and British LQTS probands resulted in the identification of the c.233C>T; p.T78M variant in a Danish family, which was already known to be affected by the Kv11.1:p.I400N mutation [6]. Caveolin-3:p.T78M, has previously been associated with a number of diseases [103-106] including LQTS [107] and SIDS [108]. However, the association with disease, in all cases, was not clear-cut and, taken together, these data do not support the hypothesis that the caveolin-3:p.T78M mutation is pathogenic in the heterozygous state.

Caveolin-3 is known to play a role in the availability of Kv11.1 at the cell membrane under hypokalaemic conditions [109]. This interdependence between hypokalaemia and caveolin-3 function may explain the highly variable phenotypic expression of *CAV3* mutations. Furthermore, *in vitro* studies showed that caveolin-3:p.T78M impairs the formation of caveolae in muscle cells [110-112] and disruption of caveolae might affect the beta-adrenergic responsiveness and the excitation–contraction coupling of cardiomyocytes [113-115]. Vaidyanathan *et al*. found that mutations in *CAV3* (including p.T78M) significantly decreased I_{K1} density [116]. It is interesting to speculate that the effect caveolin-3:p.T78M has on individual cardiac ion channels which form part of macromolecular complexes (containing several such ion channels) within caveolae [109, 117, 118] might produce a cumulative and possibly compensatory effect on the currents that make up the AP, making the effect of CAV3 mutations very difficult to assess using standard electrophysiological methods, which do not recapitulate

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the physiological environment. The use of patient-specific induced pluripotent stem-cells [119] may be the key to resolving the clinically relevant effect *CAV3* mutations have on the AP.

While experimental evidence of the functional effect of an amino acid substitution may, in many cases, be lacking *in silico* prediction servers such as Polyphen-2 [120], SIFT [121] and MutationAssessor [122] can be used to assess the possible functional significance of an amino acid substitution. Using these three prediction servers, 80 % of LQTS mutations identified in the Danish LQTS cohort, with known *in vitro* deleterious effects, were considered to have a functional effect [5].

7.3.3 GENETIC VARIANTS OF MICRORNA GENES DO NOT PLAY A ROLE IN LONG QT SYNDROME

Investigating genes involved in the regulation of cardiac ion channels is another strategy to identify the cause of disease in the ~10-20 % of LQTS cases which are currently considered mutation-negative. Animal models suggest that microRNAs (miRNAs) might be involved in the cardiac repolarisation [123] and a number of associations between SNPs in predicted miRNA binding sites on target mRNAs and phenotypic traits have previously been reported [124-127].

Pri-miRNAs undergo a two-step cleavage process to produce a small (~22 nucleotide (nt)) mature miRNA. Seed regions (short 3-8 nt regions important for target specificity) of miRNA's are highly conserved and SNPs are rarely seen in these regions. However, several reports provided evidence of clinically relevant phenotypes, in humans and mice, associated with miRNA seed region variants [128-130]. Variants outside of the seed region of mature miRNA have also been associated with clinical phenotypes; Dorn *et al.* demonstrated that a rare variant in mature MiR-499 protected against cardiomyopathy in a transgenic mouse model [131] and Ohanian *et al*. identified a genetic variant in *mir-133a-2* which altered strand abundance resulting in an accumulation of the miRNA* (the degraded/passenger strand of miRNA duplex) strand in an atrial fibrillation patient [132]. Furthermore, several SNPs in pri-miRNA genes have been reported to affect processing and expression levels of mature miRNA [127, 133]. Amin *et al*. proposed that SNPs within miRNA binding sites may modify disease severity by virtue of the effect miRNAs have on translation of these transcripts [134]. We hypothesised that variants within the miRNA genes themselves may cause disease by disrupting the regulation of cardiac ion channels.

We screened four miRNA genes in 125 Danish LQTS probands and found no putative disease-causing mutations. However, we did not evaluate strand abundance and, consequently, we cannot conclude that none of the variants identified affect strand abundance [7].

7.4 CARDIAC SODIUM CHANNEL OVERLAP SYNDROMES

The *SCN5A* gene encodes the voltage gated α-subunit of the cardiac sodium channel, which controls the influx of Na^+ into cardiomyocytes during the initial rapid depolarisation phase of the cardiac AP (phase 0). Mutations in *SCN5A* have been implicated in several cardiac diseases, including LQTS-type-3 (LQT3), BrS, cardiac conduction disease (CCD), sick sinus syndrome, atrial standstill, AF and DCM. The most common signs and symptoms of SCN5A-disease are depicted in Figure 10.

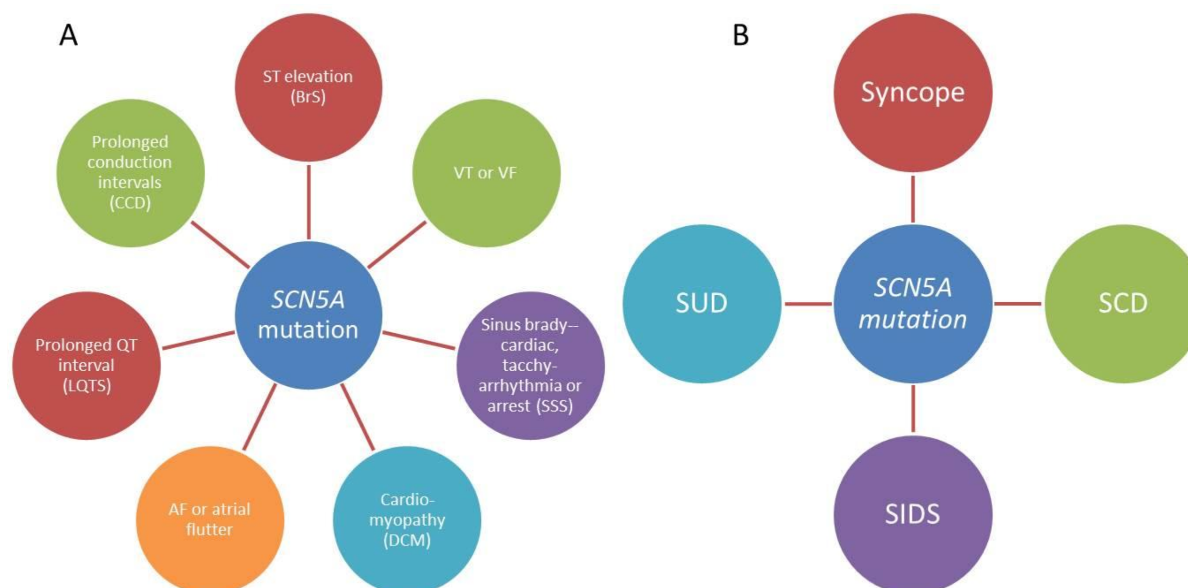

*Figure 10: **A**. Common signs and **B**. symptoms of SCN5A-disease. Figure adapted from [135]*

***AF**: atrial fibrillation, **BrS**: Brugada syndrome, **CCD**: cardiac conduction disease, **DCM**: dilated cardiomyopathy, **LQTS**: long QT syndrome, **SCD**: sudden cardiac death, **SIDS**: sudden infant death syndrome, **SSS**: sick sinus syndrome, **SUD**: sudden unexpected death.*

Initially, the various diseases associated with mutations in *SCN5A* were considered distinct clinical entities. However, reports of overlapping phenotypes, referred to as "cardiac sodium channel overlap syndromes", in which clinical and biophysical properties of these diseases are mixed within a family or even within a single individual, negates this assertion [136].

The clinical and genetic diagnosis of these overlap syndromes is further complicated by variable disease expression within an affected family. Furthermore, the clinical management of cases with LQT3/BrS overlap syndrome is particularly problematic. Beta-blockers are effective in LQTS, but are known to increase ST elevation in BrS [137]. Sodium-blockers may worsen BrS, although quinidine has been suggested to be useful in BrS due to the beneficial I_{to} blocking effect, but its I_{Kr} blocking abilities would certainly induce further QT prolongation and *torsades des pointes*. New alternatives, such as ranolazine, a late sodium channel blocker, may be

effective in treating LQT3/BrS overlap patients. The clinical problems associated with treating these overlap patients highlights the significance of identifying them.

We have reported the identification of a LQT3/BrS overlap syndrome, unmasked by flecainide testing, caused by the Nav1.5:p.L1786Q mutation [8] and we identified several known LQT3-causing mutations in an early-onset lone AF cohort [9].

Flecainide testing of the Danish *SCN5A*-mutation-positive AF cohort did not reveal concealed BrS, as expected [138], however it did reveal a greater prolongation in QT_c interval during flecainide testing than that expected for healthy individuals [139]. Indeed, three of the seven patients carrying LQT3-associated mutations had either a borderline prolonged QT_c interval at baseline or a greater than expected prolongation of the QT_c interval during flecainide testing [139].

