# Cardiac Repolarization

## Basic Science and Clinical Management

Nabil El-Sherif
*Editor*

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# Foreword

Normal cardiac repolarization is vital to maintain optimal mechanical and electrical cardiac function. Repolarization that is too long or too short, or otherwise abnormal, puts the patient at risk for developing life-threatening cardiac arrhythmias. It is essential that scientists and clinical electrophysiologists understand cardiac repolarization. The textbook *Cardiac Repolarization: Basic Science and Clinical Management*, edited by Nabil El-Sherif, provides information critical to that understanding.

Chapter 1, "Physiology and Molecular Biology of Ion Channels Underlying Ventricular Repolarization of the Mammalian Heart," reviews the key ion channels that underlie cardiac repolarization and focuses on critical potassium ion channels, while the Chap. 2, "Neuromodulation of Cardiac Repolarization and Arrhythmogenesis," explores autonomic modulation of this activity at the cardiac channel, cellular, and organ levels.

Heart failure can predispose to the development of potentially fatal arrhythmias via multiple mechanisms including altered calcium handling, stretch, and changes in myocyte electrical and metabolic properties, as explored in Chap. 3, "Repolarization Remodeling in Structural Heart Disease." A major advance in studying mechanisms impacting repolarization has been the use of pluripotent stem cell-derived cardiomyocytes to model cardiac disease states, a major advance presented in Chap. 4, "Cardiac Repolarization and Stem Cells: An Emerging Path Toward Precision Medicine."

Voltage-gated sodium channels, responsible for the rapid upstroke of the action potential, are vital to maintain electrical excitability and coordinate excitation-contraction coupling, as explored in Chap. 5, "Role of Late Sodium Current during Repolarization and Its Pathophysiology." How age, sex, and race impact cardiac repolarization and arrhythmogenesis is presented in Chap. 6, "Age, Sex, and Racial Differences in Cardiac Repolarization and Arrhythmogenesis," emphasizing that testosterone appears to shorten the QTc interval in males, while in females, there is a more complex interaction between progesterone and estrogen.

With the initial chapters serving as a scientific foundation for clinical events involving repolarization, the text now turns to the clinical arena, beginning with a consideration of the standard electrocardiogram (ECG), still one of the most useful methods for risk stratification for sudden cardiac death, and an analysis of the QT interval, as explained in Chap. 7, "ECG-derived Evaluation of Cardiac Repolarization." Microvolt T-wave analysis represents

another approach for investigating the QT interval to track T-wave alternans, a beat-to-beat fluctuation in ST-segment or T-wave morphology, and is discussed in Chap. 19, "Microvolt T-Wave Alternans: Pathophysiology and Clinical Aspects."

The next several chapters feature clinical manifestations of the long QT interval. Chapter 8 delves into the phenotype-genotype relationship of clinical presentations, ECG patterns, risk stratification for cardiac events, and therapy in "Genotype-Phenotype Correlation in Congenital LQTS: Implications for Diagnosis and Risk Stratification," concentrating on the three genes KCNQ1, KCNH2, and SCN5A underlying LQT1, LQT2, and LQT3, respectively, which account for about 95% of patients with an identified genetic cause. Chapter 9 discusses the clinical management of patients with the inherited long QT syndrome, "Clinical Management of LQTS Patients," focusing on minimizing the adrenergic response, shortening the QTc, decreasing the dispersion of refractoriness, and improving the function of dysfunctional ion channel.

Chapter 10, "Drug-Induced Long QT Syndrome and Torsades de Pointes," emphasizes drug-induced LQTS and its ventricular arrhythmia, noting that QT prolongation is a useful but imperfect predictor of patients at high risk of developing the serious and life-threatening arrhythmia, torsades de pointes. Chapter 11, "Acquired Long QT Syndrome and Electrophysiology of Torsade de Pointes," stresses the electrophysiological mechanisms of acquired LQTS, its electrocardiographic (ECG) characteristics, clinical presentation, and management of the acquired long QT interval.

New mechanisms of QT prolongation are explored in Chap. 12, "Pathogenesis of Autoimmune-Associated Long QT Syndrome," and Chap. 13, "The Role of Inflammation and Autoimmunity in Long-QT Syndrome."

The QTc interval can be too short (<340 ms) as well as too long and provoke ventricular arrhythmias, as discussed in Chap. 18, "The Short QT Syndrome."

A relatively newly described entity, the J-wave syndromes, appears to be a constellation of several clinical entities characterized by J-wave abnormalities, such as the early repolarization syndrome associated with variants in seven different genes, discussed in Chap. 14, "Genetics, Molecular Biology and Emerging Concepts of Early Repolarization Syndrome." Chapter 15, "Electrocardiographic J-wave and Cardiovascular Risk in the General Population," presents the prevalence and significance of the ECG pattern of early repolarization in the general population, while Chap. 16, "Benign Versus Malignant Early Repolarization Patterns," differentiates benign from arrhythmogenic J waves, emphasizing the J-wave proper, the ST-segment, and the ensuing T-waves.

Brugada syndrome appears to be part of the J-wave group and is discussed in Chap. 17, "Genetic Architecture, Pathophysiology, and Clinical Management of Brugada Syndrome," in which the authors stress the understanding of the pathophysiological mechanism(s), the ongoing efforts to reappraise the genetic architecture, and the advances in risk stratification and therapeutic approaches.

Chapter 20, the final chapter, "Action Potential Dynamics in Human Atrial Fibrillation," explores the action potential variability in the most common sustained arrhythmia that affects more than 30 million people worldwide, atrial fibrillation.

This textbook, ably edited by Nabil El-Sherif and written by him along with a multitude of electrophysiology experts, is a must for basic and clinical electrophysiologists interested in cardiac repolarization.

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# Preface

Cardiac repolarization is a vital aspect for understanding normal cardiac rhythm, and its abnormal modulation is the basis of many potentially lethal cardiac arrhythmias. The last book that dealt exclusively with cardiac repolarization was published in 2003. It is overdue to readdress the many significant changes in the field.

This book was designed to provide a comprehensive up-to-date coverage of clinical and research aspects of cardiac repolarization, with several sections that are the domain of practicing physicians. The 20 chapters are subgrouped into 4 parts. Part I deals with the basic, molecular biology and clinical aspects of cardiac repolarization at large, including the influence of the autonomic system, remodeling in organic heart disease, racial and gender difference in cardiac repolarization and arrhythmic risk, ECG-derived evaluation of cardiac repolarization, and future role of stem cells studies of cardiac repolarization.

Part II provides a comprehensive coverage of pathophysiology, molecular biology, and clinical aspects of long QT syndromes. While basic and clinical aspects of congenital long QT syndrome and torsade de pointes are usually the domain of academic cardiologists and cardiac electrophysiologists, acquired and drug-induced long QT syndrome should be of interest to a majority of medical professionals who prescribe drugs and take care of patients.

Part III provides updated basic and clinical coverage of the subject of early repolarization (ER), also called J-wave syndromes, and expound currently controversial aspects such as benign versus malignant ER in the 12-lead ECG and in the general population at large. Finally, Part IV covers the pathophysiology and clinical aspects of special cardiac repolarization syndromes that include short repolarization syndrome, repolarization alternans, the important role of microvolt T-wave alternans in arrhythmia risk stratification, and the recently recognized role of atrial repolarization and arrhythmogenesis.

The wide-ranging features of the book should be of interest not only to academic and clinical cardiologists and cardiac electrophysiologists but also to a much larger group of practicing internists and other medical professionals who would like to keep abreast of the important developments in this field.

Brooklyn, NY, USA — Nabil El-Sherif

# Contents

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# Part I

# Pathophysiology, Molecular Biology, and Clinical Aspects of Cardiac Repolarization

# 1 Physiology and Molecular Biology of Ion Channels Underlying Ventricular Repolarization of the Mammalian Heart

Thomas W. Comollo, Chuangeng Zhang, Xinle Zou, and Robert S. Kass

## Introduction

The proper mechanical pumping of the mammalian heart depends upon a precise, cyclic cascade of electrical events, with each player from the anatomical region, down to the ion channels responsible for each ionic current playing its exact role in this symphonic-like mechanism. Cells in the sinoatrial note, the "pacemaker" region of the heart, are first activated and propagate the electrical activity through the remainder of the heart. This signal spreads due to the coupling of cardiac cells at gap junctions [1–5], inciting "action potentials" in each of the cells (cardiomyocytes), as the cell is electrically excited. The surface electrocardiogram (ECG) reflects the differences in voltage across different regions of the heart as this electrical cascade spreads through the organ.

The "action potentials" generated in each cell as the electrical signals spread throughout the heart are the result of activation and subsequent inactivation of both depolarizing, inward sodium ($Na^+$) and calcium ($Ca^{2+}$) currents that are then countered by repolarizing, outward $K^+$ currents [4, 6]. During this action potential cascade of events, the influx of $Ca^{2+}$ triggers release of more $Ca^{2+}$ from the myocyte version of the endoplasmic reticulum, the sarcoplasmic reticulum (SR), through $Ca^{2+}$-sensitive ryanodine receptor channels [7]. This triggers the excitation-contraction (EC) coupling relationship that triggers calcium-dependent muscle contraction [7, 8]. Calcium ions bind a protein called trophin, exposing a myosin-binding site on the actin filaments, and myosin moves the actin by an adenosine triphosphate (ATP)-dependent mechanism [9]. Thus, the electrical signaling of the heart translates into mechanical pumping via rhythmic contraction of the myocardium.

Ventricular action potential waveforms reflect the coordinated activity of multiple ion channels that open, close, and inactivate on different time scales (Fig. 1.1). The rapid upstroke of the action potential (phase 0) is caused by a large inward current through voltage-gated $Na^+$ channels. It is followed by a transient repolarization (phase 1), reflecting $Na^+$ channel inactivation and the activation of voltage-gated outward potassium ($K^+$) currents (Fig. 1.1). This transient repolarization or "notch" influences the height and duration of the plateau phase (phase 2) of the action potential, which depends on the delicate balance of inward ($Ca^{2+}$ and $Na^+$) currents and outward ($K^+$) currents. Although $Ca^{2+}$ influx through high-threshold, L-type voltage-gated $Ca^{2+}$ channels is the main contributor of inward current during the plateau phase, this current declines during phase 2 as the (L-type $Ca^{2+}$) channels undergo $Ca^{2+}$ and voltage-dependent inactivation. The driving force for $K^+$ efflux through the voltage-gated

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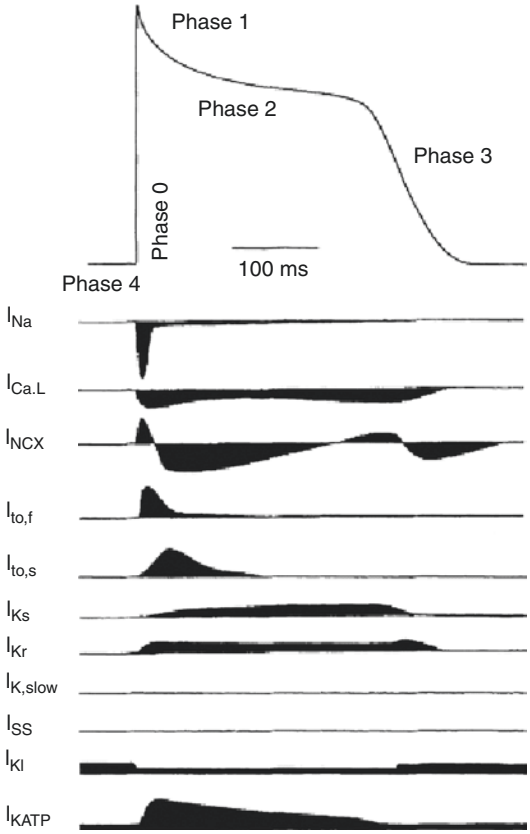


**Fig. 1.1** Schematic of the action potential and underlying ionic currents in adult human ventricular myocytes. The contributions of some $K^+$ currents, such as $I_{K,slow}$ and $I_{SS}$ which are expressed in other species, have not been defined in human ventricular cells

(and other) $K^+$ channels, however, is high during the plateau, and, as the $Ca^{2+}$ channels inactivate, the outward $K^+$ currents predominate resulting in a second, rapid phase (phase 3) of repolarization back to the resting potential (Fig. 1.1). The height and the duration of the action potential plateau, as well as the time- and voltage-dependent properties of the underlying voltage-gated $Na^+$, $Ca^{2+}$, and $K^+$ currents, therefore, also influence action potential durations. As a result, modifications in the properties or the densities of any of these channels could have dramatic effects on ventricular action potential waveforms, refractory periods, and cardiac rhythms.

Electrophysiological studies have detailed the properties of the major voltage-gated inward $Na^+$ and $Ca^{2+}$ and outward $K^+$ currents (Table 1.1). These currents determine the heights and the durations of ventricular action potentials. In contrast to the $Na^+$ and $Ca^{2+}$ currents, there are multiple types of $K^+$ currents in ventricular myocytes. Of the various $K^+$ currents, the voltage-gated $K^+$ currents are the most numerous and diverse. At least two types of transient outward currents, $I_{to,f}$ and $I_{to,s}$, and several components of delayed rectification, including $I_{Kr}$ ($IK_{(rapid)}$) and $I_{Ks}$ ($IK_{(slow)}$), have been distinguished in ventricular myocytes in a variety of different species (Table 1.1). In addition, there are marked regional differences in the expression patterns of these (voltage-gated) $K^+$ currents. These differences contribute to the observed variability in ventricular action potential waveforms [10–12]. The time- and voltage-dependent properties of the various repolarizing currents in myocytes isolated from different species and/or from different regions of the ventricles in the same species are similar, suggesting that the molecular correlates of the underlying channels are also the same [13]. A rather large number of pore-forming (α) and accessory (β, δ, and γ) subunits that coassemble as $Na^+$, $Ca^{2+}$, and $K^+$ channels have been identified in mammalian ventricles, and considerable progress has been made in defining the relationships between these subunits and functional ventricular $Na^+$, $Ca^{2+}$, and $K^+$ channels. Importantly, these studies have revealed that distinct molecular entities underlie the various ion channels contributing to ventricular action potential repolarization.

Importantly, voltage-gated channels change between at least three different conformations. These are open, closed, and inactivated [14–16]. In the closed state, the channel pore is closed. Upon activation, due to rising intracellular voltage, the pore opens due to movement of the voltage sensor and the channel enters the "open" conformation. After time that varies from channel to channel, the voltage-gated ion channel will enter the "inactivated" conformation, a nonconducting state. Eventually, the channel will recover from the inactivated state and become capable of conducting ionic current again [14–16].

The densities and the properties of voltage-gated $Na^+$, $Ca^{2+}$, and $K^+$ currents change during normal ventricular development, reshaping

**Table 1.1** Ionic currents contributing to cardiac action potential repolarization

| Current | Activation | Inactivation | Recovery | Blocker | Species |
|---|---|---|---|---|---|
| INa | Fast | Fast | Fast | TTX | Cat,dog, ferret, human, mouse, rat |
| ICa(L) | Fast | $Ca^{2+}$-dep | Fast | DHP, $Cd^{2+}$ | Cat, dog, ferret, human, mouse, rat |
| $I_{to,f}$ | Fast | Fast | Fast | mM 4-AP, flecainide, HaTX, HpTX | Cat, dog, ferret, human, mouse, rat |
| $I_{to,s}$ | Fast | Slow | Slow | mM 4-AP | Ferret, human, mouse, rat, rabbit |
| $I_{Kr}$ | Moderate | Fast | Slow | E-4031, dofetilide, lanthanum | Cat, dog, guinea pig, human, mouse, rabbit, rat |
| $I_{Kur}$ | Fast | Slow | Slow | μM 4-AP | Human |
| $I_{Ks}$ | Very slow | No | | NE-10064, NE-10133 | Dog, guinea pig, human, rabbit |
| $I_{KP}$ | Fast | No | | $Ba^{2+}$ | Guinea pig |
| $I_K$ | Slow | Slow | Slow | mM TEA | Rat |
| $I_{K,slow1}$ | Fast | Slow | Slow | mM 4-AP | Mouse |
| $I_{K,slow2}$ | Fast | Very slow | Slow | mM TEA | Mouse |
| $I_{ss}$ | Slow | No | | mM TEA, A1899 | Dog, human, mouse, rat |
| $I_{KI}$ | | | | $Ba^{2+}$ | Cat, dog, ferret, human, mouse, rabbit, rat |
| $I_{K(ATP)}$ | ATP depletion | | | SUR | Cat, dog, mouse, ferret, human, rabbit, rat |
| $I_{K(Ach)}$ | Acetylcholine | | | Tertiapin-Q | Mouse, human, |
| $I_{KCa}$ | Internal $Ca^{2+}$ | | | Maurotoxin Apamin | Mouse, rat, guinea pig, rabbit dog, human |

*TTX* tetrodotoxin, *DHP* dihydropyridine, *4-AP* 4aminopyridine, *HATX* hanatoxin, *HpTX* heteropoatoxin, *SUR* sulfonylurea, *TEA* tetraethylammonium

ventricular action potential waveforms [17] and modifying the sensitivity to antiarrhythmics. In addition, alterations in the densities and properties of voltage-gated $Na^+$, $Ca^{2+}$, and $K^+$ currents occur in a number of myocardial disease states [18–26] changes that can lead to the generation of life-threatening ventricular arrhythmias. As a result, there is considerable interest in defining the molecular mechanisms controlling the regulation, modulation, and functional expression of the channels underlying action potential repolarization in the ventricles.

## Inward $Na^+$ and $Ca^{2+}$ Currents in Mammalian Ventricles

### Voltage-Gated Ventricular $Na^+$ Currents

Voltage-gated $Na^+$ channels open rapidly on membrane depolarization and underlie the rapid rising phases of the action potentials in mammalian ventricular myocytes (Fig. 1.1). The threshold for activation of these channels is quite negative (~ −55 mV), and activation is steeply voltage dependent. The inward movement of $Na^+$ through open voltage-gated $Na^+$ channels underlies impulse conduction in ventricular muscle, as well as in the specialized conducting network of Purkinje fibers in the ventricle [27]. Voltage-gated $Na^+$ channels also inactivate rapidly, and during the plateau phase of the ventricular action potential, most of the $Na^+$ channels are in an inactivated and nonconducting state [27]. There is, however, a finite probability of channel reopening at voltages corresponding to the action potential plateau (Fig. 1.1), and present estimates are that approximately 99% of the channels are inactivated and 1% of the channels are open during the plateau phase [28–30].

The probability of $Na^+$ channel reopening at depolarized voltages (i.e., during phase 2) is determined by the overlap of the curves describing the voltage dependences of channel activation (a measure of the probability of the channel transitioning from a closed, but available, to an open state) and steady-state inactivation (a measure of the availability of channels in the closed state). This overlap has been termed the "window"

current because it is conducted over a "window" of voltages where the two curves overlap [31]. Although the $Na^+$ channel "window" current has been recognized as an important determinant of action potential duration in the heart for many years [31, 32], studies focused on exploring the mechanisms underlying inherited cardiac arrhythmias have shown that voltage-gated $Na^+$ channels play an important role in ventricular action potential repolarization. In addition, there are regional differences in the expression of the persistent component of the voltage-gated $Na^+$ current in the ventricles [33]. Together with the marked differences in voltage-gated outward $K^+$ current densities and properties, differences in $Na^+$ current densities may contribute to observed regional heterogeneities in ventricular action potential amplitudes and durations [12].

While some studies have suggested there are additional varieties of voltage-gated $Na^+$ channels in the myocardium, the predominant cardiac sodium channel is $Na_V1.5$ [16, 34–36]. $Na_V1.5$ channel α subunits are encoded by the SCN5A gene [16, 27, 34, 37, 38]. Four different types of sodium channel β subunits modulate these NaV1.5 channels [16, 34, 39]. There are four total sodium channel β subunit genes, SCN1B–SNC4B, each coding for a β subunit, all of which are expressed in cardiac tissue [16, 34, 39]. Subunit composition often modulates the exact properties of cardiac $Na^+$ current [16, 34, 39]. Ionic sodium current is also shaped by exact structures of subunits as often dictated by genetically encoded mutation and factors affecting channel trafficking and regulation, both genetic and environmental [5, 34, 40–42]. Additionally, electrical and chemical factors of the environment also affect channel function and thus voltage-gated sodium channel conductance [5, 40–42].

## Voltage-Gated Ventricular $Ca^{2+}$ Currents

A number of different subtypes of voltage-gated calcium ($Ca^{2+}$) currents/channels have been distinguished in neurons and in muscle cells based on differences in time- and voltage-dependent properties and pharmacological profiles, and two (somewhat similar) classification schemes have evolved to describe the voltage-gated $Ca^{2+}$ currents/channels in a variety of different cell types, including mammalian ventricular myocytes. In the first system, voltage-gated $Ca^{2+}$ channels are referred to as low-voltage-activated (LVA) and high-voltage-activated (HVA) $Ca^{2+}$ channels based primarily on differences in the (voltage) threshold of channel activation [43–48]. Similar to voltage-gated $Na^+$ channels, for example, the LVA $Ca^{2+}$ activate at relatively hyperpolarized membrane potentials, i.e., ~ −50 mV. In addition, LVA $Ca^{2+}$ channels activate and inactivate rapidly. HVA $Ca^{2+}$ channels, in contrast, open on depolarization to membrane potentials more positive than −20 mV and inactivate over a time course of several hundred milliseconds to seconds [47]. Only HVA $Ca^{2+}$ currents are evident in adult mammalian ventricular myocytes [47, 49] (Fig. 1.1).

There is considerable variability in the detailed kinetic and pharmacological properties of the HVA $Ca^{2+}$ channels expressed in different cell types, and it is now clear that HVA $Ca^{2+}$ are considerably more heterogeneous than LVA $Ca^{2+}$ channels. In the alternate $Ca^{2+}$ channel nomenclature, the various HVA channels are referred to by a single letter designation, i.e., L, N, P, Q, or R. In this scheme, LVA channels are referred to as T (transient)-type $Ca^{2+}$ channels. Although all HVA $Ca^{2+}$ channels exhibit relatively large single-channel conductances (13–25 pS) and have similar permeation properties, the electrophysiological and pharmacological properties of L- (long-lasting), N- (neither L- nor T-), P- (Purkinje), and Q- and R- (remaining) type channels are distinct [43–48, 50].

In mammalian ventricular myocytes, the HVA current is through L-type voltage-gated $Ca^{2+}$ channels, and the densities of the L-type $Ca^{2+}$ channel currents do not vary appreciably in different species and/or in myocytes isolated from different region of the ventricles of the same species [47]. These channels require strong depolarization for activation, generate relative long-lasting calcium currents, (ICa(L)), when $Ba^{2+}$ is the charge carrier, and are selectively

blocked by dihydropyridine $Ca^{2+}$ channel antagonists, such as nifedipine and nitrendipine [47] (Table 1.1). The opening of L-type voltage-gated $Ca^{2+}$ channels in response to membrane depolarization in ventricular myocytes is delayed relative to the voltage-gated $Na^+$ channels (Fig. 1.1). As a result, the L-type $Ca^{2+}$ channels contribute little to the rapid rising phase (phase 0) of ventricular action potentials [10–12, 51] (Fig. 1.1). $Ca^{2+}$ influx through the L-type $Ca^{2+}$ channels triggers $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores and contributes importantly, therefore, to excitation-contraction coupling. In addition, the inward $Ca^{2+}$ current through these channels counters outward $K^+$ efflux and contributes importantly, therefore, to the prominent plateau phase of the action potential in ventricular myocytes (Fig. 1.1). Nevertheless, at positive potentials, L-type $Ca^{2+}$ channels do inactivate, and the inactivation of these channels is voltage and $Ca^{2+}$ dependent. Under normal physiological conditions with $Ca^{2+}$ as the charge carrier, inactivation of ventricular L-type voltage-gated $Ca^{2+}$ currents is rapid owing to $Ca^{2+}$-dependent channel inactivation. In addition to contributing to the termination of the action potential plateau and action potential repolarization, the spontaneous ceasing of conductance (inactivation) of ventricular L-type $Ca^{2+}$ channels functions as a negative feedback system that is critical in governing intracellular $Ca^{2+}$ concentration [10–12, 51].

## Diversity of Voltage-Gated, Outward $K^+$ Currents in Mammalian Ventricles

Voltage-gated $K^+$ channel currents influence the amplitudes and durations of ventricular action potentials, and in most cells, two broad classes of voltage-gated $K^+$ currents have been distinguished: transient outward $K^+$ currents, $I_{to}$, and delayed, outwardly rectifying $K^+$ currents, $I_K$ (Table 1.1). The transient currents ($I_{to}$) activate and inactivate rapidly and underlie the early phase (phase 1) of repolarization, whereas the delayed rectifiers ($I_K$) determine the latter phase (phase 3) of action potential repolarization in the ventricular myocardium (Fig. 1.1). These are broad classifications, however, and there are actually multiple types of $I_{to}$ and of $I_K$ (Table 1.1) expressed in ventricular cells. In addition, there are species and regional differences in the densities, as well as the detailed biophysical properties, of these currents, and these are evident in the waveforms of ventricular action potentials recorded in different cell types/species [10–12, 51].

### Transient Outward $K^+$ Current Channels, $I_{to}$

Activating and inactivating quickly upon membrane depolarization, $I_{to}$ does a part of the action potential shaping controlled by the differing densities and type of $I_{to}$ present [4, 52]. Among these characteristics influenced by $I_{to}$ are action potential height and duration [4]. Multiple types of $I_{to}$ further diversify $I_{to}$ influence on the various cardiac cell type action potentials [4, 52].

Although two transient outward current components, referred to as $I_{to1}$ and $I_{to2}$, were originally distinguished in cardiac Purkinje fibers and assumed to reflect distinct $K^+$ conductance pathways [53–55], the $Ca^{2+}$-dependent $I_{to2}$ appears to be a $Cl^-$ (not a $K^+$) current [53–56]. The $Ca^{2+}$-independent, 4-aminopyridine (4-AP)-sensitive $I_{tol}$ in cardiac Purkinje fibers, in contrast, is $K^+$-selective [54, 55]. Numerous studies have described the properties of $Ca^{2+}$-independent, 4-AP-sensitive transient outward $K^+$ currents in ventricular cells (Table 1.1), although the currents have been variably referred to as $I_{to}$, $I_{to1}$, or $I_t$ [13, 57, 58]. Electrophysiological and pharmacological studies, however, have now clearly demonstrated that there are two distinct transient outward $K^+$ currents, $I_{to,fast}$ ($I_{to,f}$) and $I_{to,slow}$ ($I_{to,s}$), in ventricular cells and that these currents are differentially distributed [11, 12, 59–62].

The rapidly activating and inactivating transient outward $K^+$ currents that are also characterized by rapid recovery from steady-state inactivation are referred to as $I_{to,fast}$ ($I_{to,f}$). The rapidly activating and inactivating transient outward $K^+$ currents that recover slowly from inactivation are referred to as $I_{to,slow}$ ($I_{to,s}$) (Table 1.1) following

the nomenclature suggested by Xu and colleagues [60]. Although prominent in ventricular myocytes from most species including cat [63], dog [64, 65], ferret [57], human [66–68], mouse [60, 69–71], and rat [66, 72] (Table 1.1), $I_{to,f}$ is recorded in guinea pig ventricular myocytes only when extracellular $Ca^{2+}$ is removed [73]. The time- and voltage-dependent properties of ventricular $I_{to,f}$ in different species (Table 1.1) are similar in that activation, inactivation, and recovery from steady-state inactivation are all rapid [57, 60, 64–68, 72, 74]. In addition, $I_{to,f}$ is readily distinguished from other voltage-gated outward $K^+$ currents including $I_{to,s}$ using the spider $K^+$ channel toxins *Heteropoda* toxin-2 or toxin-3 [59–61, 75]. The fact that the properties of ventricular $I_{to,f}$ in different species are similar (Table 1.1) led to the hypothesis that the molecular correlates of functional ventricular $I_{tof}$ channels in different species are the same [13], and considerable experimental evidence in support of this hypothesis has now been provided. Indeed, all available evidence suggests that members of the $K_V4$ subfamily of α subunits encode functional ventricular $I_{to,f}$ channels [10, 11]. Nevertheless, there are differences in the biophysical properties of ventricular $I_{to,f}$ channels, suggesting that there are subtle, albeit important, differences in the molecular correlates of these channels in different species.

The transient outward $K^+$ currents in rabbit ventricular myocytes (originally referred to as $I_t$) inactivate slowly and recover from steady-state inactivation very slowly, with complete recovery requiring seconds [76–78]. The properties of rabbit ventricular $I_t$ are similar to the slow transient $K^+$ current, referred to as $I_{to,slow}$ or $I_{to,s}$, that was distinguished (from $I_{to,f}$) in mouse ventricular myocytes by the slow rates of inactivation and recovery from inactivation [60]. In adult mouse ventricle, $I_{to,f}$ and $I_{to,s}$ are differentially distributed [60–62]. In all cells isolated from the wall of the right (RV) and left (LV) ventricles, for example, $I_{to,f}$ is expressed and $I_{to,s}$ is undetectable [60–62]. There are, however, differences in $I_{to,f}$ densities in RV and LV cells [60–62]. In the ventricular septum, the currents are more heterogeneous: 80% of the cells express $I_{to,f}$ and $I_{to,s}$ and the remaining (20%) express $I_{to,s}$ alone. In addition, when present, $I_{to,f}$ density is significantly ($p < .00$ I) lower in septum, than in RV or LV cells [60, 61].

The rates of inactivation and recovery from inactivation of the transient outward $K^+$ currents are significantly slower in ferret LV endocardial than epicardial cells [59]. Interestingly, the time- and voltage-dependent properties and the pharmacological sensitivities of the transient outward $K^+$ current in these cells are similar to mouse ventricular $I_{to,s}$ and $I_{to,f}$, respectively. The properties of $I_{to}$ ($I_t$) in rabbit ventricular myocytes, in contrast, appear to be analogous to mouse ventricular $I_{to,s}$ (Table 1.1).

The greatly varying properties of $I_{to,s}$ and $I_{to,f}$ suggested that they are two different currents with different molecular determinants [13]. Evidence suggests that $I_{to,f}$ occurs as product of $K_V4.3/K_V4.2$ α subunits, with $K_V4.3$ being the prominent producer of $I_{to,f}$ in human ventricles and a mix of $K_V4.3/K_V4.2$ in rat, mouse, and ferret [52, 59, 79–85]. Alternately the cardiac $I_{to,s}$ is believed to be resultant of $K_V1.4$ α subunits [52]. This is because $K_V1.4$ in an expression systems yields currents that closely resemble $I_{to,s}$ [86–88].

## Delayed Rectifier $K^+$ Currents/ Channels, $I_K$

Delayed rectifier $K^+$ currents, $I_K$, have been characterized extensively in myocytes isolated from canine [65, 74, 89, 90], feline [91, 92], guinea pig [93–99], human [100, 101], mouse [60, 61, 71, 102, 103], rabbit [104, 105], and rat [72, 106, 107] hearts, and, in most cells, multiple components of $I_K$ (Table 1.1) are co-expressed. In guinea pig ventricular myocytes, for example, two prominent components of $I_K$, $I_{Kr}$ ($I_K$,rapid) and $I_{Ks}$ ($I_K$,slow), were distinguished based on sensitivity to block by class III antiarrhythmic agents and differences in time- and voltage-dependent properties [72, 108, 109]. $I_{Kr}$ activates rapidly, inactivates very rapidly, displays marked inward rectification, and is selectively blocked by several class III antiarrhythmics, including dofetilide, E-4031, and sotalol [96]. No inward rectification

is evident for $I_{Ks}$, and this current is blocked by class III compounds, including NE-10064 and NE-10133, which do not affect $I_{Kr}$ [110]. In human [100, 101, 111, 112], canine [74, 89], and rabbit [105, 113] ventricular cells, both $I_{Kr}$ and $I_{Ks}$ are expressed and are prominent repolarizing currents. In feline [91] and rat [107] ventricular myocytes, however, only $I_{Kr}$ is detected. $I_{Kr}$ and $I_{Ks}$ are also readily distinguished at the microscopic level [93, 94, 104, 105], and molecular genetic studies suggest that the molecular correlates of these channels are distinct [10, 11].

The distinctively differing characteristics of $I_{Kr}$ and $I_{Ks}$, both in voltage- and timing-related properties, elude to specialized roles for each in repolarization [4]. This is especially true of cells in Purkinje fibers and ventricles, though, perhaps even more indicative of specialized roles for $I_{Kr}$ and $I_{Ks,}$ in some cells only $I_{Kr}$ or $I_{Ks}$ is expressed [4]. Indeed, each is the result of a different α subunit. $I_{Kr}$ normally makes the greatest contribution to repolarization and is a product of $K_V11.1$ known as the hERG channel [109, 114]. HERG stands for "human ether-a-go-go-related gene" [114]. $I_{Ks}$ is resultant of the KCNQ1 α subunit in complex with the accessory subunit KCNE1 [4, 109]. KCNE1 confers the characteristic $I_{Ks}$ traits to the KCNQ1 α subunit complex [4, 109].

A third type of delayed rectifier current exists, known as $I_{Kur}$, but is expressed primarily in the atrial myocardia and not in the ventricles [111, 115, 116]. $I_{Kur}$ is a current conducted by the HK2 channel, also known as human $K_V1.5$ [116–118]. This channel is expressed on the protein and mRNA level in both the atria and ventricle, as well as several other organs [116, 119–121]; it is thought to only produce $I_{Kur}$ current in the atria [116, 122].

In rodent ventricles, there are additional components of $I_K$ with properties different from $I_{Ks}$ and $I_{Kr}$ (Table 1.1). In rat ventricular myocytes, for example, there are novel delayed rectifier $K^+$ currents, referred to as $I_K$ and $I_{ss}$ (Table 1.1) [72, 123]. In mouse ventricular myocytes, two voltage-gated $K^+$ currents, $I_{K,slow}$ and $I_{ss}$, have also been identified [60, 71, 102, 103, 124]. $I_{K,slow}$ is rapidly activating and slowly inactivating $K^+$ current with kinetic and pharmacological properties distinct from $I_{to,f}$ and $I_{to,s}$ (and $I_{ss}$); in addition, $I_{K,slow}$ is blocked effectively and selectively by μM concentrations of 4-AP [102, 103, 124] which do not affect $I_{to,f}$ or $I_{to,s}$ in the same cells [60, 71]. The current, $I_{ss}$, remaining at the end of long (up to 10 s) depolarizing voltage steps, in contrast, is slowly activating and 4-AP insensitive [60, 71]. In contrast to the differential distribution of $I_{to,f}$ and $I_{to,s}$, however, $I_{K,slow}$ and $I_{ss}$ appear to be expressed in all mouse ventricular myocytes [60, 61, 71].

## Developmental Changes in Voltage-Gated K Channel Expression

During postnatal development, ventricular action potentials shorten, phase 1 repolarization (Fig. 1.1) becomes more pronounced, and functional $I_{to,f}$ density is increased [123, 125–134]. In neonatal canine ventricular myocytes, for example, the "notch" and the rapid (phase 1) repolarization that are typical in adult cells are not clearly evident [126]. Action potentials in neonatal cells are insensitive to 4-AP, and voltage-clamp recordings reveal that $I_{to,f}$ is undetectable [126]. In cells from 2-month-old animals, $I_{to,f}$ is present and phase 1 repolarization is clearly evident [126].

The density of $I_{to,f}$ is also low in neonatal mouse [127, 133] and rat [123, 125, 128, 130–132, 134] ventricular myocytes and then increases several fold [13, 17] during early postnatal development. In rat, the properties of the currents in 1–2-day ventricular myocytes [134] are also distinct from those of $I_{to,f}$ in postnatal day 5 to adult cells [123] in that inactivation and recovery from inactivation are slower in postnatal day 1–2 cells. Indeed, the properties of the transient outward currents in postnatal day 1–2 rat ventricular cells [134] more closely resemble $I_{to,s}$ than $I_{to,f}$. Neither $I_{to,s}$ nor $I_{to,f}$, however, is detectable in embryonic ventricular myocytes [135].

In rabbit ventricular myocytes, transient outward $K^+$ current density increases and the kinetic properties of the currents also change during postnatal development [129]. In contrast to rat, however, the rate of recovery of the currents (mean recovery time ~100 ms) is ten times faster

in neonatal than in adult (mean recovery time ~1300 ms) cells [129]. The slow recovery of the transient outward currents underlies the marked broadening of action potentials at high stimulation frequencies in adult (but not in neonatal) rabbit ventricular myocytes [129, 136]. In rabbit ventricular cells, therefore, it appears that the change in the transient outward $K^+$ currents is opposite to that seen in other species, i.e., $I_{to,f}$ is expressed in the neonate, whereas $I_{to,s}$ is prominent in the adult.

The expression patterns of delayed rectifier $K^+$ currents also change during postnatal development. Both $I_{Kr}$ and $I_{Ks}$, for example, are readily detected in fetal/neonatal mouse ventricular myocytes [127, 137, 138], whereas these currents are difficult to detect in adult cells [60, 61, 71]. Although $I_{Kr}$ appears to be the predominant repolarizing $K^+$ current in fetal mouse ventricular cells, $I_{Kr}$ density decreases with age [60, 61, 137, 138]. The density of $I_{Ks}$ increases during late embryonic development [137] and subsequently decreases during postnatal development [127, 133, 137]. Because $I_{Kr}$ and $I_{Ks}$ are prominent repolarizing $K^+$ currents in adult cardiac cells in several species, changes in the expression and/or the properties of these conductance pathways must be distinct from those observed in the mouse heart. In contrast to the marked changes in $I_{to,f}$, the density of $I_K$ does not change significantly in rat ventricular cells after postnatal day 5 [123, 132].

## Regional Differences in $K^+$ Current Expression

There are marked differences in $I_{to,f}$ densities in different regions of the ventricles in canine [64, 139], cat [92], ferret [59], human ([66]; but, *see* also [68]), mouse [60, 61], and rat [140]. In canine left ventricle, for example, $I_{to,f}$ density is 5–6-fold higher in epicardial and midmyocardial than in endocardial cells [139]. $I_{to,s}$ density is also variable in mouse and ferret left ventricles [59–61], being detected only in (ferret) endocardial [59] and (mouse) septum [60, 61] ventricular cells.

The densities of ventricular $I_{Ks}$ and $I_{Kr}$ are also variable. In dog, for example, $I_{Ks}$ density is higher in epicardial and endocardial cells than in M cells [89]. There are also regional differences $I_{Kr}$ and $I_{Ks}$ expression in guinea pig LV [141, 142]. In cells isolated from the LV free wall, for example, the density of $I_{Kr}$ is higher in subepicardial, than in midmyocardial or subendocardial, myocytes [142]. At the base of the LV, in contrast, $I_{Kr}$ and $I_{Ks}$ densities are significantly lower in endocardial than in midmyocardial or epicardial cells. These differences in voltage-gated $K^+$ current densities contribute to the variations in action potential waveforms recorded in different regions (right vs left, apex vs base) and layers (epicardial, midmyocardial, and endocardial) of the ventricles [10–12, 57, 64, 89, 141, 142].

## Other Ionic Currents Contributing to Repolarization in Mammalian Ventricles

In addition to the voltage-gated $K^+$ channels, there are other $K^+$ channels that play a role in action potential repolarization in mammalian ventricles. The inwardly rectifying $K^+$ channels (Kir), which are readily distinguished biophysically from the voltage-gated, outwardly rectifying $K^+$ channels because these channels carry inward $K^+$ current better than outward $K^+$ current [143], contribute to myocardial action potential repolarization through $I_{K1}$ [143–145]. This biophysical property of these channels, however, is of no functional importance because the membrane potential of cardiac myocytes never reaches values more negative than the reversal potential for $K^+$ (~ −90 mV). It is the outward $K^+$ currents through these channels that are important physiologically, and these currents also play a role in action potential repolarization. Three types of inwardly rectifying $K^+$ channel currents have been described in the mammalian ventricular myocytes: the cardiac inward rectifier current, $I_{K1}$, the ATP-dependent $K^+$ current, $I_{KATP}$, and $I_{K(ACh)}$ channels mediated by muscarinic acetylcholine receptor. Although the densities of these currents vary in different regions (atria, ventricles, and conducting tissue) of the heart, in the ventricles, these currents appear to be uniformly expressed.

$I_{KCa}1$ is a small-conductance $Ca^{2+}$-activated $K^+$ current [146]. It is caused by SKCa channels, $Ca^{2+}$-activated $K^+$ channels that have a single-channel conductance of ~10 pS [147]. These channels are involved in repolarization, with SK channel blocker causing longer action potentials, most pronounced in the atria [148–150]. Some atrial fibrillation patients have been associated with a single nucleotide polymorphism in the SK channel coding KCNN3 gene [150–152].

$I_{K(ACh)}$ channels are gated through G protein-coupled mechanism, mediated by muscarinic acetylcholine receptor activation [153, 154]. $I_{K(ACh)}$ channels are activated by the binding of G protein ßγ-subunits in response to the acetylcholine released on vagal stimulation [155]. Although $I_{K(ACh)}$ channels are expressed in AVN, SAN, atrial, and Purkinje cells these channels are all activated by acetylcholine released on vagal stimulation. These channels are not thought to contribute appreciably to action potential repolarization under normal physiological conditions. Consistent with this hypothesis, targeted deletion of one of the Kir subunits (Table 1.6) encoding $I_{K(ACh)}$ channels Kir3.4 does not measurably affect resting heart rates [156]. However, atrial fibrillation is not evident in Kir3.4 null mice exposed to the acetylcholine receptor agonist carbachol, suggesting that activation of $I_{K(ACh)}$ channels is involved in the cholinergic induction of atrial fibrillation [157].

The weakly inwardly rectifying ATP-dependent $K^+$ channels, first identified in cardiac muscle, are inhibited by increased intracellular ATP and activated by nucleotide diphosphates [145]. These channels are thought, therefore, to provide a link between cellular metabolism and membrane potential [145, 158]. In ventricular myocardium, activation of $I_{KATP}$ channels is thought to be important under conditions of metabolic stress, as occurs during ischemia or hypoxia and results in shortening action potential durations [158, 159]. The activation of $I_{KATP}$ channels has also been suggested to contribute to the cardioprotection resulting from ischemic preconditioning [160, 161]. Unlike the voltage-gated $K^+$ channels expressed in the ventricular myocardium, $I_{KATP}$ channels appear to be distributed uniformly through the right and left ventricles and through the thickness of the ventricular wall. The $I_{KATP}$ channels, however, are expressed at much higher density than other sarcolemmal $K^+$ channels [161]. Although these channels are inhibited under normal physiological conditions, the high density suggests that action potentials will be shortened markedly when only a small number of $I_{KATP}$ channels are activated [162]. In mammalian ventricular myocytes, $I_{K1}$ plays a role in establishing the resting membrane potential, the plateau potential, and contributes to phase 3 repolarization (Fig. 1.1). The fact that the conductance of these channels is high at negative membrane potentials underlies the contribution of $I_{K1}$ to ventricular resting membrane potentials [163]. The voltage-dependent properties of $I_{K1}$ channels, however, are such that the conductance is very low at potentials positive to approximately −40 mV [163]. Now $I_{K1}$ channels have been found in human [164, 165], guinea pig [165, 166], and rabbit [76, 167, 168] atrial and ventricular myocytes and rabbit SAN cells [167]. The properties of $I_{K1}$ channels are similar in that all are blocked by extracellular $Ba^{2+}$, intracellular $Cs^+$ and strongly inwardly rectifying [76, 93, 164, 165]. The strong inward rectification evident in cardiac $I_{K1}$ channels is attributed to block by intracellular $Mg^{2+}$ [169] and $Ca^{2+}$ [170] and by polyamines [163, 171, 172]. Removal or depletion of intracellular polyamines, $Mg^{2+}$ and/or $Ca^{2+}$, will eliminate the steep inward rectification of $I_{K1}$ channels current and convert to a linear current-voltage relation [163, 169–172].

The fact that the strongly inwardly rectifying $I_{K1}$ channels conduct at negative membrane potentials suggests that these channels will play a role in establishing the resting membrane potentials of Purkinje fibers, as well as of atrial and ventricular myocytes. Direct experimental support for this hypothesis was provided with the demonstration that ventricular membrane potentials are depolarized in the presence of $Ba^{2+}$ [163], which blocks $I_{K1}$ channels. In addition, action potentials are prolonged, and phase 3 repolarization is slowed in the presence of extracellular $Ba^{2+}$ [143], suggesting that $I_{K1}$ channels

also contribute to repolarization, particularly in the ventricular myocardium.

Similar to the $K_V$ channels, $I_{K1}$ densities and the detailed biophysical properties of the currents do vary in different myocardial cell types. In the human heart, for example, $I_{K1}$ density is more than twofold higher in ventricular, than in atrial, cells [165]. In guinea pig, the properties of the atrial and ventricular $I_{K1}$ currents are also distinct in that ventricular $I_{K1}$ inactivates during maintained depolarizations, whereas atrial $I_{K1}$ does not [93, 166]. In addition, changes in extracellular $K^+$ modulate the magnitude of ventricular $I_{K1}$ but have little effect on atrial $I_{K1}$ [93]. At the microscopic level, (guinea pig) atrial and ventricular $I_{K1}$ channels are also distinct. Mean channel open times of ventricular $I_{K1}$ channels, for example, are approximately five times longer than those of atrial $I_{K1}$ channels, whereas the single atrial and ventricular $I_{K1}$ channel conductance are indistinguishable [93].

Taken together, these observations suggested the interesting possibility that distinct molecular entities underlie ventricular and atrial $I_{K1}$ channels, and experimental support for this hypothesis has now been provided [166].