It has been reported that LQTS mutation-carriers with QT_c intervals <440ms have an increased risk for life-threatening cardiac events [1]. Consequently, we speculate that the early-onset AF patients carrying LQT3-associated mutations may have an increased risk of life-threatening arrhythmias. Furthermore, Lemoine *et al.* demonstrated atrial action potential prolongation, atrial EAD and triggered activity in a genetically modified animal model of human LQT3 [140].

Late onset of a normal T-wave pattern as described by Zhang *et al*. [141] was observed in the Nav1.5:p.L1786Q patients described here, as well as LQTS/BrS overlap patients carrying Nav1.5:p.E1784K [142] and Nav1.5:p.1795insD [143]. This pattern is uncommon in LQT3 patients [141], and maybe indicative of LQT3/BrS overlap syndrome in individuals with *SCN5A* mutations.

However improbable these cardiac sodium channel overlap syndromes seemed when first discovered they pose an interesting clinical problem in determining the best means of handling an *SCN5A*-mutation carrier. The association of *SCN5A* mutations with DCM [144, 145] suggests that the cardiac sodium channel may play a more complex role in cardiomyocyte function than previously thought.

7.5 CHANNELOPATHY GENES CAUSE CARDIOMYOPATHY

Patients with structural heart diseases such as hypertrophic cardiomyopathy (HCM) are at risk of developing life-threatening arrhythmias. In addition to the role *SCN5A* mutations play in structural heart disease cardiac ion channel β-subunits have been hypothesised to play a role in hypertrophy. A *kcne2* null mouse model has been shown to develop cardiac hypertrophy as a consequence of hypothyroidism [146]. Furthermore, it has been suggested that mink (encoded

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by *KCNE1*), through its interaction with telethonin [147], forms part of an “electro-mechanical feedback” system which links cardiomyocyte stretching to potassium flux.

We analysed the KCNE genes (*KCNE1*, *KCNE2*, *KCNE3*, *KCNE4* and *KCNE1L*) in a Danish HCM cohort. While variants in these genes have been associated with several inherited arrhythmic disorders such as LQTS [1, 2, 148, 149], AF [150] and BrS [151], the frequency of arrhythmia-associated genetic variants was so low in the Danish HCM cohort, and did not convincingly differ from that of controls, that variants in these genes cannot explain the increased occurrence of arrhythmia in HCM [152]. However, the previously arrhythmia-associated variants minK:p.D85N [66, 153, 154] and MiRP1:p.T8A [155], as well as very rare genetic variants with likely functional significance, i.e. MiRP2:p.M1T and MiRP3:p.E141A, occurred more frequently in controls than in HCM patients. This suggests that neither *KCNE1*, despite its physical association with telethonin at the Z-disc [147], nor the other KCNE genes, are common causes of HCM. However, rare, but functionally significant variants in these genes may contribute to the risk of developing arrhythmias in the general population.

CHAPTER 8: CONCLUSION AND FUTURE DIRECTION

There appears to be a lack of clinical relevance and biological significance in the criteria applied to the characterisation of disease-causing mutations. This was highlighted in our study of caveolin-3:p.T78M, which was purported to be a disease-causing variant in a number of diseases, and yet, when evaluating the evidence, we concluded that this variant is a rare polymorphism [6]. While we cannot exclude that caveolin-3:p.T78M modifies the disease phenotype, we must question the rationale for defining *CAV3* as LQT9.

Furthermore, using standard screening methods, the genetic cause of ~20 % of LQTS cases will remain unknown. In a bid to improve this detection rate, we employed several strategies to identify other genetic causes e.g. exon rearrangements in LQTS-causing genes, but unfortunately these were largely unsuccessful. The clinical significance and diagnostic utility of CNV screening in the LQTS genes remains unresolved. It would appear that CNV screening is more useful in a cohort of mutation-negative cases, but, as we are becoming increasingly aware, we cannot exclude the possibility of double and compound heterozygotes in LQTS. Indeed, the absence of CNV data is a limitation of all the genetic screening studies reported here. In addition, the assessment of miRNA genetic variation in LQTS patients in our study is limited by solely focusing on identifying genetic variation within the miRNA genes themselves and not assessing the MiR-1 and MiR-133A binding sites of putative target transcripts [7]. This decision was made because there is very little experimental evidence indicating which transcripts are targeted by specific miRNA's.

The study of SCN5A-disease and its characteristic overlap syndromes is highly clinically relevant, as patient management decisions are extremely complicated in these cases. A proportion of LQT3 *SCN5A* mutation-carriers might harbour a concealed BrS phenotype [8], and a proportion of AF *SCN5A* mutation-carriers could harbour a LQTS phenotype [9], along with the concomitant risk of arrhythmic events. Incorrect characterisation of these diseases and consequent incorrect treatment of these patients could inadvertently harm the patient and strategies should be in place to avoid this possibility.

Significant progress has been made in understanding the genetic and biophysical aspects of LQTS. Despite this, establishing a genetic diagnosis in LQTS cases is still a complex matter as a consequence of the broad clinical heterogeneity, incomplete penetrance and the high prevalence of "private" mutations. To further complicate matters, the occurrence of double or compound heterozygotes and the involvement of modifying factors are not fully accounted for using current methodologies. This complexity raises a number of ethical issues; the clinical

relevance of a putative mutation is, in many instances, associated with a large degree of uncertainty and the implications of communicating this uncertainty have not been adequately evaluated [156]. Furthermore, the "duty-to-warn" at-risk relatives frequently outweighs the right of a family member "not-to-know" their mutation status [157].

The clinical service provided for these patients and their families could be strengthened by ensuring that the treating physicians are genetically-literate and work closely with genetic counsellors to best handle family data and disseminate genetic information into the family. As genetic analysis in LQTS should be reserved for patients with clinical signs and symptoms of disease followed by cascade screening of family members, it is necessary that these tests be ordered and interpreted by a specialist in cardiology and clinical genetics. However, clinical genetics is a fast evolving field; direct-to-consumer genetic screening in addition to limited interpretation and counselling services place a large burden on primary health physicians and nurses to keep abreast of the clinical application and interpretation of current genetic analyses [158]. Educating physicians and nurses in clinical genetics will relieve this burden and improve patient care. It is naïve to think, in this changing "landscape" of clinical genetics, that genetic testing can be entirely limited to specialised centres; in light of this, it is important to develop clear policies with respect to clinical utility and genetic counselling [159, 160]. Finally, it is very important that the clinical findings and the molecular data related to putative disease-causing mutations are made available to the scientific community, so that better interpretations of genetic data can be translated into clinical practice. This includes creating better access to negative data which is sorely missing from current scientific literature [10].

Several genetic variants within the known LQTS-causing genes have been found to be associated with acquired LQTS [70, 161], meaning that an improved understanding of the causes and mechanisms of congenital LQTS can be applied to the acquired LQTS. While the incidence of acquired LQTS is largely unknown, it is considered low for any one drug [162]. However, the significance for public health lies in the number and spectrum of drugs which potentially prolong the QT interval and the large number of patients receiving these drugs. These drugs are tightly regulated and in some cases have been withdrawn from the market [163].

The continued combination of population-based genetic studies with molecular and functional studies, along with the application of new methods to evaluate genetic causes and modifiers of disease, will help identify and categorise potentially relevant genetic variants. Additionally, a re-evaluation of the current criteria applied to characterise a disease-causing mutation and the development of improved *in silico* tools to better predict the consequence of novel mutations will facilitate better risk stratification in carriers of LQTS-causing mutations. Next generation sequencing (NGS) technologies hold great promise in the identification of novel genetic factors

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of disease. Ultimately, clinical genetic laboratories will increase the success rate of genetic screens through the use whole genome/exome sequencing. In addition to improving screening outcomes and generating insight into disease mechanisms, NGS generates vast amounts of data which requires improved bioinformatics and data-handling solutions as well as clear guideline with respect to informing patients and the role of physicians and geneticists in this process. Furthermore, the collection of whole genome/whole exome data on large families, like the South African founder families, would, in the future, provide the possibility to identify possible second-order interactions between causal and modifying genes.

CHAPTER 9: REFERENCES

1. Hedley PL, Jorgensen 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:**1486-1511.
2. Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA, Christiansen M: **The genetic basis of Brugada syndrome: a mutation update.** *Hum Mutat* 2009, **30:**1256-1266.
3. Christiansen M, Hedley PL: **Long QT testing: implications for complex diagnosis in personalized medicine.** *Personalized Medicine* 2010, **7:**125-127.
4. Hedley PL, Durrheim GA, Hendricks F, Goosen A, Jespersgaard C, Stovring B, Pham TT, Christiansen M, Brink PA, Corfield VA: **Long QT syndrome in South Africa : the results of comprehensive genetic screening : cardiovascular topic.** *Cardiovascular Journal Of Africa* 2013, **24:**231-237.
5. Christiansen M, Hedley PL, Theilade J, Stoevring B, Leren TP, Eschen O, K.M. S: **Mutations in Danish patients with long QT syndrome and identification of a large founder family with p.F29L in *KCNH2*.** *BMC Medical Genetics* 2013, **submitted**.
6. Hedley PL, Kanters JK, Dembic M, Jespersen T, Skibsbye L, Aidt FH, Eschen O, Graff C, Behr ER, Schlamowitz S, et al: **The Role of CAV3 in Long QT Syndrome: Clinical and Functional Assessment of a Caveolin-3/Kv11.1 Double Heterozygote Versus Caveolin-3 Single Heterozygote.** *Circ Cardiovasc Genet* 2013, **6:**452-461.
7. Hedley PL, Carlsen AL, Christiansen K, Kanters JK, Behr ER, Corfield VA, Christiansen M: **MicroRNA in long QT syndrome.** *Scandinavian Journal of Clinical & Laboratory Investigation* 2013, **Submitted**.
8. Kanters JK, Yuan L, Hedley PL, Stoevring B, Joens C, Bloch-Thomsen PE, Grunnet M, Christiansen M, Jespersen T: **Flecainide provocation reveals concealed Brugada syndrome in a long QT syndrome family with a novel L1786Q mutation in SCN5A.** *Circulation Journal* 2013, **Submitted**.
9. Olesen MS, Yuan L, Liang B, Holst AG, Nielsen N, Nielsen JB, Hedley PL, Christiansen M, Olesen S-P, Haunsø S: **High Prevalence of Long QT Syndrome–Associated SCN5A Variants in Patients With Early-Onset Lone Atrial FibrillationClinical Perspective.** *Circ Cardiovasc Genet* 2012, **5:**450-459.

Stellenbosch University http://scholar.sun.ac.za

10. Hedley PL, Haundrup O, Andersen PS, Aidt FH, Jensen M, Moolman-Smook JC, Bundgaard H, Christiansen M: **The KCNE genes in hypertrophic cardiomyopathy: a candidate gene study.** *J Negat Results Biomed* 2011, **10:**12.
11. McCain ML, Lee H, Aratyn-Schaus Y, Kleber AG, Parker KK: **Cooperative coupling of cell-matrix and cell-cell adhesions in cardiac muscle.** *Proc Natl Acad Sci U S A* 2012, **109:**9881-9886.
12. Severs NJ: **The cardiac muscle cell.** *Bioessays* 2000, **22:**188-199.
13. Jongbloed MR, Vicente Steijn R, Hahurij ND, Kelder TP, Schalij MJ, Gittenberger-de Groot AC, Blom NA: **Normal and abnormal development of the cardiac conduction system; implications for conduction and rhythm disorders in the child and adult.** *Differentiation* 2012, **84:**131-148.
14. Braam SR, Passier R, Mummery CL: **Cardiomyocytes from human pluripotent stem cells in regenerative medicine and drug discovery.** *Trends Pharmacol Sci* 2009, **30:**536-545.
15. Durrer D, van Dam RT, Freud GE, Janse MJ, Meijler FL, Arzbaecher RC: **Total excitation of the isolated human heart.** *Circulation* 1970, **41:**899-912.
16. Lazzara R: **Arrhythmia mechanisms in the new millennium.** *J Interv Card Electrophysiol* 2001, **5:**133-135.
17. Wellens HJ: **Cardiac arrhythmias: the quest for a cure: a historical perspective.** *J Am Coll Cardiol* 2004, **44:**1155-1163.
18. Chakrabarti S, Stuart AG: **Understanding cardiac arrhythmias.** *Arch Dis Child* 2005, **90:**1086-1090.
19. Tan HL, Hou CJ, Lauer MR, Sung RJ: **Electrophysiologic mechanisms of the long QT interval syndromes and torsade de pointes.** *Ann Intern Med* 1995, **122:**701-714.
20. Bazett HC: **The time relations of the blood-pressure changes after excision of the adrenal glands, with some observations on blood volume changes.** *J Physiol* 1920, **53:**320-339.
21. Keating MT, Sanguinetti MC: **Molecular and cellular mechanisms of cardiac arrhythmias.** *Cell* 2001, **104:**569-580.
22. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, et al: **Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee.** *Circulation* 2007, **115:**e69-171.
23. Nattel S: **New ideas about atrial fibrillation 50 years on.** *Nature* 2002, **415:**219-226.
24. Zipes DP, Wellens HJ: **Sudden cardiac death.** *Circulation* 1998, **98:**2334-2351.

Stellenbosch University http://scholar.sun.ac.za

25. Josephson M, Wellens HJ: **Implantable defibrillators and sudden cardiac death.** *Circulation* 2004, **109:**2685-2691.
26. Fishman GI, Chugh SS, Dimarco JP, Albert CM, Anderson ME, Bonow RO, Buxton AE, Chen PS, Estes M, Jouven X, et al: **Sudden cardiac death prediction and prevention: report from a National Heart, Lung, and Blood Institute and Heart Rhythm Society Workshop.** *Circulation* 2010, **122:**2335-2348.
27. Kong MH, Fonarow GC, Peterson ED, Curtis AB, Hernandez AF, Sanders GD, Thomas KL, Hayes DL, Al-Khatib SM: **Systematic review of the incidence of sudden cardiac death in the United States.** *J Am Coll Cardiol* 2011, **57:**794-801.
28. Deo R, Albert CM: **Epidemiology and genetics of sudden cardiac death.** *Circulation* 2012, **125:**620-637.
29. Rubart M, Zipes DP: **Mechanisms of sudden cardiac death.** *J Clin Invest* 2005, **115:**2305-2315.
30. Huikuri HV, Castellanos A, Myerburg RJ: **Sudden death due to cardiac arrhythmias.** *N Engl J Med* 2001, **345:**1473-1482.
31. Adamson PB, Barr RC, Callans DJ, Chen PS, Lathrop DA, Makielski JC, Nerbonne JM, Nuss HB, Olgin JE, Przywara DA, et al: **The perplexing complexity of cardiac arrhythmias: beyond electrical remodeling.** *Heart Rhythm* 2005, **2:**650-659.
32. Mohler PJ, Splawski I, Napolitano C, Bottelli G, Sharpe L, Timothy K, Priori SG, Keating MT, Bennett V: **A cardiac arrhythmia syndrome caused by loss of ankyrin-B function.** *Proc Natl Acad Sci U S A* 2004, **101:**9137-9142.
33. Priori SG, Barhanin J, Hauer RN, Haverkamp W, Jongsma HJ, Kleber AG, McKenna WJ, Roden DM, Rudy Y, Schwartz K, et al: **Genetic and molecular basis of cardiac arrhythmias; impact on clinical management. Study group on molecular basis of arrhythmias of the working group on arrhythmias of the european society of cardiology.** *Eur Heart J* 1999, **20:**174-195.
34. Drew BJ, Ackerman MJ, Funk M, Gibler WB, Kligfield P, Menon V, Philippides GJ, Roden DM, Zareba W, American Heart Association Acute Cardiac Care Committee of the Council on Clinical Cardiology tCoCN, the American College of Cardiology F: **Prevention of torsade de pointes in hospital settings: a scientific statement from the American Heart Association and the American College of Cardiology Foundation.** *Circulation* 2010, **121:**1047-1060.
35. Grilo LS, Carrupt PA, Abriel H: **Stereoselective Inhibition of the hERG1 Potassium Channel.** *Front Pharmacol* 2010, **1:**137.
36. Al-Khatib SM, LaPointe NM, Kramer JM, Califf RM: **What clinicians should know about the QT interval.** *JAMA* 2003, **289:**2120-2127.

37. Shewan LG, Coats AJS: **Ethics in the authorship and publishing of scientific artcles.** *International Journal of Cardiology* 2010.

38. Modell SM, Lehmann MH: **The long QT syndrome family of cardiac ion channelopathies: a HuGE review.** *Genet Med* 2006, **8:**143-155.

39. Goldenberg I, Moss AJ, Peterson DR, McNitt S, Zareba W, Andrews ML, Robinson JL, Locati EH, Ackerman MJ, Benhorin J, et al: **Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome.** *Circulation* 2008, **117:**2184-2191.

40. Moss AJ, Goldenberg I: **Importance of Knowing the Genotype and the Specific Mutation When Managing Patients with Long QT Syndrome.** *Circ Arrhythm Electrophysiol* 2008, **1:**213-226; discussion 226.

41. Hobbs JB, Peterson DR, Moss AJ, McNitt S, Zareba W, Goldenberg I, Qi M, Robinson JL, Sauer AJ, Ackerman MJ, et al: **Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome.** *JAMA* 2006, **296:**1249-1254.

42. Ackerman MJ: **Cardiac causes of sudden unexpected death in children and their relationship to seizures and syncope: genetic testing for cardiac electropathies.** *Semin Pediatr Neurol* 2005, **12:**52-58.