## Molecular Correlates of Voltage-Gated $Na^+$ and $Ca^{2+}$ Currents in Mammalian Ventricle

### Voltage-Gated $Na^+$ Channel (Na) Pore-Forming α Subunits

Voltage-gated $Na^+$ ($Na_V$) channel pore-forming (α) subunits (Fig. 1.2a) belong to the "S4" superfamily of genes encoding voltage-gated ion channels. Although a number of $Na_V$ α subunits (Fig. 1.2a) have been identified to date (Table 1.2), only one of these, $Na_V1.5$ (SCN5A), appears to be widely expressed in mammalian ventricular

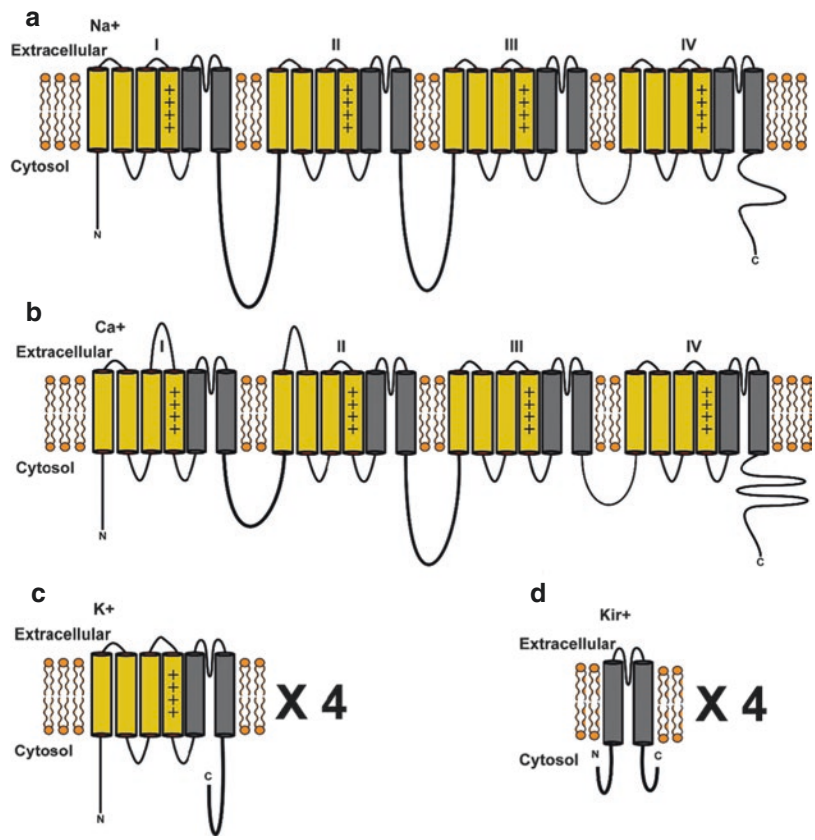


**Fig. 1.2** Pore-forming (α) subunits of cardiac ion channels. Comparisons of primary sequences and the membrane topologies of the α subunits encoding voltage-gated $Na^+$ ($Na_V$) (**a**), voltage-gated $Ca^{2+}$ ($Ca_V$) (**b**), voltage-gated $K^+$ ($K_V$) (**c**), and inwardly rectifying $K^+$ channel s (Kir) (**d**) are illustrated

**Table 1.2** Diversity of voltage-gated $Na^+$ and $Ca^{2+}$ α subunits

| Subfamily | Protein | Gene | Locus | | | Cardiac current |
|---|---|---|---|---|---|---|
| | | | Homo | Mus | Rattus | |
| $Na^+$ | | | | | | |
| $Na_V1$ | | | | | | |
| | $Na_V1.1$ | *SCN1A* | 2q24.3 | 2C1.3 | 3q21 | |
| | $Na_V1.2$ | *SCN2A* | 2q24.3 | 2C1.3 | 3q21 | |
| | $Na_V1.3$ | *SCN3A* | 2q24.3 | 2C1.3 | 3q21 | |
| | $Na_V1.4$ | *SCN4A* | 17q23.3 | 11E1 | 10q32.1 | |
| | $Na_V1.5$ | *SCN5A* | 3p22.2 | 9F3 | 8q32 | $I_{Na}$ |
| | $Na_V1.6$ | *SCN8A* | 12q13.13 | 15F1 | 7q36 | |
| | $Na_V1.7$ | *SCN9A* | 2q24.3 | 2C1.3 | 3q21 | |
| | $Na_V1.8$ | *SCN10A* | 3p22.2 | 9F3 | 8q32 | |
| | $Na_V1.9$ | *SCN11A* | 3p22.2 | 9F3 | 8q32 | |
| $Na_X$ | | | | | | |
| | $Na_V2.1$ | *SCN7A* | 2q24.3 | 2C1.3 | 3q21 | |
| $Ca^{2+}$ | | | | | | |
| $Ca_V1$ | | | | | | |
| | $Ca_V1.1$ | *CACNA1S* | 1q32.1 | 1E4 | 13q13 | |
| | $Ca_V1.2$ | *CACNA1C* | 12p13.33 | 6F1 | 4q42 | $I_{Ca(L)}$ |
| | $Ca_V1.3$ | *CACNA1D* | 3p21.1 | 14B | 16p16 | |
| | $Ca_V1.4$ | *CACNA1F* | Xp11.23 | XA1.1 | Xq12 | |
| $Ca_V2$ | | | | | | |
| | $Ca_V2.1$ | *CACNA1A* | 19p13.13 | 8C2 | 19q11 | |
| | $Ca_V2.2$ | *CACNA1B* | 9q34.3 | 2A3 | 3p13 | |
| | $Ca_V2.3$ | *CACNA1E* | 1q25.3 | 1G3 | 13q21 | $I_{Ca(R)}$ |
| $Ca_V3$ | | | | | | |
| | $Ca_V3.1$ | *CACNA1G* | 17q21.33 | 11D | 10q26 | |
| | $Ca_V3.2$ | *CACNA1H* | 16p13.3 | 17A3.3 | 10q12 | |
| | $Ca_V3.3$ | *CACNA1I* | 22q13.1 | 15E1 | 7q34 | |

Boxes denote cardiac expression

myocardium (Table 1.2) [36, 173–175]. Each $Na_V$ a-subunit, which forms the ion-conducting pore and contains channel gating components, consists of four homologous domains (I to IV) (Fig. 1.2) [27, 176]. Each domain contains six α-helical transmembrane repeats (S1–S6), for which mutagenesis studies have revealed key functional roles [27, 36, 176]. The cytoplasmic linker between domains III and IV, for example, is an integral component underlying voltage-dependent inactivation [177], and a critical isoleucine, phenylalanine, and methionine (IFM) motif within this linker [27, 174, 178] has been identified as the inactivation gate [179–181]. $Na^+$ channel inactivation is due to rapid block of the inner mouth of the channel pore by the cytoplasmic linker between domains III and IV that occurs within milliseconds of membrane depolarization [182]. NMR analysis of this linker (gate) in solution has revealed a rigid helical structure that is positioned such that it can block the pore, providing a structural interpretation of the functional studies [183]. More recently, cryo-EM solved structures of $Na_V1.4$-β1 complex from a species of electric eel and from human has confirmed the predicted structural arrangement of

sodium channels and offered insight into the structure of $Na_V1.5$ [184, 185].

During the plateau phase of the ventricular action potential, more than 99% of the voltage-gated $Na^+$ channels are in an inactivated, nonconducting state in which the inactivation gate occludes the inner mouth of the conducting pore through specific interactions with sites on the S6 segment of domain IV [186] or the S4–S5 loop of domain IV [187]. Inherited mutations (i.e., ΔKPQ) in the linker between domains III and IV in SCN5A disrupt inactivation and cause one form of long QT syndrome, LQT3 [188]. The mutation-induced enhancement of sustained $Na^+$ current activity measured during prolonged depolarization is caused by altering modes of channel gating. In a gating mode in which sustained current is enhanced, single-channel recordings revealed that channels do not enter an absorbing inactivated state but instead reopen. Enhanced sustained current caused by bursting is sufficient to prolong cellular action potentials in theoretical models [189] and in genetically modified mice [190]. Subsequent analysis of additional SNC5A mutations, linked both to LQT-3 and another inherited arrhythmia, the Brugada syndrome, however, has revealed that this is not the only mechanism by which altered $Na^+$ channel function can prolong the cardiac action potential.

Several studies have revealed a critical role for the carboxy (C)-terminal tail of the $Na_V$ channel a-subunit in the control of channel inactivation [28, 191–194]. Point mutations in the C-terminus, for example, can shift the voltage dependence of inactivation, promote sustained $Na^+$ channel activity, change the kinetics of both the onset of and recovery from inactivation, and alter drug-channel interactions [195–199]. In an investigation into the secondary structure of the $Na^+$ channel C-terminus and the roles of possible tail structures in the control of inactivation, single-channel data revealed that the C-terminus has pronounced effects on repetitive channel openings that occur in bursts during prolonged depolarization [200]. Homology modeling of the C-terminus, assuming similarity to the N-terminal domain of calmodulin, predicted that the C-terminus would adopt a predominantly α-helical structure, a prediction verified by circular dichroism (CD) of a purified C-terminus fusion protein. Only the proximal region of the C-terminus, which contains all of the helical structure, markedly modulates channel inactivation, but not activation. The distal C-terminal tail, which is largely unstructured, does not affect channel gating but affects the density of functional $Na^+$ channels in the surface membrane. Taken together, these experiments suggest that interactions occur between the structured region of the C-terminus and other components of the channel protein and that these interactions function to stabilize the channel in a pore-blocked inactivated state during membrane depolarization.

The structural data also provide a framework to interpret the mechanistic basis of a large number of mutations linked either to LQT-3 or Brugada syndrome that occurs within the structured and charged proximal region of the C-terminus of the channel [201–204]. All inherited mutations linked to LQT-3, for example, alter $Na^+$ channel activity in a manner that prolongs the QT interval of the ECG in mutation carriers, but at least three different mutations, discovered by linkage to LQT-3, have now been reported that do not result in increased sustained inward $Na^+$ current. The first mutation, D1790G, was discovered in a large Israeli family [205]. Initial expression studies revealed that this mutation changed the voltage dependence and kinetics of inactivation of mutant channels but did not promote sustained current [206], a finding questioned by subsequent investigations [207]. Computer-based simulations suggest that this mutation may prolong action potential duration through an indirect effect on the control of cytoplasmic calcium concentrations [208], a result that awaits testing in animal models.

Another LQT-3 mutation has also been reported that does not appear to result in enhanced sustained current [209]. In this case, the mutation (E1295K) alters both activation and inactivation gating by causing small but significant shifts in the voltage dependence of gating. As a result, although there is no enhanced sustained $Na^+$ current, the voltage dependence of the "window" current is changed. The peak of the "window"

current is shifted in the positive direction such that the background currents against which it is expressed are different than in the case of wild-type channels [209]. As a result, window current of the same amplitude will have a greater effect on net membrane currents, and, because the currents flowing during the plateau phase are so small, the net result will be increased inward current and action potential prolongation [162, 210, 211]. More recently, a similar mechanism has been proposed to explain the cellular consequences of another $Na^+$ channel mutation linked to sudden infant death syndrome [212].

## Voltage-Gated $Na^+$ Channel (Na) Accessory β Subunits

Functional voltage-gated $Na^+$ channels appear to be multi-subunit complexes consisting of a central pore-forming α subunit (Fig. 1.2a) and one to two auxiliary β subunits [213]. There are at least four different β subunit genes encoding a total of five proteins. SCNlb encodes β1 and β1B [214–216]. SCN2b encodes β2 [217, 218], and SCN3b encodes β3 [219]. Additionally, SCN4A encodes β4 protein [220] (Table 1.3). Structurally, most of the voltage-gated $Na^+$ channel β subunits have a type 1 topology, meaning that they have an extracellular N-terminus and an intracellular C-terminus connected by a single pass through the membrane of their one amino acid chain [221]. The exception to this is β1B that due to its incorporation of intron 3 in the SCN1b gene produces an alternate C-terminal region lacking a transmembrane domain. Thus, β1B is a secreted cell adhesion protein [222, 223].

In the heart, the functional role of these subunits remains controversial. Most studies have focused on the role of SCNlb, which has relatively minor effects on channel gating in the heart compared with its effects on gating in the skeletal muscle [224]. Nevertheless, it has been shown that co-expression of SCNlb affects inactivation kinetics and current densities [206, 225]. Although relatively few functional effects have been reported for SCN2b [226], it has been reported that this subunit plays a role in controlling the $Ca^{2+}$ permeability of voltage-gated $Na^+$ channels [227]. Recently, SCN3b has been reported to also be expressed in the heart with predominant expression in the Purkinje fibers and ventricles but little expression in the atria [225]. In addition, like SCNlb, co-expression of SCN3b increases current density and may affect inactivation gating [225].

## Voltage-Gated $Ca^{2+}$ Channels, (CaV) Pore-Forming α Subunits

The $\alpha_1$ subunit of voltage-gated $Ca^{2+}$ channels (VGCC) contains the channel pore and is the largest of the subunits [45, 47, 48, 228, 229]. Like $Na_V$ channels, voltage-gated $Ca^{2+}$ ($Ca_v$) channel pore-forming (α) subunits (Fig. 1.2b) belong to the "S4" superfamily of voltage-gated ion channel genes that also includes voltage-gated $Na^+$ (Fig. 1.2a) and $K^+$ (Fig. 1.2c) channel α subunits. Structurally, functional voltage-gated $Ca^{2+}$ ($Ca_V$) channels consist of a $Ca_V\alpha_1$ subunit, with a predicted mass of 212–273 kDa, and auxiliary, $Ca_V\beta$ and $Ca_V$ $\alpha_2\delta$ subunits (Fig. 1.3c). In most voltage-gated $Ca^{2+}$ channels, the $\alpha_1$ subunit determines the properties of the resulting channels.

Also, like $Na_V\alpha_1$, $Ca_V\alpha_1$ subunits are comprised of four homologous domains (I–IV), each of which is made up of six putative transmembrane segments (S1–S6), one of which is the "S4" voltage-sensing domain, and a region between S5 and S6, known as the selectivity filter, that contributes to the $Ca^{2+}$-selective pore (Fig. 1.2b) [4, 48, 229–231]. This schematic is supported by cryo-electron microscopy (cryo-EM) of a rabbit skeletal muscle voltage-gated calcium channel ($Ca_V1.1$) [232, 233]. Also supported by this cryo-EM is the assertion that, as also true in $Na_V$ channels, $Ca_V$ channel structurally adopts a "domain-swapped" conformation allowed by their long S4–S5 linker. In a domain-swapped conformation, the S5–S6 pore region is next to the S1–S4 region of the adjacent subunit [232, 233]. This is not, apparently, the case in channels with shorter S4–S5 linkers, such as hERG that will be discussed later [234, 235]. Similar to voltage-gated $Na_V\alpha_1$ subunits

**Table 1.3** Diversity of $Na^+$ and $Ca^{2+}$ channel accessory subunits

| Family | Gene | Locus | | | Subunit | Cardiac current |
|---|---|---|---|---|---|---|
| | | Homo | Mus | Rattus | | |
| $Na^+$ | | | | | | |
| β | | | | | | |
| | *SCN1B* | 19q13.11 | 7B1 | 1q21 | β1 | |
| | | | | | β1B | |
| | *SCN2B* | 11q23.3 | 9A5.2 | 8q22 | β2 | INa |
| | *SCN3B* | 11q24.1 | 9A5.1 | 8q22 | β3 | INa |
| | *SCN4B* | 11q23.3 | 9A5.2 | 8q22 | β4 | |
| $Ca^{2+}$ | | | | | | |
| β | | | | | | |
| | *CACNB1* | 17q12 | 11B | 10q31 | β1a | |
| | | | | | β1b | |
| | *CACNB2* | 10p12.33-p12.31 | 2A2 | 17q12.3 | β2 | $ICa_{(L)}$ |
| | *CACNB3* | 12q13.12 | 15F1 | 7q36 | β3 | |
| | *CACNB4* | 2q23.3 | 2C1.1 | 3q12 | β4 | |
| α2δ | | | | | | |
| | *CACNA2D1* | 7q21.11 | 5A2-A3 | 4q12 | α2δ-1 | $ICa_{(L)}$ |
| | *CACNA2D2* | 3p21.31 | 9F1 | 8q32 | α2δ-2 | $ICa_{(L)}$ |
| | *CACNA2D3* | 3p21.1-p14.3 | 14A3 | 16p16 | α2δ-3 | $ICa_{(L)}$ |
| | *CACNA2D4* | 12p13.33 | 6F1 | 4q42 | α2δ-4 | $ICa_{(L)}$ |
| γ | | | | | | |
| | *CACNG1* | 17q24.2 | 11E1 | 10q32.1 | γ1 | |
| | *CACNG2* | 22q12.3 | 15E1 | 7q34 | γ2 | |
| | *CACNG3* | 16p12.1 | 7F2 | 1q36 | γ3 | |
| | *CACNG4* | 17q24.2 | 11E1 | 10q32.1 | γ4 | $ICa_{(L)}$ |
| | *CACNG5* | 17q24.2 | 11E1 | 10q32.1 | γ5 | |
| | *CACNG6* | 19q13.42 | 7A1 | 1q12 | γ6 | $ICa_{(L)}$ |
| | *CACNG7* | 19q13.42 | 7A1 | 1q12 | γ7 | $ICa_{(L)}$ |
| | *CACNG8* | 19q13.42 | 7A1 | 1q12 | γ8 | |
| γ like | *Tmem37* | 2q14.2 | 1E2.3 | 13q11 | TmEM37 | |

Boxes denote cardiac expression

(Fig. 1.2a), the N- and C-termini of $Ca_V\alpha_1$ subunits are intracellular (Fig. 1.2b). The $Ca_V$ channel N- and C-termini contain binding sites for a variety of proteins, including calmodulin and other calcium-binding proteins [48, 236, 237].

Supported by experimental evidence and molecular dynamics simulations of other voltage-gated channels, it is theorized that activation of voltage-gated $Ca^{2+}$ channels caused by the cellular depolarization triggered movement of the positively charged "S4" segment in each of the four domains [232, 233, 238].

To date, three distinct subfamilies of $Ca_V$ channel pore-forming α1 subunits, Cav1, Cav2, Cav3, have been identified, and in each case, there are many subfamily members [46]

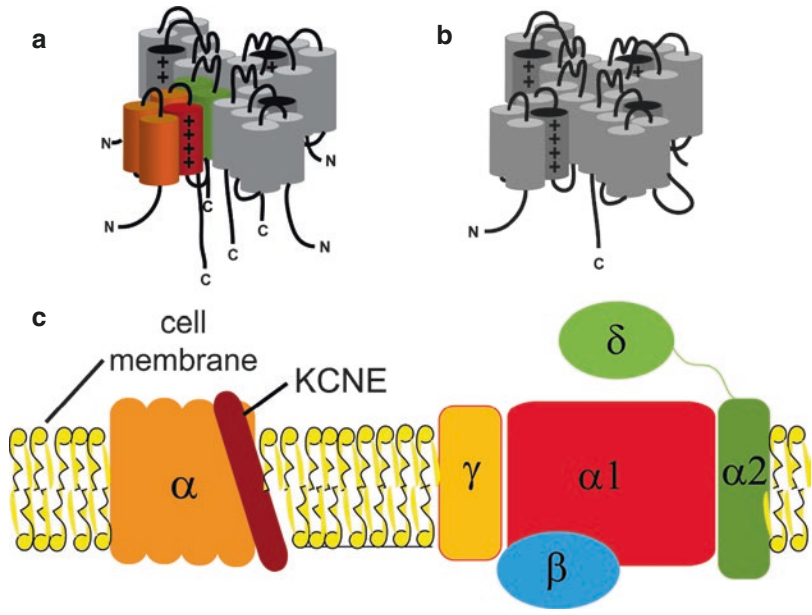


**Fig. 1.3** Molecular comparison of assembly of cardiac voltage-gated channels. $K_V$ channels assemble from four α subunits. (**a**) Each of these alpha subunits has an N-terminal region, a C-terminal region, and six transmembrane helices. The first four of these helices are the voltage-sensing domain (orange and red), where the fourth helix (red) contains charged residues for sensing voltage change. The last two helices (green) combine with their counterparts from the other three $K_V$ α subunits to form the pore. (**b**) In voltage-gated $Na^+$ and $Ca^{2+}$ channels, there is one α subunit that is comprised of four similar repeats of six transmembrane helices, that fold into a similar structure as the four $K_V$ channel α subunits, but is one continuous protein. (**c**) Voltage-gated channel complexes contain auxiliary subunits. (*left*) KCNQ1 contains a KCNE subunit in its functional complex. (*right*) An L-type voltage-gated $Ca^{2+}$ channel complex contains an α1 subunit and may contain a β, α2δ, and/or γ subunits

(Table 1.2). These numeric classifications represent the families of L-type, neuronal, and T-type $Ca_V$ channels, respectively. The L-type and neuronal families are known to be high-voltage-activated (HVA) $Ca^{2+}$ channels, and T-type are known to be low-voltage-activated (LVA) $Ca^{2+}$ channels. The various $Ca_V\alpha_1$ subunits are differentially expressed, and heterologous expression studies have revealed that these genes encode voltage-gated $Ca^{2+}$ channels with distinct time- and voltage-dependent properties and pharmacological sensitivities. As an example, functional expression of any one of the four members of the $Ca_V1$ subfamily, Cav1.1 ($\alpha_{1s}$), Cavl.2 ($\alpha_{1c}$), Cav1.3 ($\alpha_{1d}$), or Cav1.4 ($\alpha_{1f}$) (Table 1.2), reveals L-type $Ca^{2+}$ channel currents, which are long-lasting when $Ba^{2+}$ is the charge carrier. These channels activate at relative high voltages (~ −20 mV), and dihydropyridine (DHP) $Ca^{2+}$ channel antagonists block these channels.

In the human heart, at different periods of development, there expressed both L-type ($Ca_V1$) and T-type ($Ca_V3$) voltage-gated calcium channels [239, 240]. Studies in rodents have suggested T-type channels ($Ca_V3$) play a role in development of the embryonic heart but are expressed less prominently in the adult human heart [241–244]. T-type channels appear to play a role in pacemaking and cardiac remodeling in the adult human myocardium [240, 245–248].

Two types of L-type calcium channels have been observed in the human cardiomyocytes: $Ca_V1.2$ ($\alpha_{1c}$) and $Ca_V1.3$ ($\alpha_{1d}$). These are expressed from embryonic stages onward [239]. $Ca_V1.2$ expression is throughout the heart, especially the atria and ventricles [47, 239]. It is widely accepted that the $Ca_V1.2$ ($\alpha_{1c}$) is the most prevalent $Ca_V$ expressed in the mammalian myocardium and is the major $Ca_V$ involved in the $Ca^{2+}$-driven excitation-contraction coupling in

the heart [4, 231, 241, 249, 250]. Meanwhile, $Ca_V1.3$ is mainly found in the nonventricular regions: the pacemaking, sinoatrial node (SAN), the conductive Purkinje fibers and atrioventricular node (AVN), and the mechanical pumping atria [47, 239, 251, 252].

The human CACNA1C gene that encodes the $\alpha_{1c}$ subunit expansively stretches over 55 exons, 9 of which are subject to alternative splicing [253–256]. From these alternate exons, and many possible splice sites, many variations of $\alpha_{1c}$ result in many types of tissue-specific $Ca_V1.2$. Examples of these include $Ca_V1.2a$, $Ca_V1.2b$, and $Ca_V1.2c$ [4, 49, 253, 257, 258]. $Ca_V1.2$ is primarily represented by the smooth muscle isoform, $Ca_V1.2b$, and the cardiac-specific $Ca_V1.2a$ [256]. $Ca_V1.2a$ is encoded by 44 invariant and 6 alternative exons of the CACNA1C gene [257]. $Ca_V1.2a$ bears >95% amino acid sequence identity to the $\alpha_{1c}$'s of $Ca_V1.2b$ and $Ca_V1.2c$ despite being expressed in different tissues [49, 259].

## $Ca_V$ Channel Accessory Subunits

Though it is the type of $Ca_V\alpha_1$ present that the variety of the channel is determined, there are usually other subunits present in the $Ca_V$ channel complex. There are three major families of $Ca_V$ channel accessory subunits [47, 48]. One major $Ca_V$ auxiliary subunit is the $Ca_V\beta$ subunit [260, 261]. In the case of L-type calcium channels, such as $Ca_V1.2$ ($\alpha_{1c}$) and $Ca_V1.3$ ($\alpha_{1d}$), the $Ca_V\beta$ subunit is believed to be required for proper $Ca_V$ channel trafficking [262–264]. The $Ca_V\alpha_2\delta$ subunit has been shown to increase the maximal Ca2+ current and increase inactivation rates of $Ca_V$ channels [265]. A $Ca_V\gamma$ subunit may also be present in the $Ca_V$ channel complex and is the least studied of the $Ca_V$ accessory subunits with much of its role in the human myocardium still unknown [266].

Almost always found with the membrane-targeted α subunit is the β subunit [47, 48, 228, 262, 263, 267]. The β subunit is believed to be important in the trafficking and regulation of the α subunit to the membrane [262, 267]. Four different $Ca_V\beta$ subunit-encoding genes (and subunit coded for) have been identified: CACNB1 ($Ca_V\beta_1$), CACNB2 ($Ca_V\beta_2$), CACNB3 ($Ca_V\beta_3$), and CACNB4 ($Ca_V\beta_4$) [6, 260, 261, 268–270]. In these proteins amino acid sequence, there are three variable regions: the N-terminus, a small center HOOK region (~100 amino acids), and the C-terminus. Separating between the variable N-terminus and HOOK region is a highly conserved, hydrophobic SH3 domain. Between the HOOK region and the C-terminus is the highly conserved, guanylate kinase (GK) domain [271–275]. The variable regions are two conserved regions thought to mediate interaction with the $Ca_V\alpha_1$ subunit.

In heterologous expression systems, all four $Ca_V\beta$ subunits associate with $Ca_V\alpha_1$ subunits (Fig. 1.3c) and modify the time- and voltage-dependent properties, as well as the magnitude, of the expressed currents. It has been suggested that co-expression of $Ca_V\beta$ subunits with $Ca_V\alpha_1$ subunits increases the number of functional cell surface membrane channels, resulting in increases in $Ca^{2+}$ current amplitudes and densities [276–279]. Alternatively, the association of $Ca_V\beta$ subunits and the resulting increases in current amplitudes/densities could reflect increased expression of the $Ca_V\alpha_1$ subunit, an increase in the channel open probability, [277] and/or the stabilization of channel complex in the cell membrane [264, 277, 280, 281]. In addition to increasing current amplitudes, co-expression of $Ca_V\beta$ subunits modifies the kinetics and the voltage dependences of current activation and inactivation [282–285].

Detailed analysis of different $Ca_V\alpha_1$ subunits has revealed that a highly conserved sequence motif, called the alpha subunit interaction domain (or AID), mediates the interaction(s) with $Ca_V\beta$ subunits [275]. The AID sequence, QqxExxLxGYxxWlxxxE, is located at 24 amino acids from the S6 transmembrane region of domain I (Fig. 1.2b) of the $Ca_V\alpha_1$ subunit [154, 173–175, 259, 286–288]. Regions outside of AID, including low-affinity binding sites in the C-termini of the $Ca_V\alpha_1$ subunits, have also been suggested to participate in the $Ca_V\beta$-$Ca_V\alpha_1$ subunit-subunit interactions [289–291]. Nevertheless, it appears that these C-terminal regions in $Ca_V\alpha_1$also interact specifically with

the second (internal) highly conserved domain of the $Ca_V\beta$ subunits to induce the observed modulatory effects [292, 293].

Another subunit that also may be present in the VGCC complex is the $Ca_V\alpha_2\delta$ subunit [48, 228, 262, 267, 294]. This disulfide-linked, transmembrane accessory subunit, was first cloned from the skeletal muscle [295], and, to date, five different isoforms of $Ca_V\alpha_2\delta$-1 have been identified (Table 1.3). In addition, two homologous $Ca_V\alpha_2\delta$ genes, $Ca_V\alpha_2\delta$-2 and $Ca_V\alpha_2\delta$-3, have been identified in the brain [296]. $Ca_V\alpha_2\delta$-4 is found in retinal neurons and some non-neuronal endocrine cells [267, 297, 298]. Sequence comparison revealed ~55% amino acid identity between $Ca_V\alpha_2\delta$-1 and $Ca_V\alpha_2\delta$-2 and ~30% identity between $Ca_V\alpha_2\delta$-1 and $Ca_V\alpha_2\delta$-3 [296]. The $Ca_V\alpha_2\delta$ subunits are heavily glycosylated proteins that are cleaved posttranslationally to yield disulfide-linked $\alpha_2$ and δ proteins (Fig. 1.3c). The $\alpha_2$ domain is located extracellularly, whereas the δ domain has a large hydrophobic region, which inserts into the membrane and serves as an anchor to secure the $Ca_V\alpha_2\delta$ complex [299–301].

In contrast to the accessory $Ca_V\beta$ subunits, the functional roles of $Ca_V\alpha_2\delta$ are somewhat variable and depend, at least in part, on the identities of the co-expressed $Ca_V\alpha_1$ and $Ca_V\beta$ subunits and the expression environment. In general, co-expression of $Ca_V\alpha_2\delta$-1 shifts the voltage dependence of channel activation, accelerates the rates of current activation and inactivation, and increases current amplitudes, compared with the currents produced on expression of $Ca_V\alpha_1$ and $Ca_V\beta$ subunits alone [296, 300–304]. The increase in current density reflects improved targeting of $Ca_V\alpha_1$ subunits to the membrane [305]. This effect is attributed to $\alpha_2$, whereas the changes in channel kinetics reflect the expression of the δ protein [305].

Sometimes a $Ca_V$ channel complex will also contain a $Ca_V\gamma$ subunit, of which there are eight human genes that code for these subunits. These subunits are named $Ca_V\gamma_1$ through $Ca_V\gamma_8$ and contain four transmembrane helices [306–309]. These eight proteins, believed to be members of the claudin superfamily of proteins [306, 309], have been grouped into two clusters: neuronal and skeletal based on sequence similarity and tissue distribution [306]. The neuronal $Ca_V\gamma$ subunits contain $Ca_V\gamma_{2-5}$ and $Ca_V\gamma_{7-8}$ PDZ binding domain for binding AMPA receptors, and, thus, these $Ca_V\gamma$ subunits are classified as TARPs (transmembrane AMPA receptor regulatory proteins) [306, 310]. The skeletal $Ca_V\gamma$s are $Ca_V\gamma_1$ and $Ca_V\gamma_6$ and are expressed in the skeletal muscle among other tissues. $Ca_V\gamma_4$, $Ca_V\gamma_6$, $Ca_V\gamma_7$, and $Ca_V\gamma_8$ are expressed in the human heart and associated with the cardiac $Ca_V\alpha_{1C}$ subunit [311].

## Molecular Basis of Voltage-Gated $K^+$ Current Diversity in Mammalian Ventricle

### Voltage-Gated $K^+$ (Kv) Channel Pore-Forming α Subunits

Voltage-gated $K^+$ channel ($K_V$) pore-forming (α) subunits are six transmembrane-spanning domain proteins (Fig. 1.2c) with a region between the fifth and sixth transmembrane domains that contributes to the $K^+$-selective pore [11]. The positively charged fourth transmembrane domain in the $K_V$ a-subunits (Figs. 1.2c and 1.3a) is homologous to the corresponding region in voltage-gated $Na^+$ (Figs. 1.2a and 1.3b) and $Ca^{2+}$ (Figs. 1.2b and 1.3b) channel a-subunits, placing them in the "S4" superfamily of voltage-gated channels [312]. In contrast to voltage-gated $Na^+$ and $Ca^{2+}$ channels, however, functional voltage-gated $K^+$ channels comprise four a-subunits (Fig. 1.3a, b). To an even greater degree than $Na^+$ and $Ca^{2+}$ α subunits (Table 1.2), there is a great diversity of $K_V\alpha$ subunits (Table 1.4). Twelve homologous $K_V\alpha$ subunit subfamilies, $K_V$l.x, $K_V$2.x, $K_V$3.x, and $K_V$4.x, for example, have been identified, and many of these are expressed in mammalian ventricles (Table 1.4). In addition to the multiplicity of $K_V\alpha$ subunits (Table 1.4), further functional $K^+$ channel diversity can arise through alternative splicing of transcripts, as well as through the formation of heteromultimeric channels [313] between two or more $K_V$ a-subunit proteins in the same Kv subfamily [11, 314].

**Table 1.4** Diversity of $K^+$ channel α subunits

| Subfamily | Protein | Gene | Locus | | | Cardiac current |
|---|---|---|---|---|---|---|
| | | | Homo | Mus | Rattus | |
| Kv1 | | | | | | |
| | Kv1.1 | *KCNA1* | 12p13.32 | 6F3 | 4q42 | ?? |
| | Kv1.2 | *KCNA2* | 1p13.3 | 3F2.3 | 2q34 | $I_{k,slow(rat)}$ ($I_{k,DTX}$) |
| | Kv1.3 | *KCNA3* | 1p13.3 | 3F2.3 | 2q34 | |
| | Kv1.4 | *KCNA4* | 11p14.1 | 2E3 | 3q33 | $I_{to,s}$ |
| | Kv1.5 | *KCNA5* | 12p13.32 | 6F3 | 4q42 | $I_{Kur(human,rat)}$, $I_{k,slow(mousr)}$ |
| | Kv1.6 | *KCNA6* | 12p13.32 | 6F3 | 4q42 | |
| | Kv1.7 | *KCNA7* | 19p13.33 | 7B3 | 1q22 | ?? |
| | Kv1.8 | *KCNA10* | 1p13.3 | 3F2 | 2q34 | ?? |
| Kv2 | | | | | | |
| | Kv2.1 | *KCNB1* | 20q13.13 | 2H3 | 3q42 | $I_{k,slow(mousr)}$ |
| | Kv2.2 | *KCNB2* | 8q21.11 | 1A3 | 5q11 | ?? |
| Kv3 | | | | | | |
| | Kv3.1 | *KCNC1* | 11p15.1 | 7B3 | 1q22 | $I_{Kur(canine)}$ |
| | Kv3.2 | *KCNC2* | 12q21.1 | 10D2 | 7q22 | |
| | Kv3.3 | *KCNC3* | 19q13.33 | 7B3 | 1q22 | |
| | Kv3.4 | *KCNC4* | 1p13.3 | 3F2.3 | 2q34 | |
| Kv4 | | | | | | |
| | Kv4.1 | *KCND1* | Xp11.23 | XA1.1 | Xq12 | ?? |
| | Kv4.2 | *KCND2* | 7q31.31 | 6A2-A3.1 | 4q22 | $I_{to,f}$ |
| | Kv4.3 | *KCND3* | 1p13.2 | 3F2.2 | 2q34 | $I_{to,f}$ |
| Kv5 | | | | | | |
| | Kv5.1 | *KCNF1* | 2q25.1 | 12A1.1 | 6q16 | ?? |
| Kv6 | | | | | | |
| | Kv6.1 | *KCNG1* | 2q13.13 | 2H3 | 3q42 | ?? |
| | Kv6.2 | *KCNG2* | 18q23 | 18E3 | 18q12.3 | |
| | Kv6.3 | *KCNG3* | 2p21 | 17E4 | 6q12 | |
| | Kv6.4 | *KCNG4* | 16q24.1 | 8E1 | 19q12 | |
| Kv7 | | | | | | |
| | Kv7.1 | *KCNQ1* | 11p15.5-p15.4 | 7F5 | 1q42 | $I_{Ks}$ |
| | Kv7.2 | *KCNQ2* | 20q13.33 | 2H4 | 3q43 | ?? |
| | Kv7.3 | *KCNQ3* | 8q24.22 | 15D1 | 7q34 | |
| | Kv7.4 | *KCNQ4* | 1q34.2 | 4D2.2 | 5q36 | |
| | Kv7.5 | *KCNQ5* | 6q13 | 1A4 | 9q13 | |
| Kv8 | | | | | | |
| | Kv8.1 | *KCNV1* | 8q23.22 | 15B3.3 | 7q31 | |
| | Kv8.2 | *KCNV2* | 9q24.2 | 19C1 | 1q52 | |
| Kv9 | | | | | | |

**Table 1.4** (continued)

| Subfamily | Protein | Gene | Locus | | | Cardiac current |
|---|---|---|---|---|---|---|
| | | | Homo | Mus | Rattus | |
| | Kv9.1 | *KCNS1* | 20q13.12 | 2H3 | 3q42 | |
| | Kv9.2 | *KCNS2* | 8q22.2 | 15B3.1 | 7q22 | |
| | Kv9.3 | *KCNS3* | 2q24.2 | 12A1.1 | 6q15 | ?? |
| Kv10 | | | | | | |
| | Kv10.1 | *KCNH1* | 1q32.2 | 1H6 | 13q27 | |
| | Kv10.2 | *KCNH5* | 14q23.2 | 12C3 | 6q24 | |
| Kv11 | | | | | | |
| | Kv11.1 | *KCNH2* | 7q36.1 | 5A3 | 4q11 | $I_{Kr}$ |
| | Kv11.2 | *KCNH6* | 17q23.3 | 11E1 | 10q32.1 | |
| | Kv11.3 | *KCNH7* | 2q24.2 | 2C1.3 | 3q21 | |
| Kv12 | | | | | | |
| | Kv12.1 | *KCNH8* | 3p24.3 | 17C | 9q11 | |
| | Kv12.2 | *KCNH3* | 12q13.12 | 15F1 | 7q36 | |
| | Kv12.3 | *KCNH4* | 17q21.2 | 11D | 10q31 | |

Boxes denote cardiac expression

In contrast to the $K_V1.x$ – $K_V4.x$ α subunit subfamilies, heterologous expression of $K_V5.x$ – Kv9.x (Table 1.4) α subunits alone does not reveal functional voltage-gated $K^+$ channels, with the exception of $K_V7.1$, which is also known as KCNQ1 [314–318]. Interestingly, however, co-expression of any of these ($K_V5.1$, $K_V6.1$, $K_V8.1$, or $K_V9.1$) subunits with Shab ($K_V2.x$) subfamily members attenuates the amplitudes of the Shab ($K_V2.x$) modulated currents [318]. These observations have been interpreted as suggesting that the $K_V5.x$ – $K_V9.x$ subunits are regulatory $K_V$ α subunits of the $K_V2.x$ subfamily [317], although the roles of these "silent" subunits in the generation of functional voltage-gated $K^+$ channels in cardiac cells remain to be determined.

The second subfamily of voltage-gated $K^+$ channel a-subunit gene was revealed with the cloning of the *Drosophila* ether-a-go-go (eag) locus [319]. Homology screening led to the identification of human eag, as well as another subfamily, the human eag-related gene, referred to as hERG [314, 320], which was subsequently identified as the locus of mutations leading to one form of familial long QT syndrome, LQT2 [321]. Expression of hERG (human ERGl) in Xenopus oocytes revealed inwardly rectifying voltage-gated, $K^+$-selective currents [322, 323] with properties similar to cardiac $I_{Kr}$ (Table 1.4). Related ERG genes, ERG2 and ERG3, have also been identified (Table 1.4), although these appear to be nervous system specific and are not expressed in the mammalian heart [324]. Alternatively processed forms of ERG1, however, have been cloned from mouse and human heart cDNA libraries and postulated to contribute to cardiac IKr [325–327].

Another subfamily of voltage-gated $K^+$ channel a-subunits was revealed with the cloning of KvLQTI [328], the loci of mutations in another inherited form of long QT syndrome (LQTI) (Table 1.4). Although expression of KvLQT1 (KCNQI) alone reveals rapidly activating and noninactivating $K^+$ currents, co-expression with the subunit KCNE1 produces slowly activating $K^+$ currents that account for the slow component of cardiac delayed rectification, IKs [329, 330]. Additional KCNQ subfamily members, KCNQ2 and KCNQ3, although not expressed in heart, have been identified [331–333]. Interestingly, however, KCNQ2 and KCNQ3 have been identified as loci of mutations leading to benign familial neonatal convulsions [331, 333]. Heterologous expression of KCNQ2 or KCNQ3 produces slowly activating, noninactivating $K^+$-selective currents that deactivate very slowly on membrane repolarization [333, 334]. The unique kinetic and pharmacological properties of the expressed

currents suggest that functional neuronal M channels reflect the heteromeric assembly KCNQ2 and KCNQ3 [334].

## Voltage-Gated K (Kv) Channel Accessory Subunits

A number of voltage-gated $K^+$ ($K_V$) channel accessory subunits have now been identified (Table 1.5). The first of these, KCNE1, encodes a small (130 amino acids) protein with a single membrane-spanning domain [335–337]. As expected for an accessory $K_V$ channel subunit, KCNE1 does not produce functional voltage-gated $K^+$ channels when expressed alone in heterologous systems [330]. Rather, it coassembles with KCNQ1 (Fig. 1.3c) to form functional $I_{Ks}$ channels [329, 330]. KCNE1 homologues, MiRP1 (KCNE2), MiRP2 (KCNE3), MiRP3

**Table 1.5** Diversity of $K^+$ channel accessory subunits

| Family | Gene | Locus | | | Subunit | Associates with | Cardiac current |
|---|---|---|---|---|---|---|---|
| | | Homo | Mus | Rattus | | | |
| Kvβ | | | | | | | |
| | *KCNAB1* | 3q25.31 | 3E1 | 2q31 | Kvβ1.1 | Kv1.1-1.6, Kv4.3 | $I_{to,f}$??$I_{Kur}$?? |
| | | | | | Kvβ1.2 | Kv1.2, Kv1.5 | |
| | | | | | Kvβ1.3 | Kv1.5 | |
| | *KCNAB2* | 1p36.31 | 4E2 | 5q36 | Kvβ2.1 | Kv1.1-1.6, Kv4.3 | $I_{to,f}$??$I_{Kur}$?? |
| | *KCNAB3* | 17p13.1 | 11B3 | 10q24 | Kvβ3.1 | Kv1.1-1.6 | ?? |
| KCNE | | | | | | | |
| | *KCNE1* | 21q22.12 | 16C4 | 11q11 | Mink | KCNQ1 | $I_{Ks}$ |
| | *KCNE2* | 21q22.11 | 16C4 | 11q11 | MiRP1 | KCNQ1,HERG | $I_{fKs}$,$I_{Kr}$ |
| | *KCNE3* | 11q13.4 | 7E2 | 1q32 | MiRP2 | HERG/Kv4,KCNQ1 | $I_{Kr}$/$I_{to,f}$,$I_{Ks}$ |
| | *KCNE4* | 2q36.1 | 1C4 | 9q34 | MiRP3 | | |
| | *KCNE5* | Xq23 | | Xq33 | MiRP4 | KCNQ1,Kv4 | $I_{Ks}$,$I_{to,f}$ |
| KChIP | | | | | | | |
| | *KCNIP1* | 5q35.1 | 11A4 | 10q12 | KchIP1 | Kv4.1-4.3 | |
| | *KCNIP2* | 10q24.32 | 19C3 | 1q54 | KchIP2 | Kv4.1-4.3 | $I_{to,f}$ |
| | *KCNIP3* | 2q11.1 | 2F1 | 3q36 | KchIP3 | Kv4.2 | |
| | *KCNIP4* | 4p15.31-p15.2 | 5B3 | 14q11 | KchIP4 | Kv4.2, Kv4.3 | |
| KChAP[a] | | | | | | | |
| | *PIAS3* | 1q21.1 | 3F2 | 2q34 | KChAP | | $I_{to,f}$??,$I_K$?? |
| NCS | | | | | | | |
| | *NCS1* | 9q34.11 | 2B | 3p12 | NCS_1 | | $I_{to,f}$ |
| DPPLs | | | | | | | |
| | *DPP6* | 7q36.2 | 5B1 | 4q11 | DPP6 | Kv4.2, Kv4.3 | $I_{to,f}$ |
| | *DPP10* | 2q14.1 | 1E2 | 13q11-q12 | DPP10 | Kv4.2, Kv4.3 | $I_{to,f}$ |

Boxes denote cardiac expression

[a]KChAP only found interaction with Kv channels in rat

(KCNE4), and MiRP4 (KCNE5) have also been identified (Table 1.5), and it has been suggested that MiRP1 functions as an accessory subunit of ERG1 in the generation of (cardiac) $I_{Kr}$ [338–340]. Although it is unclear whether KCNE1, MiRP1, or other KCNE subfamily members [338] contribute to the formation of voltage-gated $K^+$ channels in addition to $I_{Ks}$ and $I_{Kr}$ in the myocardium, it has been reported that MiRP2 assembles with $K_V3.4$ in mammalian skeletal muscle [341] and with $K_V4.x$ a-subunits in heterologous expression systems [342]. These observations suggest the interesting possibility that members of the KCNE subfamily of accessory subunits can assemble with multiple $K_V$ a-subunits and contribute to the formation of multiple types of voltage-gated myocardial $K^+$ channels. Direct experimental support for this hypothesis, however, has not been provided to date, and the roles of the KCNE family of accessory subunits in the generation of functional ventricular $K^+$ channels need to be defined.

Another type of $K_V$ accessory subunit was revealed with the identification of low molecular weight (~ 45 kD) cytosolic β subunits in the brain [343, 344]. Three homologous $K_V$ β subunits, $K_V\beta1$, $K_V\beta2$, and $K_V\beta3$ (Table 1.5), as well as alternatively spliced transcripts, have been identified [345–350], and both $K_V\beta1$ and $K_V\beta2$ are expressed in the heart [350]. The $K_V$ β subunits interact with the intracellular domains of the $K_V$ α subunits of the $K_V1$ subfamily in assembled voltage-gated $K^+$ channels, and heterologous expression studies suggest that $K_V$ β subunit co-expression affects the functional properties and the cell surface expression of $K_V$ α subunit-encoded $K^+$ currents [345–349, 351–353]. Because $K_V$ α and β subunits coassemble in the endoplasmic reticulum [354], the increase in functional channel expression suggests that the $K_V$ β subunits affect channel assembly, processing, or stability or that they function as chaperon proteins.

Heterologous co-expression studies suggest that the effects of the $K_V$ β subunits are subfamily specific, i.e., $K_V\beta1$, $K_V\beta2$, and $K_V\beta3$ interact only with the $K_V1$ subfamily of α subunits [355, 356], whereas $K_V\beta4$ is specific for the $K_V2$ subfamily [357]. Nevertheless, it is not known which $K_V$ α subunit(s), the $K_V\beta1$and $K_V\beta2$ subunits, associate within the myocardium, and the roles of these ($K_V$ β) subunits in the generation of functional cardiac $K^+$ channels remain to be determined.