43. Bastiaenen R, Behr ER: **Sudden death and ion channel disease: pathophysiology and implications for management.** *Heart* 2011, **97:**1365-1372.

44. Tester DJ, Ackerman MJ: **Genetic testing for potentially lethal, highly treatable inherited cardiomyopathies/channelopathies in clinical practice.** *Circulation* 2011, **123:**1021-1037.

45. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, Camm AJ, Ellinor PT, Gollob M, Hamilton R, et al: **HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA).** *Heart Rhythm* 2011, **8:**1308-1339.

46. Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, et al: **Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome.** *Circulation* 2000, **101:**616-623.

47. Goldenberg I, Moss AJ, Bradley J, Polonsky S, Peterson DR, McNitt S, Zareba W, Andrews ML, Robinson JL, Ackerman MJ, et al: **Long-QT syndrome after age 40.** *Circulation* 2008, **117:**2192-2201.

48. Liu JF, Jons C, Moss AJ, McNitt S, Peterson DR, Qi M, Zareba W, Robinson JL, Barsheshet A, Ackerman MJ, et al: **Risk factors for recurrent syncope and subsequent fatal or near-fatal**

events in children and adolescents with long QT syndrome. *J Am Coll Cardiol* 2011, **57:**941-950.

49. Heart Rhythm UKFSDSSDG: **Clinical indications for genetic testing in familial sudden cardiac death syndromes: an HRUK position statement.** *Heart* 2008, **94:**502-507.
50. Hofman N, Tan HL, Alders M, van Langen IM, Wilde AA: **Active cascade screening in primary inherited arrhythmia syndromes: does it lead to prophylactic treatment?** *J Am Coll Cardiol* 2010, **55:**2570-2576.
51. Kaufman ES, Priori SG, Napolitano C, Schwartz PJ, Iyengar S, Elston RC, Schnell AH, Gorodeski EZ, Rammohan G, Bahhur NO, et al: **Electrocardiographic prediction of abnormal genotype in congenital long QT syndrome: experience in 101 related family members.** *J Cardiovasc Electrophysiol* 2001, **12:**455-461.
52. Grunnet M, Behr ER, Calloe K, Hofman-Bang J, Till J, Christiansen M, McKenna WJ, Olesen SP, Schmitt N: **Functional assessment of compound mutations in the KCNQ1 and KCNH2 genes associated with long QT syndrome.** *Heart Rhythm* 2005, **2:**1238-1249.
53. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AA, Ackerman MJ: **Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test.** *Heart Rhythm* 2009, **6:**1297-1303.
54. Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S: **Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice.** *JAMA* 2005, **294:**2975-2980.
55. Tester DJ, Will ML, Haglund CM, Ackerman MJ: **Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing.** *Heart Rhythm* 2005, **2:**507-517.
56. Wilde AA: **Long QT syndrome: a double hit hurts more.** *Heart Rhythm* 2010, **7:**1419-1420.
57. Theilade J, Kanters J, Henriksen FL, Gilsa-Hansen M, Svendsen JH, Eschen O, Toft E, Reimers JI, Tybjaerg-Hansen A, Christiansen M, et al: **Cascade Screening in Families with Inherited Cardiac Diseases Driven by Cardiologists: Feasibility and Nationwide Outcome in Long QT Syndrome.** *Cardiology* 2013, **126:**131-137.
58. Vincent GM, Zhang L: **The role of genotyping in diagnosing cardiac channelopathies : progress to date.** *Mol Diagn* 2005, **9:**105-118.
59. Koopmann TT, Alders M, Jongbloed RJ, Guerrero S, Mannens MM, Wilde AA, Bezzina CR: **Long QT syndrome caused by a large duplication in the KCNH2 (HERG) gene undetectable**

by current polymerase chain reaction-based exon-scanning methodologies. *Heart Rhythm* 2006, **3:**52-55.

60. Eddy CA, MacCormick JM, Chung SK, Crawford JR, Love DR, Rees MI, Skinner JR, Shelling AN: **Identification of large gene deletions and duplications in KCNQ1 and KCNH2 in patients with long QT syndrome.** *Heart Rhythm* 2008, **5:**1275-1281.
61. Johnson JN, Ackerman MJ: **QTc: how long is too long?** *Br J Sports Med* 2009, **43:**657-662.
62. Berge KE, Haugaa KH, Fruh A, Anfinsen OG, Gjesdal K, Siem G, Oyen N, Greve G, Carlsson A, Rognum TO, et al: **Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers.** *Scand J Clin Lab Invest* 2008, **68:**362-368.
63. Brink PA, Crotti L, Corfield V, Goosen A, Durrheim G, Hedley P, Heradien M, Geldenhuys G, Vanoli E, Bacchini S: **Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population.** *Circulation* 2005, **112:**2602-2610.
64. de Jager T, Corbett CH, Badenhorst JC, Brink PA, Corfield VA: **Evidence of a long QT founder gene with varying phenotypic expression in South African families.** *J Med Genet* 1996, **33:**567-573.
65. Zaklyazminskaya EV, Abriel H: **Prevalence of Significant Genetic Variants in Congenital Long QT Syndrome is Largely Underestimated.** *Front Pharmacol* 2012, **3:**72.
66. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC: **Compound mutations: a common cause of severe long-QT syndrome.** *Circulation* 2004, **109:**1834-1841.
67. Crotti L, Spazzolini C, Schwartz PJ, Shimizu W, Denjoy I, Schulze-Bahr E, Zaklyazminskaya EV, Swan H, Ackerman MJ, Moss AJ, et al: **The common long-QT syndrome mutation KCNQ1/A341V causes unusually severe clinical manifestations in patients with different ethnic backgrounds: toward a mutation-specific risk stratification.** *Circulation* 2007, **116:**2366-2375.
68. Priori SG, Napolitano C, Schwartz PJ: **Low penetrance in the long-QT syndrome: clinical impact.** *Circulation* 1999, **99:**529-533.
69. Kaab S, Schulze-Bahr E: **Susceptibility genes and modifiers for cardiac arrhythmias.** *Cardiovasc Res* 2005, **67:**397-413.
70. 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 (Berl)* 2004, **82:**182-188.

71. Laohakunakorn P, Benson DW, Yang P, Yang T, Roden DM, Kugler JD: **Bidirectional ventricular tachycardia and channelopathy.** *Am J Cardiol* 2003, **92:**991-995.

72. 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:**1251-1258.

73. 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:**1657-1663.

74. (EVS) EVS: **NHLBI GO Exome Sequencing Project (ESP).** Seattle, WA.

75. Andreasen C, Refsgaard L, Nielsen JB, Sajadieh A, Winkel BG, Tfelt-Hansen J, Haunso S, Holst AG, Svendsen JH, Olesen MS: **Mutations in Genes Encoding Cardiac Ion Channels Previously Associated With Sudden Infant Death Syndrome (SIDS) Are Present With High Frequency in New Exome Data.** *Can J Cardiol* 2013, **29:**1104-1109.

76. Marjamaa A, Salomaa V, Newton-Cheh C, Porthan K, Reunanen A, Karanko H, Jula A, Lahermo P, Vaananen H, Toivonen L, et al: **High prevalence of four long QT syndrome founder mutations in the Finnish population.** *Ann Med* 2009, **41:**234-240.

77. Fodstad H, Bendahhou S, Rougier JS, Laitinen-Forsblom PJ, Barhanin J, Abriel H, Schild L, Kontula K, Swan H: **Molecular characterization of two founder mutations causing long QT syndrome and identification of compound heterozygous patients.** *Ann Med* 2006, **38:**294-304.

78. Lahti AL, Kujala VJ, Chapman H, Koivisto AP, Pekkanen-Mattila M, Kerkela E, Hyttinen J, Kontula K, Swan H, Conklin BR, et al: **Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture.** *Dis Model Mech* 2012, **5:**220-230.

79. Obeyesekere MN, Sy RW, Klein GJ, Gula LJ, Modi S, Conacher S, Leong-Sit P, Skanes AC, Yee R, Krahn AD: **End-recovery QTc: a useful metric for assessing genetic variants of unknown significance in long-QT syndrome.** *J Cardiovasc Electrophysiol* 2012, **23:**637-642.

80. Giudicessi JR, Kapplinger JD, Tester DJ, Alders M, Salisbury BA, Wilde AA, Ackerman MJ: **Phylogenetic and physicochemical analyses enhance the classification of rare nonsynonymous single nucleotide variants in type 1 and 2 long-QT syndrome.** *Circ Cardiovasc Genet* 2012, **5:**519-528.

81. Jou CJ, Barnett SM, Bian JT, Weng HC, Sheng X, Tristani-Firouzi M: **An in vivo cardiac assay to determine the functional consequences of putative long QT syndrome mutations.** *Circ Res* 2013, **112:**826-830.

82. Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B, Guicheney P, Group DESIRS: **Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population.** *Eur J Hum Genet* 2005, **13:**1213-1222.

83. Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, Yamamoto S, Ozawa T, Ding WG, Toyoda F, et al: **D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome.** *J Am Coll Cardiol* 2009, **54:**812-819.

84. Hasegawa K, Ohno S, Itoh H, Makiyama T, Aiba T, Nakano Y, Shimizu W, Matsuura H, Makita N, Horie M: **A rare KCNE1 polymorphism, D85N, as a genetic modifier of long QT syndrome.** *Journal of Arrhythmia* 2013.

85. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, Lyon E, Ward BE, Molecular Subcommittee of the ALQAC: **ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007.** *Genet Med* 2008, **10:**294-300.

86. Ohanian M, Otway R, Fatkin D: **Heuristic methods for finding pathogenic variants in gene coding sequences.** *J Am Heart Assoc* 2012, **1:**e002642.

87. Levy S, Sutton G, Ng PC, Feuk L, Halpern AL, Walenz BP, Axelrod N, Huang J, Kirkness EF, Denisov G, et al: **The Diploid Genome Sequence of an Individual Human.** *PLoS Biol* 2007, **5:**e254.

88. Zhang F, Gu W, Hurles ME, Lupski JR: **Copy number variation in human health, disease, and evolution.** *Annu Rev Genomics Hum Genet* 2009, **10:**451-481.

89. Lupski JR: **Genomic rearrangements and sporadic disease.** *Nat Genet* 2007, **39:**S43-47.

90. Chan D, Weng YM, Graham HK, Sillence DO, Bateman JF: **A nonsense mutation in the carboxyl-terminal domain of type X collagen causes haploinsufficiency in schmid metaphyseal chondrodysplasia.** *J Clin Invest* 1998, **101:**1490-1499.

91. Henrichsen CN, Chaignat E, Reymond A: **Copy number variants, diseases and gene expression.** *Hum Mol Genet* 2009, **18:**R1-8.

92. Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, Aerts J, Andrews TD, Barnes C, Campbell P, et al: **Origins and functional impact of copy number variation in the human genome.** *Nature* 2010, **464:**704-712.

93. Jakobsson M, Scholz SW, Scheet P, Gibbs JR, VanLiere JM, Fung HC, Szpiech ZA, Degnan JH, Wang K, Guerreiro R, et al: **Genotype, haplotype and copy-number variation in worldwide human populations.** *Nature* 2008, **451:**998-1003.

94. Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T, Hansen N, Teague B, Alkan C, Antonacci F, et al: **Mapping and sequencing of structural variation from eight human genomes.** *Nature* 2008, **453:**56-64.

95. Matsuzaki H, Wang PH, Hu J, Rava R, Fu GK: **High resolution discovery and confirmation of copy number variants in 90 Yoruba Nigerians.** *Genome Biol* 2009, **10:**R125.

96. Pang AW, MacDonald JR, Pinto D, Wei J, Rafiq MA, Conrad DF, Park H, Hurles ME, Lee C, Venter JC, et al: **Towards a comprehensive structural variation map of an individual human genome.** *Genome Biol* 2010, **11:**14.

97. Perry GH, Ben-Dor A, Tsalenko A, Sampas N, Rodriguez-Revenga L, Tran CW, Scheffer A, Steinfeld I, Tsang P, Yamada NA, et al: **The fine-scale and complex architecture of human copy-number variation.** *Am J Hum Genet* 2008, **82:**685-695.

98. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, et al: **Global variation in copy number in the human genome.** *Nature* 2006, **444:**444-454.

99. Shaikh TH, Gai X, Perin JC, Glessner JT, Xie H, Murphy K, O'Hara R, Casalunovo T, Conlin LK, D'Arcy M, et al: **High-resolution mapping and analysis of copy number variations in the human genome: a data resource for clinical and research applications.** *Genome Res* 2009, **19:**1682-1690.

100. Zogopoulos G, Ha KC, Naqib F, Moore S, Kim H, Montpetit A, Robidoux F, Laflamme P, Cotterchio M, Greenwood C, et al: **Germ-line DNA copy number variation frequencies in a large North American population.** *Hum Genet* 2007, **122:**345-353.

101. Stattin EL, Bostrom IM, Winbo A, Cederquist K, Jonasson J, Jonsson BA, Diamant UB, Jensen SM, Rydberg A, Norberg A: **Founder mutations characterise the mutation panorama in 200 Swedish index cases referred for Long QT syndrome genetic testing.** *BMC Cardiovasc Disord* 2012, **12:**95.

102. Barc J, Briec F, Schmitt S, Kyndt F, Le Cunff M, Baron E, Vieyres C, Sacher F, Redon R, Le Caignec C, et al: **Screening for copy number variation in genes associated with the long QT syndrome: clinical relevance.** *J Am Coll Cardiol* 2011, **57:**40-47.

103. Reijneveld JC, Ginjaar IB, Frankhuizen WS, Notermans NC: **CAV3 gene mutation analysis in patients with idiopathic hyper-CK-emia.** *Muscle Nerve* 2006, **34:**656-658.

104. Ricci G, Scionti I, Ali G, Volpi L, Zampa V, Fanin M, Angelini C, Politano L, Tupler R, Siciliano G: **Rippling muscle disease and facioscapulohumeral dystrophy-like phenotype in a patient carrying a heterozygous CAV3 T78M mutation and a D4Z4 partial deletion: Further evidence for "double trouble" overlapping syndromes.** *Neuromuscul Disord* 2012, **22:**534-540.

105. Spadafora P, Liguori M, Andreoli V, Quattrone A, Gambardella A: **CAV3 T78M mutation as polymorphic variant in South Italy.** *Neuromuscul Disord* 2012, **22:**669-670.

106. Traverso M, Bruno C, Broccolini A, Sotgia F, Donati MA, Assereto S, Gazzerro E, Lo Monaco M, Modoni A, D'Amico A, et al: **Truncation of Caveolin-3 causes autosomal-recessive Rippling Muscle Disease.** *J Neurol Neurosurg Psychiatry* 2008, **79:**735-737.

107. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foell JD, Li Z, et al: **Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome.** *Circulation* 2006, **114:**2104-2112.

108. Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, Ackerman MJ: **Novel mechanism for sudden infant death syndrome: persistent late sodium current secondary to mutations in caveolin-3.** *Heart Rhythm* 2007, **4:**161-166.

109. Massaeli H, Sun T, Li X, Shallow H, Wu J, Xu J, Li W, Hanson C, Guo J, Zhang S: **Involvement of caveolin in low K+-induced endocytic degradation of cell-surface human ether-a-go-go-related gene (hERG) channels.** *J Biol Chem* 2010, **285:**27259-27264.

110. Das K, Lewis RY, Scherer PE, Lisanti MP: **The membrane-spanning domains of caveolins-1 and -2 mediate the formation of caveolin hetero-oligomers. Implications for the assembly of caveolae membranes in vivo.** *J Biol Chem* 1999, **274:**18721-18728.

111. Parton RG: **Caveolae and caveolins.** *Curr Opin Cell Biol* 1996, **8:**542-548.

112. Schlegel A, Lisanti MP: **A molecular dissection of caveolin-1 membrane attachment and oligomerization. Two separate regions of the caveolin-1 C-terminal domain mediate membrane binding and oligomer/oligomer interactions in vivo.** *J Biol Chem* 2000, **275:**21605-21617.

113. Balijepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ: **Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation.** *Proc Natl Acad Sci U S A* 2006, **103:**7500-7505.

114. Barbuti A, Terragni B, Brioschi C, DiFrancesco D: **Localization of f-channels to caveolae mediates specific beta2-adrenergic receptor modulation of rate in sinoatrial myocytes.** *J Mol Cell Cardiol* 2007, **42:**71-78.

115. Calaghan S, White E: **Caveolae modulate excitation-contraction coupling and beta2-adrenergic signalling in adult rat ventricular myocytes.** *Cardiovasc Res* 2006, **69:**816-824.

116. Vaidyanathan R, Vega AL, Song C, Zhou Q, Tan B, Berger S, Makielski JC, Eckhardt LL: **The interaction of caveolin 3 protein with the potassium inward rectifier channel Kir2.1: physiology and pathology related to long qt syndrome 9 (LQT9).** *J Biol Chem* 2013, **288:**17472-17480.

117. Balijepalli RC, Kamp TJ: **Caveolae, ion channels and cardiac arrhythmias.** *Prog Biophys Mol Biol* 2008, **98:**149-160.

118. Maguy A, Hebert TE, Nattel S: **Involvement of lipid rafts and caveolae in cardiac ion channel function.** *Cardiovasc Res* 2006, **69:**798-807.

119. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, Dorn T, Goedel A, Hohnke C, Hofmann F, et al: **Patient-specific induced pluripotent stem-cell models for long-QT syndrome.** *N Engl J Med* 2010, **363:**1397-1409.