Using a yeast two-hybrid screen, Wible and colleagues (1998) identified a novel voltage-gated $K^+$ channel regulatory protein, KChAP ($K^+$ channel accessory protein) (Table 1.5) [358]. Sequence analysis of ChAP revealed a 574 amino acid protein with no transmembrane domains and no homology to $K_V$ α or $K_V$ β subunits [358]. Co-expression of KChAP with $K_V2.1$ (or $K_V2.2$) in Xenopus oocytes, however, markedly increases functional $K_V2.x$-induced current densities without measurably affecting the time- and/or the voltage-dependent properties of the currents [358] suggesting that KChAP functions as a chaperon protein. Yeast two-hybrid assays also revealed that KChAP interacts with the N-termini of $K_V1.x$ α subunits and with the C-termini of $K_V\beta1.x$ subunits [358].

Using the intracellular N-terminus (amino acids 1–180) of $K_V4.2$ as the "bait" in a yeast two-hybrid screen, An and colleagues (2000) identified three novel K v channel interacting proteins, KChIP1, KChIP2, and KChIP3 (Table 1.5) [359]. Of these, only KChIP2 appears to be in the heart [359, 360], although there are several splice variants of KChIP2 expressed [360–362]. Sequence analysis revealed that the KChIPs belong to the recoverin family of neuronal $Ca^{2+}$-sensing (NCS) proteins, particularly in the "core" regions, which contain multiple EF-hand domains [363]. Unlike other NCS-1 proteins, however, KChIP2 and KChIP3 lack N-terminal myristoylation sites, and the N-termini of the KChIP proteins are unique [359]. Interestingly, KChIP3 is identical to the NCS protein calsenilin, a $Ca^{2+}$-binding protein that interacts with the presenilin proteins and regulates proteolytic processing [364]. In addition, the nucleotide sequence of KChIP3 is 99% identical to the $Ca^{2+}$-regulated transcriptional repressor, DREAM [365]. The expression of several genes has been shown to be regulated by downstream regulatory elements (DRE), and,

importantly, the DREAM protein binds to DRE elements in the absence of $Ca^{2+}$ and dissociates when $Ca^{2+}$ is elevated [365]. Thus, DREAM is thought to act as an activity-dependent regulator of gene expression [365]. It should be noted, however, that DREAM is predicted to have an alternative start codon resulting in a 20 amino acid N-terminal extension not present in KChIP3 [359, 365]. Nevertheless, these findings suggest the interesting possibility that the KChIP proteins may be expressed in different cellular compartments and may subserve multiple cellular functions.

When expressed in CHO cells with $K_V$4.2, the KChIPs increase the functional cell surface expression of $K_V$4.2-encoded $K^+$ currents, slow current inactivation, speed recovery from inactivation, and shift the voltage-dependence of activation [359]. Similar effects are observed when $K_V$4.2 or $K_V$4.3 is expressed with the KChIPs in Xenopus oocytes [359] or HEK-293 cells [366, 367]. In contrast, KChIP expression does not affect the properties or the densities of $K_V$1.4- or $K_V$2.1-encoded $K^+$ currents, suggesting that the modulatory effects of the KChIP proteins are specific for *ex* subunits of the K v4 subfamily [359]. In addition, although the binding of the KChIP proteins to $K_V$4 α subunits is not $Ca^{2+}$-dependent, mutations in EF-hand domains I, III, and IV eliminate the modulatory effects of KChIPl on $K_V$4.2-induced $K^+$ currents in CHO cells [359]. It has recently been shown that KChIP2 co-immunoprecipitates with $K_V$4.2 and $K_V$4.3 α subunits from adult mouse ventricles, consistent with a role for this subunit in the generation of $K_V$4-encoded mouse ventricular $I_{to,f}$ channels [366]. Interestingly, a gradient in KChIP2 message expression is observed through the thickness of the ventricular wall in canine and human heart, suggesting that KChIP2 underlies the observed differences in $I_{to,f}$ densities in the epicardium and endocardium in human and canine ventricles [360]. In rat and mouse, however, there is no gradient in KChIP2 expression [360, 366], and it appears that differences in $K_V$4.2 underlie the regional variations in $I_{to,f}$ densities in rodents [83, 366].

## Relation Between $K_V$ Subunits and Ventricular Transient Outward $K^+$ Channels

Considerable experimental evidence has accumulated documenting a role for $K_V$ α subunits of the $K_V$4 subfamily in the generation of ventricular $I_{to,f}$ channels. In rat ventricular myocytes exposed to antisense oligodeoxynucleotides (AsODNs) targeted against $K_V$4.2 or $K_V$4.3, for example, $I_{to,f}$ density is reduced by – 50% [368]. Similar results have recently been obtained in studies on mouse ventricular $I_{to,f}$ [366]. Reductions in rat ventricular $I_{to,f}$ density are also seen in cells exposed to adenoviral constructs encoding a truncated $K_V$4.2 subunit ($K_V$4.2ST) that functions as a dominant negative [369]. In ventricular myocytes isolated from transgenic mice expressing a dominant negative pore mutant of $K_V$4.2 ($K_V$4.2W362F) in the myocardium, $I_{to,f}$ is eliminated [370]. Taken together, these results demonstrate that members of the $K_V$4 subfamily underlie $I_{to,f}$ in mouse and rat ventricles. In addition, biochemical and electrophysiological studies suggest that $K_V$4.2 and $K_V$4.3 are associated in adult mouse ventricles and that functional mouse ventricular $I_{to,f}$ are heteromeric [366]. Given the similarities in the properties of $I_{to,f}$ (Table 1.1), it seems reasonable to suggest that $K_V$4 α subunits also underlie $I_{to,f}$ in other species. In dog and human, however, the candidate subunit is $K_V$4.3 because $K_V$4.2 appears not to be expressed [79]. Two splice variants of $K_V$4.3 have been identified in human [82] and rat [371, 372] heart. Although the longer version of $K_V$4.3, which contains a 19 amino acid insert in the carboxy tail, is the more abundant message [82, 371, 372], the expression levels of the two $K_V$4.3 proteins, as well as the roles of these subunits in the generation of functional cardiac $I_{to,f}$ channels, have been determined.

The kinetic and pharmacological properties of the slow transient outward $K^+$ currents, $I_{to,s}$, in ventricular myocytes are distinct from $I_{to,f}$ (Table 1.1), suggesting the molecular correlates of ventricular $I_{to,s}$ and $I_{to,f}$ channels. Direct experimental support for this hypothesis has been

provided in studies completed on ventricular myocytes isolated from (C57BL6) mice with a targeted deletion in the $K_V1.4$ gene, $K_V1.4-/-$ animals [373]. The waveforms of the outward $K^+$ currents in cells isolated from the RV or from the LV apex of Kvl.4−/− animals are indistinguishable from those recorded in wild-type (right ventricle and left ventricular apex) cells which lack $I_{to,s}$ [61]. In cells isolated from the septum of Kv 1.4−/− animals, $I_{to,s}$ is undetectable, demonstrating that $K_V1.4$ underlies $I_{to,s}$ [61].

Interestingly, $I_{to.s}$ (and $K_V1.4$ protein) is upregulated in left ventricular apex and in right ventricular cells in the $K_V4.2W362F$-expressing transgenics [62], suggesting that electrical remodeling occurs when $I_{to,f}$ is eliminated. When the dominant negative $K_V4.2W362F$ transgene is expressed in the $K_Vl.4-/-$ null background, both $I_{to,f}$ and $I_{to.s}$ are eliminated, although no further electrical remodeling is evident [62]. Indeed, electrophysiological recordings from $K_V4.2W362F$-expressing $K_V1.4-/-$ cells revealed that the waveforms of the outward $K^+$ currents in all right and left ventricular cells are indistinguishable [62]. Given the similarities in the time- and voltage-dependent properties of the slow transient outward $K^+$ currents in other species [59, 78], it seems reasonable to suggest that $K_V1.4$ also encodes $I_{to,s}$ in ferret, rabbit, and human left ventricular myocytes. Direct experimental support for this hypothesis, however, has not been provided to date.

### Relation Between $K_V$ Subunits and Ventricular Delayed Rectifier $K^+$ Channels

Human ERG1 was identified as the locus of one form of long QT syndrome, LQT2 [321], and heterologous expression of ERG1 in Xenopus oocytes revealed voltage-gated, inwardly rectifying $K^+$-selective channels that are similar to cardiac $I_{Kr}$ [322, 323]. Although these observations suggest that ERG1 encodes functional cardiac $I_{Kr}$ channels [322], AsODNS targeted against KCNE1 attenuate $I_{Kr}$ in AT-1 (an atrial tumor line) cells [374] and heterologously expressed ERG1 and KCNE1 co-immunoprecipitate [375]. These observations might be interpreted as suggesting that $I_{Kr}$ channels are multimeric, comprising of the protein products of KCNH2 and KCNEl [374, 375].

Alternatively processed forms of HERG 1 and MERG1 with unique N- and C-termini have also been identified in mouse and human heart [325–327] and suggested to be important in the generation of functional IKr channels [325, 326]. Western blot analysis of ERG1 protein expression in the myocardium, however, revealed that only the full-length ERG1 proteins are detected in rat, mouse, and human ventricles, suggesting that alternatively spliced (ERG1) variants do not play a role in the generation of functional $I_{Kr}$ channels [107].

Although heterologous expression of KCNQ1, the locus of mutations leading to LQT1 [328], reveals rapidly activating, noninactivating voltage-gated $K^+$ currents, co-expression with KCNE1 produces slowly activating $K^+$ currents similar to $I_{Ks}$ [329, 330]. These observations, together with biochemical data demonstrating that heterologously expressed KCNQ1 and KCNE1 associate [329], have provided evidence KCNE1 coassembles with KCNQ1 to form functional cardiac $I_{Ks}$ channels [329, 330]. In addition, the finding that mutations in the transmembrane domain of KCNE1 alter the properties of the resulting $I_{Ks}$ channels suggests that the transmembrane segment of KCNE1 modifies to the $I_{Ks}$ channel pore movement [376–379]. Also of interest, N-terminal truncated variants of KCNQ1 exert a dominant negative effect when co-expressed with full-length KCNQ1 [380].

## Molecular Correlates of Other Ventricular $K^+$ Currents

### Inwardly Rectifying Cardiac $K^+$ (Kir) Channel Pore-Forming α Subunits

Similar to the Kv channels, functionally distinct types of myocardial inwardly rectifying $K^+$ channels [381] are formed by the association of diverse

**Table 1.6** Inward rectifier α subunits

| | | | | Location | | |
|---|---|---|---|---|---|---|
| Family | Subfamily | Protein | Gene | Human | Mouse | Cardiac current |
| Kir | | | | | | |
| | Kir1 | | | | | |
| | | Kir1.1 | KCNJ1 | 11q24.3 | 9 A4 | ?? |
| | Kir2 | | | | | |
| | | Kir2.1 | KCNJ2 | 17q24.3 | 11 E2 | IK1 |
| | | Kir2.2 | KCNJ12 | 17q11.2 | 11 B2 | IK1 |
| | | Kir2.3 | KCNJ4 | 22q13.1 | 15 E1 | IK1 |
| | | Kir2.4 | KCNJ14 | 9q13.33 | 7B3 | |
| | Kir3 | | | | | |
| | | Kir3.1 | KCNJ3 | 2q24.1 | 2 | IKACh |
| | | Kir3.2 | KCNJ6 | 21q22.13 | 16C4 | |
| | | Kir3.3 | KCNJ9 | 1q23.2 | 1H3 | |
| | | Kir3.4 | KCNJ5 | 11q24.3 | 9A4 | IKACh |
| | Kir4 | | | | | |
| | | Kir4.1 | KCNJ10 | 1q23.2 | 1H3 | |
| | | Kir4.2 | KCNJ15 | 21q22 | 16C4 | |
| | Kir5 | | | | | |
| | | Kir5.1 | KCNJ16 | 17q24.3 | 11 E2 | |
| | Kir6 | | | | | |
| | | Kir6.1 | KCNJ8 | 12p12.1 | 6G2 | |
| | | Kir6.2 | KCNJ11 | 11p15.1 | 7B3 | IKATP |
| | Kir7 | | | | | |
| | | Kir7.1 | KCNJ13 | 2q37.1 | 1C5 | |

Boxes denote cardiac expression

inward rectifier $K^+$ (Kir) channel pore-forming α subunit genes (Fig 1.2d) [382]. Several Kir subunit subfamilies, Kir1 through Kir6, most with several members, have been identified (Table 1.6) and, like Kv α subunits, Kir α subunits also assemble as tetramers to form functional $K^+$-selective channels. Also similar to Kv channel α subunits, a unifying terminology has been developed for naming the Kir α subunit proteins (Kir1.x – Kir7.x) and the genes (KCNJ1–KCNJ16) encoding these proteins [383, 384]. Potassium channels can be classified into three groups depending on their topological structures; two, four, or six transmembrane domains [385]. Kir α subunit are composed of one pore (P) domain and two transmembrane domains (M1–M2).

Based on the properties of heterologously expressed Kir subunits, it was suggested that α subunits of the Kir2 subfamily likely encode the strongly inwardly rectifying Kir channels, $I_{K1}$, in cardiac cells [381, 382]. Three members of the Kir2 subfamily Kir2.1, Kir2.2, and Kir2.3 (Table 1.6) are expressed in the myocardium [386, 387]. Kir 2.4 are predominantly expressed in motoneurons of cranial nerve motor nuclei within the general somatic and special visceral motor cell column [388]. Interestingly, the KCNJ2 gene, which encodes Kir2.1, has been identified as the locus of mutations in Andersen's syndrome [389, 390], an inherited disorder that is often life-threatening owing to QT prolongation and cardiac (ventricular) arrhythmias, also called

long QT syndrome type 7 (LQT7). Similar to Timothy's syndrome [391], however, Andersen's syndrome is actually a multisystem disorder involving the cardiovascular, skeletomuscular, and other systems, and typically, Andersen's syndrome patients present initially with developmental abnormalities [392]. The mutations in KCNJ2 that are associated with Andersen's syndrome that have been described appear to result in mutant Kir2.1 protein that functions in a dominant negative fashion to suppress Kv2.x-encoded $I_{K1}$ currents [393–395]. Individuals carrying Andersen's syndrome mutations in KCNJ2 can display QT prolongation (long QT7), periodic paralysis, as well as craniofacial malformations [392, 393, 396, 397], alone or in combination. Because only the KCNJ2 gene appears to be affected in Andersen's syndrome, the multisystem nature of this disorder likely reflects the fact that Kir2.x-encoded channels are expressed and are functional in a variety of cells/tissues. Myocardial $I_{K1}$ (and other $K^+$ channels) have been shown to be regulated directly by phosphatidylinositol bisphosphate (PIP2) [398–401]. More than 33 mutations in KCNJ2 have been linked to Andersen's syndrome [402]. Interestingly, many of Andersen's mutations are in the PIP2-binding region of Kir2.1 [403], suggesting that the regulation/modulation of $I_{K1}$ channels by PIP2 is altered and that it is the alterations in the modulatory effects of PIP2 that underlie the phenotypic consequences of Andersen's syndrome mutations. It was reported that Kir2.1 gain-of-function mutations (V93I and D172N) are also associated with increased $I_{K1}$, short QT syndrome type 3, and familial atrial fibrillation [404–408]. The mutant of Kir2.1 channels showed larger outward currents than wild type. In addition to the short QT interval, absence of inward rectification in mutant can lead to familiar atrial fibrillation [408].

The first direct molecular evidence that Kir2 α subunits encode cardiac $I_{K1}$ channels was provided in studies completed on myocytes isolated from mice bearing a targeted disruption of the coding region of Kir2.1 (Kir2.1−/−) or Kir 2.2 (Kir2.2−/−) [409, 410]. Although the Kir2.1−/− mice have cleft palate and die shortly after birth, thereby precluding electrophysiological studies on adult cells [410], experiments completed on isolated newborn Kir2.1−/− ventricular myocytes revealed that $I_{K1}$ is absent [409]. Interestingly, however, an inwardly rectifying current, with properties distinct from the wild-type $I_{K1}$, is evident in Kir2.1−/− myocytes, suggesting either that an additional Kir current component is present, but difficult to resolve in wild-type cells in the presence of $I_{K1}$, or, alternatively, that a novel $I_{K1}$ is upregulated in Kir2.1−/− hearts. In contrast to the findings in Kir2.1−/− cells, voltage-clamp recordings from adult Kir2.2−/− ventricular myocytes revealed that $I_{K1}$ densities are reduced, compared with $I_{K1}$ densities in wild-type cells and that the properties of the residual $I_{K1}$ currents (in Kir2.2−/− cells) are indistinguishable from wild-type $I_{K1}$ [409]. These results were interpreted as suggesting that both Kir2.1 and Kir2.2 contribute to (mouse) ventricular $I_{K1}$ channels, and subsequent studies provided biochemical and molecular evidence to support this hypothesis [411, 412]. The observation that the inwardly rectifying channels remaining in the absence of Kir2.1 have properties distinct from the endogenous $I_{K1}$ channels further suggests that functional cardiac $I_{K1}$ channels are heteromeric. Consistent with this hypothesis, detailed comparisons of the properties of heterologously expressed Kir2.1, Kir2.2, and Kir2.3 α subunits and endogenous guinea pig and sheep atrial and ventricular myocytes suggest marked regional and cell type-specific differences in the molecular composition of $I_{K1}$ channels [166]. Hetero-tetramerization of Kir2.1 channels with wild-type Kir2.2 and Kir2.3 channels in HEK cell or oocytes can present the molecular basis of the extraordinary pleiotropy of Andersen's syndrome [395]. Further studies focused on defining the molecular compositions of myocardial $I_{K1}$ channels in different cell types and in different species, including humans, are needed to define the molecular diversity and the functioning of these channels.

$K_{ATP}$ channels were first identified in cardiac myocytes [145] and later were also found in vascular smooth muscle [413], neuron [414], skeletal muscle, and ß cells [415]. $K_{ATP}$ channels

open spontaneously in inside-out patch-clamp experiments, and the opening can be inhibited by ATP and activated by NDP analogs such as ADP [416]. In the heart, weakly inwardly rectifying $I_{KATP}$ channels are thought to play a role in both myocardial ischemia and preconditioning [145, 158, 163]. In heterologous systems, $I_{KATP}$ channels can be reconstituted by co-expression of Kir6.x subunits with ATP-binding cassette proteins that encode the sulfonylurea receptors, SURx, composed with 4 Kir6.x subunits for pore and 4 SURx for auxiliary [417, 418]. Although previous pharmacological and molecular studies suggest that cardiac sarcolemmal $I_{KATP}$ channels reflect the heteromeric assembly of Kir6.2 and SUR2A subunits, Kir6.1 is also expressed in the heart [419], and exposure of isolated (rat neonatal) ventricular myocytes to antisense oligodeoxynucleotides against SUR1 reduces $I_{KATP}$ channel densities [420]. These observations suggest the interesting possibility that there may be some molecular heterogeneity among cardiac $I_{KATP}$ channels. The absolute requirement for the Kir6.2 subunit in the generation of cardiac $I_{KATP}$ channels, however, was unequivocally demonstrated in studies completed on mice in which the Kir6.2 gene was disrupted by homologous recombination [418, 421, 422]. Voltage-clamp recordings from ventricular myocytes isolated from these (Kir6.2−/−) animals revealed no detectable $I_{KATP}$ channel activity [421, 422]. These findings clearly suggest that Kir6.1 alone (i.e., in the absence of Kir6.2) cannot generate functional myocardial $I_{KATP}$ channels. Nevertheless, it is certainly still possible that Kir6.1 coassembles with Kir6.2 to form Kir6.1/Kir 6.2 heteromeric cardiac $I_{KATP}$ channels.

The suggestion that SUR2 plays a pivotal role in the generation of cardiac $I_{KATP}$ channels is supported by the finding that $I_{KATP}$ channel density is reduced in myocytes from animals (SUR2−/−) in which SUR2 has been deleted [423]. In contrast, there are no measurable cardiac effects of the targeted disruption of SUR1 [418, 424]. Nevertheless, it is interesting to note that $I_{KATP}$ channel density is reduced, i.e., the channels are not eliminated, in SUR2−/− ventricular myocytes, and the properties of the residual $I_{KATP}$ channels in SUR2−/− ventricular myocytes are similar to those of the channels produced on heterologous co-expression of Kir6.2 and SUR1. These findings strongly suggest that SUR1 likely also coassembles with Kir6.2 in ventricular myocytes to produce functional $I_{KATP}$ channels (at least in the absence of SUR2). Immunohistochemical studies suggest that Kir6.2 and SUR2A assemble to form plasma membrane cardiac $I_{KATP}$ channels, whereas Kir6.1, Kir6.2, and SUR2A are expressed in mitochondria. These observations interpreted suggest that the molecular compositions of functional $I_{K1}$ channels in different cellular compartments are distinct [425]. Similar to $I_{K1}$ channels, myocardial $I_{KATP}$ channels are also modulated by the binding of PIP2 and other membrane lipids [398, 400].

The waveforms of action potentials recorded from isolated Kir6.2−/− ventricular myocytes are indistinguishable from those recorded from wild-type cells [422]. These observations clearly suggest that $I_{KATP}$ channels do not play a role in shaping action potential waveforms (in mouse ventricles) under normal physiological conditions. The action potential shortening typically observed in wild-type ventricular cells during ischemia or metabolic blockade, however, is abolished in Kir6.2−/− ventricular cells [422]. In addition, the protective effect of ischemic preconditioning is abolished in Kir6.2−/− hearts [418, 426], and infarct size in Kir6.2−/− animals, with and without preconditioning, is the same [426]. These observations are consistent with the hypothesis that cardiac $I_{KATP}$ channels play an important role under pathophysiological conditions, particularly those involving metabolic stress [90, 427]. Interestingly, however, it has been demonstrated that action potential durations are also largely unaffected in transgenic animals expressing mutant $I_{KATP}$ channels with markedly (40-fold) reduced ATP sensitivity [428]. The mutant $I_{KATP}$ channels would be expected to be open (owing to the reduced sensitivity to closure by ATP) at rest and to markedly affect cardiac membrane excitability. The fact that action potentials are unaffected in ventricular myocytes expressing mutant $I_{KATP}$ channels clearly suggests that additional inhibitory regulatory mechanisms play a role in

the physiological control of cardiac $I_{KATP}$ channel activity in vivo [428]. Further studies focused on defining and characterizing these regulatory mechanisms will be of considerable interest.

## Two Pore Domain $K^+$ Channels

In addition to the many voltage-gated $K^+$ (Kv) and the inwardly rectifying $K^+$ (Kir) (Tables 1.4 and 1.6) channel α subunits, a novel type of $K^+$ α subunit with four transmembrane-spanning domains and two pore domains was identified, with the cloning of TWIK-l, referred to as KCNK1 [429], the first member of the K2P family (Table 1.7). Studies in heterologous systems suggest that functional K2P channels, unlike Kv and Kir channels that assemble as tetramers, assemble as dimers. K2P channels possess three special characteristics compared

**Table 1.7** Two-pore $K^+$ channel α subunits

| | | | Location | | | |
|---|---|---|---|---|---|---|
| Family | Subfamily | Protein | Gene | Human | Mouse | Cardiac current |
| Two pore | | | | | | |
| | TWIK | | | | | |
| | | TWIK -1 | KCNK1 | 1q42.2 | 8 E2 | ?? |
| | | TWIK - 2 | KCNK6 | 19q13.2 | 7 B1 | |
| | | TWIK-3 | KCNK7 | 11q13.1 | 19A | |
| | TREK | | | | | |
| | | TREK -1 | KCNK2 | 1q41 | 1 H6 | Iss?? |
| | | TREK - 2 | KCNK10 | 14q31.3 | 12 E | |
| | TASK | | | | | |
| | | TASK -1 | KCNK3 | 2p23.3 | 5 B1 | Ikp?? |
| | | TASK - 3 | KCNK9 | 8q24.3 | 15D3 | |
| | | TASK - 5 | KCNK15 | 20q13.12 | 2 H3 | |
| | TALK | | | | | |
| | | TALK -1 | KCNK16 | 6p21.2 | 14 | |
| | | TALK - 2 | KCNK17 | 6p21.2 | 8 | |
| | | (TASK-4)[a] | | | | |
| | | TASK - 2[b] | KCNK5 | 6p21.2 | 14A3 | |
| | TRAAK | | | | | |
| | | TRAAK -1 | KCNK4 | 11q13.1 | 19A | |
| | TRESK | | | | | |
| | | TRESK-1 | KCNK18 | 10q25.3 | 19D3 | |
| | THIK | | | | | |
| | | THIK -1 | KCNK13 | 14q32 | 12 E | |
| | | THIK - 2 | KCNK12 | 2p21 | 17 E4 | |

Boxes denote cardiac expression
[a]TASK2 and TALK4 have been found to be the same protein
[b]TASK2 has been found to actually be part of the TALK family

to other potassium channels. (1) Each subunit of K2P channels contributes with two pore-forming domains to the ion pore after being assembled as a dimer. The other potassium channels subunits have only one pore-forming domain. (2) The mechanisms of K2P channel regulation are multiple. Their activity can be regulated by pH, membrane stretch, temperature, or intracellular signaling pathway [430]. (3) K2P channels show a unique extracellular "helical cap" structure between the M1 and M2 domain. This structure could be a potential drug target to treat diseases related to K2P channel dysfunction [431, 432]. Heterologous expression of either of these subunits alone gives rise to instantaneous, noninactivating $K^+$ currents that display partly voltage dependence [430, 433]. These observations have led to suggestions that these subunits contribute to myocardial "background" or "leak" $K^+$ currents [434]. Interestingly, however, the properties of the currents produced on expression of TREK-1 or TASK-1 are similar to those of the current referred to as plateau current $I_{Kp}$ identified in guinea pig ventricular myocytes [434, 435], as well as to Iss in mouse ventricular myocytes [60, 436].

The K2P family has 15 members that are subdivided into 6 subfamilies, which are TWIK, TREK, TASK, TALK, THIK, and TWIK-related spinal cord $K^+$ channels (TRESK), and a subset of these appears to be expressed in the myocardium (Table 1.7). In 1996, the first K2P channel TWIK-1 was identified [429]. In the next 7 years (1996–2003), 14 other K2P members were identified [437]. Similar to the Kv and Kir channels, a systematic terminology has been developed for naming the (KCNK) genes encoding K2P subunits.

Heterologous expression studies have demonstrated that the members of various K2P subunit subfamilies give rise to $K^+$-selective currents with distinct time- and voltage-dependent properties and differential sensitivities to a variety of modulators, including pH, membrane stretch, fatty acids, and anesthetics and so on [430, 438]. It seems likely that K2P channels could be important in regulating the normal physiological functioning of the adult mammalian heart, as well as influencing myocardial responses to pathophysiological stimuli. Direct experimental support for this hypothesis was provided with the demonstration that the pathophysiological effects of platelet-activating factor on the myocardium are directly linked to inhibition of KCNK3 (TASK-1)-encoded (or closely related) $K^+$ channels in ventricular myocytes [439]. The effects of both platelet-activating factor and TASK-1 are dependent on protein kinase C [439, 440]. Although it has been demonstrated that different KCNK-encoded K2P subunits can coassemble to form heterodimer channels [441, 442], it is presently unclear whether heterodimeric K2P subunit assembly is physiologically relevant in the myocardium. Similarly, there is very little information presently available about the role(s) of accessory subunits and/or other regulatory molecules in the generation of K2P subunit-encoded myocardial channels, looks like K2P channels do not need any accessory subunits.

The fact that there are so many K2P α subunits (Table 1.7), that some of them are ubiquitously expressed, and that the properties of K2P channels are regulated by a variety of potentially relevant physiological and pathophysiological stimuli suggests that K2P channels likely subserve a variety of important physiological functions. Experimental support for this hypothesis has been provided by the demonstration that mice bearing a targeted disruption of the KCNK2 gene (TREK-1, Table 1.7) display increased sensitivity to epilepsy and ischemia [443]. It is unclear, however, whether there is a cardiac phenotype in the KCNK2−/− mice, primarily because this possibility appears not to have been addressed [443]. TREK-1, TALK-2, and TASK-1 are all expressed in the heart [444, 445]. We now know that TASK-1 channel modulation of TASK-1 can alter human atrial action potential duration, suggesting that this channel might be a drug target for the treatment of atrial fibrillation [446]. In our lab, we also discovered loss of function mutation in the KCNK3 gene (TASK-1) in patients with idiopathic and familial PAH [447]. TASK-4 (KCNK17, also named TALK-2) is also expressed in the atrioventricular node and cardiac Purkinje

fibers. Also, a gain of function by TASK-4 in the conduction system has suggested TASK-4 may be a novel arrhythmia gene [448].

## $Ca^{2+}$-Activated and $Na^{+}$-Activated $K^{+}$ Channels

$Ca^{2+}$-activated $K^{+}$ channels have three classifications based on their conductance. They are big conductance (BKCa), intermediate conductance (IKCa), and small conductance (SKCa) (Table 1.8) [449].

The first described $K^{+}$ channel found to be sensitive to both $Ca^{2+}$ and transmembrane voltage is a BKCa channel termed the BK channel (big conductance and $Ca^{2+}$-dependent potassium channel). It is a tetramer of α subunit *slo1* (KCNMA1) gene products that assemble to form the channel pore [450, 451]. The BK channel is $K_{Ca}1.1$ in standard nomenclature [451, 452] and is expressed in cell membranes of most mammalian tissue types [450]. The BK channel may also be modulated by one of four (1–4) β or a γ subunit [450].

Following the discovery of $K_{Ca}1.1$ (BK), screening for *slo1(KCNMA1)* gene ($K_{Ca}1.1$), other $Ca^{2+}$-dependent $K^{+}$ channels with similar sequences were discovered [451]. The next two classes were $K_{Ca}2$ and $K_{Ca}3$ and were found to be sensitive to $Ca^{2+}$, but unlike $K_{Ca}1.1$, they are insensitive to membrane voltage. The $K_{Ca}2$ family has three members $K_{Ca}2.1$, (SK1) $K_{Ca}2.2$ (SK2), and $K_{Ca}2.3$ (SK3) [453]. The $K_{Ca}3$ family has one member, $K_{Ca}3.1$ [454, 455]. Two other families of channels, containing three total members, given names $K_{Ca}4.1$, $K_{Ca}4.2$, and $K_{Ca}5.1$, have been identified [451], but it has become evident that these channels are instead regulated by intracellular $Na^{+}$ and $Cl^{-}$ [451, 456–458].

A version of the BK channel known as mitoBKCa has been found to be present in the inner membrane of mitochondria in adult cardiomyocytes [449, 459]. BKCa channels are not believed to be present in the cell membrane of adult cardiomyocytes [449, 459]. The presence of the mitoBKCa channel in the cardiomyocyte mitochondrial inner membrane is believed to be cardioprotective [460]. NS1619, a potent BKCa opener, has been shown to cause a $Ca^{2+}$-induced $K^{+}$ flux that increases ROS production while maintaining membrane potential [461]. MitoBKCa-induced depolarization of the mitochondrial membrane potential has been reported to help stop mitochondrial $Ca^{2+}$ overload, also cardioprotective [462].

**Table 1.8** Calcium-activated potassium channel (Kca) pore-forming α subunits

| Subfamily | Protein | Gene | Locus | | | Cardiac current |
|---|---|---|---|---|---|---|
| | | | Homo | Mus | Rattus | |
| $K_{Ca}1$ | | | | | | |
| | $K_{Ca}1.1$, BK | *KCNMA1* | 10q22.3 | 14A3 | 15p16 | |
| $K_{Ca}2$ | | | | | | |
| | $K_{Ca}2.1$,SK1 | *KCNN1* | 19p13.11 | 8B3.3 | 16p14 | $I_{K(Ca)}$ |
| | $K_{Ca}2.1$,SK2 | *KCNN2* | 5q22.3 | 18B3-C | 18q11 | $I_{K(Ca)}$ |
| | $K_{Ca}2.1$,SK3 | *KCNN3* | 1q21.3 | 3F1 | 2q34 | $I_{K(Ca)}$ |
| $K_{Ca}3$ | | | | | | |
| | $IK_{Ca}1$, IK | *KCNN4* | 19q13.31 | 7A3 | 1q21 | |
| $K_{Ca}4$ | | | | | | |
| | $K_{Ca}4.1$, Slack | *KCNT1* | 9q34.3 | 2A3 | 3p13 | |
| | $K_{Ca}4.2$, Slick | *KCNT2* | 1q31.3 | 1F | 13q21 | |
| $K_{Ca}5$ | | | | | | |
| | $K_{Ca}5.1$, Slo3 | *KCNU1* | 8p11.23 | 8A2 | 16q12.4 | |

Boxes denote cardiac expression

$K_{Ca}2$ family channels are SKCas (small-conductance apamin-sensitive $Ca^{2+}$-activated $K^+$ channels) and have been shown to have a constitutively bound calmodulin that confers the $Ca^{2+}$ sensitivity to the channel α subunit via the calmodulin's $Ca^{2+}$ binding rather than direct $Ca^{2+}$ binding to the α subunit [449, 463]. These channels have been found to be important to modulating cardiac action potential duration via this conferred $Ca^{2+}$ sensitivity [464]. These channels, encoded by the KCNN gene, are responsible for the IKAS current found in cardiac cells, but most prominently in atrial, and not ventricular cells [451].

Demonstrated in mouse cardiomyocytes, SKCa channels are activated by increases in intracellular, cytosolic $Ca^{2+}$ such as via ryanodine receptor 2 (RyR2) release of $Ca^{2+}$ from the sarcoplasmic reticulum [465]. The hyperpolarization that occurs as a result of this $Ca^{2+}$-activated $K^+$ current inhibits and slows the firing frequency of repeated action potentials primarily in atrial cells.

Additionally, $K_{Ca}4.2$, also now known as $K_{Na}1.2$, is a $Na^+$-sensitive channel found in cardiac cells, among other tissues [451]. It has been proposed that activation of this channel is cardioprotective in mouse [466]. There are no reported human mutations in $K_{Ca}4.2$ ($K_{Na}1.2$), so there is much unknown about its role in human physiology [451].

## Summary and Conclusions

Electrophysiological studies have clearly identified multiple types of voltage-gated inward and outward currents that contribute to action potential repolarization in mammalian ventricular myocardium (Table 1.1). Interestingly, the outward currents are more numerous and more diverse than the inward currents, and mammalian ventricular myocytes express a repertoire of voltage-gated and inwardly rectifying $K^+$ channels (Table 1.1) that all contribute importantly to shaping the waveforms of action potentials, as well as influencing automaticity and refractoriness. Nevertheless, the voltage-gated inward $Ca^{2+}$ channel currents and the $Na^+$ channel "window" current [31, 32, 467] also contribute to ventricular repolarization. The pivotal role played by the $Na^+$ channel "window" current has been elegantly demonstrated in studies characterizing mutations in SCN5A that underlie long QT-3. These studies have revealed novel mechanism whereby altered $Na^+$ channel function impacts ventricular action potential durations.

Computer-based simulations of cellular electrical activity have also been helpful in linking altered $Na^+$ (and other) channel functioning to possible/likely effects on action potential waveforms [190, 210, 211, 468]. The results of these studies demonstrate that subtle changes in $Na^+$ channel gating can have profound effects on repolarization because the plateau phase of the action potential is maintained by the balance of very small currents, a lesson learned more than 70 years ago from the elegant experiments of Silvio Weidmann [469]. The insights gleaned from the analysis of inherited $Na^+$ (and other) channel mutations also underscore the importance of the interplay of all of the ionic currents contributing to repolarization in determining ventricular action potential waveforms.

Molecular cloning studies have revealed an unexpected diversity of voltage-gated ion channel pore-forming a-subunits (Tables 1.2, 1.4, 1.6, 1.7, and 1.8) and accessory subunits (Tables 1.3 and 1.5) that contribute to the formation of the various inward and outward current channels (Table 1.1) identified electrophysiologically. Similar to the electrophysiological diversity of ventricular $K^+$ channels (Table 1.1), the molecular analyses have revealed that multiple voltage-gated ($K_V$) (Table 1.4) and inwardly rectifying ($K_{ir}$) (Table 1.6) $K^+$ channel pore-forming a-subunits, as well as the accessory subunits of these channels (Table 1.5), are expressed in the myocardium. A variety of in vitro and in vivo experimental approaches have been exploited to probe the relationship(s) between these subunits and functional ventricular $K^+$ channels. Important insights into these relationships have been provided through molecular genetics and the application of techniques that allow functional channel

expression to be manipulated in vitro and in vivo. The results of these efforts have led to the identification of the pore-forming α subunits contributing to the formation of most of the $K^+$ channels expressed in mammalian ventricular myocytes (Tables 1.4, 1.5, and 1.6). In contrast to the progress made in defining the Kv and the Kir a-subunits encoding the functional voltage-gated and inwardly rectifying $K^+$ currents expressed in mammalian ventricular myocytes, presently very little is known about the functional roles of the two-pore domain $K^+$ channel a-subunits (Table 1.7). In addition, the roles of the various accessory subunits (Table 1.5) in the generation of functional cardiac $K^+$ channels remain to be clarified.

It seems reasonable to suggest that defining the molecular correlates/compositions of the channels underlying ventricular action potential repolarization will facilitate future efforts focused on delineating the molecular mechanisms controlling the properties and the functional expression of these channels. Numerous studies have documented changes in functional ion channel expression during normal ventricular development, as well as in damaged or diseased ventricular myocardium. Importantly, it has also been shown that electrical remodeling occurs in the ventricles in response to changes in cardiac electrical activity or cardiac output, and most of the effects observed can be attributed to changes in the expression and/or the properties of the channels underlying ventricular repolarization.

Numerous possible (transcriptional, translational, and posttranslational) mechanisms could be involved in regulating the expression and the properties of the inward and outward current channels underlying repolarization. However, we are just beginning to learn about the underlying molecular mechanisms that are important in mediating the changes in channel expression evident during normal development, as well as in conjunction with myocardial damage, disease, and/or electrical remodeling. Clearly, a major focus of future research will be on exploring these mechanisms in detail.

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# 2 Neuromodulation of Cardiac Repolarization and Arrhythmogenesis

Fabrice Extramiana and Pierre Maison-Blanche

## Introduction

The relationship between neuropsychological influences and cardiac clinical symptoms has been long and consistently recognized. The most extreme illustration is being "scared to death" as demonstrated by the increase in sudden cardiac death (SCD) occurrence during intense psychological stress [1–6]. Physical stress and exercise are also classical triggers for SCD [7, 8]. But, on the other side of the same coin, SCD may also occur during sleep [9, 10]. These caricatures obviously point to autonomic factors precipitating ventricular fibrillation (VF) [11, 12]. Pioneer experimental studies have shown that sympathetic stimulation at different anatomical levels decreases the threshold for VF and that this effect is prevented by parasympathetic stimulation [12–16].

The classic Coumel's triangle has raised awareness about the three ingredients required for the occurrence of a spontaneous clinical arrhythmia, an arrhythmogenic substrate, a trigger factor, and modulation factors of which the most common is the autonomic nervous system [17, 18].

There have been dramatic advances in the last decades in understanding the links between the autonomic nervous system (ANS), arrhythmogenesis, and ventricular repolarization (VR). In the present chapter, we intend to synthetize current knowledge of the physiology and pathophysiology of autonomic influences on VR and the related clinical aspects of this interaction and finally to describe the current and potential future antiarrhythmic therapies based on neuromodulation of ventricular repolarization.

## Physiology and Pathophysiology of Autonomic Nervous System (ANS) Influences on Ventricular Repolarization

### A Short Description of the Neural Control of the Heart

Physiological cardiac properties are key determinants of blood pressure regulation and adaptation to stress and exercise, and their neural control has been long identified [19, 20].

The description of the complex anatomy of the ANS is beyond the scope of this chapter and not perfectly understood [21, 22]. It has been described as a three-component system with central, intrathoracic, and intrinsic cardiac components, each with a feedback loop resulting in a complex fine-tuning [23].

At the end-effector level, both sympathetic and parasympathetic autonomic receptors influence G proteins and, respectively, lead to an increase or a

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decrease in PKA-dependent phosphorylation [24]. However, the two limbs of the ANS cannot be considered as independent from each other. A parasympathetic feedback will antagonize a sympathetic stimulation [25], and at the ventricular level, the effect of parasympathetic stimulation would be to antagonize any preexistent sympathetic stimulation but would be inefficient in the absence of sympathetic stimulation [14].

## ANS Effects on Ventricular Repolarization (VR) Duration

The effects of the ANS on VR have been described at the channel, cellular, and organ levels.

Beta-1 adrenergic receptor stimulation is associated with a rate-independent increase of the slow calcium (ICaL) and slow component of the delayed potassium (IKs) currents [26–29]. In addition to these direct effects, IKs is also calcium-dependent and is also increased by the calcium concentration increase following adrenergic-dependent calcium channel phosphorylation.

Conversely, type 2 muscarinic receptor stimulation by acetylcholine will antagonize ICaL but only in the presence of β-adrenergic stimulation [29].

Adrenergic stimulation has been long recognized as increasing heart rate through affecting both the membrane and calcium clocks [20, 30]. The shortening of the cardiac cycle will impact the kinetics of ionic channels. Higher heart rates will be associated with less time for de-inactivation, thereby decreasing the INa, ICaL, and Ito1.

The global impact of heart rate-dependent and rate-independent effects of sympathetic stimulation is a shortening of ventricular APD and refractory periods [31–34].

## Proarrhythmic Effects of Sympathetic Stimulation on Ventricular Repolarization

Although the link between sympathetic stimulation and ventricular arrhythmias is supported by overwhelming clinical data (see section "Clinical Syndromes" of the present chapter), the underlying mechanisms are not fully understood.

Conduction properties [35–37] and calcium handling [38–40] are also influenced by sympathetic stimulation in a proarrhythmic manner. We will here schematically describe proarrhythmic effects mediated by changes in VR properties induced by sympathetic stimulation.

These effects might be summed up at cellular, tissue, and organ levels.

At the cellular level, early afterdepolarizations (EADs) may result in triggered activity arising from abnormal repolarization. Mathematical models show that EADs may result from secondary activation of the L-type Ca2+ current during the plateau of an action potential or from activation of sodium-calcium exchanger by increased intracellular calcium due to spontaneous Ca2+ release from the SR during the late repolarization phase [41, 42]. Increasing the L-type Ca2+ current and intracellular calcium by adrenergic stimulation promotes the occurrence of EADs via a complex kinetic mismatch of PKA targets [43]. This proarrhythmic effect at a cellular level seems to be particularly involved in case of decreased IKs current as seen in type 1 LQTS [44, 45].

Although triggered activities are critical for arrhythmia initiation, the maintenance of arrhythmias is related to self-sustained propagation of the depolarization front in a tissue structure. In most cases, the underlying mechanism is reentry which requires a match between propagation speed and refractory periods [46, 47]. Theoretically, the APD shortening induced by adrenergic stimulation [31–34] would decrease the wavelength of potential reentrant circuits (or the critical mass required for the reentry) and hence promote arrhythmia maintenance.

Beyond duration, VR heterogeneities, either spatial or temporal, have been recognized as key players in arrhythmogenesis.

Pioneer studies have shown the spatial heterogeneity of ventricular refractory periods and the potential role of increased spatial dispersion as a mechanism for transient (unidirectional) conduction block serving as the substrate for functional reentry [48, 49]. This arrhythmia mechanism has

been evidenced in experimental models of myocardial infarction, long QT syndrome, heart failure, Brugada syndrome, and short QT syndrome [50–57]. It is noteworthy that epicardial steep spatial ventricular repolarization gradient has been noninvasively evidenced (using ECG imaging) in the long QT syndrome when compared to normal subjects [58].

As discussed above, adrenergic stimulation is associated with a shortening of ventricular refractory periods. However, this effect is not spatially homogeneous due to (1) the spatial heterogeneities of ionic channel densities across the ventricular wall and different parts of the ventricles [59, 60] and (2) the heterogeneity in regional distribution and activity of sympathetic nerves [61]. And it has been shown that beta-adrenergic stimulation increases spatial VR heterogeneities in experimental models [51, 57] as well as in patients using invasive endocardial ventricular mapping in patients with myocardial infarction [62].

Finally, the spatial heterogeneity of ventricular repolarization not only may serve as a substrate for functional reentries. Steep local ventricular repolarization gradients may generate an electrotonic current leading to phase 3 EADs [63].

Temporal VR heterogeneities also play a critical role in arrhythmogenesis. VR duration is dependent on heart rate in steady-state condition but also on the duration of the preceding diastolic interval [64]. The former is named repolarization restitution and is displayed as a graphical curve which steepness would determine the evolution of the system [65]. Steep restitution curves (above 1) would induce APD alternans which when spatially discordant would lead to functional conduction blocks, hence promoting the transition from a stable to an unstable rhythm, ultimately to fibrillation [65–68]. The relationship between the ventricular restitution slope and the probability of inducing VF during premature ventricular stimulation has been found in patients with the Brugada syndrome [69].

Adrenergic stimulation has been shown to increase the steepness of the APD restitution in experimental models as well as in humans [70, 71]. Conversely, the steepness of the APD restitution is decreased by vagal nerve stimulation [71] in a muscarinic receptor-independent manner, through neuronal nitric oxide release [72, 73].

A very recent provocative study suggests, however, that APD alternans would be associated with a greater regional expression of calsequestrin and ryanodine receptor but not with restitution properties [74]. If this concept hold true, this mechanism would be a major link between adrenergic stimulation and arrhythmogenesis.

Finally, the proarrhythmic effect of sympathetic stimulation on VR at the organ level should not be overlooked.

The adrenergic-induced increase in inotropy, chronotropy, dromotropy, and lusitropy is associated with a steep increase in myocardial oxygen demand potentially leading to ischemia defined as an imbalance in the supply and demand. Acute ischemia is associated with hyperkalemia and acidosis and will affect conduction velocity but also result in IKATP activation leading to APD shortening. Looking only to the consequences of altered repolarization, steep APD gradients at the ischemic border zone may promote triggered activity via phase 2 reentry and/or electrotonically triggered EADs [75, 76].