120. Adzhubei I, Jordan DM, Sunyaev SR: **Predicting functional effect of human missense mutations using PolyPhen-2.** *Curr Protoc Hum Genet* 2013, **Chapter 7:**Unit7 20.

121. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC: **SIFT web server: predicting effects of amino acid substitutions on proteins.** *Nucleic Acids Res* 2012, **40:**W452-457.

122. Reva B, Antipin Y, Sander C: **Predicting the functional impact of protein mutations: application to cancer genomics.** *Nucleic Acids Res* 2011, **39:**e118.

123. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D: **Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2.** *Cell* 2007, **129:**303-317.

124. Borel C, Antonarakis SE: **Functional genetic variation of human miRNAs and phenotypic consequences.** *Mamm Genome* 2008, **19:**503-509.

125. Kim J, Bartel DP: **Allelic imbalance sequencing reveals that single-nucleotide polymorphisms frequently alter microRNA-directed repression.** *Nat Biotechnol* 2009, **27:**472-477.

126. Ryan BM, Robles AI, Harris CC: **Genetic variation in microRNA networks: the implications for cancer research.** *Nat Rev Cancer* 2010, **10:**389-402.

127. Saunders MA, Liang H, Li WH: **Human polymorphism at microRNAs and microRNA target sites.** *Proc Natl Acad Sci U S A* 2007, **104:**3300-3305.

128. Mencia A, Modamio-Hoybjor S, Redshaw N, Morin M, Mayo-Merino F, Olavarrieta L, Aguirre LA, del Castillo I, Steel KP, Dalmay T, et al: **Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss.** *Nat Genet* 2009, **41:**609-613.

129. Lewis MA, Quint E, Glazier AM, Fuchs H, De Angelis MH, Langford C, van Dongen S, Abreu-Goodger C, Piipari M, Redshaw N, et al: **An ENU-induced mutation of miR-96 associated with progressive hearing loss in mice.** *Nat Genet* 2009, **41:**614-618.

130. Kunej T, Skok DJ, Horvat S, Dovc P, Jiang Z: **The glypican 3-hosted murine mir717 gene: sequence conservation, seed region polymorphisms and putative targets.** *Int J Biol Sci* 2010, **6:**769-772.

131. Dorn GW, 2nd, Matkovich SJ, Eschenbacher WH, Zhang Y: **A human 3' miR-499 mutation alters cardiac mRNA targeting and function.** *Circ Res* 2012, **110:**958-967.

132. Ohanian M, Humphreys DT, Anderson E, Preiss T, Fatkin D: **A heterozygous variant in the human cardiac miR-133 gene, MIR133A2, alters miRNA duplex processing and strand abundance.** *BMC Genet* 2013, **14:**18.

133. Han SJ, Marshall V, Barsov E, Quinones O, Ray A, Labo N, Trivett M, Ott D, Renne R, Whitby D: **KSHV encoded microRNA Single Nucleotide Polymorphisms Identified in Clinical Samples can affect microRNA Processing, Level of Expression and Silencing Activity.** *J Virol* 2013.

134. Amin AS, Pinto YM, Wilde AA: **Long QT syndrome: beyond the causal mutation.** *J Physiol* 2013.

135. Chockalingam P, Wilde A: **The multifaceted cardiac sodium channel and its clinical implications.** *Heart* 2012, **98:**1318-1324.

136. Remme CA, Wilde AA, Bezzina CR: **Cardiac sodium channel overlap syndromes: different faces of SCN5A mutations.** *Trends Cardiovasc Med* 2008, **18:**78-87.

137. Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Aizawa Y, Ogawa S: **Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome.** *J Am Coll Cardiol* 1996, **27:**1061-1070.

138. Pappone C, Radinovic A, Manguso F, Vicedomini G, Sala S, Sacco FM, Ciconte G, Saviano M, Ferrari M, Sommariva E, et al: **New-onset atrial fibrillation as first clinical manifestation of latent Brugada syndrome: prevalence and clinical significance.** *Eur Heart J* 2009, **30:**2985-2992.

139. Shimizu W, Antzelevitch C, Suyama K, Kurita T, Taguchi A, Aihara N, Takaki H, Sunagawa K, Kamakura S: **Effect of sodium channel blockers on ST segment, QRS duration, and corrected QT interval in patients with Brugada syndrome.** *J Cardiovasc Electrophysiol* 2000, **11:**1320-1329.

140. Benito B, Brugada R, Perich RM, Lizotte E, Cinca J, Mont L, Berruezo A, Tolosana JM, Freixa X, Brugada P, Brugada J: **A mutation in the sodium channel is responsible for the association of long QT syndrome and familial atrial fibrillation.** *Heart Rhythm* 2008, **5:**1434-1440.

141. Zhang L, Timothy KW, Vincent GM, Lehmann MH, Fox J, Giuli LC, Shen J, Splawski I, Priori SG, Compton SJ, et al: **Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes.** *Circulation* 2000, **102:**2849-2855.

142. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, Schulze-Bahr E, Fukuhara S, Mochizuki N, Makiyama T, et al: **The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome.** *J Clin Invest* 2008, **118:**2219-2229.

143. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van Der Hout AH, et al: **A single Na(+) channel mutation causing both long-QT and Brugada syndromes.** *Circ Res* 1999, **85:**1206-1213.

144. McNair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, Mestroni L, Familial Cardiomyopathy Registry Research G: **SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia.** *Circulation* 2004, **110:**2163-2167.

145. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, Horton SC, Rodeheffer RJ, Anderson JL: **Sodium channel mutations and susceptibility to heart failure and atrial fibrillation.** *JAMA* 2005, **293:**447-454.

146. Roepke TK, King EC, Reyna-Neyra A, Paroder M, Purtell K, Koba W, Fine E, Lerner DJ, Carrasco N, Abbott GW: **Kcne2 deletion uncovers its crucial role in thyroid hormone biosynthesis.** *Nat Med* 2009, **15:**1186-1194.

147. Furukawa T, Ono Y, Tsuchiya H, Katayama Y, Bang ML, Labeit D, Labeit S, Inagaki N, Gregorio CC: **Specific interaction of the potassium channel beta-subunit minK with the sarcomeric protein T-cap suggests a T-tubule-myofibril linking system.** *J Mol Biol* 2001, **313:**775-784.

148. Bianchi L, Shen Z, Dennis AT, Priori SG, Napolitano C, Ronchetti E, Bryskin R, Schwartz PJ, Brown AM: **Cellular dysfunction of LQT5-minK mutants: abnormalities of IKs, IKr and trafficking in long QT syndrome.** *Hum Mol Genet* 1999, **8:**1499-1507.

149. Duggal P, Vesely MR, Wattanasirichaigoon D, Villafane J, Kaushik V, Beggs AH: **Mutation of the gene for IsK associated with both Jervell and Lange-Nielsen and Romano-Ward forms of Long-QT syndrome.** *Circulation* 1998, **97:**142-146.

150. Ravn LS, Aizawa Y, Pollevick GD, Hofman-Bang J, Cordeiro JM, Dixen U, Jensen G, Wu Y, Burashnikov E, Haunso S, et al: **Gain of function in IKs secondary to a mutation in KCNE5 associated with atrial fibrillation.** *Heart Rhythm* 2008, **5:**427-435.

151. Delpon E, Cordeiro JM, Nunez L, Thomsen PE, Guerchicoff A, Pollevick GD, Wu Y, Kanters JK, Larsen CT, Burashnikov E, et al: **Functional Effects of KCNE3 Mutation and its Role in the Development of Brugada Syndrome.** *Circ Arrhythm Electrophysiol* 2008, **1:**209-218.

152. McKenna WJ, England D, Doi YL, Deanfield JE, Oakley C, Goodwin JF: **Arrhythmia in hypertrophic cardiomyopathy. I: Influence on prognosis.** *Br Heart J* 1981, **46:**168-172.

153. 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:**182-188.

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154. Van Laer L, Carlsson PI, Ottschytsch N, Bondeson ML, Konings A, Vandevelde A, Dieltjens N, Fransen E, Snyders D, Borg E, et al: **The contribution of genes involved in potassium-recycling in the inner ear to noise-induced hearing loss.** *Hum Mutat* 2006, **27:**786-795.

155. Abbott GW, Goldstein SA: **Potassium channel subunits encoded by the KCNE gene family: physiology and pathophysiology of the MinK-related peptides (MiRPs).** *Mol Interv* 2001, **1:**95-107.

156. Aatre RD, Day SM: **Psychological issues in genetic testing for inherited cardiovascular diseases.** *Circ Cardiovasc Genet* 2011, **4:**81-90.

157. Hall AE, Burton H: **Legal and ethical implications of inherited cardiac disease in clinical practice within the UK.** *J Med Ethics* 2010, **36:**762-766.