Although the pathophysiology of the Takotsubo cardiomyopathy is not fully understood and probably multifactorial, the common emotional stress before the event and the occurrence in patients with pheochromocytoma and after administration of catecholamines suggest an important role for adrenergic stimulation [77]. The Takotsubo cardiomyopathy is associated with profound surface ECG VR changes reflecting an increase in inhomogeneity and dispersion of ventricular repolarization and has been shown to lead to torsades de pointes [78, 79].

Last but not the least, chronic adrenergic stimulation has a profound impact on the myocardial structure as it promotes fibrosis [80] which in turn with cellular uncoupling will increase repolarization gradients, hence paving the way for an arrhythmogenic substrate [81–83].

Figure 2.1 provides a summary of the proarrhythmic effects of sympathetic stimulation effects on ventricular repolarization.

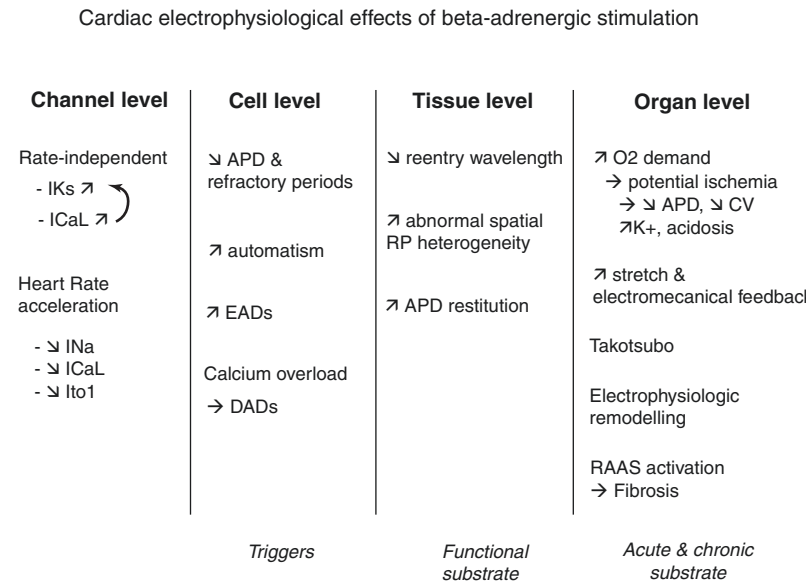


**Fig. 2.1** Diagram of the electrophysiological effects of beta-adrenergic receptor stimulation at channel, cell, tissue, and organ levels. APD action potential duration, EADs early afterdepolarizations, DADs delayed afterdepolarization, RP refractory period, CV conduction velocity, RAAS renin-angiotensin-aldosterone system

## Clinical Aspects

In the next paragraphs, We will first take a closer look into the interplay between body surface ECG markers of VR and ANS. Then, for the main ventricular arrhythmia syndromes, we will review the clinical features that provide evidence of the ANS influences.

### Neuromodulation of ECG Correlates of Ventricular Repolarization

Availability of implantable cardiac defibrillators (ICDs) made precise cardiac arrhythmia risk assessment very useful. Reduced left ventricular ejection fraction (LVEF) is without question a strong predictor of the risk of SCD, but soon it was understood that LVEF has major drawbacks, mainly because the majority of SCD cases occur in patients with preserved or moderately reduced LVEF [84, 85]. Then a reduced LVEF is a risk factor for both sudden and non-sudden death. Currently, only relatively few patients with reduced LVEF will benefit from an ICD (most will never experience a threatening arrhythmic event).

Because of the direct relationship between VR abnormalities and arrhythmogenesis as reported above, vast clinical research programs have been deployed to validate VR-based risk markers [86].

Due to length constraint, we will discuss only the role of global QT and Tpeak-Tend ECG time intervals, QT hysteresis, and QT rate dependency. Pathophysiology of microvolt T wave alternans will be presented in another chapter.

#### Global QT Duration

Autonomic nervous system influences on QT/QTc interval in humans are poorly understood. In particular, the effects of an autonomic intervention, sympathetic stimulation, or parasympathetic blockade will impact both the heart rate and the QT interval. Therefore, any drugs that affect the ANS can modulate the QT interval directly or only by changing heart rate. Actually, autonomic modulation of QT interval has produced conflicting results, with among other methodological issues the limitations of any QT rate correction formula. In an elegant pioneer clinical study, Browne [87] compared QT intervals that had similar RR interval durations during sleep and awake states. At 60 beats per minute, the duration of the QT interval was longer during sleep in all 15 patients (19 ± 7 ms). In another study, Ahnve showed that in healthy subjects, when the heart rate was kept constant by cardiac pacing, propranolol did not induce any

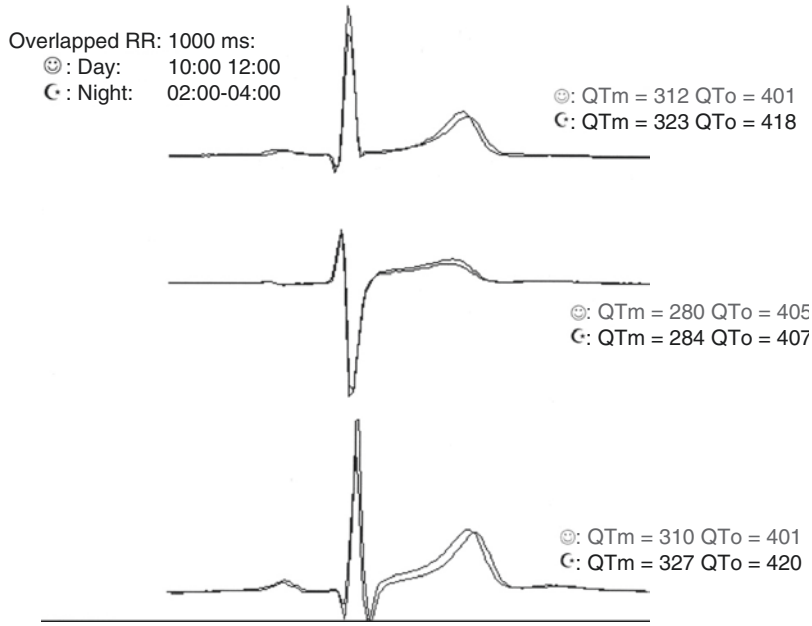


**Fig. 2.2** Circadian modulation of the QT intervals, a rate-independent approach. Using the rate-independent approach as introduced by Browne [88], the individual ECG waveforms collected at 60 beats per minute (RR = 1000 ms) separately at day and night were averaged. The ECG recording was a digital 24-hour recording with an orthogonal lead configuration (upper traces as X lead and bottoms traces as Z lead). ECG intervals were manually measured using screen calipers

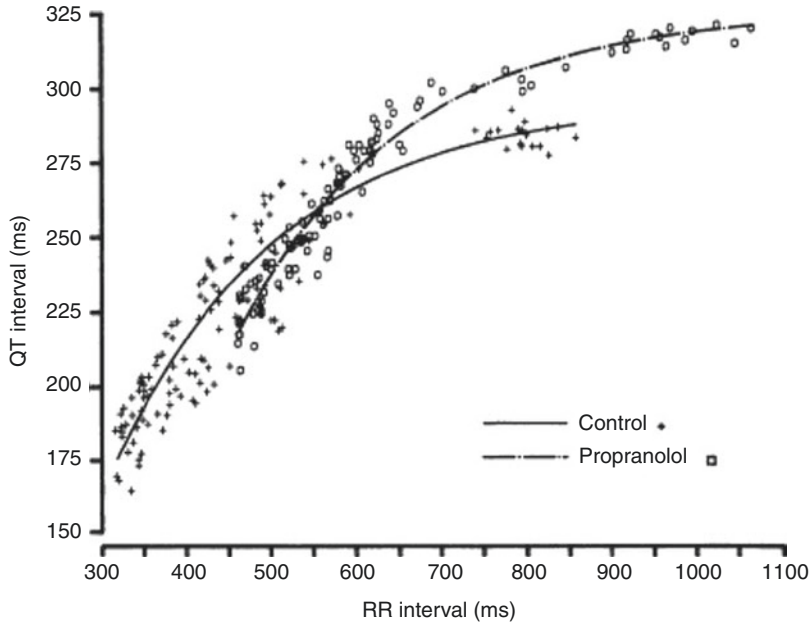


**Fig. 2.3** Modulation of QT rate dependency by beta-blockers. In this study, the modulation of the QT/RR slope by a beta-blocker leads to a biphasic effect on the QT interval: a significant prolongation at longer cycle and a shortening at shorter cycle lengths. In this study, Bazett's formula did not show any significant effect when the same raw data were used. (Adapted from Sarma et al. [89])

significant change in the QT interval [88]. Figure 2.2 shows in a healthy individual two ECG waveforms collected at identical heart rate, but one during the day and the other at night. This figure provides a simple illustration of the QT circadian pattern. Using electrocardiograms continuously recorded, Sarma estimated the effects of propranolol on the QT interval at three predefined RR intervals (400, 700, and 1000 ms). This study showed that the effect of propranolol on the QT intervals is biphasic (Fig. 2.3). It significantly prolonged the interval at longer cycle lengths but shortened it at shorter cycle lengths. It is noteworthy that Bazett's formula did not

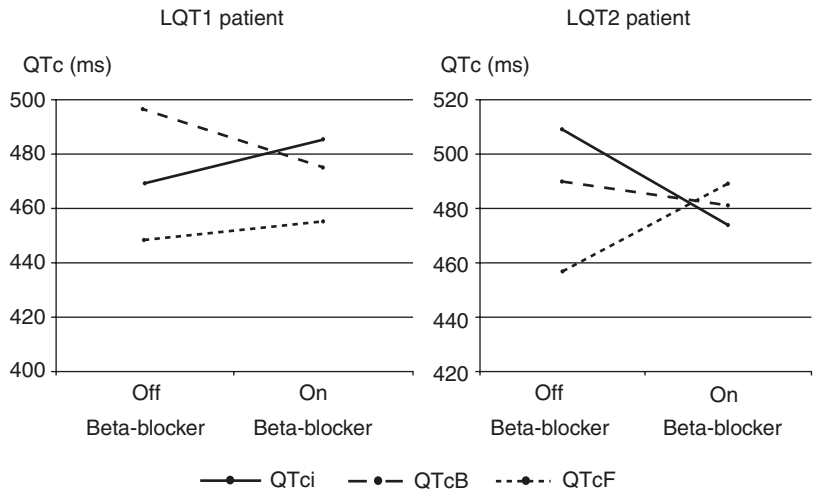


**Fig. 2.4** The importance of the QT rate correction coefficient to determine the effects of pharmacologic beta-adrenergic blockade. QTc changes from baseline under beta-blocker treatment using different rate correction coefficients (individual, Bazett, or Fridericia) in a LQT1 (left) and a LQT2 patient (right). Depending on the mathematical factor, one can conclude in either QTc shortening or QTc lengthening after beta-blockade. (Adapted from Extramiana et al. [90]. Used with permission from John Wiley and Sons)

show any significant effect when the same raw data were used [89].

Therefore, it is likely that the QT rate correction formula is a confounding factor in clinical trials, but the use of a rate-independent QT approach based on continuous ECG recordings is not suitable in most patient populations (Fig. 2.4).

## QT Interval and Risk Stratification in the General Population

Montanez and co-authors [91] conducted a literature search to identify all published prospective cohort studies evaluating the association between prolonged QTc interval and risks of total and cardiovascular mortality as well as sudden death. The seven prospective cohort studies identified included Framingham, Amsterdam, and two Rotterdam studies and the Zutphen, Finnish, and Danish studies. In this review article, there was no consistent association between prolonged QTc interval and total and cardiovascular mortality or morbidity, with the exception of patients with prior cardiovascular disease. Even in this subgroup, however, the data are not entirely consistent. This study outcome needs to be interpreted, keeping in mind the methodological limitations described above.

## Inherited Arrhythmia Syndromes: Long and Short QT Syndromes

Mazzanti and co-authors recently reviewed database findings from 1710 patients affected by long QT syndrome (LQTS) and a follow-up time of 7.1 years. Based on QTc duration, the aim of the study was to estimate the 5-year risk of life-threatening arrhythmic events and to assess the antiarrhythmic efficacy of beta-blockers. The risk of arrhythmic events increased by 15% for every 10 ms increment of QTc duration for all genotypes. The risk for patients with LQT2 and LQT3 increased by 130% and 157% at any QTc duration versus patients with LQT1 [92].

Regarding LQT3 population, Wilde and co-authors analyzed electrocardiographic and genetic parameters accumulated by seven participating LQT3 registries. The study population included 406 LQT3 patients. In this study, the risk of a first cardiac event was also directly related to the degree of QTc prolongation. Each 10 ms increase in QTc duration up to 500 ms was associated with a 19% increase in cardiac events. Interestingly in this LQTS subgroup, beta-blocker therapy was associated with an 83% reduction in cardiac events in females but not in males [93]. In our LQT3 cohort of 111 genotyped

LQT3 patients (median follow-up 7 years, IQR 10.3) [7], beta-blockade was not associated with a decrease in severe rhythmic event occurrences (defined as documented polymorphic VT/VF or torsades de pointes, ACA, and SCD) but rather with a statistically nonsignificant increase (HR 3.87 95%CI 0.92–16.2, $p = 0.06$) [94].

Short QT syndrome (SQTS) is an inherited cardiac disease characterized by malignant ventricular tachyarrhythmias leading to syncope and sudden cardiac death [95]. Due to the low number of patients with SQTS, risk stratification represents the main current challenge in clinical characterization. So far, the only reliable predictor of cardiac arrest identified in SQTS population has been a previous history of cardiac arrest. In contrast with LQTS, a clear relationship between clinical risk and QT/QTc duration in patients with SQTS has not been proven. There are some trends in some studies toward increased events based on shorter absolute QT or QTc intervals. In a pediatric cohort of SQTS patients [96], Villafane reported that a Gollob score may be useful in identifying patients at higher risk for unexplained syncope or aborted SCD. QTc duration is embedded in the Gollob score (the shorter QTc rated a large number of points), and most symptomatic patients had high scores. However, Giustetto in a study with long-term follow-up of patients with SQTS did not report that the QTc values could differentiate between asymptomatic subjects and those with cardiac arrest [97].

## QT Rate Dependency

The dynamic relationship between QT and RR intervals in humans includes two distinct phenomena: first, the so-called QT/RR dependency, representing the magnitude of QT interval variations at steady state following changes of RR intervals, and then the QT/RR hysteresis, i.e., the time course of the QT interval following an abrupt variation in RR interval and the time spent to reach steady state.

Many studies have assessed the effect of changes in heart rate on ventricular repolarization. Rather than using a rate correction formula and a "rate corrected" QT interval, the key clinical marker in those studies is the slope of the QT/RR relationship. The magnitude of QT changes following changes in cardiac in cycle length is modulated by intrinsic factors that differ between subjects [98, 99]. QT-RR rate dependency varies among individuals, being not only population specific but patient specific. In addition, the lag adaptation times are also patient specific [100].

A neuromodulation of the QT rate dependency is demonstrated by many reports. When considering the analysis of the QT/RR slope separately at day- and nighttimes, studies have shown that the slope is steeper during daytime [99]. This circadian pattern is blunted in diabetic patient with diabetic autonomic neuropathy [101]. The effects of beta-blockade on the rate dependency of the QT interval depend on the time of the day. In healthy subjects, at daytime, the QT rate dependency is reduced by atenolol, whereas during the night, the QT rate dependency remains unchanged [102, 103]. Oppositely, in healthy male volunteers, dobutamine increases the QT/RR slopes [104].

Not surprisingly, dynamic parameters of VR and expressed as the QT/RR slope have been used to identify patients with increased risk for ventricular arrhythmias. Steep QT/RR slopes, reflecting inadequate adaptation of ventricular repolarization to heart rate changes, and in particular both exaggerated lengthening of repolarization at long RR cycle lengths and shortened QT intervals at short RR cycle are frequently observed in patients at risk for sudden cardiac death [105–114].

Our group assessed QT/RR slopes in a group of 68 patients with a previous history of Q-wave myocardial infarction, including a VT subgroup of 34 patients who had a recent history of VT and a control group consisting of 34 patients with a previous Q-wave myocardial infarction but without a history of VT events, an arrhythmia-free survival for 3 years during follow-up [105]. From Holter recordings, QT/RR slopes were computed during three different periods, a diurnal period (eight consecutive daily hours with fastest heart rate (awake period), a sleep period (four consecutive sleeping hours with lowest heart rate), and finally the 2 hours around

the awakening heart rate acceleration (awakening period). A circadian pattern was observed in both groups; the lowest QT rate dependence was observed during sleep, whereas the awakening period was characterized by the steepest QT/RR slope. Interestingly, within the three circadian periods, QT/RR slopes were significantly stronger in the VT group, and the difference was more pronounced after awakening. In other words, the QT shortening with increasing heart rate is maximum during the awakening period and in patients prone to VTs (Fig. 2.5a). Interestingly, the QT/RR slopes were further increased in patients with VF when compared to patients with VT (Table 2.1). The higher incidence of arrhythmic events and sudden cardiac death in this period is well established (see section "Neuromodulation of Cardiac Arrhythmias in Ischemic and Non-ischemic Cardiomyopathy").

Congenital long QT syndrome type 1 patients are at high risk for arrhythmic events during high adrenergic states such as exercise and emotion, and β-blockers are the first-line therapy for these patients (see section "Congenital Long QT Syndrome"). It has been shown that patients with missense mutations in the cytoplasmic loop (C-loops) regions had a higher risk for events than patients with mutations outside of the C-loop regions [116]. Furthermore (Fig. 2.5b), β-blockers were associated with greater benefit in patients with mutations located in the C-loop regions. Analysis of the QT-RR dynamicity in 24-hour Holter recordings acquired in healthy individuals and LQT-1 patients with and without mutation in the C-loop region showed stronger QT adaptation to heart rate in non-C-loop mutation LQT-1 patients regardless of the period of the day, whereas C-loop mutation LQT-1 patients presented abnormal adaptation (flat slopes) during the day [115]. The lack of QT shortening leads to prolonged QT interval at elevated heart rate, and consequently one expects C-loop LQT-1 patients to be at higher risk for cardiac events during elevated heart rate.

### QT Hysteresis

The QT-RR hysteresis during an exercise test has been previously described by Sarma and coauthors in 1985 [89]. In this setting, QT-RR hysteresis is a phenomenon marked by longer QT intervals at a given RR interval, while heart rates are increasing (RR intervals decreasing) during exercise and shorter QT intervals at the same RR interval when heart rates are decreasing (RR intervals increasing) during recovery. The ECG variable is the "ΔQT" in milliseconds, an estimate of the difference between QT intervals measured at a predetermined RR observed both during rate acceleration and deceleration. Evaluation of this QT-RR relationship during

**Fig. 2.5** Rate dependency in post-MI patients and in LQTS1 patients. (panel **a**) QT/RR slopes in the awakening period in post-myocardial infarction (MI) patients without (control group) or with ventricular tachycardia (VT group) during follow-up. For cardiac cycle lengths spanning from 740 to 960 ms RR intervals, QT intervals are not different between control and VT groups. At RR intervals >960 ms, QT intervals are significantly longer in the VT group. However, at RR intervals <740 ms, QT intervals are significantly shorter in the VT group (at RR intervals = 600 ms, the mean QT interval duration is 353 ms in the VT group versus 372 ms in the control group, $p < 0.05$). (Adapted from Extramiana et al. [105]. Used with permission from Elsevier). (panel **b**) QT-RR slopes in LQTS1 patients off β-blocker and in controls. QT-RR lines describing the QT-interval duration for the controls (black dots), CL QT-1 (white dots), and NCL LQT-1 (squares) groups for the diurnal (lines) and nocturnal (dotted lines) periods. We superimposed the curve describing QTc = 500 ms and grayed the areas corresponding to the range of RR and QT values associated with higher risk for cardiac events. CL C-loop LQT1 mutation, NCL non-C-loop LQT1 mutation. (Adapted from Couderc et al. [115])

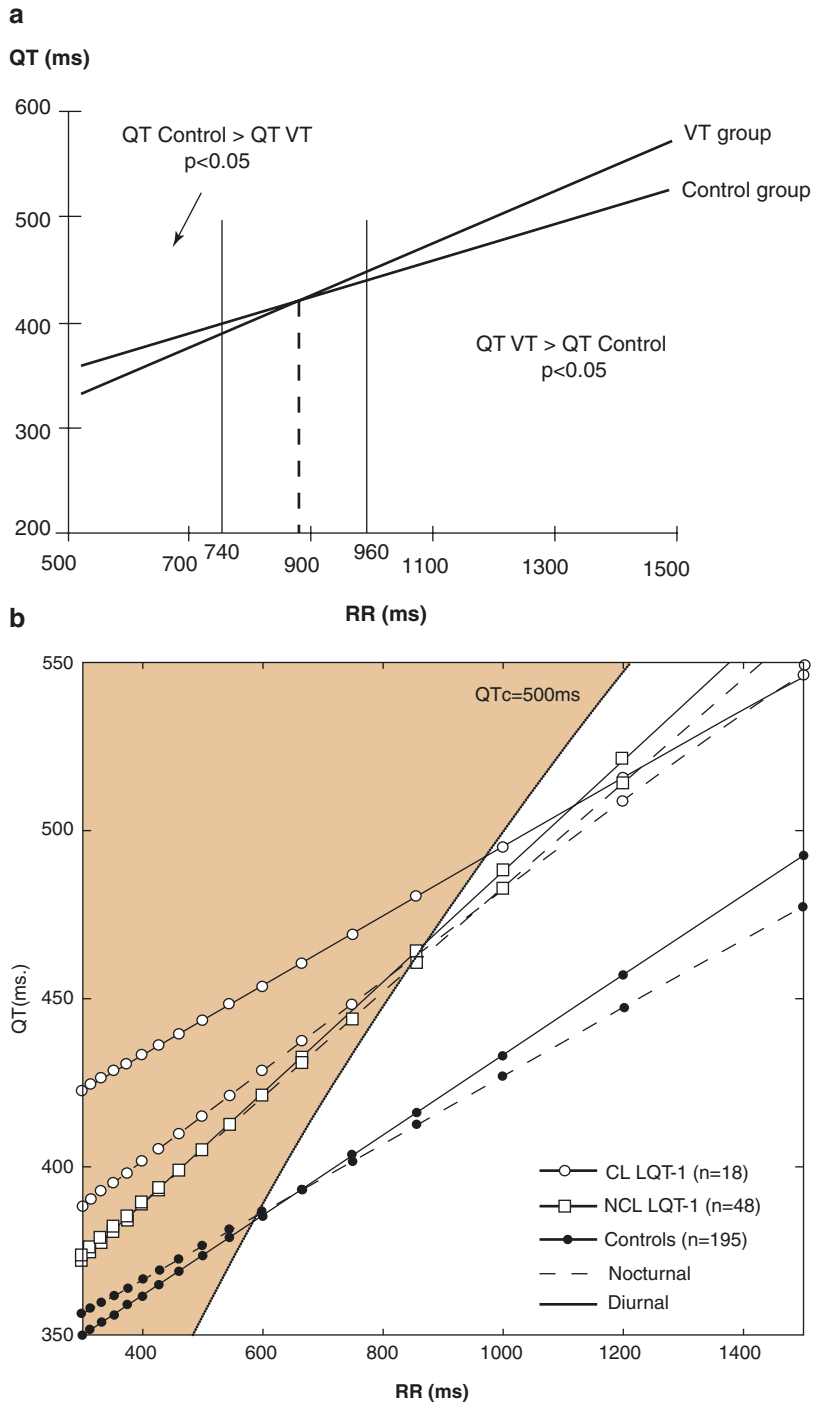
a
QT (ms)
600
500
400
300
200
QT Control > QT VT
p<0.05
VT group
Control group
QT VT > QT Control
p<0.05
500
700
740
900
960
1100
1300
1500
RR (ms)
b
550
500
450
400
350
QT(ms.)
QTc=500ms
CL LQT-1 (n=18)
NCL LQT-1 (n=48)
Controls (n=195)
Nocturnal
Diurnal
400
600
800
1000
1200
1400
RR (ms)

**Table 2.1** QT/RR slopes in patients with VT or VF after myocardial infarction

| | VT | VF | Difference [95% CI] |
|---|---|---|---|
| **QT/RR** During the day | 0.172 [0.145; 0.199] | 0.229 [0.193; 0.265] | −0.057 **[−0.101; −0.012]** |
| **QT/RR** At night | 0.175 [0.125; 0.226] | 0.188 [0.143; 0.233] | −0.013 [−0.081; 0.055] |
| **QT/RR** After awakening | 0.180 [0.134; 0.226] | 0.338 [0.292; 0.385] | −0.158 **[−0.224; −0.093]** |

Unpublished data from Ref. [105]

exercise and recovery has shown clinical value in certain situations, such as differentiating patients with long QT syndrome from patients with borderline QT duration [117, 118] and as a marker of myocardial ischemia [119, 120].

In long QT syndrome, Krahn showed that an exaggerated hysteresis of the QT interval was seen in patients with long QT syndrome at baseline compared with controls (46 ms vs. 19 ms). In this study, beta-blockers markedly reduced hysteresis [117]. In another study, this group reported that LQT2 patients had an exaggerated QT hysteresis compared with LQT1 and control subjects [118].

However, the mechanism of QT-RR hysteresis is not fully understood. It has been speculated that autonomic balance contributes to differences in repolarization during exercise and following recovery period. Specifically, late exercise is characterized by a shifted sympathovagal balance with enhanced sympathetic tone, whereas at early recovery, the parasympathetic tone quickly increases, while the sympathetic tone is declining. [117]. Pelchovitz evaluated QT-RR hysteresis in three groups of subjects, the first group consisted of normal subjects, the second group consisted of subjects with type 2 diabetes mellitus with no known cardiovascular disease, and the last group consisted of subjects with coronary artery disease. In this study protocol, subjects underwent exercise test with and without parasympathetic blockade [121]. The study demonstrates that parasympathetic blockade reverses the expected QT-RR hysteresis that would be due to directional changes in the RR interval, suggesting that differential parasympathetic inputs during exercise and recovery are responsible in large part for this phenomenon (for instance, ΔQT intervals without parasympathetic blockade were 6.8 ms in the control, and after parasympathetic blockade, ΔQT intervals were − 19.1 ms).

## Tpeak to Tend Interval

Tpeak to Tend (TpTe) interval has been found to be an independent risk factor for SCD. In the Oregon Sudden Unexpected Death Study, a population-based study of SCD, TpTe interval on the resting 12-lead ECG was prolonged in SCD cases compared to control subjects and was significantly associated with SCD independently of age, gender, QTc, QRS duration, and left ventricular dysfunction [122].

In 2017, Tse and co-authors performed a systematic review and a meta-analysis to evaluate the significance of the TpTe interval in predicting arrhythmic and/or mortality end points. A total of 33 observational studies involving 155,856 patients were included in the meta-analysis [123].

The main findings of this study were the following, supporting the Oregon study findings:

- A prolonged TpTe interval is associated with a 1.14-fold increased risk in VT/VF, SCD, cardiovascular death, or all-cause mortality when data from all pathological conditions were pooled with significant heterogeneity among studies.
- In the general population, a prolonged TpTe interval was also predictive of arrhythmic or mortality outcomes with an OR of 1.59.

The link from TpTe interval to mechanisms of ventricular arrhythmia has been investigated in many nonclinical trials. Using a canine myocardial wedge preparation model, Antzelevitch and co-workers have explored the genesis of TpTe.

In this study, TpTe corresponds to ventricular transmural dispersion of repolarization, a period during which the epicardium has repolarized and is fully excitable, but the M cells are still in the process of repolarization [124, 125]. According to this model, a prolonged TpTe corresponds to a vulnerable period during which EAD can lead to reentry and therefore could increase risk of ventricular arrhythmias. However, the surface ECG T wave also reflects other spatial heterogeneities, such as APD differences observed between the base and the apex of the heart [126].

Vaseghi showed that during sympathetic stimulation, there is an increase in dispersion of myocardial repolarization and increase in TpTe interval [127]. The global epicardial dispersion of repolarization as measured using activation recovery intervals (ARI) at baseline and during right stellate ganglion (RSG) and left stellate ganglion (LSG) stimulation correlated with TpTe interval. Vaseghi concludes that TpTe is a proarrhythmic ECG marker related to the underlying myocardial mechanisms that explain the proarrhythmia.

Recently, Srinivasan and co-authors also examined the association between intracardiac ventricular repolarization and the T wave on the body surface ECG. They positively concluded that the precordial ECG repolarization indices reflect regional repolarization differences between the right and left heart [128]. Interestingly, this article triggered a debate among the scientific community. On behalf of the e-Rhythm group of EHRA, a group of scientists stated that "Electrocardiography is certainly a very valuable tool and we believe that important information in ECG signals remains to be deciphered including assessment of repolarization heterogeneity in terms of both spatial and temporal dispersion. Nevertheless, it seems rather unlikely that obtaining such information could be based only on simple measurements made with the naked eye and ruler" [129].

We cannot conclude this paragraph without showing a superimposed view ("butterfly") of a 12-lead ECG in a normal subject and in a subject with a long QT syndrome type 2. Figure 2.6 shows interlead variation of the Tpeak location supporting the concerns of the e-Rhythm group. It is also fair to recall an excellent editorial from Pentti Rautaharju "QT and dispersion of ventricular repolarization: the greatest fallacy in electrocardiography in the 1990s" [130].

### Beat-to-Beat Evaluation

In 2014, the European Heart Rhythm Association (EHRA) together with the ESC Working Group on Cardiac Cellular Electrophysiology released a consensus guideline concerning the beat-to-beat QT interval variability (QTV) measurement, physiological background, and clinical utility [131].

From this consensus report, we have selected and quote the three main points related to neuromodulation of ventricular repolarization and arrhythmogenesis.

- Actually, there are many remaining technical issues: "While dedicated computer programs for QTV measurement are readily available, the current level QTV measurement standardization is insufficient. Data acquisition requirements, minimum signal-to-noise levels, recording duration, pre-processing modalities, and beat and artefact rejection techniques require further investigation."
- Then regarding neuromodulation of QTV: "The physiological basis of QTV is currently insufficiently explored. Although evidence suggests that QTV may be useful for quantifying relative changes in sympathetic ventricular outflow during states of heightened activity, it remains to be established whether QTV indices can be used to infer absolute values of sympathetic activity in normal subjects or whether QTV magnitude correlates with changes of sympathetic activity only. Future investigations should differentiate neural control directed to the sinus node from that directed to ventricles and research how this regulation contributes to the coupling/decoupling between heart rate variability and QTV."
- Finally, regarding clinical applications: "The pathophysiology of increased QTV is poorly understood, although reduced repolarization

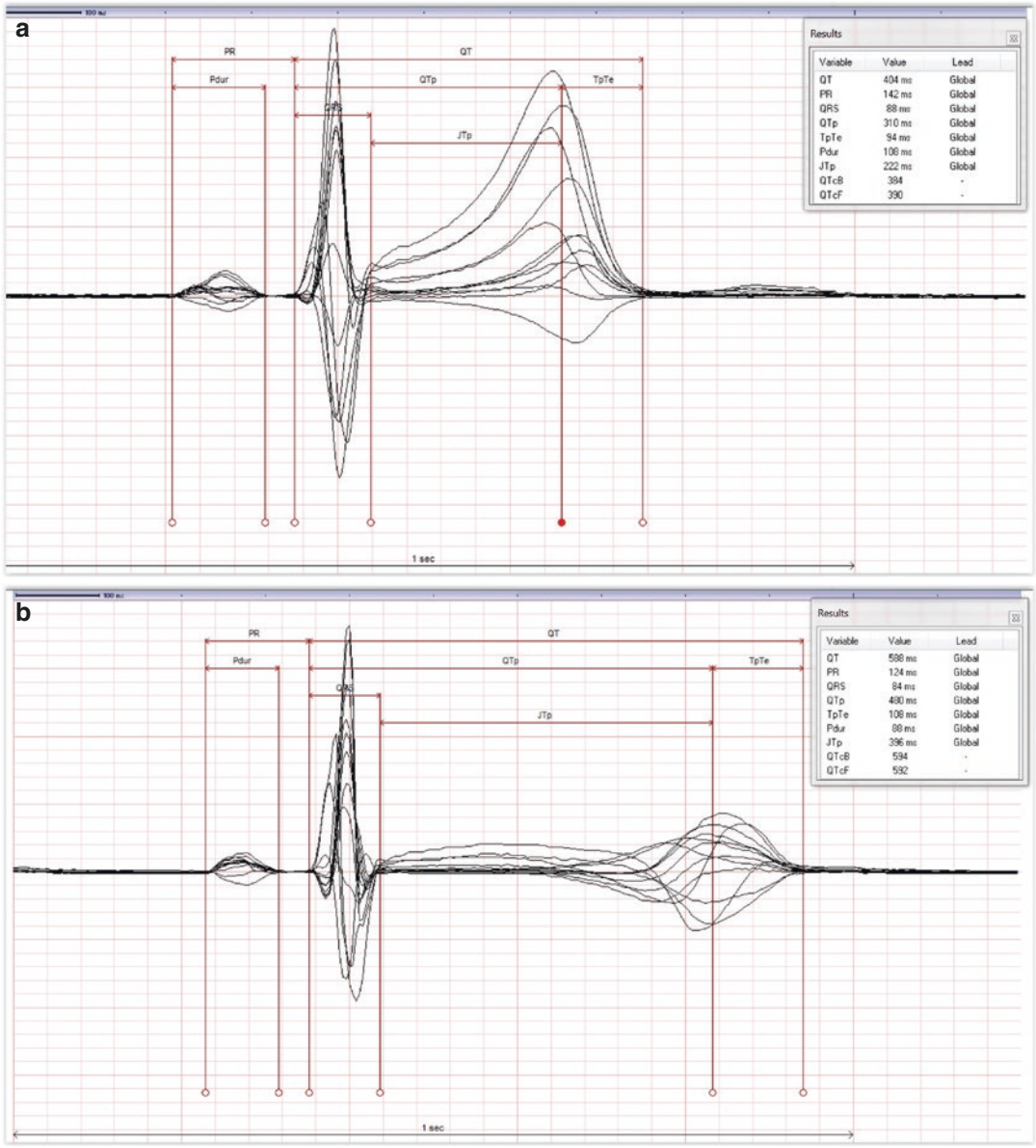


**Fig. 2.6** The 12-lead superimposed view ("butterfly") from two digital 10-second ECG. ECG collected in a normal subject (panel **a**) and in a LQTS patient (panel **b**). Tables on the right show results calculated from the vertical cursor position. The interlead variation of the Tpeak position is large in B as well as in A

reserve, causing more variable regulation responses, may play a role. The relation between autonomic dysfunction and QTV in cardiac patients is not well established. The main clinical use of QTV may lie in SCD risk stratification. Although several studies have demonstrated independent predictive value of QTV, most of the evidence is based on retrospective data analyze. Prospective trials are needed to prove the usefulness of QTV."

To the best of our knowledge, the somewhat negative comment released in 2014 remains valid in 2019.

## Clinical Syndromes

Several acquired cardiomyopathies and inherited channelopathies, including long QT syndrome (LQTS), catecholaminergic polymorphic ventricular cardiomyopathy (CPVT), hypertrophic cardiomyopathy (HCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC), are associated with exercise-related sudden cardiac death. Syncope and cardiac arrest occur during exercise because of ventricular arrhythmias, which are mainly managed with oral β-blockade. The transvenous ICD has been in clinical use for >3 decades, and robust data from high-quality RCTs support its use in various patient populations including survivors of cardiac arrest, patients with VT and structural heart disease, and patients with significant LV dysfunction.

In this chapter, we will expose the main clinical data supporting neuromodulation of ventricular arrhythmias (Table 2.2). In particular, these are as follows:

- The mode of onset of spontaneous ventricular tachyarrhythmias and their circadian distribution, during the day, or at night, and during exercise, or at rest, at fast or low heart rates
- The daily timing of ICD therapy, early morning or at night, and the weekday peaks
- And finally, the effects of pharmacologic beta-blockade

Autonomic influences on arrhythmogenesis are not limited to VR changes. Calcium handling, DADs, conduction velocity, and rate-dependent conduction block are also modulated by ANS. But at bedside, there is no easy way of dealing with the respective contribution of each arrhythmic mechanism.

## Neuromodulation of Cardiac Arrhythmias in Ischemic and Nonischemic Cardiomyopathy

There has been accumulating evidence that the sympathetic nervous system plays a considerable role in heart failure patients as well as in patients after myocardial infarction [132]. Because of their excellent safety profile and effectiveness in treating ventricular arrhythmias and reducing the risk of sudden cardiac death, beta-blockers are often the first-line antiarrhythmic therapy [84, 133–136]. Their antiarrhythmic efficacy is related to the effects of adrenergic-receptor blockade on sympathetically mediated triggering mechanisms.

**Table 2.2** Neuromodulation of ventricular arrhythmias: clinical features

| Clinical syndrome | Onset mode | Circadian pattern | Beta-blocker efficacy | Cardiac denervation |
|---|---|---|---|---|
| ICM, NICM | Exercise<br>Mental stress<br>Tachycardia<br>HRV decrease | Early morning<br>Monday | +++ | +++<br>(electrical storm) |
| Type 1 LQTS | Exercise<br>Stress<br>Tachycardia<br>Swimming | Unknown | +++ | ++ |
| Type 2 LQTS | Exercise<br>Stress<br>Tachycardia<br>Auditory stimulus | Unknown | ++ | ++ |
| Type 3 LQTS | Rest<br>Bradycardia<br>Pause | Nocturnal | ± | – |
| Short QTS | None | None | ± | – |
| Brugada syndrome | Bradycardia<br>Meal<br>Fever | Nocturnal | –<br>Isoproterenol + | – |
| ERS | Bradycardia | Nocturnal | –<br>Isoproterenol + | – |

Sudden death and implantable cardioverter-defibrillator (ICD) shocks have been reported to occur with higher frequency in the early morning hours together with increased event rates on Mondays, in both ischemic and nonischemic cardiomyopathies. Mechanisms such as physical activity or mental stress that could stimulate sympathetic activity or cause ischemia were suggested to account for the morning peak and increase in events on Mondays. The higher risk for sudden death in the first hours after awakening may in part be due to the morning increase in blood pressure and heart rate, increased vascular tone, changes in heart rate variability, elevated blood viscosity, and platelet aggregability.

Analysis of the timing of ICD therapy for life-threatening ventricular arrhythmias in the Sudden Cardiac Death-Heart Failure Trial (SCD-HeFT) cohort also confirmed that the time distribution of ICD therapies shows an early morning nadir in occurrence of ventricular arrhythmia, but no overall typical morning peak or Monday increase in events. However, subjects not on beta-blocker therapy demonstrated an increased event rate on Mondays. These findings may indicate suppression of neurohormonal triggers with current cardiac therapies. This has also led to the hypothesis that a subset of episodes of ventricular arrhythmias and sudden cardiac death corresponding to these circadian surges may be aborted by treating patients with beta-blockers [137].

The Oregon SUDS is an ongoing, prospective community-based study of out-of-hospital SCA in the Portland, Oregon, metropolitan area. In brief, cases of SCA are identified prospectively from among all residents of the region. For this analysis, cases identified between February 1, 2002, and January 31, 2014, were included. The authors did not observe a morning peak in SCA in this community-based prospective observational study. Additionally, during the week, they did not observe any peak of SCA occurrence on Mondays. Instead, they found a nadir in SCA occurrence on Sundays. Although the exact mechanisms need further investigation, the authors speculated about a potential interference of the Internet and smartphones use, which allow any activities 24 hours a day, 7 days a week [138].

A direct temporal relationship has been established between sympathetic activity and spontaneous ventricular tachycardia using heart rate and as a surrogate of sympathetic tone. Results regarding heart rate variability are more conflicting. Shusterman reported that before the onset of VT recorded in Holter recordings, heart rate increases, whereas index of heart rate variability decreases [139]. The HAWAI registry evaluated the role of sinus tachycardia and heart rate variability in predicting the occurrence of ventricular tachycardia and fibrillation (VT/VF) in patients with an implantable cardioverter-defibrillator [140]. A significant decrease of the mean value of all RR intervals was observed in the period prior to VT. The standard deviation of RR intervals (SDNN) and the power at low frequency (LF) also showed significant changes prior to VT/VF. Au-Yeung explored the use of ICD data to automatically predict ventricular arrhythmia using heart rate variability in patients enrolled in the ICD arm of the Sudden Cardiac Death-Heart Failure Trial (SCD-HeFT). The AUC for the prediction models were above 0.8, and the authors conclude that moderate classification accuracy can be achieved to predict ventricular tachyarrhythmia with machine learning algorithms using HRV features from ICD data [141].

Finally, the results of sympathetic neural ablation procedures in patients with ventricular tachyarrhythmias. This will be discussed in the section "Antiarrhythmic Neuromodulations of VR: beyond oral Beta-adrenergic blockade".

## Congenital Long QT Syndrome

The diagnosis of LQTS is made clinically by combining patient and family history and the 12-lead ECG. The LQTS syndrome is caused by mutations in any of 17 LQTS genes, but the most common genotypes are LQT1, 2, and 3, and here we will only discuss the neuromodulation of those three genotypes. On the surface ECG, a prolonged QT interval is key [142–145].

LQT1 is the most common, representing 30–35% of all congenital LQTS. The hallmark of LQT1 is the failure of the QT interval to adapt to increases in heart rate, as often seen during diagnostic exercise stress testing [144, 145]. Cardiac

events in patients with IKs-associated LQTS are often triggered by stress and exercise [144, 145]. β-Blockers have been demonstrated as a particularly effective therapy for LQT1 patients, who are more sensitive to stress- and exercise-induced arrhythmia than other LQT subtypes [144–146]. The stronger the blockade, the stronger the clinical efficacy [147].

However, the efficacy of β-blockers is strongly genotype-specific. Adrenergic trigger seems less important in LQT2, and events more frequently occur during sleep or at rest in type 3 LQTS [148, 149]. Occurrence of arrhythmic events with bradycardia, as well as potential concomitant Brugada syndrome, has raised concerns about the use of β-blockers in LQT3 patients [148, 149]. β-Blockers produce greatest symptom relief and cardiac event reduction in LQT1 patients, followed by LQT2 patients, and are least effective in LQT3 patients [26, 148, 149]. The efficacy of beta-blockade in LQT1 and LQT2 is not related to the effects on QTc interval duration. Actually, we reported that the effect of beta-adrenergic blockade on QTc duration is different in LQT1 and LQT2 patients. In LQT1 patients, the beneficial effect of beta-blockade is associated with a prolongation of QTc duration, whereas beta-blockade therapy in LQT2 leads to QTc shortening [90].

In selected cases, left cervicothoracic sympathetic denervation (LCSD), which surgically interrupts adrenergic input to the heart, can be considered. This will be discussed in depth in section "Vagus Nerve Stimulation".

## Congenital Short QT Syndrome

Short QT syndrome (SQTS) is a rare disease known for less than 20 years. It is an inherited cardiac channelopathy characterized by an abnormally short QT interval and increased risk for atrial and ventricular arrhythmias. Diagnosis is based on the evaluation of symptoms (syncope or cardiac arrest), family history, and electrocardiogram (ECG) findings [150–152].

In SQTS, arrhythmic events occur both at rest and during effort, and as of today, it is not possible to identify a consistent trigger for those events. There is lack of data to support a clinically significant neuromodulation of ventricular repolarization on SQTS patients [150–152]. In the study published by Mazzanti and co-authors [153], 20 patients experienced cardiac arrest, of whom 10 had multiple events. A total of 25 events occurred during rest, sleep, or quiet routine activities, 3 during emotional stress, and 2 during effort. The arrhythmic triggers in SQTS appear more similar to Brugada syndrome (see below).

Based on the family history of individuals with SQTS and their relatives, early studies of patients with SQTS suggested a very high risk of SCD. An ICD was considered the first-choice therapy, but alternative therapy was necessary, especially in small children [29, 153, 154]. The first clinical results of testing of hydroquinidine as a QT-prolonging drug and reducing arrhythmic events in patients with SQTS were reported as early as 2006 [155, 156]. So far, treatment with hydroquinidine remains to be associated with a lower incidence of arrhythmic events, and the therapy seems to be safe [153].

## J-Wave Syndromes

Both Brugada syndrome (BrS) and early repolarization syndrome (ERS) are clinical syndromes occurring in subjects with no apparent structural heart disease but affected with severe ventricular arrhythmias, ventricular fibrillation (VF) and polymorphic ventricular tachycardia, and sudden cardiac death [145, 157, 158].

Among clinical similarities in BrS and ERS, there is a male predominance in both syndromes, with a peak incidence of VF or sudden cardiac death occurring in the third or fourth decade of life. Most interestingly for this review on neuromodulation of arrhythmogenesis, the accentuated J waves and ST-segment elevation are generally associated with bradycardia or pauses, and VF events have been proposed to occur mostly during sleep or at low levels of physical activity [145, 157, 158].

The pathophysiological mechanisms underlying the J-wave syndromes remain a matter of debate. Sympathovagal balance, among other factors, modulates not only ECG morphology in BrS but could also explain the development of ventricular arrhythmias under certain conditions [159]. Analysis of the mode of onset and

circadian distribution of spontaneous episodes of ventricular tachyarrhythmia indicates that the appearance of accentuated J waves and ST-segment elevation is generally associated with bradycardia or pauses. This can explain why VF in both syndromes often occurs during sleep or during a low level of physical activities [145, 157, 158].

Kamakura investigated the onset mode and circadian distribution of ventricular tachyarrhythmia episodes in ERS and BrS, respectively. They reported that most VTAs occurred nocturnally in patients with ERS and BrS [160, 161]. Kim reported similar findings in patients who underwent implantable cardioverter-defibrillator implantation. Circadian distribution of appropriate shocks showed a significant nocturnal peak in patients with ERS and a nonsignificant trend toward a nocturnal peak in patients with BrS [162]. Ikeda in patients with a Brugada-type ECG assessed 12-lead ECGs before and after a large meal (full stomach test). The full stomach test was positive in 17 of the study patients. A positive test outcome was characterized by a higher incidence of a history of life-threatening events [163]. Mizumaki evaluated the relationship between augmentation of J-wave elevation and changes in RR interval or autonomic nervous activities in patients with idiopathic VF. The authors concluded that J-wave elevation was more strongly augmented during bradycardia and was associated with an increase in vagal activity [164]. Aizawa assessed the circadian pattern of VF occurrence in 64 patients with idiopathic ventricular fibrillation, excluding Brugada syndrome. The overall distribution pattern of VF occurrence showed two peaks at approximately 6:00 AM and around 8:00 PM. Nocturnal VF was observed in 20 patients (31.3%), and J waves were present in 14 of these 20 individuals (70.0%), whereas J waves were less frequent in the 44 non-nocturnal patients with VF. The authors concluded that the presence of J waves may characterize a higher nocturnal incidence of VF and a higher acute and chronic risk of recurrence [165].

### Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

The CPVT is the most caricatural presentation of the link between exercise and/or mental stress and ventricular arrhythmias leading to syncope and SCD [166]. Key clinical features include 1) bidirectional ventricular premature beats or Tachycardia, 2) normal resting ECG, 3) structurally normal heart, 4) occurrence of arrhythmias and symptoms (syncope or cardiac arrest) under increased sympathetic activity conditions, 5) first symptoms during the 2 first decades of life [167]. CPVT is a genetic disease, and all the mutations found are located in genes coding for proteins involved in reticulum calcium handling [168, 169].

The mechanism of arrhythmias is believed to be related to diastolic calcium leakage from the sarcoplasmic reticulum, with a subsequent increase in the cytosolic calcium concentration leading to electrogenic sodium/calcium exchanger activation and DADs [170, 171]. Accordingly, VR is not a major player in arrhythmogenesis in CPVT. It was, however, difficult not to reference CPVT syndrome in this review.

Beta-blocker treatment is pivotal, although not fully effective, in CPVT management, but more recently sodium channel blockade has been shown to improve outcome [172–174].

## Antiarrhythmic Neuromodulations of Ventricular Repolarization: Beyond Oral Beta-Adrenergic Blockade

Despite medications and ICD, a significant proportion of patients with heart disease continue to experience arrhythmias. Non-pharmacological intervention such as catheter ablation can be useful for reducing recurrent VT and ICD shocks [175]. Non-pharmacologic modulation of the autonomic nervous system for the purpose of preventing arrhythmias is an emerging new therapeutic modality [84].

## Modulation of the Sympathetic Nervous System

The autonomic modulation can be achieved either through interruption of sympathetic outflow to the heart or through stimulation of the parasympathetic pathway [84]. Surgical autonomic modulation has proven efficacy for certain long QT syndrome and catecholaminergic polymorphic ventricular tachycardia [84].

### Cardiac Sympathetic Denervation

Early studies in anginal patients treated with sympathectomy were highly successful in preventing anginal attacks and improving performance during an exercise stress test. But with the availability of oral β-blockers, sympathectomy became outdated despite its clear efficacy [176]. Interest for the antiarrhythmic potential has been rapidly expanding. The antifibrillatory effect of left cardiac sympathetic denervation (CSD) has clearly been demonstrated in LQTS patients and in patients with catecholaminergic polymorphic ventricular tachycardia [84, 145, 177–181]. Traditionally, a thoracotomy or cervical incision has been used as the standard surgical approach for performing left cardiac sympathetic denervation, but more recently video-assisted thoracoscopic surgery allows a minimally invasive technique [182].

Early studies demonstrated that only left stellate ganglion block increased the ventricular fibrillation threshold and protected against ischemic ventricular arrhythmia. Usually it has been admitted that blockade of the right stellate ganglion had a potentially arrhythmogenic effect. The different effects of left versus right cardiac sympathetic denervation were explained by the fact that the left-sided cardiac sympathetic nerves are quantitatively dominant at the ventricular level. However, bilateral cardiac sympathetic denervation does appear to be at least non-inferior and potentially superior to left CSD for treatment of ventricular arrhythmia in humans [183, 184].

For cardiovascular disease, CSD has been used primarily as a treatment modality in young adults and children with life-threatening arrhythmias due to inherited channelopathies, such as LQTS and CPVT [145]. Recent international guidelines recommend left CSD for LQTS and CPVT when ICD or beta-blocker therapy is not able to be implemented effectively [84]. The value of CSD for ventricular arrhythmias secondary to ischemia and ischemic cardiomyopathy is less well established, but more recently, a significant decrease in ICD shocks in patients with refractory ventricular arrhythmias following either left or bilateral CSD has been reported [185, 186]. Efficacy has also been demonstrated for general anesthesia in the acute management of refractory ventricular arrhythmias [187].

### Cardiac Sympathetic Blockade

Cardiac sympathetic denervation is typically a surgical resection of the lower half of the stellate ganglion (thoracotomy or cervical incision and more recently video-assisted thoracoscopic surgery), but another approach of neuromodulation is stellate ganglion block, so-called stellate ganglion block (SGB), performed by injecting local anesthetic agents percutaneously to stellate ganglion, which is less invasive than CSD and can be performed at bedside in emergent setting in patients with hemodynamic instability [188–191]. To better understand the role of SGB in electrical storms, Meng and co-authors performed dedicated literature searches [189]. SGB was achieved by administering local anesthetic percutaneously to the SG. Most patients only had left SGB and in few of them both left and right SGB. The major findings of this review on the efficacy of SGB for electrical storm are threefold: GB is effective in reducing the number episodes and therapies for VA; this efficacy was independent of the subtype of ventricular arrhythmia, the presence or absence of cardiomyopathy, and the degree of LV dysfunction in the patients studied.

## Vagus Nerve Stimulation

The finding that decreased vagal activity could be as important as increased sympathetic activity in jeopardizing cardiovascular morbidity and mortality drew attention to the possibility to implement the enhancement of vagal tone in clinical practice. However, it is fair to admit that there are few drugs that can be used for vagus nerve activation in the clinic.

Endurance exercise training is well established to modify autonomic nervous system balance, with a coupling effect, increase in cardiac parasympathetic tone and decrease in sympathetic activity [192, 193]. The effects of endurance exercise training exercise on ventricular repolarization abnormalities have not been extensively investigated. Some data suggest that endurance exercise training can reduce ischemically induced heterogeneities in repolarization and thereby could protect against ventricular fibrillation [192, 193].

By increasing parasympathetic drive, direct vagal nerve stimulation (VNS) has been shown to have beneficial cardiac effects. VNS reduces slope of APD restitution, lengthen ventricular refractory periods, and raise VF threshold in various animal models including rats, rabbits, pigs, cats, and dogs [194, 195]. A reduction in sudden cardiac death from ventricular arrhythmias has been demonstrated with vagal stimulation in a healed infarct canine model subjected to repeat ischemia. Vanoli showed that direct electrical vagal stimulation is feasible in conscious animals and that, when commenced shortly after the onset of an episode of acute myocardial ischemia, it provides significant protection from ventricular fibrillation [196].

Recently VNS was also shown to reduce ventricular tachyarrhythmia inducibility in the setting of chronic myocardial infarction. Kolman studied the effect of VNS upon ventricular vulnerability in 30 mongrel dogs subjected to varying levels of adrenergic stimulation [197]. Vulnerability was assessed both by determining the minimum current required to produce ventricular fibrillation (VF threshold). VNS was without significant effect on the VF threshold, but during left SGS, however, VNS was associated with a 93% increase in VF threshold. After beta-adrenergic blockade with propranolol, VNS was without effect on VF threshold. Authors concluded that increased sympathetic tone is a precondition for a VNS-induced elevation in VF threshold. According to Kolman, the vagal effect is indirect and is expressed by opposing the effects of heightened adrenergic tone on ventricular vulnerability [197].

In another study, Yamaguchi reported that the electrophysiological and hemodynamic effects of bilateral VNS were not mitigated by CSD, involving bilateral stellectomy and removal of T2–T4 thoracic ganglia. The authors concluded that much of the beneficial effects of VNS are driven by activation of efferent vagal fibers acting directly at the neural-myocyte interface rather than through central sympathetic inhibition. Furthermore, CSD and VNS could have synergistic effects in the treatment of ventricular arrhythmias [198].

The first human cardiac application was described in eight patients for the indication of heart failure using CardioFit stimulators [199]. Subsequent human trials for heart failure have shown conflicting results. ANTHEM-HF, a nonblinded trial for NYHA II–III patients with EF <40%, showed improvements in NYHA class and EF [200]. NECTAR-HF was a randomized blinded study, which showed no improvements with VNS with respect to objective parameters, such as EF, but improved clinical parameters such as NYHA class [201]. INOVATE-HF was a randomized study that further showed no benefit of mortality or worsening HF in NYHA III patients with EF ≤40% [202]. As mentioned above, the vago-sympathetic trunk contains both parasympathetic and sympathetic and afferent and efferent nerves. Different stimulation parameters can differentially engage these fibers, and the stimulation parameters used can significantly affect the outcomes of VNS and may account for the mixed human clinical trial results. With better characterization of the optimal dose of stimulation, VNS could remain a promising option to apply to reduce VT/VF.

## A Black Cat Crossing the Path: Isoproterenol as Antiarrhythmic Drug

Although adrenergic stimulation is proarrhythmic in most cases, the antiarrhythmic effects of beta-adrenergic stimulation deserve to be described.

The chronotropic effect of beta-adrenergic stimulation has long been used to prevent ventricular arrhythmias arising during severe bradycardia [203]. Bradycardia-induced QT prolongation and drug-induced torsades de pointes are the hallmarks of the beneficial effects of adrenergic stimulation that can be found in any cardiology textbook. It is however important to underline that isoproterenol may promote torsades de pointes immediately after the start of the infusion, before heart rate acceleration, and when QT prolongation is related to a congenital form (specifically, type 1 LQTS).

More recently, the description of arrhythmia mechanisms related to abnormal epicardial ventricular repolarization in Brugada syndrome models has provided a rationale for the use of adrenergic stimulation in order to prevent arrhythmias [56]. Specifically, the adrenergic-induced increase in ICaL will counterbalance the prominent Ito current, hence preventing the early repolarization at the base of both phase 2 reentry and increased spatial dispersion of ventricular repolarization duration.

The efficacy in clinical practice has been subsequently documented in patients with a Brugada syndrome during electrical storms [204, 205].

Isoproterenol infusion has also been shown to prevent VF recurrences during electrical storms in patients with the early repolarization syndrome (ERS) [206]. The efficacy of adrenergic stimulation in ERS patients supports a similar pathophysiological background with the Brugada syndrome and the so-called repolarization hypothesis as the underlying mechanism of arrhythmias in J-wave syndromes [207].

Finally, isoproterenol has been also reported to prevent ventricular arrhythmia recurrences in the short QT syndrome [208]. The underlying mechanism remains to be determined.

## Future Aspects

Renal denervation has been proposed as a way to decreased central sympathetic activity, initially to decrease blood pressure in patients with refractory hypertension. It has also shown antiarrhythmic effects at the ventricular level in different animal models as well as in limited cases in patients [209–212]. This approach definitely warrants further validation.

Spinal cord stimulation reduces stellate ganglia activity and increases vagal tone. It has been shown to reduce regional myocardial sympathoexcitation, decrease ventricular arrhythmias, and improve myocardial function in a porcine model of acute ventricular ischemia [213]. Clinical evaluation in patients with reduced left ventricular ejection fraction heart failure showed however no improvement in ejection fraction [214].

Recent experimental studies have addressed the proarrhythmic consequences of heterogeneous spatial sympathetic innervation after myocardial infarction. Both innervation restoration [215] and homogenization by local denervation [216] have been shown to prevent ventricular arrhythmias. These innovative approaches underline the complexity of electrophysiologic effects of cardiac innervation [217], the gap in understanding but also the opportunity for future experimental research that will hopefully lead to clinical validation.

## Conclusions

Adrenergic stimulation strongly influences ventricular repolarization with a proarrhythmic potential. Repolarization changes are not a single player, and changes in calcium handling induced by adrenergic stimulation are also potent proarrhythmic factors. However, as illustrated with action potential alternans mechanisms, these two determinants interact each other [218–220]. We still probably fail to reach a deep understanding of the complex relationship between repolarization dynamics and calcium handling, in the

search to elucidate the mechanisms and timing leading to ventricular fibrillation.

Despite the flaws in our understanding, counteracting adrenergic stimulation by using beta-blocking drugs has been one of the best success stories in cardiology. Indeed, as stated by Douglas Zipes: "the greatest reduction in cardiovascular mortality (including SCD) in patients with clinically manifest heart disease has resulted from the use of beta blockers and non-antiarrhythmic drugs" [38, 221].

The strong guideline recommendation and widespread use of beta-blockers in patients with cardiomyopathies do not mean that the proarrhythmic potential of adrenergic stimulation has been solved. Conversely, the concept of "the 'adrenergic paradox' that implies that "the more marked the autonomic nervous system dependence of tachyarrhythmias, the less obvious its evidence [222]" is strongly supported by the already proven efficacy of non-pharmacological reduction in sympathetic stimulation described above. Improving our understanding and ability to interfere with the complex tuning of the autonomic nervous system is most probably an avenue to further decrease the burden of sudden cardiac death.

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# 3 Repolarization Remodeling in Structural Heart Disease

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## Introduction

Heart failure (HF) is highly prevalent, affecting 5.7 million adults in the United States alone, and the incidence and prevalence has continued to increase with the aging of the population [1]. Despite remarkable improvements in medical therapy, the prognosis of patients with heart failure remains poor with almost 50% of patients dying within 5 years of initial diagnosis. Of the deaths in patients with heart failure, up to 50% are sudden and unexpected. In the Framingham Study, HF was associated with a 2.6- to 6.2-fold increased risk of sudden cardiac death (SCD) [2].

Increased electrical instability of the failing heart is multifactorial, and, depending on etiology, different mechanisms including ischemia in coronary heart disease or structural alterations such as fibrosis or myocardial scarring after myocardial infarction may be prominent. However, one of the most important predictors of SCD is depressed left ventricular systolic function [3, 4], suggesting that, despite etiological heterogeneity, common mechanisms are involved in the propensity of failing myocardium to develop potentially fatal arrhythmias. Altered intracellular and transmembrane calcium handling [5, 6], stretch-induced mechanisms due to altered ventricular loading conditions [7, 8], and changes in myocyte electrical [9, 10] and metabolic [11, 12] properties have been implicated in the increased electrical instability of the failing heart. Several lines of evidence indicate an important role of abnormalities in repolarization for the increased risk of SCD in HF. Prolongation of action potential duration (APD), one of the most consistent findings in myocytes from hypertrophied and failing hearts is associated with exaggerated lability of the repolarization process [10, 13]. In addition to alteration of individual currents, spatial and temporal inhomogeneity of repolarization, measured as dispersion of repolarization [14], has been described in patients with increased risk of SCD. This review is to give an overview on available evidence of alterations of ion currents that mediate cardiac repolarization in cardiac hypertrophy and heart failure.

## Repolarization: A Labile Process

An elementary and distinctive signature of any given excitable tissue is its action potential profile. Myocardial cells possess a characteristically long action potential (Fig. 3.1). After an initial rapid upstroke, there is a plateau of maintained depolarization before repolarization occurs. APD prolongation in left ventricular hypertrophy or failure is caused by either a decrease in outward

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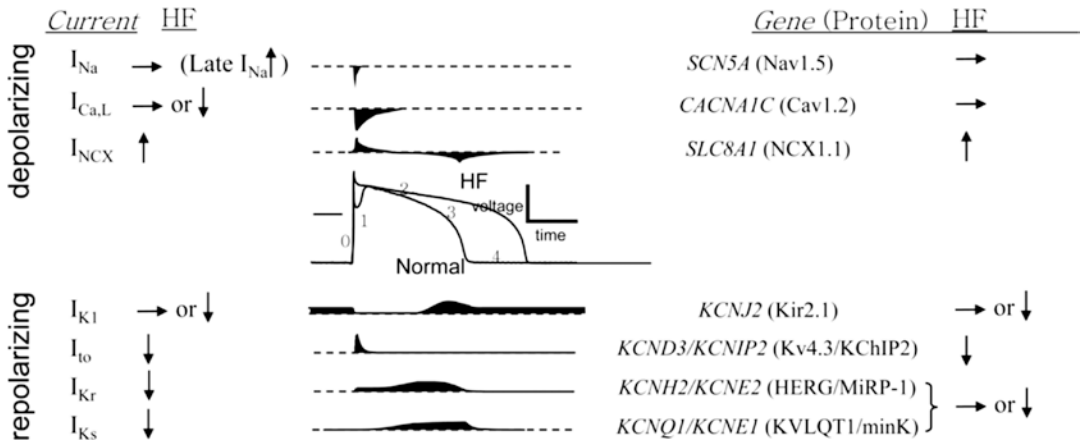


**Fig. 3.1** Schematic of inward and outward currents, pumps, and exchanges which inscribed the ventricular action potential. A schematic of the time course of each current is shown on the left, while the gene product that underlies the current is shown on the right. (Adapted from Tomaselli and Marbán [9], by permission of Oxford University Press)

(mostly potassium) currents or an increase in depolarizing inward currents (sodium or calcium; Fig. 3.1). In animal models of pressure overload-induced HF, this prolongation is progressive and generally occurs in the absence of other electrophysiological abnormalities such as changes in resting membrane potential, action potential amplitude, or upstroke velocity [10]. The plateau phase of the action potential is known to be quite labile: this is a time of high membrane resistance during which small changes in current can easily tip the balance either toward repolarization or toward maintained depolarization. In general, the longer the action potential, the more labile is the repolarization process. This lability may be manifest as variability in duration and/or secondary depolarizations that interrupt action potential repolarization, including early afterdepolarizations (EADs) that can initiate torsade de pointes.

In addition to action potential prolongation on a cellular level, regional differences in the action potential duration represent an important aspect of arrhythmogenesis in hypertrophy and heart failure. Action potential durations vary across the myocardial wall [15–17] and in different regions [18] of the mammalian heart. Data from experimental animal models of hypertrophy and human heart failure suggest regional inhomogeneity in action potential prolongation [19, 20]. The finding of enhanced spatial and temporal dispersion of APD, refractoriness, and electrocardiographic QT intervals in humans [21, 22] and animals with heart failure [23] is consistent with an exaggerated dispersion of action potential duration. Heterogeneous APD prolongation within the ventricular wall amplifies dispersion of repolarization, an established mechanism contributing to reentry.

Regional differences in electrical properties also play an important role in dyssynchronous heart failure and cardiac resynchronization therapy (CRT) [24]. In a large animal model of dyssynchronous heart failure (DHF), action potential durations were significantly prolonged in DHF, especially in late-activated lateral vs. early-activated anterior cells, and CRT abbreviated action potential duration in lateral as compared to anterior cells [24]. Early afterdepolarizations were more frequent in DHF than in control cells and were reduced with CRT [24].

## Decrease in Outward Potassium Currents Leads to Prolongation of the Action Potential Duration

Downregulation of many repolarizing potassium currents during heart failure is well documented [9, 25]. While the major inward currents (sodium

and L-type calcium current) appear to be rather consistent among different species, a large diversity of potassium channels has been observed with expression of different potassium channel types and densities in different species and myocardial regions. Currently, more than 20 potassium channels are known to be expressed in the heart [26]. Thus, depending on the species studied, different channels may be involved in a similar phenotypic prolongation of the action potential in cardiac hypertrophy or failure. One of the potassium currents most consistently reduced in cardiac hypertrophy and heart failure is the transient outward potassium current ($I_{to}$).

## Transient Outward Potassium Current ($I_{to}$)

In ventricular myocytes and Purkinje fibers, the initial depolarization of the membrane potential is generated by $Na^+$ influx ($I_{Na}$) from coordinated opening of voltage-gated sodium channels. This depolarization rapidly activates the calcium-independent $I_{to}$ which is primarily responsible for phase 1 or notch of the action potential. $I_{to}$ then rapidly inactivates with a time constant of ~50 ms, thus, shaping the rapid phase 1 repolarization of the cardiac action potential and setting the height of the initial plateau (phase 2), thereby playing a key role in excitation-contraction (EC) coupling [27, 28], as well as in arrhythmogenesis [29].

Since $I_{to}$ is an early transient current, it may not directly affect the ventricular APD in large mammalian hearts as it does in rodent ventricle. $I_{to}$ downregulation is the most consistent change of any ion current in failing hearts [25, 30, 31]. $I_{to}$ displays a large transmural gradient with a three to fivefold higher current density and faster recovery from inactivation kinetics in the subepicardium compared to the subendocardium [15]. In human myocardium, $I_{to}$ is encoded by *KCND3* ($K_V4.3$) subunits in subepicardial and midmyocardial cell layers, while *KCNA4* encodes a slowly recovering current ($K_V1.4$) in the subendocardium, contributing to regional heterogeneities in action potential waveforms [32]. In non-failing human hearts, the transmural gradient of $I_{to}$ contributes to the slightly longer action potential duration in the subendocardium vs. subepicardium, which enables subendocardial myocytes to provide more coupling current to depolarize adjacent cells. As such, conduction of excitability is facilitated in the physiological direction of the endocardium to the epicardium but disfavored in the opposite direction [33]. Downregulation of $I_{to}$ in cells isolated from terminally failing human hearts affects mostly *KCND3* ($K_V4.3$) [30, 31], leading to selective downregulation of the $I_{to}$ in the subepicardium and midmyocardium and a diminished transmural gradient of $I_{to}$ in HF. Therefore, the directional preference of conduction from the subendocardium to the subepicardium is blunted due to a HF-related decline in the transmural gradient of $I_{to}$. As conduction of abnormal impulses originating in the subepicardium is relatively facilitated, these changes may contribute to proarrhythmia [33].

The molecular mechanism of $I_{to}$ downregulation in HF is likely to be multifactorial. Reduced steady-state levels of *KCND3* mRNA are highly correlated with functional downregulation of $I_{to}$ in human HF [24, 30, 31, 34, 35]. In a canine HF model, tachycardia downregulates $I_{to}$ expression, with the $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and calcineurin/NFAT systems playing key $Ca^{2+}$-sensing and signal-transducing roles in rate-dependent $I_{to}$ control [36].

## Delayed Rectifier Currents ($I_{Kr}$ and $I_{Ks}$)

The delayed rectifier $K^+$ currents $I_{Kr}$ and $I_{Ks}$ (encoded by *KCNH2/KCNE2* and *KCNQ1/KCNE1*, respectively) play a prominent role in the late phase of repolarization [37]; therefore changes in either the slow ($I_{Ks}$) or fast ($I_{Kr}$) activating components of this current could contribute significantly to prolongation of the APD in HF. Reduced $I_K$ density, slower activation, and faster deactivation kinetics have been observed in hypertrophied feline ventricles [38]. Downregulation of both $I_{Kr}$ and $I_{Ks}$ have

been reported in a rabbit model of rapid ventricular pacing HF [39], whereas $I_{Ks}$, but not $I_{Kr}$, was downregulated in all layers of the left ventricular myocardium in a canine model of tachypacing HF [40].

In human hearts, a greater inhibition of $I_{Kr}$ by the specific inhibitor E-4031 indexed by greater APD prolongation in perfused left ventricular wedges was observed in non-failing compared with wedges isolated from failing hearts [41]. The reduced functional $I_{Kr}$ expression was attributed to a shift in the ratio between the two protein isoforms of mammalian *KCNH2*/hERG1 channel subunits, hERG1a and hERG1b that conduct the $I_{Kr}$ current in the heart, as no differences in steady-state mRNA levels of hERG1a or hERG1b were found between non-failing and failing human hearts [41].

There is increasing evidence to suggest that the neurohormonal dysregulation observed in HF contributes to downregulation of $I_{Kr}$, as angiotensin II (Ang II) has been shown to produce an inhibitory effect on $I_{Kr}$ currents via angiotensin 1 receptors, linked to the PKC pathway in ventricular myocytes [42, 43]. Of note, Ang II has also been shown to inhibit $I_{Ks}$ in a concentration-dependent manner in native guinea pig cardiomyocytes [44].

Importantly, $K^+$ current changes are often measured in nonphysiological conditions (voltage clamp, low pacing rates, cytosolic $Ca^{2+}$ buffers). A recent study compared $I_{Kr}$, and $I_{Ks}$, and their $Ca^{2+}$- and β-adrenergic dependence in rabbit ventricular myocytes in chronic pressure/volume overload-induced HF (versus age-matched controls). Intriguingly, both $I_{Kr}$ and $I_{Ks}$ were significantly *upregulated* in HF under action potential clamp, but *only* when cytosolic $Ca^{2+}$ was not buffered. CaMKII ($Ca^{2+}$/calmodulin-dependent protein kinase II) inhibition abolished $I_{Ks}$ upregulation in HF, but it did not affect $I_{Kr}$. $I_{Ks}$ response to β-adrenergic stimulation was also significantly diminished in HF. Thus, under β-adrenergic stimulation, reduced $I_{Ks}$ responsiveness severely limits its integrated repolarizing $K^+$ current and repolarization reserve in HF. This would increase arrhythmia propensity in HF, especially during adrenergic stress [45].

### Inward Rectifier Current ($I_{K1}$)

Reduced inward $I_{K1}$ density in HF may contribute to prolongation of APD and enhanced susceptibility to spontaneous membrane depolarizations including delayed afterdepolarizations (DADs) [40, 45–47]. Changes in functional expression of $I_{K1}$ are more variable than $I_{to}$ and controversial. Even within the same experimental HF model (e.g., pacing-induced), inconsistencies have been observed across species: reduced $I_{K1}$ density in canine [40, 48] and rabbit [45], whereas other investigators did not observe changes in rabbit [49]. In terminal human HF, $I_{K1}$ is significantly reduced at negative voltages [30], but the underlying basis for such downregulation appears to be posttranscriptional in light of the absence of changes in the steady-state level of *KCNJ2*/Kir2.1 mRNA [31].

## Increase in Inward Current Leads to Prolongation of the APD

### Late Inward Sodium Current

The cardiac voltage-dependent $Na^+$ channel (SCN5A) generates a large inward current ($I_{Na}$) within the first milliseconds of excitation and mediates the upstroke of the cardiac action potential in ventricular myocardium. Inactivation of $I_{Na}$ has both fast and slow components, which range from several milliseconds to hundreds of milliseconds, respectively. The current associated with the slow inactivating phase has been referred to as late or persistent sodium current ($I_{Na,L}$) to distinguish it from the peak transient $I_{Na}$ [50]. In non-failing hearts, the amplitude of late $I_{Na}$ is less than 1% that of peak $I_{Na}$.

Whereas peak $I_{Na}$ has been reported to be decreased [51], unchanged [48], or increased [52–54] in animal models of HF, changes in $I_{Na,L}$ are more consistent, as $I_{Na,L}$ as a percentage of peak $I_{Na}$ can be significantly (up to tenfold) increased in HF due to a slower decay of $I_{Na,L}$ in failing canine and human hearts [55–57]. Therefore, increased $I_{Na,L}$ likely plays an important role in APD prolongation in heart failure and

may contribute to arrhythmogenesis. $I_{Na,L}$ inhibition decreased beat-to-beat AP variability and eliminated early afterdepolarizations in failing cardiomyocytes [57]. Likewise, dyssynchronous HF markedly increased $I_{Na,L}$ compared with nonfailing hearts. CRT dramatically reduced DHF-induced enhanced $I_{Na,L}$, abbreviated the APD, and suppressed early afterdepolarizations. CRT was associated with a global reduction in phosphorylated $Ca^{2+}$/calmodulin protein kinase II, which has distinct effects on inactivation of cardiac $Na^+$ channels [56].

## Altered $Ca^{2+}$ Currents and Calcium Homeostasis

Altered $Ca^{2+}$ homeostasis underlies abnormalities in excitation-contraction coupling and arrhythmic risk in HF. Intracellular $Ca^{2+}$ and APD are intricately linked by a variety of $Ca^{2+}$-mediated cell surface channels and transporters such as $I_{Ca\text{-}L}$, $I_K$, $Ca^{2+}$-activated Cl-current, and NCX. $I_{Ca\text{-}L}$ density is unchanged or reduced in HF, the latter typically occurring in more advanced disease [38]. In human HF, baseline $I_{Ca\text{-}L}$ density is consistently unchanged, although single channel studies suggest a reduction in channel number with an increase in open probability perhaps due to altered phosphorylation or subunit composition [58, 59].

The amplitude of the calcium transient (CaT) and its rate of decay are reduced in intact preparations and cells isolated from failing ventricles [60]. Systematic comparisons of the CaT profile and dynamics in cells isolated from different regions of the failing heart are limited. Sarcoplasmic reticulum $Ca^{2+}$-ATPase (*ATP2A2*/SERCA2A), its inhibitor phospholamban (PLN), and NCX are primary mediators of $Ca^{2+}$ removal from the cytoplasm. In HF, ventricular myocytes exhibit a greater reliance on NCX for removal of $Ca^{2+}$ from the cytosol and an increase in NCX function [61], which leads to defective sarcoplasmic $Ca^{2+}$ loading. Altered NCX function in HF significantly influences CaT and AP dynamics [62]. Specifically, a sodium outward current through the $Na^+$-$Ca^{2+}$ exchanger operating in reverse mode likely contributes to early repolarization. In canine hearts, administration of KB-R7943, a selective inhibitor of the reverse mode of the $Na^+/Ca^{2+}$ exchanger, significantly increased QTc value and APD and lead to action potential alternans [63].

## Repolarization: A Systems Biology View

Action potential prolongation, abnormal $Ca^{2+}$ handling, fibrosis, and aberrant adrenergic signaling are hallmarks of left myocardial hypertrophy and failure. In a canine HF model (tachycardia-induced cardiomyopathy), we noted that the prolongation of the APD occurred early during the development of HF and coincided with profound transcriptional changes characterized by the downregulation of metabolic transcripts (Fig. 3.2) [64]. We then studied transcriptional co-expression patterns of >130 myocardial ion channels, transporters, and pumps across a whole range of physiological and pathophysiological conditions in order to test the hypothesis that altered expression of these ion channel transcripts occurs in the context of coordinated changes in metabolic gene expression. In human myocardium, we found that major repolarizing (e.g., *KCNH2*) ion channels were positively correlated with transcriptional activity of the oxidative phosphorylation pathway.

The coordinated transcriptional expression of metabolic and ion channel genes suggests that many ion channel genes expressed in the myocardium share common promoter regulatory regions with metabolic genes. Specifically, we found that transcription factor binding sites for estrogen-related receptor alpha (*ESRRA*, a key regulatory partner of PGC1α, implicated in the downregulation of mitochondrial metabolic capacity in HF) and steroidogenic factor 1 (SF1 a member of the nuclear receptor family of intracellular transcription factors, encoded by the *NR5A1* gene) are shared by metabolic genes and major myocardial ion channel genes [65].

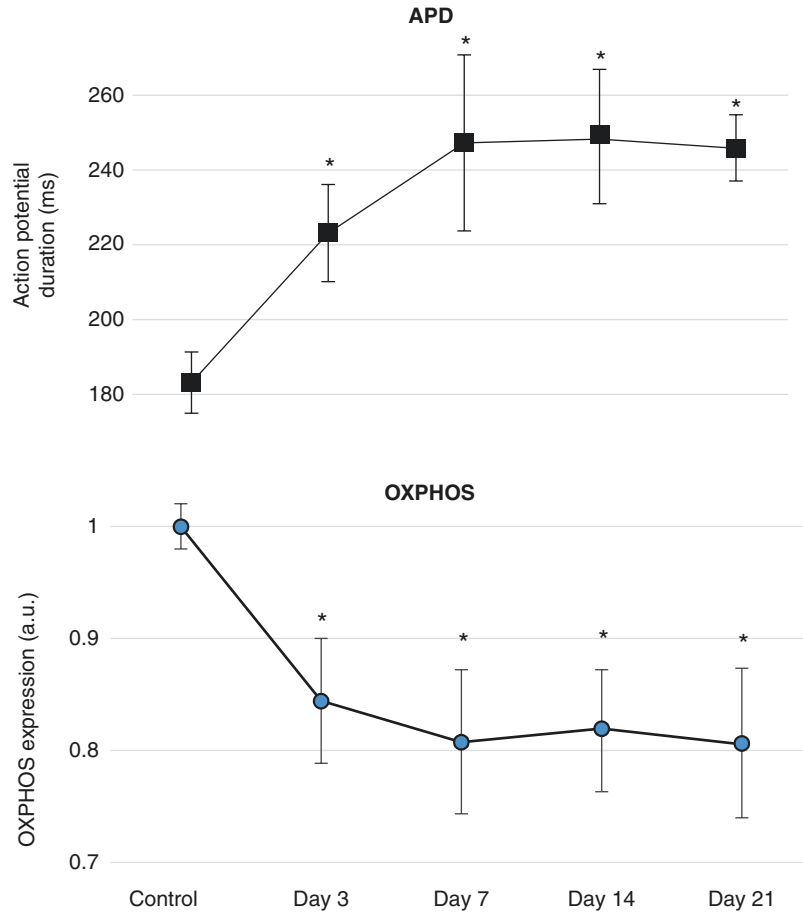


**Fig. 3.2** Temporal correlation between APD prolongation (top) and expression of transcripts of oxidative phosphorylation (OXPHOS, bottom) during HF progression. ∗ indicates $p < 0.05$ compared to control. (Adapted from data in [64])

## Conclusion

Downregulation of potassium currents and upregulation of inward currents delay repolarization and can give rise to early afterdepolarizations and increased repolarization heterogeneity in different cell layers, thereby creating a substrate for ventricular tachyarrhythmia. A variety of extracardiac conditions (hypokalemia, antiarrhythmic drugs, autonomic tone) further reduce repolarization reserve and increase the arrhythmic risk in patients with organic heart disease. Antiarrhythmic pharmacotherapy targeting specific ion channel activity has shown promising antiarrhythmic effects in the basic science laboratory, but has not translated into clinical effectiveness due to proarrhythmia. Recent insights into complex mechanisms of electrical remodeling have raised the prospect of targeting disease-related events upstream of ion channels which constitute the final common pathway of electrical activity. In fact, one of the most effective antiarrhythmic strategies so far has been targeting the early processes of the complex cascade leading to sudden arrhythmic death by non-antiarrhythmic drugs (i.e., beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor-blocker agents, and aldosterone antagonists). As our understanding of the role of the cellular context of cardiac repolarization continues to evolve, these advances are likely to lead to better treatment of ventricular arrhythmias.

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# 4 Cardiac Repolarization and Stem Cells: An Emerging Path Toward Precision Medicine

Massimiliano Gnecchi, Luca Sala, and Peter J. Schwartz

## Introduction

The days when classic textbooks were sanctimoniously preaching that "the measurement of the QT interval has no clinical value" [1] are forever gone. The recognition that prolongation of the QT interval, as a marker of ventricular repolarization, is associated with an increased risk for sudden death—both in young patients with a genetic disorder [2, 3] and in patients surviving a myocardial infarction [4]—has determined the shift by which, during the last 40–50 years, studies of cardiac repolarization are endlessly growing.

Here, we review the progress made in these types of studies by using stem cells and their health-related implications for channelopathies, with a special focus on the relevance for the long QT syndrome (LQTS).

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## Classic Methods to Study Cardiac Repolarization

The study of cardiac repolarization is of fundamental importance to characterize the pathophysiology underlying cardiac arrhythmias. Even though significant progress has been made in the understanding of clinically relevant arrhythmias, there are still several unanswered questions in the field, and every experienced cardiologist recognizes the need for new and more targeted approaches. The major limitation that has hindered the study of cardiac arrhythmias at cellular level has been the lack of patient-specific cardiomyocytes (CMs) since biopsies cannot be easily obtained from patients. To circumvent this obstacle, complex experimental models have been introduced over the years, and they have contributed to improve our knowledge.

For instance, heterologous systems have been successfully used to model genetic repolarization disorders and channelopathies [5]. This technology consists in transfecting cancer cell lines with specific ion channels of interest to evaluate mutation-related changes in ion channel function and to predict the *in vivo* relevance of these changes. This approach is simple and inexpensive and has a potentially high yield, since heterologous systems are adaptable to robotic procedures [6]. However, these models lack important constituents of cardiac ion channel macromolecular complexes necessary to reproduce the molecular and electrophysiological


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features investigated. Hence, the translational relevance of heterologous systems is often limited.

Animal models have also been used to model repolarization disorders, but their translational relevance is modest. The characteristics of the action potential (AP) of small animals, particularly the repolarization phase, are profoundly different from those of humans both in terms of duration and ion currents contribution [7, 8]. Consequently, drugs tested with positive results in small animal models often turned out ineffective in humans only in late stages of drug development, with huge losses in terms of costs and clinical advancement [9, 10]. Conversely, large animal models do show consistent similarities with humans [11] but also have important limitations that restrict their use only to more advanced stages of research and development [12]. Costs, particularly those associated with the generation, validation, and use of transgenic animals, represent a burden for laboratories and institutions, often caught between data reliability and budget limitations. Furthermore, growing ethical issues and the introduction of restrictive regulations are discouraging the use of animal models, with a profound negative impact on research.

Thus, methods that have allowed an undeniable amount of discoveries seem now limited and incapable of driving future innovation at a sustained pace.

## Generation of Pluripotent Stem Cell-Derived Cardiomyocytes

Human stem cells as a tool in disease modeling and drug discovery represent a fairly recent concept, based on the hypothesis that cells of human origin may help to bridge the gap between preclinical research and clinical trials. In the late 1990s, the isolation of human embryonic stem cells (hESC) and the introduction of protocols to differentiate them into CMs (hESC-CMs) represented pivotal moments for the advancement of this novel approach. With time, the optimization of the differentiation protocols allowed to obtain spontaneously beating hESC-CMs that roughly recapitulated several typical human CM properties as cardiac ion currents expression and the presence of typical pharmacological responses [13–16]. Afterward, co-culturing systems with the mouse endoderm-like cell line (END2) and chemically driven protocols to induce cardiac differentiation were introduced and led to the reproducible generation of hESC-CMs resembling human CMs [17]. Since then, it became possible to generate ventricular-like CMs in virtually unlimited numbers. Even though this breakthrough was only partially embraced, particularly in Europe, due to ethical bans concerning the use of embryonic-derived material, this technology profoundly innovated the whole scientific field to a point that the core activity of multiple laboratories still relies on hESC-CMs for developmental studies, cardiac disease modeling, and drug testing.

The use of embryos as a source of pluripotent stem cells was made largely redundant by the discovery of induced pluripotent stem cells (iPSCs). It was in 2006 that Shinya Yamanaka, awarded with the Nobel Prize in Physiology or Medicine in 2012 for his discovery, identified four factors, *OCT4*, *SOX2*, *KLF4*, and *cMYC*—subsequently named *Yamanaka factors*—that, once transfected into somatic cells, are able to promote their reprogramming to a pluripotent state [18, 19]. Since iPSCs were initially obtained from skin dermal fibroblasts, thus not limited by ethical restrictions, their use literally exploded. Improvements in reprogramming methodologies have allowed the generation of iPSCs from even less invasive biological specimens as urine [20] or blood-derived mononuclear cells [21]. Furthermore, differently from hESCs, iPSCs can be generated from virtually any individual, opening the possibility to derive cell lines from healthy donors and patients to recreate *in vitro* more personalized approaches in disease modeling and therapy (Fig. 4.1) [22]. So far, iPSCs have been differentiated in multiple cell culture formats (Fig. 4.2) [23]: the generation of embryoid bodies (EBs) has been the first culture method used to generate iPSC-derived CMs (iPSC-CMs) and is still used in many laboratories, largely because these structures are easily formed. Subsequently, to reduce the workload and improve throughput and reproducibility, differentiation

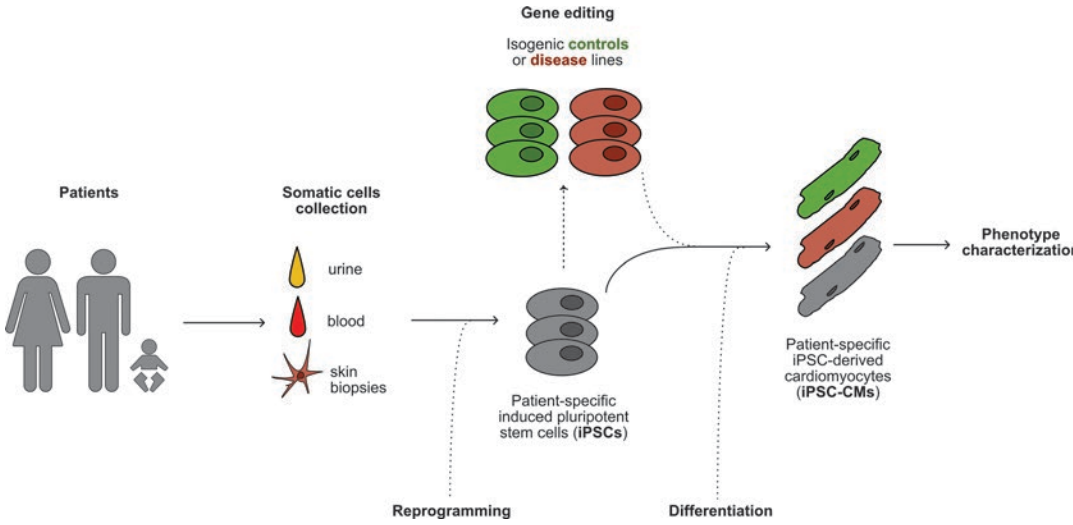


**Fig. 4.1** iPSC generation. Somatic cells from multiple lineages can be collected from patients and reprogrammed to induced pluripotent stem cells (iPSCs) by a transfection with the Yamanaka factors. Patient-derived iPSCs can undergo gene editing approaches for the generation of the respective isogenic controls or isogenic mutated iPSC line. iPSCs are then differentiated to iPSC-derived cardiomyocytes (iPSC-CMs) through multiple diverse differentiation protocols. iPSC-CMs, in different cellular formats, will be then used for phenotype characterization. Specific features of the phenotype of iPSC-derived cardiomyocytes (iPSC-CMs) will be then assessed and quantified

strategies based on supplementation of well-defined cytokine cocktails led to the generation of spontaneously beating monolayers of iPSC-CMs. However, these differentiation methods generate structures constituted by a variable and uncontrolled proportion of the different cell types, often exhibiting unpredictable line-to-line variability in differentiation efficiency. Accordingly, several groups are attempting to move toward standardized and controlled culture systems with the aim to generate monotypic cell cultures, constituted by a single cell type (i.e., CMs) embedded into extracellular matrix proteins or organoids [24–26]. This approach also allows to consciously mix different cell types (Fig. 4.2), for instance, CMs, endothelial cells, and fibroblasts, in fixed and reproducible ratios with consequent improvements in maturation and data reliability.

## The Role of Pluripotent Stem Cells in Cardiac Arrhythmias

The field of cardiac repolarization disorders has been a pioneering playground for scientists after the discovery of iPSCs [27, 28]. In particular, monogenic disorders as cardiac channelopathies were among the first to be modeled with iPSCs, given their generally straightforward disease mechanism and the fact that clear-cut phenotypes can be reproduced. Genetic mutations in ion channel genes may alter the ion channel biophysics and change key electrophysiological properties of the heart, with often devastating consequences for patients. The first iPSC line derived from a patient affected by LQTS was documented in 2010 by Alessandra Moretti and collaborators [29]. This seminal study opened the possibility to quantify the downstream effects of a specific ion channel mutation in terms of channel biophysics and allowed to determine complex and multiparametric features, typically restricted to primary CMs, such as AP, calcium transients, contraction, protein trafficking, protein-protein interaction, and pharmacological responses in cells having the same genotype of the patient. Multiple conditions were modeled in the following years using iPSC-CMs, with the main findings described below.

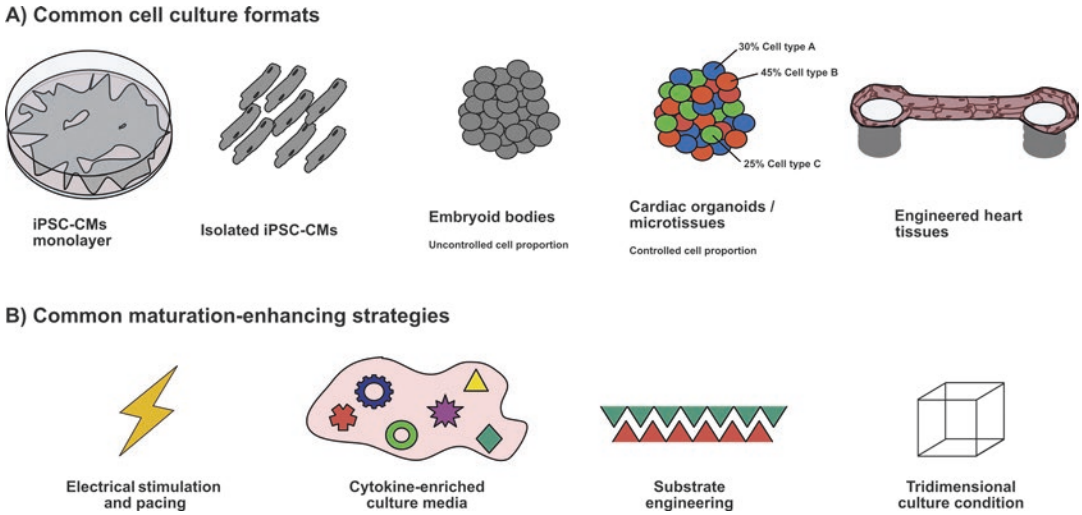


**Fig. 4.2** Common cell culture formats and maturation strategies. (**a**) The most commonly used cell configurations for iPSC-CMs: (i) monolayers, (ii) isolated iPSC-CMs, (iii) embryoid bodies, (iv) cardiac organoids/microtissues, or (v) engineered heart tissues. (**b**) Strategies used to obtain more mature CMs generally simulate conditions that do occur during development and include (i) electrical pacing, (ii) cytokine- or small molecules-enriched media, (iii) substrate engineering with more physiological strain or attachment properties, and (iv) tridimensional culture conditions

## Cardiac Repolarization Disorders Modeled with Pluripotent Stem Cell-Derived Cardiomyocytes

### Long QT Syndrome

Long QT syndrome is the best characterized arrhythmogenic disorder of genetic origin and a leading cause of sudden cardiac death (SCD) in young subjects [30, 31]. LQTS is a familial arrhythmogenic syndrome characterized by delayed repolarization that may lead to a life-threatening polymorphic ventricular tachycardia known as "torsades de pointes." The congenital form of LQTS is caused by a mutation in one of at least 16 genes encoding for key cardiac ion channels or excitation-contraction coupling proteins [32], while the acquired form manifests QT interval prolongation mainly in response to pharmacological compounds blocking $I_{Kr}$, the rapid delayed rectifier potassium current [33–35]. LQTS was the first repolarization disorder linked to precise genetic bases [36–38], and this has markedly changed the approach to diagnosis, risk stratification, and management. Indeed, already in 1995, the first gene-specific therapy was proposed based on the understanding that the genetic subtype caused by an increased sodium current (LQT3) could respond well to the sodium channel blocker mexiletine [32]. This has now become part of the standard therapy for LQTS [31, 39]. Recent data show that mexiletine can often shorten QTc also in LQT2 patients [40].