158. Leachman SAM, D.G; Angrist, M.; Gray, S.W.; Bradbury, A.R.; Vorhaus, D.B.: **Direct-to-Consumer Genetic Testing: Personalized Medicine in Evolution.** In *2011 Educational Book*. Edited by University A. American Society of Clinical Oncology; 2011

159. van El CG, Cornel MC, Borry P, Hastings RJ, Fellmann F, Hodgson SV, Howard HC, Cambon-Thomsen A, Knoppers BM, Meijers-Heijboer H, et al: **Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics.** *Eur J Hum Genet* 2013, **21:**580-584.

160. Harris A, Kelly SE, Wyatt S: **Counseling customers: emerging roles for genetic counselors in the direct-to-consumer genetic testing market.** *J Genet Couns* 2013, **22:**277-288.

161. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, et al: **Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes.** *Circulation* 2002, **105:**1943-1948.

162. Yap YG, Camm AJ: **Drug induced QT prolongation and torsades de pointes.** *Heart* 2003, **89:**1363-1372.

163. Lasser KE, Allen PD, Woolhandler SJ, Himmelstein DU, Wolfe SM, Bor DH: **Timing of new black box warnings and withdrawals for prescription medications.** *JAMA* 2002, **287:**2215-2220.

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CHAPTER 10: CANDIDATE'S CONTRIBUTIONS

CHAPTER 1

1.5.2 Review 1: The Genetic Basis of Long QT and Short QT Syndromes: A Mutation Update

- First author
- Analysis and Interpretation of data
- Writing of manuscript

1.5.3 Review 2: The Genetic Basis of Brugada Syndrome: A Mutation Update

- First author
- Analysis and Interpretation of data
- Writing of manuscript

1.5.4 Editorial: Long QT Testing: Implications for Complex Diagnosis in Personalised Medicine

- Co-author
- Planning, Discussing and Writing Manuscript

CHAPTER 2

Paper 1: Long QT Syndrome in South Africa: The Results of Comprehensive Genetic Screening

- First author
- Planning of study
- Development of laboratory protocols and lab work
- Analysis and Interpretation of data
- Writing of manuscript

CHAPTER 3

Paper 2: Mutations in Danish Patients with Long QT Syndrome and Identification of a Large Founder Family with p.F29L in KCNH2

- Shared first author
- Data acquisition
- Analysis and Interpretation of data
- Critical revision of manuscript

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CHAPTER 4

Paper 3: The Role of CAV3 in Long QT Syndrome: Clinical and Functional Assessment of a Caveolin-3/Kv11.1 Double Heterozygote versus Caveolin-3 Single Heterozygote

- First author
- Planning and coordinating study
- Analysis and Interpretation of data
- Writing of manuscript

Paper 4: MicroRNAs in Cardiac Arrhythmia: The Sequence Variation of miR-1 and miR-133a in Long QT Syndrome

- First author
- Planning and coordinating study
- Analysis and Interpretation of data
- Writing and corresponding of manuscript

CHAPTER 5

Paper 5: Flecainide Provocation Reveals Concealed Brugada Syndrome in a Long QT Syndrome Family with a Novel L1786Q Mutation in SCN5A

- Shared first author
- Project planning and discussion
- Analysis and Interpretation of data

Paper 6: High Prevalence of Long QT Syndrome–Associated SCN5A Variants in Patients with Early-Onset Lone Atrial Fibrillation

- Co-author
- Conceptualisation of the study
- Data collection
- Project planning and discussion

CHAPTER 6

Paper 7: The KCNE Genes in Hypertrophic Cardiomyopathy: a Candidate Gene Study

- First author
- Planning and coordinating study
- Analysis and Interpretation of data
- Writing of manuscript

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CHAPTER 11: SUPPLEMENTARY MATERIALS

11.1 LIST OF ABBREVIATIONS

Abbreviation	Definition
AA	African American
aa	amino acid
AF	atrial fibrillation
aLQTS	acquired long QT syndrome
AP	action potential
APD	action potential duration
AS	Andersen syndrome
ATS	Andersen-Tawil syndrome
AVN	atrioventricular node
AVNRT	atrioventricular nodal reentrant tachycardia
AVRT	atrioventricular reentrant tachycardia
BrS	Brugada syndrome
CA	cardiac arrest
CAD	coronary artery disease
CAE	capillary array electrophoresis
CCD	cardiac conduction disease
CCS	cardiac conduction system
cDNA	coding DNA
CEU	Utah residents with ancestry from northern and western Europe
CHF	congestive heart failure
CHO	Chinese hamster ovary
cLQTS	congenital long QT syndrome
CNV	copy number variant
DAD	delayed-afterdepolarisation
DCM	dilated cardiomyopathy
DHPLC	denaturing high performance liquid chromatography
DK	Denmark / of Danish origin
dLQTS	drug-related long QT syndrome

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DM	distal myopathy
EA	European American
EAD	early-afterdepolarisation
ECG	electrocardiogram
EMD	electromechanical dissociation
EP	electrophysiology
EPS	programmed electrical stimulation
ESP	exome sequencing project
EVS	exome variant server
FBS	foetal bovine serum
FSHD	Facioscapulohumeral muscular dystrophy
gDNA	genomic DNA
GFP	green fluorescent protein
HB	bundle of His
H-CK	hyperCKemia
HCM	hypertrophic cardiomyopathy
HEK	human embryonic kidney
HWE	Hardy-Weinberg equilibrium
IB	immunoblot
ICD	implantable cardioverter defibrillator
IP	immunoprecipitation
JLNS	Jervell-Lange-Nielsen syndrome
LBB	left bundle branch
LGMD	limb girdle muscular dystrophy
LQTS	long QT syndrome
LVEF	left ventricular ejection fraction
MAF	minor allele frequency
MCS	morphology-combination-score
MEF2	myocyte enhancer factor-2
MEM	minimal essential medium
MFE	minimum free energy
MI	myocardial infarction
miRNA	micro ribonucleic acid
miRNA*	the degraded/passenger strand of miRNA duplex

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MLPA	multiplex-ligation-dependent amplification
mRNA	messenger ribonucleic acid
multi-CE-SSCP	multiplex-capillary electrophoresis - single strand conformation polymorphism
MVT	monomorphic ventricular tachycardia
NGS	next generation sequencing
nt	nucleotide
PAS	Per-Arnt-Sim
PCR	polymerase chain reaction
ped	pedigree
PF	Purkinje fibre
PIP2	Phosphatidylinositol 4,5-bisphosphate
pre-miRNA	preliminary microRNA
pri-miRNA	primary microRNA
PVS	programmed ventricular stimulation
PVT	polymorphic ventricular tachycardia
QTc	corrected QT interval
RBB	right bundle branch
RISC	RNA-induced silencing complex
RMD	rippling muscular dystrophy
RVD	right ventricular dysplasia
RVOT	right ventricular outflow tract
RWS	Romano-Ward syndrome
SADS	sudden arrhythmia death syndrome
SAN	sinoatrial node
SCD	sudden cardiac death
SD	standard deviation
SEM	standard error of the mean
SIDS	sudden infant death syndrome
SNP	single nucleotide polymorphism
SNP	single nucleotide polymorphism
SQTS	short QT syndrome
SRF	serum response factor
SSCP	single strand conformation polymorphism

SUNDS	sudden unexpected nocturnal death syndrome
SVT	supraventricular tachycardia
TdP	torsades de pointes
TDR	transmural dispersion of repolarisation
TS	Timothy syndrome
TTX	tetrodotoxin
UTR	un-translated region
VF	ventricular fibrillation
VT	ventricular tachycardia
WPW	Wolff-Parkinson-White
WT	wild type
Y2H	yeast-2-hybrid

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11.2 TABLE OF GENES EXAMINED IN THE DISSERTATION

Genes	NCBI ID	Chromosome	Gene product	NCBI ID
KCNQ1	NM_000218	11p15.5	Kv7.1	NP_000209
KCNH2	NM_000238	7q35-7q36	Kv11.1	NP_000229
SCN5A	NM_198056	3p21	Nav1.5	NP_932173
KCNE1	NM_000219	21p22	minK	NP_000210
KCNE2	NM_172201	21p22	MiRP1	NP_75195
KCNE3	NM_005472	11q13.4	MiRP2	NP_005463
KCNE4	NM_080671	2q36.1	MiRP3	NP_542402
KCNE1L	NM_0122822	Xq22.3	MiRP4	NP_036414
CAV3	NM_001234	3p25	caveolin-3	NP_001225
miR-1-1	NR_029780	20q13.33	MIR-1	
miR-1-2	NR_029662	18q11.2	MIR-1	
miR-133A-1	NR_029675	18q11.2	MIR-133A	
miR-133A-2	NR_029676	20q13.33	MIR-133A	