As said, Moretti et al. approached the disease modeling of LQTS with the iPSC technology, assuming that patient-specific cells, thus sharing the same genotype with the donor, should also reproduce the clinical phenotype *in vitro* [29]. They studied a patient with LQT1 caused by a heterozygous point mutation in the *KCNQ1* gene causing a loss of function of $I_{Ks}$. Skin fibroblasts were collected from the patient and from a healthy individual, expanded, reprogrammed to iPSCs using the four Yamanaka factors, and differentiated to iPSC-CMs with a chemically driven differentiation protocol. The APs of patient-specific iPSC-CMs were markedly prolonged (Fig. 4.3), and the magnitude of $I_{Ks}$ was reduced compared to iPSC-CMs from the control. When they added catecholamines, a known arrhythmogenic trigger in LQT1 patients [41], arrhythmic activity (early afterdepolariza-

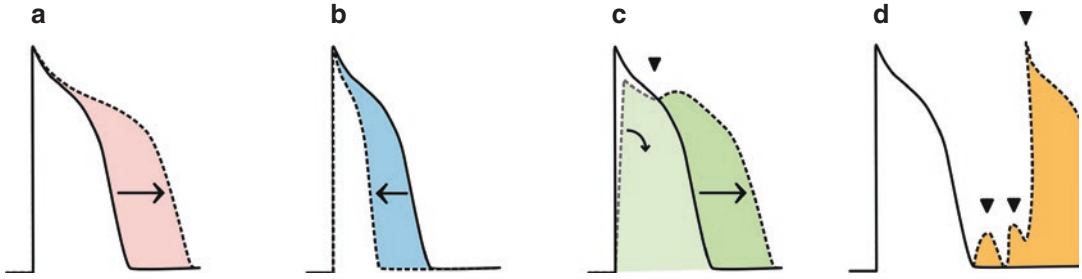


**Fig. 4.3** Phenotype exhibited by repolarization disorders in iPSC-CMs. Normal (solid line) and diseased (dashed line) action potentials from (**a**) long QT syndrome, (**b**) short QT syndrome, (**c**) Brugada syndrome, (**d**) catecholaminergic polymorphic ventricular tachycardia (CPVT)

tions) developed only in iPSC-CMs derived from the patient. These experiments demonstrated that a virtually unlimited source of patient-derived CMs could be generated to study disease traits and to validate targeted/personalized therapeutic approaches [28]. Subsequently, several iPSC lines were generated from patients with LQT1 [42–47], LQT2 [43, 45, 46, 48–59], LQT3 [60–65], LQT7 [66], LQT8 [67], LQT14 [68, 69], and LQT15 [68, 70, 71]. Phenotypic features as prolongation of AP were recapitulated as well as gain- or loss-of-function of ion currents, correctly reproducing the expected allelic stoichiometry (i.e., heterozygosity vs homozygosity).

A representative case to understand the importance of iPSC-CMs comes from the modeling of severe forms of LQTS (LQT14–16) caused by mutations in calmodulin [72], a ubiquitous and highly conserved protein that acts as calcium sensor within cells and is essential for the functioning of multiple organs, including the heart. Calmodulin is so important that identical proteins are encoded in humans by three different highly conserved genes (*CALM1–3*) [73] and a mutation in just one of the six alleles can cause life-threatening arrhythmias [72]. Heterologous overexpression of mutant calmodulin fails to preserve its native stoichiometry, while animal models are poorly predictive as the copy number and chromosomal localization of their calmodulin genes are different from those of humans. The only experimental model currently available in which gene stoichiometry can be conserved and reproduced is represented by iPSC-CMs [69]. Using patient-specific F142L iPSC-CMs we were able not only to confirm a severe impairment in $Ca^{2+}$-dependent inactivation (CDI) of L-type calcium currents (LTCC) as the cause of the action potential prolongation but also to exclude other possible causes of LQTS and ventricular arrhythmia not previously predicted by heterologous systems [69]. For example, we found that alterations in the slowly activating delayed rectifier current or the late sodium current, both of which regulated by CaM, were not responsible for the prolonged action potentials of F142L iPSC-CMs. This iPSC-based approach, in parallel with the development of gene editing techniques, already allowed the first targeted studies to test novel therapeutic approaches with pharmacological [69] or biotechnological [70, 71] strategies that have already obtained preliminary translation to patients [74].

## Short QT Syndrome

The key phenotypical manifestation of the short QT syndrome (SQTS) is an excessive shortening of ventricular repolarization due to gain- or loss-of-function mutations in genes encoding for ion channels carrying repolarizing or depolarizing currents, respectively [75]. SQTS type 1 (SQT1) is caused by mutations in the *KCNH2* gene, encoding for the voltage-gated potassium channel Kv11.1 (hERG) and conducting $I_{Kr}$. SQT1 has been modeled with iPSC-CMs, and patient-specific CMs exhibited a short AP duration (Fig. 4.3) as a consequence of an increased $I_{Kr}$ magnitude, with pro-arrhythmic events recorded under baseline conditions and

pharmacological stimulation with carbachol [76]. The disease traits of SQTS were also recently reproduced at tissue level in iPSC-CMs monolayers, which further extends the capabilities of the iPSC technology to simulate complex arrhythmogenic patterns *in vitro* at both cellular and tissue levels [77].

## Brugada Syndrome

The Brugada syndrome (BrS) is an autosomal dominant channelopathy with variable penetrance associated with premature APs in the right ventricular outflow tract which may result in SCD. ECG patterns are characterized by incomplete right bundle-branch block and ST-segment elevations in the anterior precordial leads V1-V3 [78].

The BrS type 1 (BrS1), the first type modeled with iPSC-CMs, may arise as a consequence of mutations in the *SCN5A* gene, encoding for the voltage-gated cardiac sodium channel $Na_v1.5$, causing a loss of function in the peak sodium current ($I_{Na}$) and a delay in the cardiac electrical impulse conduction. Approximately 20% of patients affected by BrS carry mutations in the *SCN5A* gene [33, 79]. Patient-specific iPSC-CMs reproduced the decreased $I_{Na}$ magnitude and exhibited short APs (Fig. 4.3) and arrhythmogenic features as sustained triggered activity with incomplete and asynchronous $Ca^{2+}$ transients [80]. The use of gene-edited isogenic controls confirmed that such peculiar features were inherent of iPSC-CMs from BrS donors and indicated that iPSC-CMs are suitable experimental models for BrS [80]. Importantly, iPSC-CMs proved useful to identify novel disease mechanisms. For instance, Veerman and coworkers identified three patients with a BrS type 1 ECG pattern who tested negative for the known BrS-related genes [81]. Their iPSC-CMs did not exhibit functional alterations in the $I_{Na}$, transient outward potassium current ($I_{to}$), or the L-type calcium current ($I_{CaL}$), suggesting the existence of a still unveiled disease mechanism underlying BrS, likely attributable to genetic modifiers or to external factors (e.g., fibrosis, structural defects, age, etc.) difficult to reproduce in iPSC-CMs at the present state of technology [82]. Moreover, although an attempted pharmacological rescue strategy, based on innovative read-through compounds, failed to revert disease traits in iPSC-CMs from *SCN5A* nonsense mutation carriers with a mixed LQT3/BrS phenotype [83], it contributed to demonstrate the plethora of applications for iPSC-CM models to study repolarization disorders. Importantly, Okata et al., through a refined gene knockout targeting strategy, demonstrated that embryonal or neonatal isoforms of the sodium channel, absent in human adult CMs but expressed in iPSC-CMs, can mask the effect of BrS mutations present in the adult isoform of the gene [64]. This raises warning signs for the modeling of certain diseases with immature iPSC-CMs.

## Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is another genetic arrhythmogenic condition characterized by ventricular tachycardia induced by beta-adrenergic activation [84, 85]. Multiple forms exist and all relate to mechanisms controlling intracellular $Ca^{2+}$ homeostasis. Mutations in the *RyR2* gene, encoding for the cardiac ryanodine receptors, the first to be linked with CPVT, are associated with the CPVT type 1 (CPVT1) [86, 87]. Mutations in *CASQ2*, encoding for calsequestrin 2, an important protein that acts as $Ca^{2+}$ buffer within the SR, have been associated with CPVT type 2 (CPVT2) [88]. The majority of CPVT cases are caused by these two genotypes, but minor genes have been identified and linked, respectively, to CPVT3–5. The immaturity of iPSCs-CMs may profoundly hinder the disease modeling of CPVT, since the absence of a properly formed T-tubules network, the lack of sarcomere organization, and the differential expression levels of ion channels and $Ca^{2+}$ handling proteins could represent a hurdle difficult to circumvent. Nonetheless, several laboratories attempted to model CPVT with iPSC-CMs, surprisingly recapitulating multiple clinical

features observed in patients as aberrant $Ca^{2+}$ transients, altered $Ca^{2+}$-store stability, arrhythmic APs (Fig. 4.3), and beta-adrenergic sensitization [89–92]. Drug screening strategies were also attempted using these models, and, in addition to the validation of current treatments, they also opened new possibilities for innovative pharmacological therapies [93–97]. Of note, iPSC-CMs allowed the identification of a novel gene (*TECRL*), which mutations are associated to a mixed LQTS/CPVT phenotype and provided an efficient platform for a pharmacological rescue of the phenotype [98].

## Techniques to Quantify the Phenotype

Along with gold standard and established techniques, the use of stem cell-derived CMs for the study of cardiac disease allowed the introduction of innovative tools or technologies for the investigation of the functional phenotype. The leading and more widespread techniques are presented below.

### Patch Clamp

After almost 50 years, the patch clamp technique remains the gold standard for single-cell electrophysiology studies. A pulled glass capillary filled with a high potassium solution resembling the ionic composition of the cytosol allows the recording of ionic currents flowing across the cell membrane (Fig. 4.4) [99]. Two main modes can be used to assess a pathological phenotype: in ***current clamp***, a controlled current square pulse is used to trigger APs from isolated CMs. Depending on the characteristics of the glass pipettes and intracellular solutions, this mode can be used to record APs from multicellular structures. In the ***voltage clamp*** mode, the operator controls the membrane potential to take advantage of the voltage-gated kinetics of ion channels and to investigate changes in their biophysical properties due to ion channel mutations. This mode can also be used in the ***AP clamp*** configuration to simulate the kinetics of specific ion currents during one AP and has been extremely useful to gather biophysical data from specific ion currents to be incorporated later into computational models [45, 100, 101].

### Multielectrode Array

The multielectrode array (MEA) technology has gained an amazing momentum after iPSC-CMs became of routine use. Unlike primary CMs, iPSC-CMs can spontaneously contract, have a high attachment capacity, can effectively form functional syncytia, and tolerate well long-term culturing conditions. This high flexibility allowed iPSC-CMs to access platforms usually considered a prerogative for immature cell types (e.g., chicken embryos CMs or murine neonatal CMs) or neurons. The MEA technology allows the recording of extracellular signals, called field potentials (FP), from excitable cells plated on extracellular microelectrode arrays. The FP is the net result of extracellular ion fluxes acquired by the tiny electrodes, and its trajectory is also affected by cell distance from the electrode, vector direction of the electrical signal, and spatial organization of the cells; the main quantitative parameters of the MEA technology applied to iPSC-CMs are FPD and beating frequency, respectively, rough equivalents of the QT and RR intervals [102] (Fig. 4.4). Although MEA data need a careful interpretation because of the high number of variables that can influence these measurements, their high-throughput predictive potential in cardiac disease modeling has been clearly established, to the point that pharma companies have begun to use this technology for their preclinical drug screening pipelines [45, 102–104]. Recent technological improvements also allowed the combined measurement of impedance and FPs [105], the recording of intracellular signals through grids of sharp nanoelectrodes [106], and the investigation of the effect of strain with stretchable MEAs [107]. Finally, MEAs can be easily combined to pacing technologies as stimulators [108] or optogenetics [109] to improve their predictivity for cardiac disease modeling and drug screening.

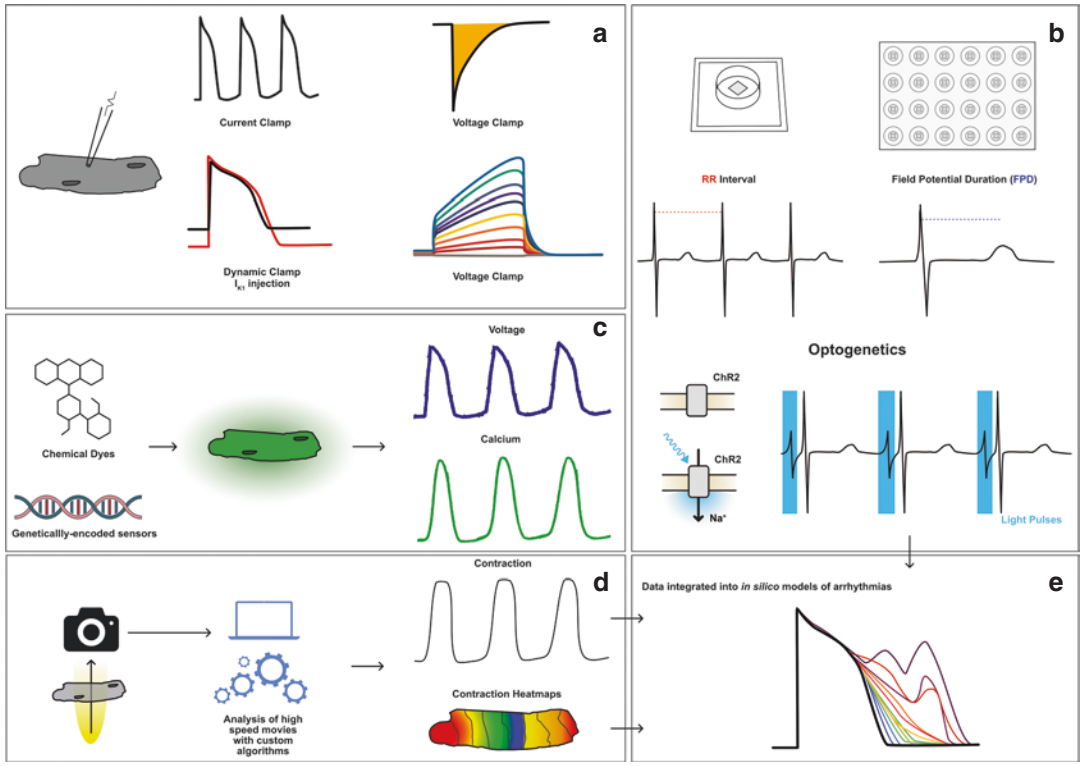


**Fig. 4.4** Technologies used for phenotype evaluation. (**a**) *Patch clamp electrophysiology*: a glass capillary allows the recording of action potentials (current clamp mode) in native conditions or with the injection of a computer-simulated current ($I_{K1}$, in the example; dynamic clamp mode) and ionic currents (voltage clamp mode) in iPSC-CMs. (**b**) *Multielectrode arrays*: iPSC-CMs are plated on single-well (top, left) or multi-well MEA chips (top, right). The recorded signals, called field potentials (FP), remind an electrocardiographic recording; the beating frequency (RR interval, mid-left) and the FP duration (FPD, mid-right) are the most relevant parameters that can be extracted and quantified with this platform. The MEA technology can be easily coupled with optogenetic actuators such as the channelrhodopsin 2 (ChR2, bottom left). This light-sensitive ion channel opens when hit by blue light, and this allows an inward flow of $Na^+$ ions to depolarize the membrane potential and trigger an action potential, recorded by the MEA as FP (bottom, right). (**c**) *Fluorescent reporters*: iPSC-CMs can be incubated with chemical dyes or transfected with genetically encoded voltage or ion sensors. These will allow the recording of multiple parameters such as voltage profiles or calcium transients in medium-high-throughput formats. (**d**) *Optical methods*: bright-field movies of contracting cells are recorded through high-speed digital cameras and analyzed by custom-made algorithms. These software tools, through different mathematical strategies, will output quantitative contraction profiles or heat maps that can be used to quantify and estimate the effect of a repolarization disorder on cardiac contraction. (**e**) *Computational approaches*: data recorded from the aforementioned techniques are combined and integrated into mathematical in silico models of arrhythmias; these models will offer tools to predict the effect of disease mutations on the phenotype of iPSC-CMs and will contribute to the generation of more accurate models of repolarization disorders

## Fluorescent Dyes

The abundant availability of iPSC-CMs boosted their integration into large-scale analysis platforms, with increasing demand from industry and academia for high-throughput technologies to quantify pharmacological effects and disease phenotypes. Technical advancements in imaging, fluorescent dyes synthesis, and genetic engineering have led to a fast optimization of chemical and genetically encoded fluorescent indicators capable to track membrane potential and intracellular ion concentrations with sufficiently rapid kinetics (Fig. 4.4) [110]. Incubation with chemi-

cal dyes is cheaper and easier than the stable integration in the genome of plasmids carrying voltage- or cation-sensitive probes; on the other hand, chemical dyes cannot be used in long-term repeated recordings and carry the inherent disadvantage of a higher phototoxicity. The relatively low signal-to-noise ratio and the low sensitivity to changes in fluorescence upon rapid voltage- or ion-concentration shifts represent general limitations of fluorescent dyes; this is why more efficient and brighter probes have been recently developed [111, 112]. The benefits offered by the high-throughput capabilities of these platforms and by the possibility to perform multiparametric measurements [113] significantly overweight their lower accuracy. This has enhanced the diffusion of fluorescent dyes for the automated characterization of diseased cell lines and for drug testing performed using large screening platforms [114–116].

## Video-Based Analyses

Recent advancements in image acquisition and processing allowed the generation of platforms capable to extract quantitative information of cardiac repolarization from movies recorded with high-speed cameras. These techniques quantify properties associated with CM contraction and relaxation such as amplitude, force, speed, and duration (Figs. 4.4 and 4.5); these measurements are possible due to the combination of sophisticated recording devices [117–119] and analysis

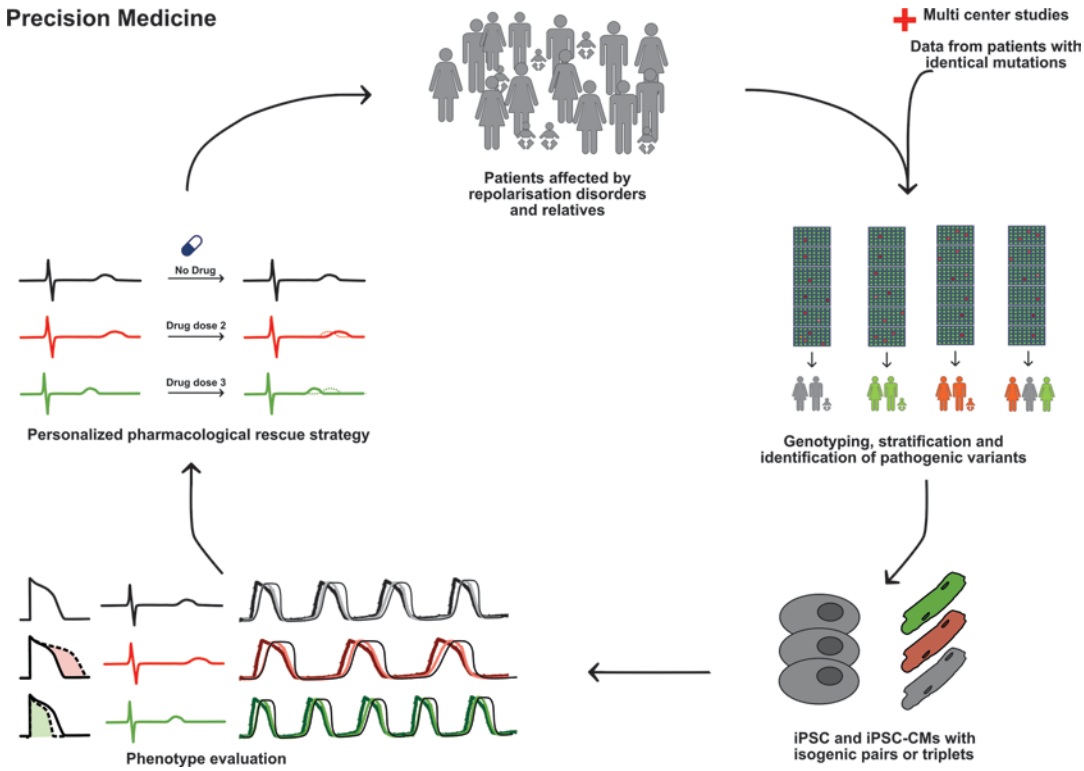


**Fig. 4.5** Precision medicine. Patients with repolarization disorders are genetically screened to evaluate the presence of disease-causing mutations or polymorphisms, and they are stratified according to their genotype or other key features as ethnicity, gender, age, etc. iPSC lines are generated from these cohorts of subjects and differentiated towards iPSC-CMs. Functional *in vitro* evaluations on patient-derived iPSC-CMs will prove the putative pathogenic role of the identified variants and will contribute to stratify patients according to the *in vitro* phenotypes. *In vitro* individual drug tests are performed, and specific patterns of response are identified and correlated to clinical features to deliver the most effective treatment to each patient

algorithms [120, 121]. Although some of these platforms may be limited by costs or by their low flexibility toward different cell configurations or substrates, the most recent ones offer open-source solutions to counterbalance the costs of proprietary systems and advanced algorithms to cope with multiple cell formats and attachment conditions [120, 121]. Of relevance, the latest trend is to move toward the determination of multiple parameters within a single measurement; e.g., quantification of contraction in combination with MEA, patch clamp, or fluorescence analyses allows simultaneous insights from multiple aspects of cardiac repolarization as contraction, membrane potential, or calcium handling [121], which is quite important for gathering insights from disorders of cardiac repolarization or pharmacological responses.

## Technological Improvements

Current differentiation strategies have approached maturation from different angles, but, despite the recent great progress, standardized protocols for large production of fully mature iPSC-CMs are not yet available. The exponential success of iPSC-CMs for disease modeling and drug testing has promoted the convergence of multiple disciplines, with some of the most representative technological advancements harnessed to obtain CMs with a more mature electrophysiological phenotype or to generate genetically engineered cell lines. Moreover, advancements in substrate engineering and computational power ensured the possibility to generate and analyze large amount of data at high throughput or to simulate consequences of disease or pharmacological challenges in silico. We report here some of the most recent applications.

### Dynamic Clamp

The electrophysiological phenotype immaturity of iPSC-CMs can be mitigated with an elegant computational approach known as dynamic clamp [122]. This technique allows the injection of a simulated ion current of interest in the cell to compensate the lack of the endogenous counterpart (Fig. 4.4). This requires the real-time coupling of an in silico mathematical model of ion currents with the *in vitro* measurement in patched cells. The voltage contour of the AP recorded in the cell is processed by a mathematical model, and the generated ion current profile is outputted and reinjected into the cell. Examples in adult CMs include the simulation of pathogenic conditions in $I_{Kr}$ [101], $I_{to}$ [100], and $I_{Na}$ [123]. Specifically for hESC- and iPSC-CMs, the efforts so far aimed to counterbalance their low $I_{K1}$ magnitude that is typical of immature CMs [69, 124, 125]. Recently, this technology has been implemented in high-throughput systems on automated patch clamp platforms [126], and, once optimized, this strategy will allow drug tests on stem cell-derived CMs in more physiological conditions.

### Optogenetics

The possibility to derive CMs from cultured iPSCs has eased the generation of cell lines carrying the insertion of stable constructs, and one of the most promising application is the use of optogenetic sensors, i.e., light-sensitive ion channels whose characteristics can be exploited to control CM's behavior [127]. The most widespread sensor is the channelrhodopsin 2 (ChR2), which encodes for an ion channel equipped with a photoreceptor unit (Fig. 4.4). Stimulation with blue light modifies the ion channel structure triggering a depolarizing current and a consequent AP [128]. Human iPSC-CMs expressing ChR2 have been generated, and they exhibit a normal cardiac phenotype with the exception that their beating frequency can be controlled by precisely triggered light pulses. As demonstrated by Sala and coworkers [45], the frequency dependence of iPSC-CMs appears, conversely to adult CMs, to be cell line specific regardless of the formulae used for rate correction; this impacts the reliability of disease phenotype assessment and pharmacological effect quantification. Algorithms partially mitigate this limitation [129], but they

often require complex stimulation platforms that are sometimes poorly compatible with many of the most used recording technologies. These limitations are circumvented through optogenetics, which can be promptly integrated into current platforms for drug screening and cardiac safety pharmacology [109].

## Computational Approaches

The exponential increase in computational capabilities of bioinformatic tools has significantly empowered these systems and their usage as predictive systems for in silico mathematical modeling of disease phenotypes, pharmacological responses, and clinical trials [130, 131]. These tools should be used in combination with experimental methodologies and will likely evolve, in the near future, into more sophisticated multiscale human models capable to simulate single cells [132–136] or even the whole heart [137] that could be used as ethical, safe, and cost-effective platform to integrate, or replace where possible, current animal or *in vitro* studies (Fig. 4.4). In silico models of stem cell-derived CMs have allowed the recapitulation of key features of healthy and diseased cells as APs [132, 133, 136], ion currents [134], phenotypic variability [135], and pharmacological responses. Computational approaches have shown an enormous potential especially when they were integrated into data analysis procedures. The ability of artificial neural networks to identify complex patterns, invisible to the human eye, has been harnessed for the classification of cell morphology [138], pharmacological responses [139], or disease patterns. Machine learning approaches have recently demonstrated the ability to automatically discriminate, with a remarkable accuracy (78–88%), cardiac disorders such as LQTS, CPVT, or hypertrophic cardiomyopathy by the profile and kinetics of $Ca^{2+}$ transients of patient-specific iPSC-CMs [140]. Computational-based strategies have been also proposed as one of the key pillars of the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative [141], a joint effort of academia, regulatory agencies, and pharmaceutical companies which aims to overcome current guidelines of safety pharmacology and drug testing through a multilevel approach including iPSC-CMs and computational models.

## Genome Editing

Shortly after the first investigation of cardiac disease phenotypes, it emerged that iPSC-CMs from different human donors, regardless of the presence of pathogenic mutations, may exhibit different, sometimes opposite, electrophysiological phenotypes. This has been related to variations in the genetic background of each donor which may influence the *in vitro* phenotype of iPSC-CMs. These variations can be attributed to unique allelic variants among individuals and epigenetic variability, on top of which relies the experimental variability associated to clonal selection and culturing conditions [142]. Thus, iPSC-CMs suffer from cell line-dependent variation which may influence their functionality and hamper the reproducibility of the results. This heterogeneity may be markedly visible when studying repolarization disorders and, specifically, the interline variations of APD and FPD, two parameters investigated during the characterization of LQTS or SQTS mutations and pivotal for safety pharmacology. As previously demonstrated [143], it is often difficult to cluster cell lines into specific disease types by relying only on APD. One solution to partially reduce background genetic variation is to generate, when feasible, a control line from a first-degree relative. Although the concept behind this approach is reasonable, its effectiveness compared to a random control selection has still to be experimentally validated. Genetic engineering now offers a smart, albeit complex, solution to this problem: the generation of *isogenic controls*. These cell lines are obtained by gene editing technologies that allow the precise insertion of point mutation in the genome without altering the overall background (Fig. 4.1). Currently, the CRISPR/Cas9 gene editing approach is the most

widely used [144], albeit its remarkable activity has also the downside to potentially leave off-target "scars" in the host DNA and might restrict the implementation of this technology to *in vitro* or ex vivo applications. Gene editing techniques allow the derivation of isogenic pairs or isogenic triplets to investigate disease phenotypes in an identical genetic background environment and with the additional key contribution of gene stoichiometry. They proved essential for the characterization of disease phenotypes [42, 43, 52, 70] and drug discovery approaches [45], even though their use becomes challenging when insertions or corrections of heterozygous mutations, rather than gene knockouts, are required. Although too complex to be performed by non-experts, inefficient, time-consuming, and expensive to achieve at present state of technology, which restricts their benefits to a minority of laboratories worldwide, these strategies do offer undeniable advantages in terms of results reproducibility and data reliability.

## Limitations

### Cell Maturity

The level of maturation is the main limitation of iPSC-CMs, but it represents an acceptable trade-off between reliability of results and the massive amount of information that can be obtained with high-throughput systems. Due to technical limitations, high-throughput electrophysiology screenings can be used mostly with immature CMs, similar to those derived from stem cells, because these cells are relatively easy to generate and handle, and can be adaptable to robotic procedures. Conversely, mature primary CMs are rod shaped, do not form monolayers *in vitro*, cannot be processed with fluorescence-activated cell sorting, and tend to rapidly dedifferentiate while in culture [145]. In the last few years, the characterization of iPSC-CMs has demonstrated that their phenotype resembles that of CMs at an early stage of development [146] and this aspect must be kept into consideration when analyzing the results of disease modeling studies. Several strategies, aimed to recreate conditions that occur during embryogenesis, have been attempted over the years to increase the maturation of iPSC-CMs and generate more predictive systems for disease modeling, drug screening, and cardiac toxicity (Fig. 4.2). Those include prolonged time in culture [147]; electrical stimulation [148]; media enriched with cytokines or growth factors [149]; the presence of physical stimuli as shear stress and mechanical stretch [148, 150, 151]; the presence of substrates of different stiffness, composition, and pattern [152]; or the assembly into more physiological tridimensional structures [24, 25, 153, 154]. All these techniques dramatically increase the costs, but the generation of more mature iPSC-CMs will guarantee more reproducible and predictive results.

## Translational Perspective

The translational capabilities of iPSC-CMs have still to be fully explored. Unlike what happens in human hearts, repolarization duration in iPSC-CMs is highly variable and often may not reflect a pathological manifestation per se. Even in the presence of putatively healthy genotypes, repolarization duration may span across values that can be considered pathological [143]. Thus, the use of appropriate controls is absolutely mandatory to avoid misinterpretations (Fig. 4.1). Differentiation conditions play a major role in such a high variability, and only the use of very strict culture conditions can guarantee homogeneous results across different research centers [103, 104], although an individual susceptibility to growth factors and cytokines exists across different cell lines [155]. The generation of paired transgenic and iPSC mouse, canine, or human models [156] to study the *in vivo* relevance of *in vitro* observations will identify the presence of recurrent patterns of similarity between preclinical and clinical observations and clarify the boundaries of iPSC-CM technology. In particular, efforts are needed to better correlate the baseline parameters of pharmacological responses recorded *in vitro* to their *in vivo* counterparts and to validate whether the observed *in vitro* values

can be sufficiently predictive and representative of the *in vivo* condition.

## A Glimpse of the Future

One of the most intriguing uses of iPSC-CMs is the possibility to generate experimental models for personalized approaches to disease modeling and therapy. Although the dream of personalized medicine might be still considered overambitious and cannot be pursued at the present state of technology, precision medicine approaches will be pushed forward thanks to the availability of patient-specific and isogenic iPSC-CM lines [157]. In agreement with the goals of precision medicine, individuals are clustered according to specific population classifiers as age, gender, ethnicity, disease conditions, etc., to identify genetic or molecular targets and mechanisms of disease through a combination of large-scale population data with *in vitro* experimental models tailored on precise population characteristics. These, in combination to a scrutinized clinical history and multiple population-specific iPSC lines, can be used to identify molecular or functional differences in disease severity, to better stratify the population, or to detect specific patterns of response to therapy, with potential implications on drug discovery and development. As a representative example of precision medicine, Burridge and coworkers were able to reproduce, in patient-derived iPSC-CMs, the different grades of cardiotoxicity observed in patients treated with doxorubicin [158]. The high similarity between the clinical phenotype and that recreated in iPSC-CMs allowed to identify new genetic causes underlying doxorubicin-induced cardiotoxicity with immediate implications for clinical handling of cancer patients. If extended, this strategy holds promises for the identification of novel compounds tailored on specific population classifiers and will allow the determination of patterns of drug response in populations previously considered identical and thus being wrongly administered identical therapies.

In this line of thought, new therapeutic possibilities are emerging through drug repurposing strategies, i.e., the identification of new clinical applications for compounds already approved for commercialization [159]. Since the safety profiles of these compounds have already been assessed, the long and expensive final stages of preclinical drug development could be skipped, allowing these drugs, once they give positive results *in vitro*, to be validated in patients within few months. This certainly represents a revolution that guarantees fast feedbacks compared to classical drug discovery approaches. The first example of application of iPSCs to drug repurposing came from amyotrophic lateral sclerosis. Wainger and coworkers reproduced the patient features *in vitro* using neurons derived from iPSCs and were able to demonstrate that retigabine, approved on the market for the treatment of epilepsy, reverted the pathological phenotype of amyotrophic lateral sclerosis *in vitro*. Based on these results, shortly after this discovery, a phase II randomized clinical trial (NCT02450552) including ~ 200 patients was launched [160, 161].

A similar approach recently emerged also for repolarization disorders; our group has demonstrated that lumacaftor, a drug approved for the treatment of a cystic fibrosis subtype, can rescue hERG trafficking defect and decrease the arrhythmogenic activity of iPSC-CMs derived from patients affected by a specific LQT2 mutation class [51]. This finding opens new possibilities for the management of LQT2 patients not fully protected by the current therapies. Also in this case, the feasibility of a clinical trial to assess the therapeutic efficacy of lumacaftor in LQTS patients will be greatly facilitated by the fact that the safety of this drug has already been proven [162, 163]. Indeed, very recently we very rapidly translated this drug repurposing strategy by treating the two LQT2 patients whose iPSC-CMs were previously tested *in vitro* and proved that, besides the expected discrepancies between the clinical and *in vitro* conditions, the data obtained in patient-specific iPSC-CMs may offer a reasonable degree of predictability in the clinical setting [164]. Time will tell us if this approach will decrease the overall duration and costs of research and development in pharmacology.

The concept of precision medicine can be also extended to the identification of new polymorphisms or variants able to shape the phenotype and the clinical severity of a disease. Several research groups have demonstrated that mutations or polymorphisms in specific genes, defined *modifier genes*, can profoundly impact the severity of cardiac arrhythmias [165–172]. Unfortunately, this kind of clinical studies require the enrollment of a high number of patients in order to reach a statistically relevant power, which might be challenging as these are often rare diseases. iPSC-CMs may then offer an easier way to investigate, in more controlled experimental conditions, the putative contribution of these genetic modifiers. A recent work from Chai and coworkers [171], by combining innovative approaches as whole exome sequencing and gene editing of iPSC-CMs derived from LQT2 patients with different clinical phenotype despite an identical disease-causing mutation, identified two novel gene variants able to exacerbate a preexisting repolarization deficiency. This study significantly improved the current knowledge on the limitations and applicability of the iPSC-CMs technology to cardiac disease modeling and also proved that genotype-phenotype discordance (i.e., minimal vs severe disease phenotype), unlike any other experimental model, can be reproduced *in vitro* with iPSC-CMs. Furthermore, the dramatic reduction in costs for whole genome sequence data has led to the identification of a plethora of rare variants of unknown significance (VUS) that have been linked to a certain degree of pathogenicity for multiple disorders. As recently demonstrated by Ma and coworkers in the case of cardiomyopathies [173], a VUS can be inserted into a healthy iPSC line to define its level of pathogenicity through a phenotypic characterization (Fig. 4.5). As the number of VUSs is rapidly increasing, we anticipate that this approach will be quickly extended to other diseases and will soon embrace repolarization disorders.

In conclusion, few years after the publication of the first study describing the discovery of iPSC-CMs, it is now clear that the road leading to an unlimited source of mature adult human CMs is and will be very demanding. Novel and promising technologies in biomaterials and organs-on-chip will certainly facilitate this path by generating better and more physiological environments for CMs, likely including the contribution of multiple cell types and the presence of metabolic byproducts or physiological stimuli. On the other side, more innovative platforms for data acquisition and phenotype characterization will be implemented due to technological leaps in bioengineering, computational power, and artificial intelligence that will lead, in the near future, to more integrative and predictive techniques to comprehensively model and study repolarization disorders at high-throughput level. The exciting times are not over.

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# 5 Role of Late Sodium Current During Repolarization and Its Pathophysiology

Mohamed Chahine

## Introduction

The heart is a rhythmic electromechanical pump that requires optimal action potential (AP) generation and propagation through the myocardium to work properly. The electrical functioning of individual cardiomyocytes is distinct and coordinated, reflecting differences in the expression levels, biophysical properties, and distribution of various types of voltage-gated ion channels in the myocardium. These ion channels underlie the different phases of APs. Changes in their densities or in their function can lead to AP prolongation, dispersion of ventricular repolarization, and early afterdepolarizations (EADs), which are substrates for polymorphic ventricular tachycardia.

Voltage-gated sodium channels are a critical determinant of cardiac excitability and play a central role in the initiation and propagation of cardiac APs. They are also involved in determining the duration of APs. In functional terms, these channels are minimally characterized by three processes: closing, activation, and inactivation. There is much evidence for the existence of additional channel conformations, modal gating, for instance, which may underlie the persistent activity of these channels. Slow cycling of the channels between the different modes over a period of many seconds is a characteristic of modal gating and is seen in many different voltage-gated channel families, including those in cardiac myocytes [1–4]. These findings were confirmed using single channel recordings of skeletal muscle and cardiac sodium channels [4, 5]. Model gating is thought to underlie the persistent or late sodium current, which is an intrinsic property of sodium channels and, when exacerbated, causes an increase in intracellular sodium and may result in calcium overload, a precipitator of myocyte abnormal electrical activity [1–4].

These mechanisms are involved in several inherited (e.g., type 3 long QT syndrome) and acquired (e.g., associated with heart failure) cardiac arrhythmic disorders. The dysfunction of sodium channels in these disorders has profound physiological consequences, including the development of life-threatening cardiac arrhythmias and even sudden death.

In this chapter, we review basic cardiac electrophysiology, with a focus on the role of voltage-gated sodium channels, their biophysical properties, and their pathophysiology, in particular the role that the late sodium current plays in cardiac repolarization. An increase in the late sodium current is a major cause of AP duration (APD) prolongation and EADs. We also review the pharmacology of persistent currents as a potential target for treating cardiac arrhythmogenesis in failing

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hearts. In this regard, ranolazine, which is an anti-anginal drug that specifically targets persistent sodium currents, shortens APDs and eliminates EADs. We review several clinical trials that established the beneficial role this drug plays in managing several related ischemic heart diseases.

## Cardiac Action Potential

Following threshold depolarization, sodium channel opening causes a large and rapid influx of sodium ions (INa) that depolarizes the myocardial membrane (phase 0) (Fig. 5.1). This is followed by a rapid repolarization due to the efflux of potassium ions through outward potassium channels (e.g., Ito). In *phase 1*, sodium channels remain inactivated, and Ito is transiently activated. Membrane depolarization also activates voltage-gated calcium inward currents (L-type, ICa-L and T-type, ICa-T). ICa-T plays an important role in automaticity, while ICa-L is essential for the contraction of myocardial cells by triggering the excitation-contraction coupling mechanism during phase 2. In *phase 2*, also known as the plateau phase, there is a delicate balance between inward (ICa-L) and outward (IKr and IKs) currents. In this phase, three sodium ions move into the cell for each calcium ion that moves out. This occurs via the sodium-calcium exchanger. In *phase 3*, sustained repolarization occurs via delayed rectifier currents (IKs and IKr). Calcium channels become inactivated during this phase. Lastly, in *phase 4*, the IK1 inward potassium current ends the repolarization phase, and the net potassium efflux restores the resting intracellular gradient to −80/90 mV. The sodium channel makes a major contribution to two phases of the AP, that is, the period of rapid depolarization (phase 0) and the AP plateau (phase 2) and thus to the repolarization of APs. The height of APs increases with each increment in the extracellular concentration of sodium ions. A dysfunction of depolarization during phase 2 can trigger arrhythmia, which is maintained by a reentry mechanism, leading to a regenerative circuit of electrical activity around relatively unexcitable tissue (Fig. 5.1) [6].

## Voltage-Gated Sodium Channels (Structure)

Given that the focus of this chapter is sodium channels, we will briefly review their structure and function.

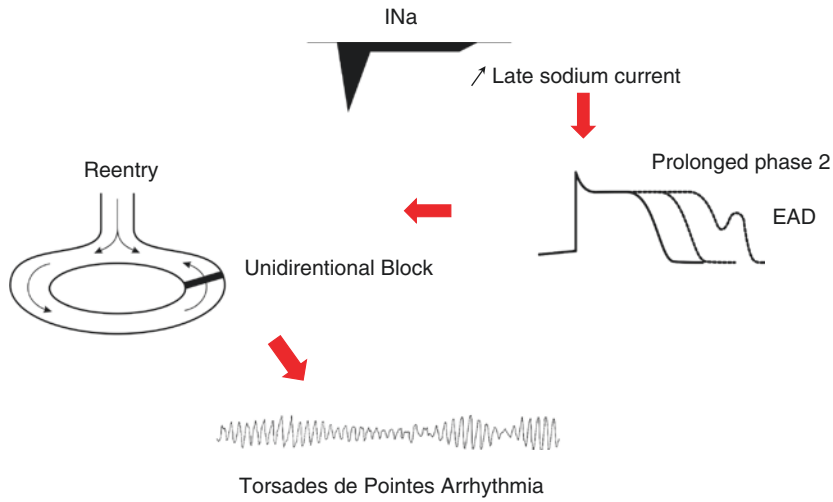


**Fig. 5.1** A sodium current activity during action potential. Incomplete inactivation gate (top panel) resulting in late sodium current activity during action potential duration. A cardiac action potential comprising phase 0 through phase 4 (top). Late sodium current may delay cardiac repolarization and QT-interval prolongation, triggering ventricular tachycardia or ventricular fibrillation (torsades de pointes) through phase 2 reentry mechanism

The *SCN5A* gene encodes the pore-forming α-subunit of the human cardiac voltage-dependent sodium channel now known as $Na_v1.5$, which activates the production of a depolarizing inward current INa, with prompt inactivation within milliseconds [7, 8]. The gene locus, which has been mapped to chromosome 3p21, consists of 28 exons and spans 80 kbp. It encodes a 2016-amino-acid protein and has a putative structure comprised of four homologous domains (DI–DIV), each containing six transmembrane-spanning segments (S1–S6) [7]. Transmembrane segments S1–S4 form the voltage sensor domain (VSD), and S5–S6 constitute the pore permeation pathway or pore domain. Nav1.5 VSD is characterized by its S4 segment, which contains highly conserved positively charged residues (arginine or lysine) and several highly conserved acidic residues on the S2 and S3 segments.

## Voltage-Gated Sodium Channels (Function)

Voltage-gated sodium channels pass through many conformational states during the time course of their activity (open and activated states, fast and slow inactivated states, recovery from inactivation, etc.). Other conformations have been reported also. In sodium channels, transitions between fast and slow conformations have been referred to as modal gating. Excessive residency times in slow conformations lead to channel malfunction and, as a consequence, type 3 long QT syndrome (LQTS3), heart failure (HF), and cardiac arrhythmias.

At almost the same time as activation, depolarization initiates fast or N-type inactivation. Sodium influx gradually comes to a halt during this process. With inactivation disabled, sodium currents exhibit a plateau of a persistent inward current during sustained depolarization. A fast inactivation process prevents channels from reopening until they have had enough time to recover. The fast inactivation of voltage-gated sodium channels is also linked to the outward movement of an S4 segment and a ball-and-chain or hinged lid mechanism. The III–IV linker acts as a lid, in which the inactivation particle (IFM) occludes the inner pore by binding to the docking site like a latch. The docking site consists of several regions, including the cytoplasmic linkers connecting S4–S5 in DIII and DIV and the cytoplasmic end of S6 in DIV.

Sodium channels also enter a slow inactivated state with prolonged depolarization, which is also called C-type inactivation. Previous studies have shown that the structural basis for slow inactivation involves the P-segment and the III–IV linker [9–11]. The duration of this more stable and non-conducting state ranges from hundreds of milliseconds up to several seconds. A dysfunction of this process contributes to pathophysiologic events associated with reduced cellular excitability, prolonged membrane depolarization, and cardiac arrhythmias.

The overlap of steady-state activation and inactivation of sodium channels defines a range of voltages (i.e., window) where the likelihood of sodium channels being open is significant, which could result in an inward sodium current that could potentially depolarize the resting membrane potential and increase myocyte excitability [12]. This is a time-independent current that can contribute to increasing excitability.

Several studies have shown that late sodium currents play an important role during the time course of APs. Low concentrations of TTX, a specific sodium channel blocker, reduce the duration of APs without affecting the Vmax [2, 13, 14], highlighting the role of the persistent activity of sodium channels during AP repolarization.

## Molecular Mechanisms and Pathophysiology of the Late Sodium Current

When the transmembrane potential reaches a certain voltage, the activation and inactivation states of the sodium channel overlap. Following the rapid depolarization of a myocyte, the sodium current does not recover as a small portion of sodium channels is inactivated slowly during the plateau phase. Although, under normal conditions, this current is very small

(0.5–1%) compared to the transient sodium current, it can contribute to delaying AP repolarization. Reactive oxygen species (ROS) are generated in ischemic hearts and contribute to left ventricular remodeling, cardiac myocyte disarray, and abnormal cardiac electrical activity. It has been shown that ROS can activate the Ca/calmodulin-dependent protein kinase IIδ via oxidation and that this may enhance late INa, causing a cellular sodium and calcium overload [15]. In this regard, $H_2O_2$ (50–200 μM), which is one of the ROS products, has been shown to induce a large late sodium current and yield a marked prolongation of the AP [16]. Furthermore, sodium entry during the few-hundred-millisecond duration of the plateau phase may cause an elevation of intracellular sodium, a condition that predisposes to calcium overload and cardiac arrhythmogenesis.

This flimsy persistent current opposes repolarizing potassium currents, delaying repolarization. Excessive residency time in abnormal conformations leads to channel malfunction and, as a consequence, severe pathologies known as channelopathies, in particular LQTS3 or HF. In certain cardiovascular pathologies, including HF, and in some genetic dysfunctions such as LQTS, this increase in the persistent sodium current may lead to the failure of repolarization. When certain repolarizing ionic currents compensate for the increase in depolarizing currents, there is a slight decrease in the repolarization reserve that, however, does not delay repolarization. However, if the magnitude of the late sodium current is substantial, a significant prolongation of repolarization can occur and can trigger abnormal impulses such as EADs.

Although the main properties of cardiac sodium channels can be expressed by the α-subunit, they are also associated with multiprotein complexes composed of auxiliary subunits such as SCNB4, cytoskeletal proteins, and caveolin-3 (CAV3). In particular, dysfunctional interacting proteins can exacerbate the magnitude of the persistent current. In this regard, mutations in SCNB4 and CAV3 have been reported to exacerbate the persistent sodium current [17–19].

Several mechanisms have recently been proposed to explain the pathophysiological role of sodium channels.

## Channelopathies Associated with Sodium Channels

Normal cardiac cellular excitation and relaxation depend on normal depolarization and repolarization processes. Defective genes and structural abnormalities can influence these two processes, leading to a variety of clinical manifestations ranging from asymptomatic to symptomatic. Many inheritable cardiac diseases involve a dysfunction of, for example, potassium, sodium, and calcium channels, leading to LQTS, BrS, progressive cardiac conduction disorders (PCCD), and overlap syndrome. LQTS is an inherited cardiac disease that can cause syncope and even sudden cardiac death associated with polymorphic ventricular tachycardia (torsades de pointes). Patients with LQTS present with an increase in the QTc value over 0.460 s for men and 0.470 for women s on a 12-lead ECG.

LQTS3 is caused by mutations in *SCN5A* genes mapped to chromosome 3q21–24, which encodes the α-subunits of the cardiac sodium channel [20]. *SCN5A* mutations have been identified in approximately 10% of genotyped LQTS patients. In many cases, the development of arrhythmias is often associated with bradycardia during sleep or relaxation when the QTc value increases.

LQTS3 is considered an inherited disorder due to abnormal myocardial repolarization. As mentioned above, cardiac repolarization is a delicately balanced process between inward currents (INa and ICa-L) and outward potassium currents (IKr and IKs) during phase 2 of the APs. Shifts in the balance of these inward and outward currents can either increase or decrease the duration of APs, resulting in QT interval prolongation or shortening, respectively. The most common cause of QT interval prolongation in LQTS3 is a late sodium current leading to AP prolongation in phase 2. During

the kinetic gating process, fast inactivation is disrupted, which causes incomplete closure of the inactivation gate during sustained depolarization. This induces a small but persistent sodium current during the plateau that delays myocyte repolarization, leading to QT interval prolongation on ECGs at slow heart rates. This kinetic gating phenomenon is thus called a gain-of-sodium function [21, 22]. This sustained inward current is extremely small, accounting for 1–2% of the peak inward sodium current. However, the relationship between this sustained current and AP prolongation has been confirmed in computational modeling by a highly nonlinear relationship between sodium channel function and cardiac excitability [21]. Although most mutations cause LQTS3 by a persistent inward current, there are other mechanisms associated with QT interval prolongation. These include faster recovery from inactivation due to the destabilization of the inactivation state, with shifts in the voltage dependence of activation and inactivation [21, 23, 24]. These kinetic gating abnormalities may lead to a loss of the delicate balance between the inward and outward currents that is required to maintain the plateau of the AP, provoking QT interval prolongation.

## Late Sodium Current and Heart Failure

HF is a major public health problem in industrialized countries, in particular because of its frequency and its consequences in terms of morbidity and mortality [25, 26]. Cardiomyocytes isolated from failing human hearts and animal models of HF both display a prolonged AP duration [27, 28]. In elegant work using a cell-attached macropatch technique in post-MI rat cardiomyocytes, Huang et al. showed that sodium current bursting activity increases during sustained depolarization, resulting in a large slow component of the sodium current decay [29]. The authors also reported an increase in nervous system sodium channel subtypes in post-MI myocytes, with a reversion of the fetal to the adult variant of the sodium channel. One of the mechanisms proposed to explain the slow kinetics of the sodium channel is the increase in a late sodium current flowing through dysfunctional sodium channels. This would have two main consequences: (1) a delay in repolarization, leading to EADs and the initiation of torsades de pointes arrhythmia, and (2) an increase in intracellular sodium, favoring a calcium overload, oscillatory calcium release, and the initiation of late (delayed) afterdepolarizations generated by the sodium-calcium exchange mechanism. We and others confirmed the involvement of these two mechanisms using computer model simulations [30, 31].

## Pharmacology of the Late Sodium Current

### Class 1 Antiarrhythmic Drugs

Because of the central role sodium channels play in initiating and propagating cardiac APs, these channels have proved to be effective targets for antiarrhythmic (AA) drug therapy. Inhibiting these channels using AAs reduces the rate of myocardial depolarization and intracardiac conduction velocity, properties that have proved beneficial in the treatment of cardiac arrhythmias. Several studies [32, 33] have shown that class I AAs are effective inhibitors of late sodium currents associated with LQTS3 mutations and have proved beneficial in the treatment of patients carrying these mutations (Fig. 5.2). We have investigated and compared the effects of ranolazine and class I AAs on the Y1767C mutation, which is associated with QT prolongation and sudden death in a family. We found differential effects of the Y1767C mutation on ranolazine, quinidine, mexiletine, and flecainide, with ranolazine exhibiting a more potent effect [34]. Our data suggest that ranolazine primarily inhibits $Na_v1.5$ channels via an open-channel blocking mechanism. This contrasts with conventional class I AAs, which inhibit sodium channels by preferentially binding with high affinity to inactivated states of the channel [35].

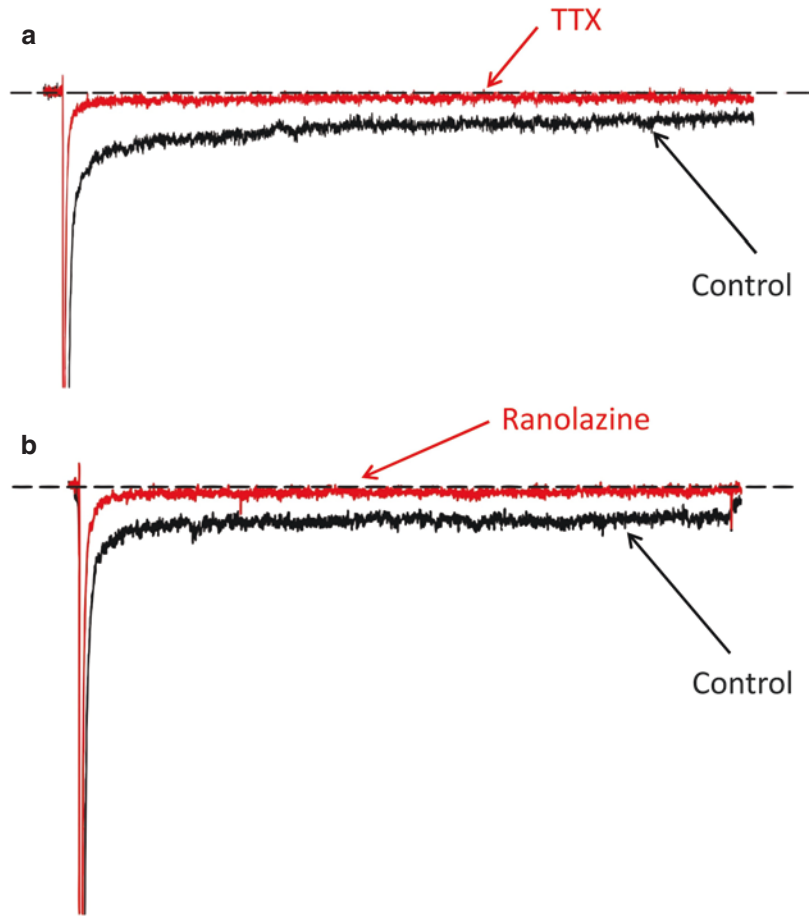


**Fig. 5.2** Effects of tetrodotoxin (TTX, 10 μM) and ranolazine (50 μM) on the cardiac late sodium currents. Sodium currents were elicited at −10 mV from −140 mV holding potential. (**a**) Effects on late sodium current of channels treated with 10 μM TTX a sodium channel blocker. (**b**) Effects on late sodium current of channels treated with ranolazine. In both cases, both TTX and ranolazine were efficient in inhibiting the late sodium current

## Ranolazine

Ranolazine is an antianginal agent that shares a similar structure with class I AAs [36] and is an effective inhibitor of late sodium currents of both LQTS3 mutant and native cardiac sodium channels [37, 38]. Increases in late INa due to the progression of ischemia contribute to the elevation of intracellular sodium concentrations in cardiomyocytes, which in turn activates the cell membrane sodium-calcium exchanger in an attempt to correct the sodium overload at the expense of a calcium overload [39, 40]. Prolonged activation of the sodium-calcium exchanger leads to an intracellular $[Ca^{2+}]i$ overload and induces further ischemia [40]. It has been suggested that ranolazine inhibits and reverses the deleterious effects of late INa. Sea anemone toxin ATX-II (ATX-II) was used as an enhancer of late INa in a preclinical study [41] because it mimics the effect of ischemia by increasing intracellular $[Na^{+}]i$ [41] and intracellular $[Ca^{2+}]I$ [42]. This study showed that ranolazine is able to attenuate the ATX-II-induced increase in late Ina [41] and $[Na^{+}]$ i-dependent calcium overload [42]. Ranolazine has also been reported to reversibly shorten the AP duration of ventricular myocytes isolated from canine failing hearts and to be more potent than lidocaine and amiodarone [37].

Although research in animal models has demonstrated that ranolazine could potentially be used to treat arrhythmias and HF, it is important that the beneficial effect of ranolazine be confirmed in clinical trials. Several clinical trials have assessed ranolazine use in patients with coronary artery diseases. In the Monotherapy Assessment of Ranolazine in Stable Angina (MARISA) trial [43], the authors showed that

ranolazine significantly improves exercise performance and delays or prevents the symptoms and ECG evidence of myocardial ischemia during exercise. In the Combination Assessment of Ranolazine in Stable Angina (CARISA) randomized trial [44], the authors showed that ranolazine increases exercise capacity and provides additional antianginal relief to symptomatic patients with severe chronic angina taking standard doses of atenolol, amlodipine, or diltiazem, without evident adverse long-term survival consequences over 1–2 years. In the Observational Study in Patients with Chronic Angina Under Ranolazine Treatment in Greece (OSCAR-GR) study [45], the authors investigated the relationship between ranolazine therapy and patient satisfaction, quality of life, physicians' assessment of therapy, and safety in patients with chronic stable angina (CSA) treated in a routine clinical setting. They concluded that ranolazine was associated with decreased angina frequency and severity and improved the quality of life. In the TERISA randomized clinical trial (Type 2 Diabetes Evaluation of Ranolazine in Subjects With Chronic Stable Angina) [46], the authors reported that ranolazine significantly reduces angina frequency among patients with type 2 diabetes, coronary artery disease, and persistent chronic angina despite a treatment with 1–2 antianginals. In the ERICA (Efficacy of Ranolazine in Chronic Angina) trial [47], the investigators reported that ranolazine significantly reduces the frequency of angina and nitroglycerin consumption when combined with amlodipine.

By inhibiting late INa using ranolazine, sodium accumulation can be reduced. Hence, ranolazine would be expected to prevent calcium accumulation that may originate from the sodium/calcium exchanger mechanism and thus improve diastolic pressure and relaxation. Using an HF dog model, the authors showed that ranolazine preserves left ventricular (LV) function and attenuates LV remodeling [48]. The authors also reported that ranolazine, when combined with an ACE inhibitor or a β-blocker, improves LV systolic and diastolic function and that the reversal of LV remodeling is superior than ranolazine alone. In a recent proof-of-concept trial, the authors reported that ranolazine improves diastolic function in HF and preserves the ejection fraction [49]. However, based on echocardiography findings, no improvements in relaxation parameters were observed. Further studies are warranted to determine whether ranolazine is beneficial for patients with diastolic heart failure.

Based on the studies described above, it seems reasonable to prescribe ranolazine to patients with chronic angina. It could also be considered a first-line drug because of its low hemodynamic effects.

## Conclusion

Persistent sodium channel currents exacerbated by different pathologies such as LQT3 syndrome or HF can delay repolarization which per se can lead to intracellular sodium overload and EADs. These EADs if of sufficient amplitude can provide the trigger for initiation of ventricular arrhythmias such as torsades de pointes and sudden cardiac death. Ranolazine demonstrated some beneficial effects in reducing late sodium channel currents in animal models. Based on the clinical trials described above, it seems reasonable to prescribe ranolazine to patients with chronic angina. It could also be considered a first-line drug because of its well tolerance and low hemodynamic effects.

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# 6 Age, Sex and Racial Differences in Cardiac Repolarization and Arrhythmogenesis

Arja Suzanne Vink, Sally-Ann B. Clur, Pieter G. Postema, Nico A. Blom, and Arthur A. M. Wilde

## Introduction

Willem Einthoven recorded the first electrocardiogram (ECG) in a healthy man in 1905, long before the understanding of the role of ion channels and the cardiac action potential. Over the last decades, extensive progress has been made in our understanding of the relationship between structure and function of the cardiac ion channels and the effects of changes in expression and gating of these channels on the electrical substrate and the ECG. The function of ion channels is significantly modified by the subunit assembly and environmental conditions (i.e. hormones, electrolyte concentrations and pH), which have substantial effects on the cardiac depolarization and repolarization. Profound ECG changes in individuals with primary arrhythmia syndromes have helped to discover the role of subunits of ion channels in causing different types of cardiac channelopathies. Indeed, pathogenic mutations in the genes encoding these subunits are causal to some of these rare arrhythmia syndromes. However, despite this improved understanding of the physiology of the cardiac ion channels, there is still a lack of knowledge on age, sex and racial differences seen in the human ECG especially regarding the QTc-interval. This is due to incomplete and controversial information on age, sex and racial differences in the expression and properties of ion channels and the regulation of ion channels, i.e. by sex hormones.

This chapter outlines the recent developments in the study of age, sex and racial differences in QTc-interval in healthy individuals.

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## General Aspects of Cardiac Repolarization

The role of cardiac ion channels in the generation of the ventricular cardiac action potential is


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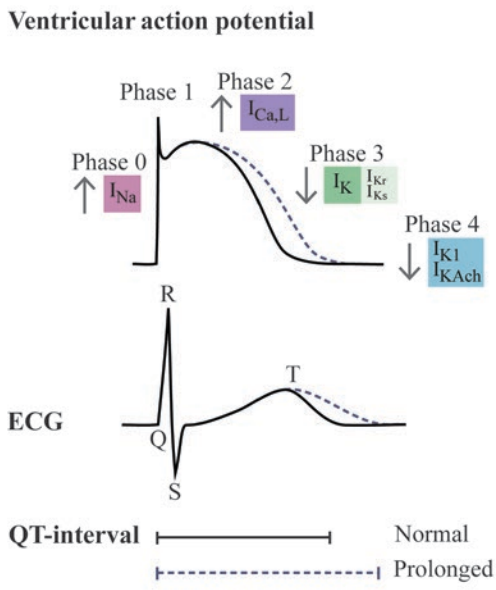


**Fig. 6.1** Electrophysiological basis of the ventricular action potential and prolongation of the QT-interval. See text for explanation

outlined in depth in Chap. 1. In short, the rapid depolarization (phase 0) is caused by the influx of sodium ions into the cell through voltage-gated sodium channels ($I_{Na}$) (Fig. 6.1). Phase 1 repolarization is mainly caused by activation of the transient outward potassium currents ($I_{to}$) together with a corresponding rapid decay of the sodium current, which is followed by phase 2. In this plateau phase, continued L-type late calcium ($I_{Ca,L}$) and a small amplitude late sodium current into the cell balance the effect of potassium currents out of the cell. The decay of the calcium current and the increase in delayed rectifier potassium current ($I_{Ks}$), in the rapid activation component of the delayed rectifier potassium current ($I_{Kr}$), and particularly the late activation of the inward rectifier potassium current ($I_{K1}$), are together responsible for the repolarization (phase 3) with ultimate return to the resting potential (phase 4).

The vectorial sum of the complex interactions of these different electrical currents in all the cardiomyocytes results in the physical manifestation of the cardiac waveform morphologies on the ECG that consist of a QRS complex and a T-wave. The QT-interval is measured from the beginning of the QRS complex to the end of the T-wave and represents the duration of activation and recovery of the ventricular myocardium. The QT-interval is most affected by alterations in phase 2 and phase 3 of the ventricular action potential [1]. Upregulation of the $I_{Ca,L}$ channel currents prolongs the QT-interval, whereas downregulation shortens the QT-interval. During phases 2 and 3, upregulation of $I_{Ks}$, $I_{Kr}$ and $I_{K1}$ channel currents shorten the QT-interval, and downregulation prolong the QT-interval. Finally, an increased amplitude of the late sodium current lengthens the action potential and thus the QT-interval.

In the normal heart, the QT-interval shortens with an increase in heart rate and lengthens with a decrease in heart rate. Since the QT-interval adapts to the heart rate, the QT-interval should be corrected for heart rate using the preceding RR-interval (QTc-interval), which can be done using several formulas [2]. None of these formulas gives an optimal correction, but the Bazett correction formula is most frequently used in daily practice [3]. A prolonged QTc-interval is a marker for an increased risk of torsades de pointes (TdP), a malignant polymorphic ventricular tachyarrhythmia that precipitates syncope, sudden cardiac arrest (SCA) or sudden cardiac death (SCD) [4, 5].

## Age-Related QTc-Interval Changes in Healthy Individuals

The QTc-interval is remarkably long after birth, in both males and females [6–8]. The QTc-interval then decreases during the first weeks after birth in both sexes [6–8] and then remains relatively consistent over the years until the age of approximately 16 years [6, 8–14]. However, when individual age trends are taking into account, both males and females have longer QTc-intervals at the age of 12 years compared to the ages of 6 and 15 years [15, 16]. After puberty, the QTc-interval shortens in males but not in females [6, 17–21]. During adulthood, the QTc-interval gradually increases with age [11, 22–33]

in both sexes [17–20, 22, 34–45]. In contradiction, there is one study that showed QTc-interval shortening in older age groups (>60 years) [46].

## Sex Differences in QTc-Interval in Healthy Individuals

Nearly 100 years ago in the early ECG recordings by Bazett [3], differences in QTc-interval were described between healthy adult males and females. Decades later, it became clear that these sex differences are not present during the first month of life [6, 8, 47, 48] but arise at a later age. At the age of 1–3 months, females have a slightly longer QTc-interval compared to males [6, 13, 14]. Thereafter, no sex difference is seen until approximately the onset of puberty [6, 9, 10, 12–14, 16, 18, 20, 49, 50]. Fukushige et al. [15] described a shorter QTc-interval in 6-year-old females compared to males, but this difference was very small (384 ms versus 386 ms; $P < 0.05$). After puberty, females have a longer QTc-interval compared to males [6, 9, 14–17, 20, 21, 50–59]. This difference has also been reported in adulthood by several studies [3, 15, 17, 19, 20, 22–27, 29–33, 35–46, 57, 60–74], although some other studies found no difference [34, 66] or even a longer QTc-interval in males [75]. The difference in QTc-interval between males and females decreases with age [25] because the QTc-interval increases more in time in males compared to females [37]. As a result, no clear sex differences are present in the highest age groups of approximately >60 years [19, 20, 27, 34, 37].

## Racial Differences in QTc-Interval in Healthy Individuals

The presence of racial differences in QTc-interval still requires clarification as the effect of race has been looked at in only a limited number of studies. Most studies report no clear racial differences [29, 48, 70, 76], although some small differences may be present. Blacks tend to have slightly shorter QTc-intervals compared to Caucasians [8, 31, 32, 74], and Asians, especially females [31, 62, 72, 73, 77], have slightly longer QTc-intervals compared to Caucasians [30, 48, 72, 78]. In addition, collective consideration of available pharmacogenetic and clinical information suggests that there may be inter-race differences in QT-prolonging effects of drugs and that Caucasians may be more sensitive than other populations [79]. These possible differences are most likely the result of the presence of considerable heterogeneity among race/ethnicity for multiple genetic loci that have an impact on the QT-interval. Some of these loci show a striking difference (i.e. order of magnitude 40–50%) between the highest and lowest frequencies between ethnicities [80]. Unfortunately, there is no data on racial-specific age-related sex differences.

## Possible Role of Sex Hormones on the QTc-Interval and Arrhythmogenesis

The age- and sex-related differences in QTc-interval, especially the change post-puberty, most likely are due to changes in sex hormones levels. The mechanisms underlying the influence of sex hormones on the repolarization are complex and still unresolved; however, mechanistic studies suggest that sex hormone has varying effects on the $I_{Ca,L}$, $I_{Kr}$, $I_{Ks}$ and $I_{K1}$ channel currents. Testosterone decreases the $I_{Ca,L}$ current and increases the potassium channel currents, resulting in a shorter QTc-interval observed in both animal and human studies [81]. Progesterone decreases the $I_{Ca,L}$ current and increases the $I_{Ks}$ current and may therefore shorten the QTc-interval [81]. Conflicting results of endogenous oestrogen on the QTc-interval have been described. Oestrogen lengthens the QTc-interval in animals; however, this has not been supported by human studies. In animal studies, oestrogen decreases the potassium channel currents and may lengthen the QT-interval through this mechanism [81].

In children, concentrations of sex hormones are influenced by the activity of the hypothalamic-pituitary-gonadal (HPG) axis. The HPG axis is active during the (I) mid-gestational period in the

foetus, (II) first months of life and (III) pubertal period [82], and therefore higher concentrations of testosterone and oestrogen are found during these periods. As a consequence, during periods of sudden changes in sex hormone concentrations (i.e. the first months of life and the onset of puberty), a marked QTc-interval shortening in males would be expected based on the higher level of testosterone compared to females. This could explain the shorter QTc-intervals in males between 1 and 3 months and after the onset of puberty compared to females.

In adulthood, the level of testosterone gradually decreases with age in males [83], potentially explaining why with ageing, the QTc-interval in males gradually lengthens and approximates that of females. Changes in sex hormone levels in females are more complex due to the influence of the menstrual cycle, pregnancy and the menopause.

## Menstrual Cycle

Oestrogen and progesterone levels fluctuate during the menstrual cycle and are lowest at the onset of menses (menstrual phase). After the cessation of the menstrual flow, there is a gradual increase in oestrogen (follicular phase). Oestrogen levels peak in the middle of the menstrual phase during the ovulation (ovulation phase), and after the ovulation, the oestrogen levels gradually decrease during the luteal phase. Progesterone levels, in contrast, are low during the menstrual and follicular phase and increase after the ovulation through the luteal phase (Fig. 6.2).

In females, there are no clear QTc-interval differences between the different phases of the menstrual cycle [84–89]. In addition, there are also no distinct differences in heart rate [86, 87, 89–92], so there is probably no effect of the method chosen to correct the QT-interval for the heart rate. Hulot et al. [84] found no relationship between the level of oestrogen and the length of the QTc-interval ($P = 0.92$). In the study by Nakagawa et al. [85], the uncorrected QT-interval was shorter in the luteal phase compared to the follicular phase, although no difference in QTc-interval was seen. They found no statistically significant difference in oestrogen level between the luteal and follicular phase, while progesterone ($P < 0.001$) and noradrenaline levels ($P < 0.05$) were higher in the luteal phase suggesting the role of progesterone and/or autonomic tone on the uncorrected QT-interval. When double autonomic blockade was given, e.g. atropine and propranolol administration, QTc-interval differences between the phases of the menstrual cycle were seen. Under these circumstances, Burke et al. [86] found a shorter QTc-interval in the luteal phase compared to the menstrual and follicular phase, whereas Endres et al. [87] observed a significantly longer QTc-interval during the follicular phase compared to the menstrual and luteal phase. Both studies indicate the important role of autonomic tone in alternations in the QTc-interval during the menstrual cycle. Rodriguez et al. [88] showed that ibutilide infusion, a known $I_{Kr}$ blocker mimicking long QT syndrome (LQTS), causes differences in QTc-interval between the phases of the menstrual cycle, whereas the luteal phase seemed to be protective against the drug-induced QTc-interval prolongation. Hence, since there is an effect of the menstrual cycle on the QTc-interval during administration of QTc-interval prolonging medication, it could be argued that this effect is also present in patients with LQTS.

Despite the unclear changes in the QTc-interval between the phases of the menstrual cycle in the normal situation, there seems to be a cyclic variation in the occurrence of episodes of arrhythmia with the menstrual cycle. During the luteal phase, an increase in the number and duration of paroxysmal supraventricular tachycardia (SVT) has been reported compared to other phases of the menstrual cycle [93, 94], with a correlation with plasma concentrations of progesterone and an inverse correlation with plasma concentrations of oestradiol [93]. Also ventricular ectopic beats seems to be more frequent during the luteal phase of the menstrual cycle [93]. So it seems that there can be significant changes in the arrhythmogenic substrate of the heart throughout the menstrual cycle without dramatic changes in the QTc-interval. As we know, there is no simple relationship between absolute QTc-

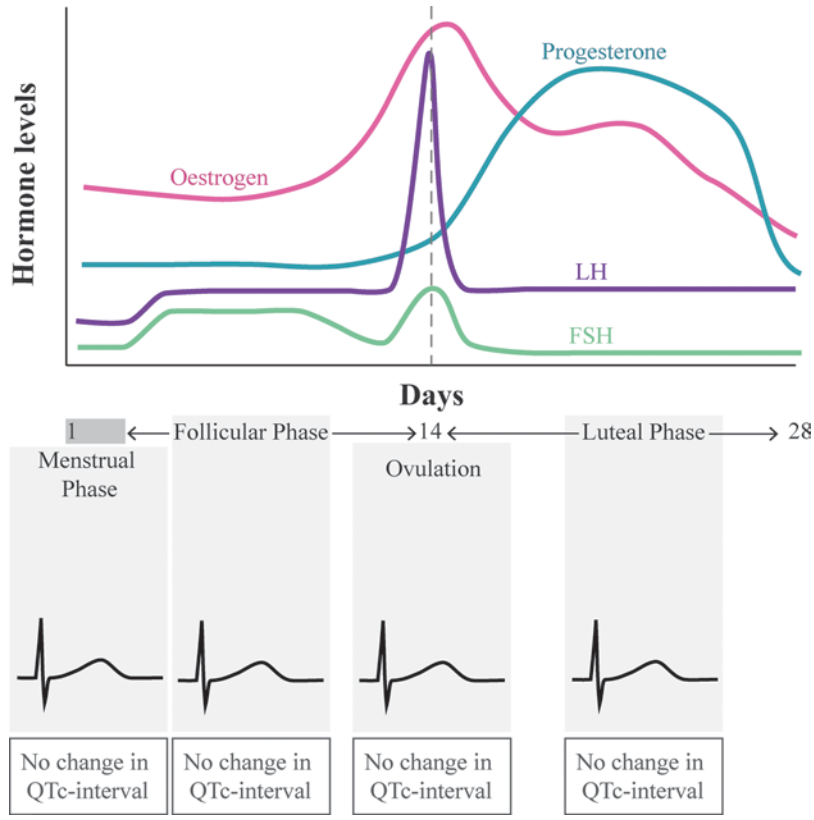


**Fig. 6.2** Oestrogen, progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels over a single menstrual cycle in females together with a schematic representation of the QTc-intervals during the phases of the menstrual cycle (i.e. menstrual phase, follicular phase, ovulation and luteal phase). See text for explanation. (Derived from data in: Sedlak et al. [81])

interval and arrhythmogenic potential or SCD [63, 88, 95], so perhaps the autonomic tone also plays an important role which is influenced by sex hormones [85, 96].

The fluctuations of oestrogen and progesterone levels during the menstrual cycle are controlled by the gonadotropin hormones, e.g. luteinizing hormone (LH) and follicle-stimulating hormone (FSH). A peak in LH and FSH is seen prior to ovulation in the normal menstrual cycle which is recognizable by an increase in body temperature (Fig. 6.2). Abehsira et al. [97] showed recently that the QTc-interval is probably influenced by a complex interaction between sex hormones and gonadotropins. In both males and females, FSH was positively correlated to QTc-interval ($r = 0.39$ and $r = 0.38$, respectively, in males and females), while free testosterone in males ($r = -0.34$) and progesterone/oestrogen ratio in women ($r = -0.38$) were negatively correlated. LH was only correlated in females to the QTc-interval ($r = 0.30$).

## Pregnancy

During gestation, there is a complex and varying combination of sex hormones. Oestrogen and progesterone levels gradually increase during pregnancy until labour when the levels drop sharply reaching pregravid levels by the fifth postpartum day (Fig. 6.3) [98–102].

Studies regarding changes in QT/QTc-interval during pregnancy used either longitudinal data during pregnancy [103], the postpartum state as a control [104] or a control group of non-pregnant women [105, 106]. There is a longer QTc-interval during the first trimester and late pregnancy, with a shortening of the QTc-interval after delivery [104–106]. The QTc-interval just after delivery is however longer compared to the postpartum period [104]. The heart rate is, on the contrary, higher during pregnancy and delivery compared to the control group [104–106] and seems to increase with gestation [103]. Due to these changes in heart rate, it is not unlikely that the

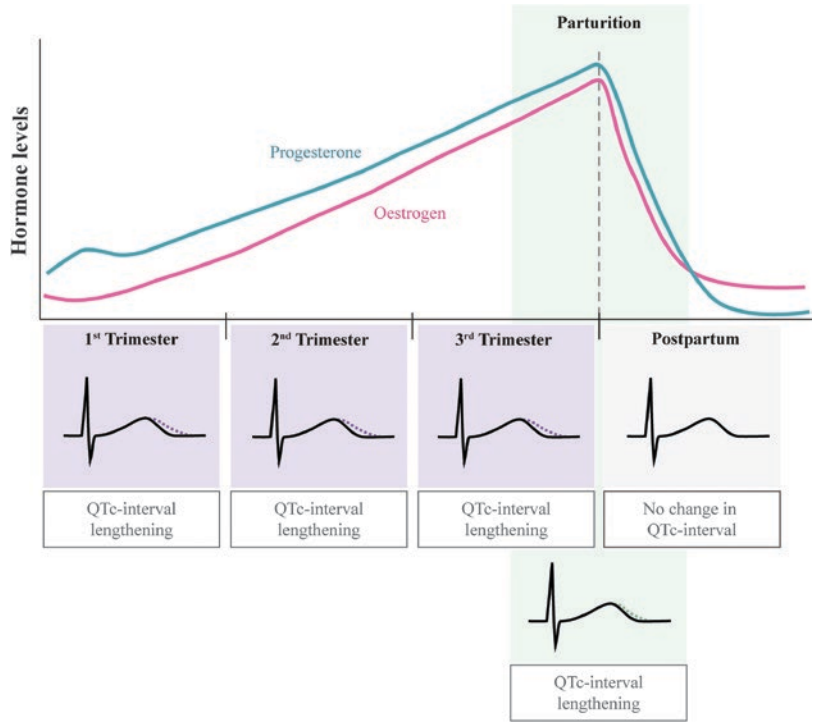


**Fig. 6.3** Oestrogen and progesterone levels during pregnancy and after parturition together with a schematic representation of the QTc-intervals during the trimesters, parturition and the postpartum period. See text for explanation. (Derived from data in: Bett [115])

observed differences in the QTc-interval are influenced by the correction method used to correct the QT-interval for the heart rate [107]. Anneken et al. [108] recently studied five healthy women who became pregnant after stimulation by clomiphene citrate therapy for infertility, observing shorter QTc-intervals during higher oestrogen levels. Progesterone did not affect the QTc-interval significantly in that study.

The gestational prolongation of the QTc-interval does not precipitate widespread fatal cardiac arrhythmias in pregnant women. However, there is a slight increase in arrhythmias during pregnancy regarding SVTs and even ventricular tachycardia (VT) compared to the postpartum period or non-pregnant controls [109, 110]. Although the occurrence of VT is usually uncommon, there presence should raise a suspicion of underlying cardiovascular disease.

## Menopausal Period

The menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 months of amenorrhoea without any other obvious pathological or physiological cause. The menopause is a reflection of complete, or near-complete, ovarian follicular depletion resulting in very low levels of oestrogen and progesterone and high levels of FSH concentrations (Fig. 6.4). The menopausal transition, or perimenopause, occurs after the reproductive years and before the menopause. This period is characterized by irregular menstrual cycles and significant hormonal variability [111, 112].

Two studies comparing premenopausal females to postmenopausal females showed no differences in QTc-interval and heart rate [113, 114]. One of the studies also measured the hormone levels and found no difference in oestrogen levels but a lower level of progesterone in the postmenopausal phase compared to the premenopausal phase [114].

The data regarding postmenopausal arrhythmogenesis is lacking; however the autonomic tone may also play a role in the peri-/postmenopausal period. Hence, the QTc-interval is shorter in women with hot flushes compared to those without, and the absence of menopausal hot flushes is associated with an elevated activity of the sympathetic nervous system.

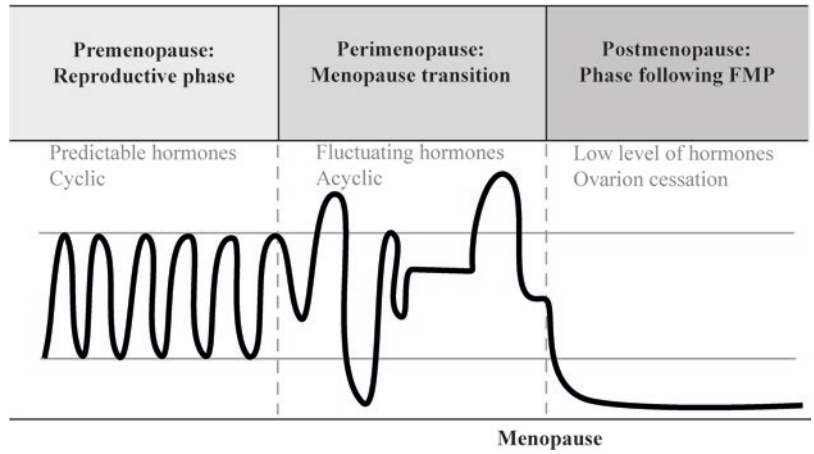


**Fig. 6.4** Menstrual cycle patterns during menopause. FMP = Final menstrual period. See text for explanation. (Reprinted by permission from Springer Nature: Deecher and Dorries [116])

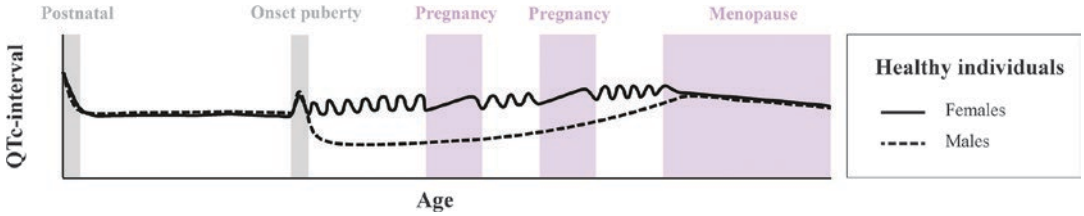


**Fig. 6.5** Schematic representation of hypothetical changes in QTc-interval in healthy individuals

## Conclusion

Age, sex and race have an influence on the QTc-interval. Although data regarding racial differences is lacking, the small differences seen are probably the result of the presence of considerable heterogeneity among race/ethnicity for multiple genetic loci that influence the QT-interval. Age- and sex-related differences in QTc-interval (Fig. 6.5) are most likely the result of changes in sex-specific hormones. Although the exact mechanisms and pathophysiology of the effect of sex hormones on the QTc-interval and the arrhythmogenesis are not known, testosterone appears to shorten the QTc-interval in males. In females, however, there is a more complex interaction between progesterone and oestrogen. In addition, the autonomic nervous system and gonadotropins may play an important part in this complex interaction.

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# 7 ECG-Derived Evaluation of Cardiac Repolarization

Gioia Turitto and Nabil El-Sherif

## Introduction

The resting 12-lead electrocardiogram (ECG) is the oldest tool and the most widely adopted clinical test for detection of heart disease. Today, it is still one of the most useful methods for risk stratification for sudden cardiac death and arrhythmic events. This is based on a large body of experimental and clinical data, which support the contention that both depolarization and repolarization analyses on the 12-lead ECG and its derivatives can provide a measure of arrhythmic risk.

Repolarization is the summation of the intrinsic characteristics of the individual cells ensuing from ion channel properties, channel density and distribution, autonomic modulation, and cell-cell coupling effects. The differences observed in ventricular cells mediate the distinct repolarization times of varying regions in the myocardium, which affect the electrocardiographic T wave [1]. The left ventricle exhibits gradients in repolarization time in both transmural and apicobasal directions. These gradients, caused by the varying strengths of cellular ionic currents in the different regions, affect the morphology of the electrocardiographic T wave. Abnormal gradients, observed clinically by alterations in the ECG, can increase susceptibility to arrhythmias.

A number of ECG repolarization measures have been identified and assessed in large population studies, as well as in research focused on high-risk cohorts, and will be discussed in this chapter. They include static parameters, such as QT interval duration, as well as dynamic markers, such as QT variability, and computationally derived variables, such as T-wave alternans. The reader is also advised to consult other chapter of this book, for additional discussion of specific methods to assess cardiac repolarization, as well as clinical syndromes associated with cardiac repolarization abnormalities.

## QT Interval Duration

In clinical practice, studies on QT and corrected QT (QTc) interval represent archetypal examples of research on abnormal repolarization for the purpose of risk stratification for sudden cardiac death [2]. Since the QT interval shortens with an increasing heart rate, its interpretation requires correction, which can be made using different formulae (Table 7.1). The most popular method is the Bazett's formula, which is given by the QT interval divided by the square root of the RR

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**Table 7.1** Commonly utilized formulae for QT correction

| Author | Formula |
|---|---|
| Bazett | $QT/RR^{1/2}$ |
| Fridericia | $QT/RR^{1/3}$ |
| Framingham | QT + 0.154 (1000 – RR) |
| Hodges | QT + 105 (1/RR – 1) |

interval. The disadvantage of this method is that QT interval is overestimated at high heart rates and underestimated at low heart rates. Fridericia's formula divides the QT interval by the cubic root of the RR interval and is preferred for slow heart rates. The ESC Guidelines published in 2015 propose an upper normal limit for the QTc interval of 480 msec and a lower limit of 340 msec for both genders [3]. The risk of developing malignant ventricular arrhythmias increases at either extreme of the QT interval, as exemplified by the long and short QT syndromes.

Different genetic types of the long QT syndrome (LQTS) are associated with distinct ECG manifestations, which relate to the type and magnitude of ion channel dysfunction. The QTc duration does not differentiate LQTS types, and therefore other static and dynamic ECG parameters reflecting changes in T-wave morphology are used to describe phenotypic expression of different LQTS genotypes. LQT1 carriers usually have broad-based T waves, LQT2 carriers show low-amplitude T waves with high incidence of notches, and LQT3 carriers frequently have extended ST segment, with relatively narrow peaked T wave [4]. QTc duration was found to predict cardiac events in LQTS. A QTc greater than 470 msec, but especially a QTc greater than 500 msec, was associated with a significant increase in the risk of cardiac events. When analyzing the predictive value of QTc by genotype, there were no differences in risk associated with QTc prolongation among patients with LQT1, LQT2, and LQT3. Furthermore, stratifying this analysis by age, sex, and genotype confirmed that QTc is uniformly predictive for cardiac events and does not have different prognostic significance in tested subgroups [4].

The diagnostic hallmark for the short QT syndrome (SQTS) is a short ECG QT interval on the 12-lead ECG; however, consensus on an appropriate cutoff value and diagnostic criteria sufficient to establish a diagnosis of SQTS have yet to emerge in the literature. The diagnosis of SQTS is further complicated by the presence of an overlapping range of QT intervals between affected cases and apparently healthy subjects. This concept is highlighted in a long-term follow-up study of healthy Finnish individuals with short (<340 msec) and very short (<320 msec) QTc values, who had no documented arrhythmic events after an average follow-up of 29 years [5]. Additional studies examining subjects with short QT intervals from the general population have revealed similarly benign outcomes with no evidence of increased arrhythmic risk. These findings suggest that the presence of a short QT interval in isolation is not always predictive of an increased arrhythmic risk and therefore should not invariably lead to a diagnosis of SQTS [6]. On the other hand, an abnormal QTc interval on a resting 12-lead ECG has not been found to be independently correlated with mortality or arrhythmic outcomes in patients with myocardial infarction or with left ventricular dysfunction [7].

## Beyond QT Interval Duration

Several novel indices of abnormal repolarization have been proposed during the past two decades (Table 7.2). While some have been validated in clinical practice, only a handful of data is available on the clinical usefulness of other parameters. The impetus for this research stemmed from the recognition that QTc provided no information on the heterogeneity of repolarization across the heart, yet the presence of such heterogeneity in repolarization may elevate arrhythmic risk. This can occur when prolongation of the action potential duration is nonuniform across the myocardium. These limitations of QTc in predicting malignant ventricular arrhythmias and sudden cardiac death have led to the development of additional markers, such as QT dispersion (QTD), the interval from the peak to the end of the T-wave (Tpeak-Tend), and Tpeak-Tend/QT ratio, among others [2].

**Table 7.2** Commonly used variables to quantify cardiac repolarization abnormalities

| Variable | Definition | Experimental correlate |
|---|---|---|
| Corrected QT interval | QT interval duration corrected for heart rate | Action potential duration (APD) |
| QT dispersion | Difference between the maximum and minimum QT intervals measured on all 12 ECG leads | Spatial differences in APD values |
| Tpeak-Tend | Interval from the peak of the T wave to its offset | Spatial dispersion of repolarization (DR) |
| Tpeak-Tend/QT | Interval from the peak to the end of the T wave divided by QT interval | DR divided by APD |
| QT dynamicity | Slope of the linear regression of QT/RR | Temporal APD variations |
| QT variability | *SDQT* standard deviation of QT intervals | Temporal APD variations |
| T-wave alternans | T-wave duration difference between alternate beats at the microvolt level | APD alternans |

Experimental work has suggested that QTD showed a significant correlation with dispersion of monophasic action potential. QTD is calculated as the difference between the maximum and minimum QT intervals measured on all 12 leads of the ECG. Reported values of QTD vary widely, with studies showing normal values between 10 and 71 msec, with a mean value of 33 msec. In otherwise healthy individuals, QTD values >58 and >80 msec were shown to increase the risk of cardiovascular mortality by three- and fourfold, respectively, when compared with subjects with QTD values <30 msec [2]. Other authors have argued, however, that elevated values for QTD may largely be due to the unreliable localization of the T-wave offset in patients with abnormal T waves [8].

Tpeak-Tend is calculated by measuring the interval from the peak of the T wave to its offset. The offset of the T wave is frequently defined as the intersection of the tangent to the steepest portion of the terminal portion of the T wave and the isoelectric line [2, 8]. It was initially suggested as a marker for transmural dispersion of repolarization, based on observations in coronary-perfused canine wedge preparations that the end of repolarization at the epicardium coincided with the Tpeak and at the M-cells coincided with Tend. Subsequent experiments showed that Tpeak coincided not with full epicardial repolarization but rather with the earliest end of repolarization, whereas Tend coincided with the latest end of repolarization rather than full M-cell repolarization. In other words, Tpeak-Tend may be a marker for global, rather than transmural, dispersion of repolarization [2]. Validation of Tpeak-Tend as a direct measure of transmural dispersion of repolarization is still debated, but most studies agree that Tpeak-Tend provides at least some measure of spatial dispersion or repolarization heterogeneity. A prolonged Tpeak-Tend may be seen in patients with LQTS1 and LQTS2, as well as in patients with SQTS and Brugada syndrome. Furthermore, Tpeak-Tend predicted mortality in both ST elevation and non-ST elevation myocardial infarction [2]. Additionally, repolarization heterogeneity, as measured by Tpeak-Tend, may be associated with adverse outcomes, including mortality, in the general population [8]. Other investigators have proposed dividing Tpeak-Tend by the QT interval. This yields the Tpeak-Tend/QT ratio, which has a relatively constant normal range between 0.17 and 0.23. Theoretically, an increased ratio represents a higher dispersion of repolarization, which should be proarrhythmic; its predictive value for arrhythmic events remains controversial [9].