11.3 CANDIDATE'S PUBLICATION LIST

11.3.1 CARDIOVASCULAR DISEASES

- Hedley PL, Kanters JK, Dembic M, Jespersen T, Skibsbye L, Aidt FH, et al. The role of CAV3 in long QT syndrome: clinical and functional assessment of a Caveolin-3/Kv11. 1 double heterozygote versus Caveolin-3 single heterozygote.
  Circ Cardiovasc Gen. 2013;6(5):452-461
- Hedley PL, Durrheim GA, Hendricks F, Corfield VA, Jespersgaard C, Støvring B, et al. Long QT syndrome in South Africa: the results of comprehensive genetic screening. Cardiovascular Journal of Africa. 2013;24(6):231-237
- Asferg CL, Nielsen SJ, Andersen UB, Linneberg A, Møller DV, Hedley PL, et al. Metabolic rather than body composition measurements are associated with lower serum natriuretic peptide concentrations in normal weight and obese men. Am J Hypertens. 2013; published online doi: 10.1093/ajh/hpt145
- Hagen CM, Aidt FH, Hedley PL, Jensen MK, Havndrup O, Kanters JK, et al. Mitochondrial haplogroups modify the risk of developing hypertrophic cardiomyopathy in a Danish population. PloS one. 2013;8:e71904.
- Asferg CL, Nielsen SJ, Andersen UB, Linneberg A, Moller DV, Hedley PL, et al. Relative atrial natriuretic peptide deficiency and inadequate renin and angiotensin II suppression in obese hypertensive men. Hypertension. 2013;62:147-153.

- Hagen CM, Aidt FA, Havndrup O, Hedley PL, Jespersgaard C, Jensen M, et al. MT-CYB mutations in hypertrophic cardiomyopathy. Molecular Genetics & Genomic Medicine. 2013 1(1),54-65.
- Godiksen MTN, Kinnear C, Ravnsborg T, Højrup P, Granström S, Laursen IA, Hedley PL, et al., Feline hypertrophic cardiomyopathy associated with the p.A31P mutation in cMyBP-C is caused by production of mutated cMyBP-C with reduced binding to Actin. Open J Vet Med, 2013, 3, 95-103.
- Olesen MS, Yuan L, Liang B, Holst AG, Nielsen N, Nielsen JB, Hedley PL, et al. High prevalence of long QT syndrome-associated SCN5A variants in patients with early-onset lone atrial fibrillation. Circ Cardiovasc Genet. 2012;5:450-459.
- Nyegaard M, Overgaard MT, Sondergaard MT, Vranas M, Behr ER, Hildebrandt LL, Lund J, Hedley PL, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. AJHG. 2012;91:703-712.
- Andersen PS, Hedley PL, Page SP, Syrris P, Moolman-Smook JC, Mckenna WJ, et al. A novel myosin essential light chain mutation causes hypertrophic cardiomyopathy with late onset and low expressivity. Biochem Res Int. 2012;2012:685108.
- Dembic M, Brusich S, Hedley PL, de Villiers CP, Čubranić Z, Kanters JK, et al. Long QT syndrome - a cause of sudden death. Cardiologia Croatica, 2012 7(11-12), 263-275.
- Olesen MS, Jespersen T, Nielsen JB, Liang B, Moller DV, Hedley P, et al. Mutations in sodium channel beta-subunit scn3b are associated with early-onset lone atrial fibrillation. Cardiovasc Res. 2011;89:786-793.
- Moller DV, Hedley PL, Olesen M, Kanters J, Svendsen JH, Christiansen M. [short QT syndrome as an inherited condition]. [Article in Danish]. Ugeskr Laeger. 2011;173:420-424.
- Hedley PL, Haundrup O, Andersen PS, Aidt FH, Jensen M, Moolman-Smook JC, et al. The KCNE genes in hypertrophic cardiomyopathy: A candidate gene study. J Negat Results Biomed. 2011;10:12.
- Szeliga MA, Hedley PL, Green CP, Moller DV, Christiansen M. Long QT syndrome - a genetic cardiac channelopathy. Kardiologia polska. 2010;68:575-583.
- Moller DV, Pecini R, Gustafsson F, Hassager C, Hedley P, Jespersgaard C, et al. Hereditary hemochromatosis (HFE) genotypes in heart failure: Relation to etiology and prognosis. BMC medical genetics. 2010;11:117.
- Christiansen M, Hedley PL. Long QT testing: Implications for complex diagnosis in personalized medicine. Personalized Medicine. 2010;7:125-127.

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- Bastiaenen R, Hedley PL, Christiansen M, Behr ER. Therapeutic hypothermia and ventricular fibrillation storm in early repolarization syndrome. Heart Rhythm. 2010;7:832-834.
- Moller DV, Pham TT, Gustafsson F, Hedley P, Ersboll MK, Bundgaard H, et al. The role of Lamin A/C mutations in Danish patients with idiopathic dilated cardiomyopathy. Eur J Heart Fail. 2009;11:1031-1035.
- Moller DV, Andersen PS, Hedley P, Ersboll MK, Bundgaard H, Moolman-Smook J, et al. The role of sarcomere gene mutations in patients with idiopathic dilated cardiomyopathy. EJHG. 2009;17:1241-1249.
- Hedley PL, Jorgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, et al. The genetic basis of long QT and short QT syndromes: A mutation update. Hum Mutat. 2009;30:1486-1511.
- Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA, et al. The genetic basis of Brugada syndrome: A mutation update. Hum Mutat. 2009;30:1256-1266.
- Andersen PS, Havndrup O, Hougs L, Sorensen KM, Jensen M, Larsen LA, Hedley PL, et al. Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. Hum Mutat. 2009;30:363-370.
- Schwartz PJ, Vanoll E, Crotti L, Spazzolim C, Ferrandl C, Goosen A, Hedley P, et al. Neural control of heart rate is an arrhythmia risk modifier in long QT syndrome. JACC. 2008;51:920-929.
- Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, et al. High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of SCN5A: A rapid genetic approach to cardiac arrhythmia. Clin Genet. 2006;69:504-511.
- Brink PA, Crotti L, Corfield V, Goosen A, Durrheim G, Hedley P, et al. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. Circulation. 2005;112:2602-2610.

11.3.2 PRENATAL DIAGNOSIS AND REPRODUCTIVE MEDICINE

- Svendsen PF, Christiansen M, Hedley PL, Nilas L, Pedersen SB, Madsbad S. Adipose expression of adipocytokines in women with polycystic ovary syndrome. Fertility and sterility. 2012;98:235-241

- Jorgensen JM, Hedley PL, Gjerris M, Christiansen M. Ethical issues related to screening for preeclampsia. Bioethics. 2012 Sep 20. doi: 10.1111/j.1467-8519.2012.02005.x.
- Hedley PL, Placing S, Wojdemann K, Carlsen AL, Shalmi AC, Sundberg K, et al. Free leptin index and papp-a: A first trimester maternal serum screening test for pre-eclampsia. Prenatal diagnosis. 2010;30:103-109.
- Christiansen M, Pihl K, Hedley PL, Gjerris AC, Lind PO, Larsen SO, et al. Adam 12 may be used to reduce the false positive rate of first trimester combined screening for Down syndrome. Prenatal diagnosis. 2010;30:110-114.
- Laigaard J, Pedersen NG, Larsen SO, Hedley PL, Wojdemann K, Gjerris AC, et al. Adam12 in first trimester maternal serum from pregnancies conceived by assisted reproduction techniques (ART). Prenatal diagnosis. 2009;29:628-629.
- Laigaard J, Larsen SO, Pedersen NG, Hedley PL, Gjerris AC, Wojdemann KR, et al. Adam 12-S in first trimester: fetal gender, smoking and maternal age influence the maternal serum concentration. Prenatal diagnosis. 2009;29:525-527
- Hedley P, Pihl K, Krebs L, Larsen T, Christiansen M. Leptin in first trimester pregnancy serum: No reduction associated with small-for-gestational-age infants. Reproductive biomedicine online. 2009;18:832-837.
- Hedley P, Christiansen M. Serum leptin in first-trimester down syndrome pregnancies. Prenatal diagnosis. 2008;28:475-477.

11.3.3 IMMUNOGENETICS

- Adu B, Dodoo D, Adukpo S, Hedley PL, Arthur FK, Gerds TA, et al. Fc gamma receptor IIIB (FcγRIIIB) polymorphisms are associated with clinical malaria in Ghanaian children. PloS one. 2012;7:e46197.
- Adu B, Dodoo D, Adukpo S, Gyan BA, Hedley PL, Goka B, et al. Polymorphisms in the RNASE3 gene are associated with susceptibility to cerebral malaria in Ghanaian children. PloS one. 2011;6:e29465.

11.3.4 PHARMACOGENETICS

- Steinmetz J, Jespersgaard C, Dalhoff K, Hedley P, Abildstrom H, Christiansen M, et al. Cytochrome p450 polymorphism and postoperative cognitive dysfunction. Minerva anestesiologica. 2012;78:303-309.