## Dynamic Changes in QT Interval

An important component of arrhythmogenesis relates to dynamic changes in cardiac repolarization, which may lead to increased vulnerability to ventricular arrhythmias. Several kinds of software have been developed in order to continuously analyze the QT interval from 24-hour ECG recordings. Compared to the ECG static evaluation, dynamic assessment of ventricular repolarization allows the analysis of the relationship between the duration of the QT interval and heart

rate changes [10]. The most studied dynamic measures of abnormal repolarization on 24-hour ECG are QT dynamicity and QT variability [2, 7, 11]. The automatic assessment of QT dynamicity is based on the measure of: QT apex (QTa, the interval between the Q wave start and the T wave apex), and QT end (QTe, the interval between the Q wave start and the T wave end). QT dynamicity is generally assessed on 24-hour ECG recordings, which are analyzed by a specific software able to automatically calculate, in a template of 30 sec, the QTa, the QTe, and the corresponding RR interval. By interpolating each measure, the software also computes the slopes of the linear regressions between QTe and QTa and the corresponding RR interval. QT-RR slope is considered an index of QT dynamicity related to arrhythmic events.

Whereas QT dynamicity is based on the analysis of QT/RR relationship, QT variability is based on the analysis of beat-to-beat changes in duration and morphology of ventricular repolarization. QT variability can be measured during a short recording (256 sec or 30 beats) or over a 24-hour period, distinguishing short-term from long-term variability. Dedicated QT variability measurement techniques match complete or partial ECG waveforms with one or several templates, either user defined or automatically computed [11]. Several studies have demonstrated the independent predictive value of QT variability for risk stratification for sudden cardiac death; however, most of the evidence is based on retrospective data analyses. Prospective trials are needed to prove the usefulness of QT variability. Additionally, cutoff values or hazard ratios need to be defined for QT variability to become an integral part of risk stratification strategies [7, 11].

Periodic repolarization dynamics is a novel ECG phenomenon that refers to previously unknown oscillations of cardiac repolarization instability. Those oscillations take place in the low-frequency range (<_0.1 Hz), occur independently from underlying heart rate variability, and can be assessed by means of a multipolar high-resolution resting ECG. Although the exact mechanisms still need to be identified, electrophysiological and other studies indicated that periodic repolarization dynamics most likely reflects the effect of phasic sympathetic activation on the myocardial cells. Periodic repolarization dynamics has been shown to be a significant predictor of mortality in cohorts of postinfarction patients with either preserved or reduced left ventricular ejection fraction [12].

The most recent electrocardiographic marker for cardiac repolarization abnormalities, T-wave area dispersion, is a measure of repolarization heterogeneity, obtained by assessing interlead T-wave areas during a single cardiac cycle. The ability of this marker to identify patients at risk for sudden cardiac death in the general population was recently tested. Low T-wave area dispersion, reflecting increased heterogeneity of repolarization, in standard 12-lead resting ECGs, was shown to be a powerful and independent predictor of sudden cardiac death in the adult general population, when assessed in the Health 2000 Study—an epidemiological survey representative of the Finnish adult population. Independent replication was performed in 3831 participants of the KORA S4 Study (Cooperative Health Research in the Region of Augsburg) [13].

According to the American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society Scientific Statement on Noninvasive Risk Stratification Techniques for Identifying Patients at Risk for Sudden Cardiac Death, the present data do not support the use of QT interval, QT dispersion, or QT-interval variability for risk stratification for sudden cardiac death in patients without the long QT syndrome. This statement may not apply to the more recent risk stratification techniques based on cardiac repolarization analysis, but there is no definite conclusion about the usefulness of such techniques for screening of the general population or cohorts at high risk [14].

## T-Wave Alternans

Microvolt T-wave alternans (TWA) was deemed to be a promising measure of dynamic electrical instability. The value of TWA in estimating risk

for cardiovascular mortality and sudden cardiac death is based on the assessment of the degree of heterogeneity of repolarization and of abnormalities in intracellular calcium cycling, which provoke a beat-to-beat alternation of action potential duration culminating in alternating changes in the size and shape of the T wave. TWA can be either spatially concordant, when action potentials in neighboring cell regions alternate in phase, or discordant, when they are out of phase. The transition from concordant to discordant TWA is likely a harbinger of increased risk for malignant arrhythmias [15]. The association of TWA with spatiotemporal heterogeneity of repolarization (i.e., transient or lasting repolarization differences among neighboring myocardial regions) is supported by a number of investigations, including in vitro optical mapping, intact large animal experimental laboratory studies, computer simulations, and clinical studies [16].

Two main techniques have been commercialized to measure microvolt levels of TWA for risk stratification for sudden cardiac death: the spectral method and the modified moving average (MMA) method [15, 16]. TWA is a rate-dependent phenomenon that can be assessed by exercise testing or by evaluating spontaneous changes during 24-hour ECG recording. In the spectral method, which requires the patient to achieve a target heart rate of 105–110/min, a composite power spectrum of the ST–T segment amplitude fluctuations is formed by applying fast Fourier transform technique to the beat-to-beat series of amplitude measurements along the JT interval in 128 consecutive QRS-aligned ECG complexes [17]. The alternans voltage is defined as the square root of the spectral power occurring at the alternans frequency (0.5 cycles/beat) after noise reduction, which corresponds to the difference in voltage between the overall average beat and either the averaged even or odd-numbered beat (i.e., half of the difference between the averaged even and odd beats). A TWA level $\geq$1.9 μV with sufficient signal-to-noise ratio for >2 min is defined as a positive test result, while a TWA level <1.9 μV is considered negative. However, as the spectral method requires a stable target heart rate to be achieved, a relatively large proportion (approximately 20–40%) of tests are classified as "indeterminate," either due to patient-related factors such as failure to achieve the target heart rate, excessive ventricular ectopy, atrial fibrillation, or non-sustained TWA or technical problems such as noise in the recording.

The MMA method is time-domain based and employs recursive averaging. The algorithm continuously streams odd and even beats into separate bins and creates averaged complexes for both bins. These complexes are then superimposed, and the maximum difference between the odd and even complexes at any point within the JT-segment is averaged for every 10 or 15 seconds and reported as the TWA value. The MMA method allows TWA analysis during routine exercise stress testing and also during 24-hour ECG recordings. Risk stratification is based on the peak TWA value using the MMA; cutoff levels of $\geq$47 μV and $\geq$60 μV have been most commonly used to define abnormal and severely abnormal TWA, respectively [18]. The four- to tenfold difference in TWA voltage output between the two methods can be accounted for largely by differences in the degree of signal averaging and filtering. Another factor accounting for the lower values reported by the spectral method as compared to MMA is that with the former, the average TWA value across the ST–T wave is calculated, whereas with the latter, the peak TWA value is provided.

Microvolt TWA has been associated with cardiovascular mortality and sudden cardiac death in several clinical studies involving several thousand subjects with reduced as well as preserved left ventricular function. Although TWA appears to be a useful marker of susceptibility for lethal ventricular arrhythmias and cardiovascular death, so far there is no sufficient evidence from randomized clinical trials to support its use in guiding therapy. In particular, the use of TWA was proposed to select patients with a reduced left ventricular ejection fraction, who may be unlikely to benefit from implantation of a cardioverter-defibrillator, in the presence of a negative TWA, but this has not been validated [15, 16, 18].

## J-Wave Syndromes: ECG Characteristics

Early repolarization, Brugada syndrome, and pathologic J waves have been described for decades, but only recently experimental and clinical data have allowed reconciliation of Brugada and early repolarization under the common definition of J-wave syndromes [19]. The J-wave syndromes are so named because they involve accentuation of the electrocardiographic J wave. Experimental evidence indicates that the J wave is inscribed as a consequence of a transmural voltage gradient caused by the manifestation of an action potential notch in the epicardium but not in the endocardium, due to a heterogeneous transmural distribution of the Ito current. The region generally most affected in Brugada syndrome is the anterior right ventricular outflow tract; in early repolarization, it is the inferior region of the left ventricle. As a consequence, Brugada syndrome is characterized by accentuated J waves appearing as a coved-type ST-segment elevation in the right precordial leads V1-V3, whereas early repolarization is characterized by J waves, notch or slur of the terminal part of the QRS, and ST-segment or Jp elevation >0.1 mV in ≥2 contiguous leads, in the lateral (type I), inferolateral (type II), or inferolateral + anterior or right ventricular leads (type III), with the peak of an end QRS notch and/or the onset of an end QRS slur being designated as Jp [19, 20]. An early repolarization pattern is often encountered in ostensibly healthy individuals, particularly in young males, black individuals, and athletes. Such pattern may also be observed in acquired conditions, including hypothermia and ischemia. When associated with malignant ventricular arrhythmias in the absence of organic heart disease, the early repolarization pattern is referred to as early repolarization syndrome.

According to the 2013 Consensus statement on inherited cardiac arrhythmias and the 2015 Guidelines for the management of patients with ventricular arrhythmias and prevention of sudden cardiac death, Brugada syndrome is diagnosed in patients with type 1 ECG features, i.e., coved ST-segment elevation ≥2 mm in ≥1 lead among the right precordial leads V1 and V2, positioned in the 2nd, 3rd, or fourth intercostal space, occurring either spontaneously or after provocative drug test with intravenous administration of Class I antiarrhythmic drugs [19]. Brugada syndrome is diagnosed in patients with type 2 or type 3 ECG features, only when a provocative drug test with intravenous administration of Class I antiarrhythmic drugs induces a type 1 ECG morphology. Type 2 is characterized by ST-segment elevation of ≥0.5 mm (generally ≥2 mm in V2) in ≥1 right precordial lead (V1-V3), followed by a convex ST. The ST segment is followed by a positive T wave in V2 and variable morphology V1. Type 3 is characterized by either a saddleback or coved appearance with an ST-segment elevation of <1 mm. The placement of the right precordial leads in more cranial positions (in the third or second intercostal space) in a 12-lead resting ECG or 12-lead Holter ECG increases the sensitivity of the ECG. It is recommended that ECG recordings be obtained in standard and superior positions for the V1 and V2 leads.

The cellular mechanisms underlying the J-wave syndrome have long been a matter of debate. In the case of Brugada syndrome, two principal hypotheses have been advanced: (1) the repolarization hypothesis, proposing that an outward shift in the balance of currents in right ventricular epicardium can lead to repolarization abnormalities resulting in the development of phase 2 reentry, which generates closely coupled premature beats capable of precipitating polymorphic ventricular tachycardia/fibrillation, and (2) the depolarization hypothesis, suggesting that slow conduction in the right ventricular outflow tract, secondary to fibrosis, plays a primary role in the development of the electrocardiographic and arrhythmic manifestations of the syndrome [19].

## Conclusion

The standard ECG is a potentially attractive tool for large-scale screening and risk assessment,

due to its relative low cost and ubiquitous availability [7]. It is increasingly apparent that repolarization heterogeneity is present in a variety of cardiovascular diseases and syndromes and may also be predictive of sudden cardiac death in the general population [8]. However, at the present time, there is no consensus on how to best measure repolarization heterogeneity in human subjects. Currently available ECG techniques are still seldom used in clinical practice, partly due to their unclear diagnostic accuracy, as well as to the lack of consensus about the appropriate computational signal processing implementation [8, 21, 22].

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# Part II

# Pathophysiology, Molecular Biology, and Clinical Aspects of Long QT Syndromes

# 8 Genotype-Phenotype Correlation in Congenital LQTS: Implications for Diagnosis and Risk Stratification

Ilan Goldenberg

## Overview

The hereditary long QT syndrome (LQTS) is a genetic channelopathy with variable penetrance and expressivity. Clinically, LQTS is recognized by abnormal QT interval prolongation on the ECG and is associated with increased risk of polymorphic ventricular tachycardia, syncope, and sudden cardiac death (SCD). It has an estimated prevalence of 1:2000 to 1:5000 [1, 2]. Mutations in the three genes *KCNQ1*, *KCNH2*, and *SCN5A* underlying LQT1, LQT2, and LQT3, respectively, account for about 95% of patients with an identified genetic cause, although mutations in a total of 17 genes have been reported to cause LQTS [1–4]. Prior studies by our group and others have investigated the phenotype-genotype relationship regarding clinical presentation, electrocardiogram (ECG) patterns, risk stratification for cardiac events, and therapy [1, 3, 5–10]. LQT1 and LQT2 genotypes caused by potassium ion-channel dysfunction account for about 80–85% of the reported LQTS cases, whereas LQT3, caused by impaired closing of the cardiac sodium channel, accounts for about 5–13% of LQTS patients [2, 4]. In 1995 [10], we provided the first evidence for a significant phenotype-genotype relationship in LQT1, LQT2, and LQT3 patients of ECG repolarization morphology describing a normal broad-based T-wave morphology in LQT1 patients, flat and frequently notched T waves in LQT2 patients, and a delayed and peaked T wave in LQT3 patients. Subsequently, we have shown a significant relationship between LQTS genotype and risk of cardiac events in LQT1–3 patients, with the lethality of cardiac events (likelihood of dying suddenly during the first syncopal episode) being fivefold higher in LQT3 than in LQT1 and LQT2 patients [1]. Accordingly, an understanding of the relationship between the genotype and phenotype characteristics of LQTS can lead to improved risk stratification and management of this hereditary arrhythmogenic disorder. However, there is substantial variation within LQT subtypes even within families segregating the same variants in penetrance and expression of disease and important sex differences in the clinical course of the disease, prompting a search for disease-modifying factors.

## Genetics and Molecular Mechanisms

### Genetic Forms of LQTS

To date, more than 600 mutations have been identified in 17 LQTS genes. The LQT1, LQT2, and LQT3 genotypes comprise more than 95% of the patients with genotype-positive LQTS and

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**Table 8.1** Mutations in ion-channel genes causing the main types long QT syndrome

| Genotype | Chromosome | Affected gene | Channel protein | Ion-channel current | Frequency in mutation-identified LQTS patients |
|---|---|---|---|---|---|
| LQT1 | 11 | KCNQ1 (Kv7.1) | 4 α-subunits each with 6 membrane-spanning segments | $\downarrow I_{Ks}$ | 45% |
| LQT2 | 7 | KCNH2 (hERG) ($K_v$11.1) | 4 α-subunits each with 6 membrane-spanning segments | $\downarrow I_{Kr}$ | 45% |
| LQT3 | 3 | SCN5A ($Na_v$1.5) | 1 α-subunit with 24-membrane spanning segments | ↑late $I_{Na}$ | 7% |

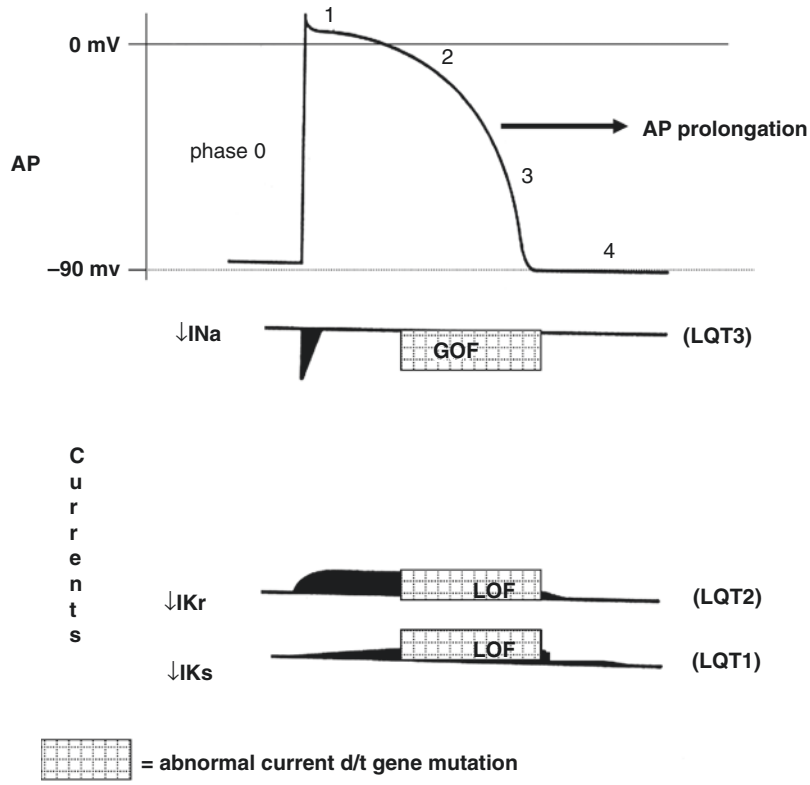



**Fig. 8.1** Schematic diagram of the influence of altered ion-channel currents on the action potential duration in LQTS. Inward currents are indicated below the line and outward currents above the line. The hatched rectangles denote the timing location of the effect of mutations in LQT1, LQT2, and LQT3 genes on sodium and potassium ion-channel currents. The action potential is prolonged (horizontal arrow) when there is inappropriate gain of function (GOF) in late sodium current $I_{Na}$ or loss of function (LOF) in slowly ($I_{Ks}$) or rapidly ($I_{Kr}$) acting repolarizing potassium currents

approximately 75% of all patients with LQTS (Table 8.1) [4, 11]. The most common pattern of inheritance of LQTS is autosomal dominant (Romano-Ward syndrome), but there is also an autosomal recessive form of inheritance. The presence of two mutations in either the KCNQ1 (LQT1) or KCNE1 (LQT5) gene results in the Jervell and Lange-Nielsen syndrome, a severe form of LQTS with sensory hearing loss. Mutations in LQTS genes affect different ion channels and produce gain or loss of function that determine phenotypic manifestations of the disease. Most commonly, QT prolongation arises from either a decrease in repolarizing potassium current during phase 3 of the action potential ("loss of function") or a prolonged depolarization due to late inward entry into the myocyte sodium or calcium current ("gain of function") (Fig. 8.1) [11].

LQTS type 1 (LQT1) is caused by a mutation in the KCNQ1 gene leading to dysfunction of the KvLQT1 potassium channel and reduction in the slowly repolarizing cardiac potassium current ($I_{Ks}$) (Fig. 8.1). The mutations associated with the KCNQ1 genes act mainly through a dominant-negative mechanism when the normal and mutant subunits create the defective channel protein resulting in dysfunctional channel with more

than 50% reduction in the channel current. The other common biophysical mechanism leading to decreased potassium current is haploinsufficiency in which potassium channel mutant subunits are incapable in co-assembling with the normal subunits to form a tetramer channel. Therefore, only subunits encoded by a normal allele form a functional channel resulting in 50% or less reduction in channel function [4].

In LQTS type 2 (LQT2), a mutation in the KCNH2 gene leads to dysfunction of the hERG potassium channel and reduction in the rapidly repolarizing cardiac potassium current ($I_{Kr}$) (Fig. 8.1).

LQTS type 3 (LQT3) is caused by a mutation in the SCN5A gene leading to dysfunction of the Nav1.5 protein, increasing the cardiac sodium current influx ($I_{Na}$) (Fig. 8.1). Reduced repolarizing potassium current or increase in the late sodium current leads to prolongation of the ventricular action potential and early afterdepolarizations, which are the potential triggers for ventricular tachyarrhythmias [11].

LQTS types 4–17 are rare, representing less than 5% of patients with genotype-positive LQTS (Table 8.2). These include (1) mutation in the KCNJ2 gene results in a reduction in Kir2.1 current, QT prolongation, and a phenotype dominated by skeletal abnormalities (Andersen-Tawil syndrome) (LQT7) [12]; (2) mutation in the CACNA1C gene results in an increase in $Ca_v1.2$ current, QT prolongation, and a phenotype characterized by syndactyly in both hands and feet and multiorgan dysfunction (Timothy syndrome) (LQT8) [13]; and (3) mutation in the SCN4B gene (LQT10), one of the β-subunits of SCN5A, results in an increase late entry of sodium current into the cell with associated QT prolongation [14].

It is now appreciated that mutations in non-ion-channel genes can affect ion-channel currents with resultant prolongation in ventricular repolarization and the QT interval. LQT4 is caused by mutations in the ankyrin-B gene [15] that produces a protein that functions as a cytoskeletal membrane adapter and is involved in the cellular organization of the sodium pump, the sodium/calcium exchanger, and the inositol-1,4,5-triphosphate receptors [16]. Mutations in caveolin-3 have been labeled LQT9 [17]. Electrophysiologic studies have revealed altered gating kinetics in the cardiac sodium channel with resultant increase in sustained late sodium current ($Na_v1.5$) probably due to direct protein-protein interactions [18]. Thus, LQT4 and LQT9 may be considered "channelopathy-related disorders" (Table 8.2), and it is likely that mutations in other channelopathy-related genes will be identified in the future to account for some of the 25% or so LQTS patients who are negative for mutations in the eight classical channelopathy genes.

**Table 8.2** Mutations in ion-channel genes causing the rare types of long QT syndrome

| Gene[1,2,3] | LQTS phenotype | Comments |
|---|---|---|
| *AKAP9* | LQTS type 11 | Limited |
| *ANK2* | LQTS type 4 | <1% |
| *CACNA1C* | LQTS type 8 | <1% |
| *CALM1* | LQTS type 14 | <1% |
| *CALM2* | LQTS type 15 | <1% |
| *CAV3* | LQTS type 9 | <1% |
| *KCNE1* | LQTS type 5 | <1% |
| *KCNE2* | LQTS type 6 | <1% |
| *KCNJ2* | LQTS type 7 | <1% |
| *KCNJ5* | LQTS type 13 | <1% |
| *SCN4B* | LQTS type 10 | Limited |
| *SNTA1* | LQTS type 12 | <1% |

## Ion-Channel Structure and Function

Ion channels are formed by transcription of exonic DNA from the gene into mRNA with translation into a sequence of amino acids that make up channel protein subunits [4, 11]. The subunits are co-assembled into a structural channel protein that is transported to the cell surface through a process called trafficking. The protein is then anchored into the myocyte membrane so it can function as an ion channel. The protein channel undergoes controlled degradation and appropriate replacement. Thus, a mutation in the genetic DNA can produce an abnormal or deficient ion channel through a variety of processes that may include altered subunit assembly, trafficking deficiencies, impaired anchoring of the channel in the myocyte membrane, abnormal amino acid sequence in the protein channel, or an imbalance in the degradation-formation rates of

the channel protein [4, 11]. Trafficking deficiencies are especially common in LQTS patients with mutations in the KCNH2 (LQT2) gene [19].

The putative topology of each voltage-gated cardiac ion channel consists of a pore-forming α-subunit and one or more attached β-subunits. The protein structures of the α-subunits of the LQT1 and LQT2 potassium channels (KCNQ1, KCNH2) are similar and consist of a series of amino acids with an N-terminus region, six membrane-spanning segments (S1–S6) with connecting intracellular cytoplasmic loops (S2–S3 and S4–S5) and a pore area (S5-loop-S6), and a C-terminus region (Fig. 8.2a). Four α-subunits join together to form a tetrameric potassium channel around a central pore (Fig. 8.2b). The

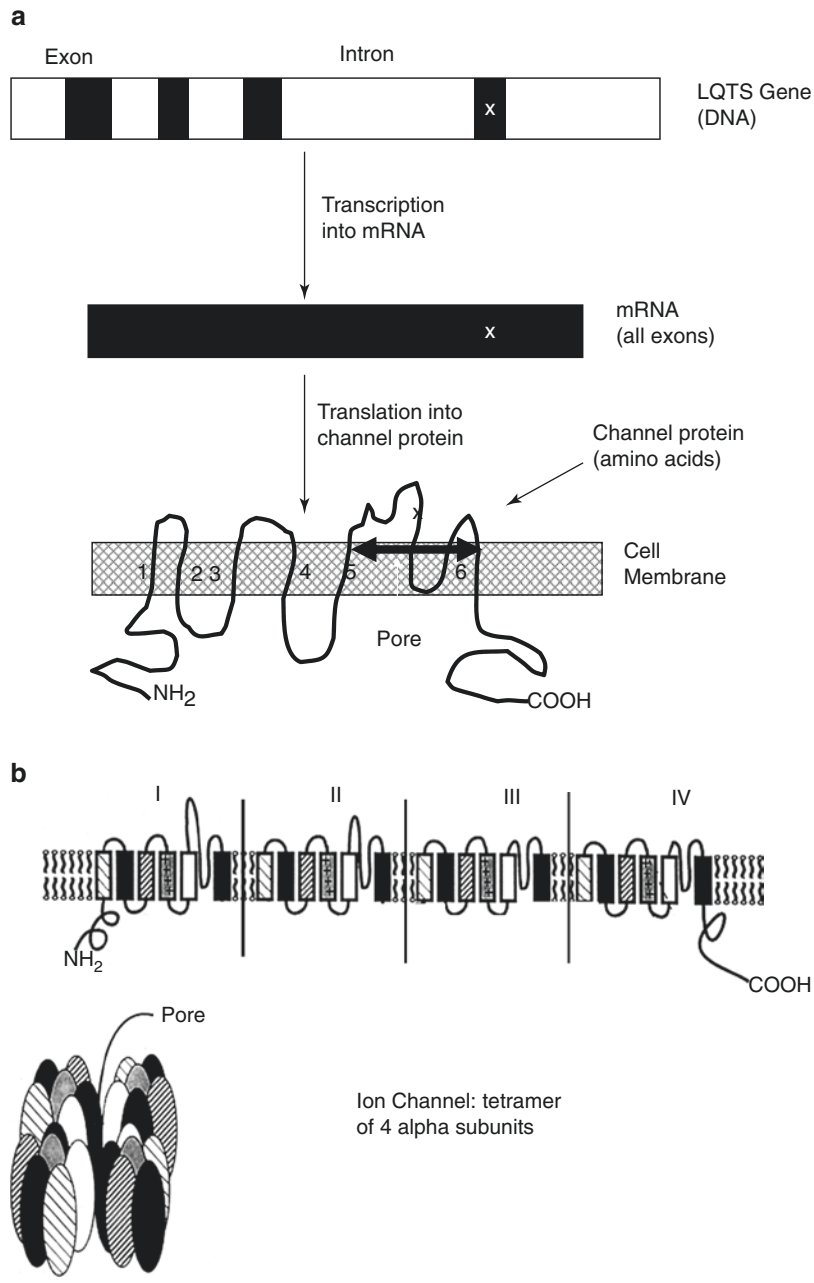



**Fig. 8.2** (**a**) Schematic diagram of ion-channel formation. Exons (black) are transcripted into messenger RNA (mRNA) that translates the information into a channel protein involving a chain of amino acids consisting of an N-terminus region, six membrane-spanning segments, and a C-terminus region. The pore area is located between the fifth and sixth membrane-spanning segments. The X in the LQTS gene denotes a DNA mutation that appears as an altered amino acid sequence in the channel protein. (**b**) Schematic topology of a prototypic **α**-subunit of a potassium channel with six membrane-spanning segments. Four **α**-subunits (I–IV) join together to form a tetrameric potassium channel around a central pore

LQT3 sodium channel (SCN5A) consists of a single protein involving four conjoined, homologous repeat α-subunits (24 membrane-spanning segments) assembled around a central permeation pathway plus one β-subunit. The LQT7 potassium channel (KCNJ2) is a simpler channel with just two transmembrane segments that embrace a pore region. The LQT8 calcium channel (CACNA1C) consists of $\alpha_1$-subunit (24 membrane-spanning segments) plus four subunits. The voltage gating, selectivity filter, inactivation plugging of the pore, role of the N- and C-terminus regions, and the modifying function of the attached subunits vary among and between the potassium, sodium, and calcium ion channels resulting in their unique and specific functional characteristics. Resolution X-ray crystallographic analysis of the ion channels has provided unique insight into the structural alterations associated with specific ion-channel mutations. It is clear from the complexity of the structure of the individual ion channels that LQTS mutations that result in altered amino acid sequences in various portions of the structural ion-channel protein can have a spectrum of effects on the channel function with mild to severe alterations in ion-channel kinetics and the phenotypic manifestations of the disorder.

## Diagnosing LQTS Patients

### QTc Duration in Standard 12-Lead ECG

An LQTS patient may present with a QTc interval duration that is in the normal range, borderline, or prolonged, following the criteria proposed by Moss and Robinson [5, 20]: normal QTc (<440 ms in children 1–15 years old, <430 ms in adult males, and <450 ms in adult females); borderline QTc (440–460 ms in children 1–15 years old, 430–450 ms in adult males, and 450–470 ms in adult females); and prolonged QTc (>460 ms in children 1–15 years old, >450 ms in adult males, and >470 ms in adult females). The values of QTc (corrected using Bazett's formula) are gender and age dependent with adult women and children showing longer values than male adults [20]. Rautaharju et al. [21] studied over 13,000 subjects representing both males and females across ages from birth till elderly, and they demonstrated that heart rate decreasing with age in males at the time of puberty contributes to gender-related difference in QTc values. The changes in heart rate (and consequently in QTc) were observed between ages 17 and 55 years, whereas in younger individuals and in individuals >55 years of age, both heart rate and QTc values were similar in both males and females, indicating the potential underlying role of sex hormones.

The major challenge of LQTS diagnosis is related to the substantial overlap observed in genetically affected and unaffected individuals. In 1991, Vincent et al. [22] demonstrated that QTc durations ranging from 410 to 470 ms may be observed among both carriers and noncarriers in LQT1 families. This substantial overlap relates to a number of factors, including varying penetrance of genes, possible effect of modifying genes, and inadequacy of the Bazett's correction formula that overcorrects QTc duration during slower heart rates that are frequently observed in LQTS patients. With increasing knowledge regarding the genetic types of LQTS, we are learning that about 50% of carriers and noncarriers may present with QTc in the gray-zone range. These patients are particularly difficult to diagnose. Such patients may benefit from analyses of multiple ECG recordings (if available), for they may show consistency of QTc prolongation or clear evidence of QTc prolongation at somewhat faster heart rates.

The diagnosis of LQTS based on QTc duration is particularly difficult in newborns. The question whether a newborn child inherited the disease is crucial for parents and for physicians taking care of LQTS families. As demonstrated by Schwartz et al. [23], healthy newborns up to about 1 month of age frequently show a QTc interval duration that exceeds 470 ms, partially due to immaturity of the ion channels but also because Bazett's formula may overestimate QTc at fast heart rates in the newborn. Fridericia's formula works somewhat better at fast heart rates.

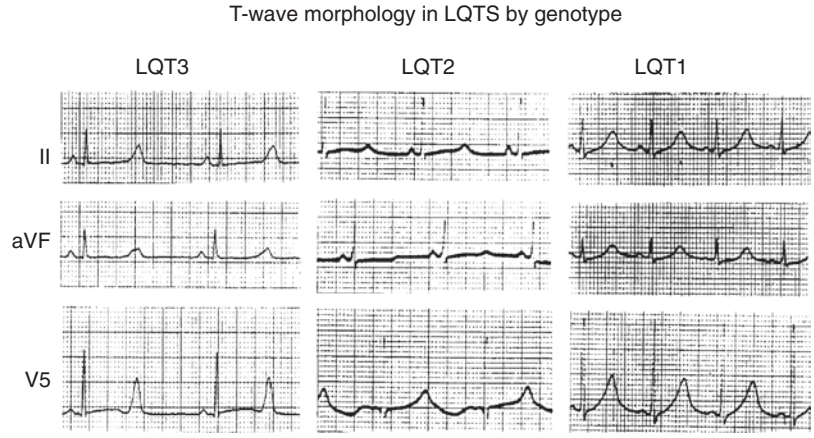



**Fig. 8.3** ECG recordings from leads II, aVF, and V5 from patients with LQT1, LQT2, and LQT3. The three LQTS genotypes are each associated with QT prolongation, yet the morphologic patterns are distinctively different. LQT1 is associated with a broad-based prominent T wave, LQT2 with low-amplitude T wave, and LQT3 with a prolonged isoelectric ST segment before the inscription of a large, somewhat peaked, T wave. (Reproduced with permission from Moss et al. [10])

## T-Wave Morphology

In 1995, Moss et al. [10] evaluated the relationship between T-wave morphology and genotype and, as shown in Fig. 8.3, specific patterns were associated with distinct genetic types of the disorder. LQT1 was found to be associated with wide, broad-based T waves, LQT2 patients usually had low-amplitude and frequently notched T waves, and LQT3 patients were categorized with a relatively long ST segment followed by a peaked, frequently tall, T wave. These initial observations were confirmed and further expanded on by Zhang and coworkers [24] who emphasized that LQT1 patients may present with normally looking T waves. Following these findings, the identification of T-wave morphology has been used to predict genotype in the era of limited genetic testing. The genotype suggested by the T-wave morphology may provide useful information to laboratories involved in screening genes for LQTS mutations, for it could save time and effort in the genetic screening process. Currently, most laboratories favor sequential testing of all major LQTS genes.

LQTS patients frequently show abnormal T-wave morphology, and careful evaluation of all 12 leads is recommended to determine the presence or absence of even subtle changes in T-wave shape. This is particularly important and useful in patients with QTc durations in the gray zone of 420–470 ms, where the diagnosis is uncertain based on just QTc. In patients with QTc <440 ms (which is considered as normal by clinicians), abnormal T-wave morphology may provide an important hint regarding the possibility of LQTS. Abnormal, mostly flat, and notched T waves are the most pathognomonic for LQT2, especially in adolescents and adults. However, it is worth stressing that T-wave notches can occur in unaffected young children. Similar to the point made regarding QTc duration, access to multiple ECGs and careful evaluation of T-wave morphology in these ECGs may help in the diagnosis of suspected individuals.

## LQTS Score

The above changes in QTc duration and T-wave morphology should be analyzed in light of the clinical history of the evaluated individual. The meaning of a QTc duration of 460 ms will be different if there is a history of frequent syncopal episodes in the past or if there is sudden death at young age in the family. This clinical approach is reflected by a LQTS diagnostic score published by Schwartz, Moss and colleagues in pre-genetic

**Table 8.3** Diagnostic criteria for long QT syndrome[a]

| Findings | Score |
|---|---|
| *Electrocardiographic*[b] | |
| Corrected QT interval, ms | |
| *≥480* | 3 |
| *460–470* | 2 |
| *450 (in males)* | 1 |
| Torsade de pointes[c] | 2 |
| T-wave alternans | 1 |
| Notched T-wave in 3 leads | 1 |
| Low heart rate for age[d] | 0.5 |
| *Clinical history* | |
| Syncope[c] | |
| *With stress* | 2 |
| *Without stress* | 1 |
| Congenital deafness | 0.5 |
| *Family history*[e] | |
| Family members with definite LQTS | 1 |
| Unexplained SCD in immediate family members <30 years | 0.5 |

[a]Reprinted with permission from Schwartz et al. [25]. Scoring ≤1 point, low probability of LQTS; 2 to 3 points, intermediate probability of LQTS; and ≥4 points, high probability of LQTS

[b]Findings in the absence of medications or disorders know to affect these electrocardiographic findings. The corrected QT interval (QTc) is calculated by Bazett's formula: $QT/RR^{1/2}$

[c]Torsade de pointes and syncope are mutually exclusive

[d]Resting heart rate below the second percentile for age

[e]The same family member cannot be counted in both categories

era of LQTS diagnosis [25]. The score, shown in Table 8.3, proposes that a high probability of LQTS diagnosis is present if it reaches a value of at least 4, whereas in the case of score values of 2–3, the likelihood of diagnosis is lower. It is important to stress that the score not only relies on QTc duration but also accounts for documented torsade de pointes, T-wave alternans, T-wave notches, as well as for bradycardia, findings frequently seen in LQTS patients. A personal history of cardiac events, diagnosis of LQTS in first-degree family members, as well as a sudden unexpected death <30 years of age further enrich the system based on ECG findings.

Tester et al. [26] studied the correlation between the LQTS score and results of genetic testing in 541 patients. In patients with a score >4, indicating a high probability of LQTS diagnosis, 72% of patients were found to be genotype positive, whereas among patients with a score <4, indicating lower probability of the diagnosis, 44% were positive by genetic testing. These data emphasize important limitations of phenotypic evaluation using the LQTS score.

## Other ECG Modalities for the Diagnosis of LQTS

In the case of clear-cut QTc prolongation >500 ms, usually there is not much doubt regarding the diagnosis. However, individuals with QTc <500 ms are frequently referred for exercise ECG testing, 24-hour Holter monitoring, or sometimes event monitoring. The diagnostic value of these modalities in borderline cases is still controversial. Krahn et al. [27] demonstrated that QT hysteresis, evaluation based on a response of QT to changing heart rate during exercise testing, may be helpful in the diagnosis of suspected cases. A distinct adaptation to increased heart rate on the upslope of exercise activity compared with the early recovery period at a similar heart rate was found useful. The authors postulated that a difference in QT >21 ms between 1 minute into recovery and early exercise measured in matched heart rates may be indicative of LQTS.

Takenaka et al. [28] analyzed exercise induced repolarization changes in a cohort of 51 LQT1 and 31 LQT2 patients. The QTc and TpTec (the interval between the peak and the end of the T wave) were 510 ± 68 ms and 143 ± 53 ms in LQT1 and 520 ± 61 ms and 195 ± 69 ms in LQT2, respectively, at baseline. These measures were both significantly larger than those observed in control subjects (402 ± 36 ms and 99 ± 36 ms). Both QTc and TpTec were significantly prolonged during exercise in LQT1 patients (599 ± 54 ms and 215 ± 46 ms) in whom a morphological change into a broad-based T-wave pattern was observed. In contrast, in LQT2 patients, exercise produced a prominent notch on the descending limb of the T wave, with no significant changes in the QTc and TpTec (502 ± 82 ms and 163 ± 86 ms). QT adaptation to changing heart rate was recognized as another method indicative of LQTS. As shown by Takenaka et al. [28], LQT2 patients

have steeper QT-RR slope than LQT1 patients during exercise. In addition, TpTe dynamics were shown to be different among subgroups. In LQT2 patients and controls, the TpTe was reduced in response to shortening of the R-R interval, thereby producing a positive TpTe/R-R slope; among LQT1 patients, the TpTe significantly prolonged when the R-R interval shortened, resulting in a negative TpTe/R-R slope [28].

For diagnostic purposes, it is believed that the presence of QTc >500 ms at heart rates <100 beats per minute during exercise testing or Holter recordings may be indicative of LQTS, whereas values below 500 ms are within physiologic range (again using Bazett's formula which is inadequate at faster heart rates).

Holter recordings may also provide information regarding adaptation of T-wave amplitude morphology with changing heart rates. Nemec et al. [29] studied the heart rate dependence of the QT interval duration in different LQTS genotypes and control subjects using computerized QT measurements obtained from Holter recordings. The dependence of the QT duration on heart rate was steeper in LQTS than in control subjects (0.347 ± 0.263 vs. 0.162 ± 0.083 at a heart rate of 100 beats/min; $p < 0.05$). In addition, the QT interval was significantly longer in LQT2 and LQT3 patients than in those with the LQT1 genotype at slow (533 ± 23 ms vs. 468 ± 30 ms at heart rate 60 beats/min; $p < 0.0001$), but not at rapid, heart rates. The heart rate dependence of QT interval was steeper in LQT2 and LQT3 than in LQT1 (0.623 ± 0.245 vs. 0.19 ± 0.079 at a heart rate of 100 beats/min; $p < 0.05$). For a given heart rate, the QT intervals varied more in LQT2 and LQT3 patients than in LQT1 patients (25.98 ± 11.18 ms vs. 14.39 ± 1.55 ms; $p < 0.01$).

In a study by Couderc et al. [30], the T-amplitude/RR slope was significantly flatter in LQT2 patients (0.31 ± 0.27 $\mu$V/ms) than in both LQT1 patients (0.62 ± 0.40 $\mu$V/ms) and healthy individuals (0.55 ± 0.29 $\mu$V/ms). The above tests evaluating dynamics of repolarization are still considered more useful as research tools than in clinical practice. Nevertheless, the additive information obtained from these tests is valuable when diagnosing patients with LQTS.

Kaufman et al. [31] studied a series of ECG parameters including exercise-induced T-wave alternans in a cohort of LQTS gene carriers and healthy controls. Standard resting QTc duration was found to be most predictive among individuals with a genotype-positive status; however, there was a 78% overlap between gene carriers and noncarriers. A T-wave alternans test did not contribute to improve LQTS diagnosis.

Epinephrine testing is considered as another test in the diagnosis of LQTS. Experimental studies using pharmacological models of LQTS tests in perfused wedge preparations by Antzelevitch and Shimizu [32] demonstrated a differential response of repolarization to epinephrine in different types of LQTS. Clinical evidence subsequently came from studies by Noda et al. [33] and Ackerman et al. [34]. The study of Noda et al. [33] showed that in LQT1 patients, QTc was prolonged remarkably (477 ± 42 to 631 ± 59 ms; $P < 0.0005$, % delta prolongation = +32%) as the RR was maximally decreased (at peak of epinephrine) and remained prolonged at steady-state conditions of epinephrine infusion (556 ± 56 ms; $P < 0.0005$ vs. baseline, +17%). In LQT2 patients, epinephrine also prolonged the QTc dramatically (502 ± 23 to 620 ± 39 ms; $P < 0.0005$, +24%) at peak epinephrine, but this shortened to baseline levels at steady state (531 ± 25 ms; $P$ = ns vs. baseline, +6%). The QTc was much less prolonged at peak epinephrine infusion in LQT3 patients (478 ± 44 to 532 ± 41 ms; $P < 0.05$, +11%) and controls (394 ± 21 to 456 ± 18 ms; $P < 0.0005$, +16%) than in LQT1 and LQT2 patients and shortened to baseline levels (LQT3: 466 ± 49 ms, −3%; controls: 397 ± 16 ms, +1%; $P$ = ns vs. baseline) at steady state.

Shimizu reported the responses of 12-lead ECG parameters to epinephrine examined in 31 LQT1, 23 LQT2, 6 LQT3, and 30 control subjects. The sensitivity by ECG diagnostic criteria was lower in LQT1 (68%) than in LQT2 (83%) or LQT3 (83%) before epinephrine and improved with steady-state epinephrine in LQT1 (87%) and LQT2 (91%) but not in LQT3 (83%), without the expense of specificity (100%) [35].

The above findings indicate that epinephrine testing may be useful, particularly in differentiating borderline cases of LQTS. However, responses to epinephrine infusion must be interpreted with caution in view of the limited amount of data available involving normal controls.

## Genetic Testing for LQTS Diagnosis

Genetic testing adds significantly to the diagnosis of LQTS. The diagnosis may be straightforward in patients showing QTc >500 ms, and one could argue that genetic testing may offer little diagnostic or prognostic value in such clearly affected individuals. Nevertheless, there are data indicating that the clinical course is different by genotype [1, 3] and the effectiveness of therapy with beta-blockers also differs in LQT1 than LQT2 and LQT3 patients [36]. Furthermore, knowing the genetic type (mutation) in a proband provides an opportunity to conduct genotyping of family members who frequently cannot be diagnosed just based on ECG findings. Genetic testing of a proband is excessively costly, and these high cost and reimbursement issues are factors limiting much wider application of the testing in clinical practice. Testing of family members for mutation(s) found in a proband should become routine to diminish overall costs of unnecessary multiple clinical testing frequently used for diagnosing individuals.

Genetic testing of individuals suspected for LQTS with QTc <500 ms is particularly useful since an overlap between affected and unaffected individuals in this category of patients is very substantial. Standard genotyping usually focuses on the most frequent LQTS genes, LQT1, LQT2, LQT3, LQT5, and LQT6, whereas the remaining genes (LQT4, LQT 7–17) are tested less frequently since mutations of these genes are very infrequent. Current state of genotyping LQTS patients leads to identification of gene mutations in about three quarters of those tested. The fact that a majority of patients can be identified with specific mutation(s) should be encouraging to both physicians and patients who should pursue this testing. Splawski et al. [4] reported that among genotyped patients, about 45% of tested patients were identified with LQT1, another 45% of patients were identified with LQT2, and about 5–8% of patients were identified with LQT3; the remaining 1–3% percent were in LQT5 and LQT6 genes. Napolitano et al. [37] evaluated the yield of genetic testing in 430 consecutive patients, with 310 (72%) patients having 235 different mutations, 138 of which were novel. The distribution of detected LQTS genotypes was as follows: 49% LQT1, 39% LQT2, 10% LQT3, 1.7% LQT5, and 0.7% LQT6. Importantly, they showed that by using the sex-based cutoff values for QTc, 40% of affected individuals (i.e., carriers of a genetic defect) were not identified by clinical assessment, further reinforcing the need for genetic testing.

Recent data from Taggart et al. [38] demonstrate that genetic testing supported by expert clinical evaluation may improve the diagnostic strategy of identifying LQTS patients. The authors have found that about 40% of individuals referred as having LQTS were found to be unaffected based on miscalculation of QTc or misinterpretation of symptoms. Again, genetic testing may be helpful in avoiding misdiagnoses, which carry lifelong consequences for LQTS-labeled individuals.

An additional benefit of comprehensive genetic testing may come from testing other non-LQTS genes of interest in suspected individuals. Tester et al. [39] found ryanodine receptor gene mutations responsible for the catecholaminergic polymorphic ventricular tachycardia in 17 (6.3%) of 269 patients with negative LQTS genetic testing.

The cost-effectiveness of genetic testing for LQTS is of concern. Philips et al. [40] found that genetic testing is more cost-effective than not testing for symptomatic index cases at an estimated cost of $2500 US dollar per life-year saved.

## Genotype-Phenotype Correlations in LQTS

LQTS genes affect different ion-current mechanisms; thus, the type of ion-channel mutation can affect phenotypic expression**.** As described

above, distinct genotype-phenotype correlations have been recognized in LQTS (Fig. 8.3) [10]. ST-T-wave patterns on the ECG and the triggers for cardiac events vary between the three genotypes. In addition, gene-specific differences in risk for cardiac events have also been described, with the genotype-specific risk factors including the type of trigger for cardiac event, age, gender, resting heart rate, mutation location, and response to beta-blockers. It has been shown that life-threatening arrhythmias in LQTS patients occur under specific circumstances (Fig. 8.4). In a study of 670 symptomatic LQTS patients with one of the three main LQTS genotypes [41], LQT1 patients were shown to experience 62% of cardiac events (including syncope, aborted cardiac arrest [ACA], and SCD) during exercise and only 3% during rest/sleep. By contrast, LQT3 patients experienced 13% of the events during exercise and 39% occurred at sleep/rest. LQT2 patients had an intermediate pattern, with only 13% occurring during exercise and most of the remainder (43%) occurring with emotional stress (i.e., fear, anger, startle, or sudden noise during sleep/rest). Similar trigger distribution pattern was found for the lethal cardiac events (ACA and SCD) (Fig. 8.4). This study and others [41–44] have shown that patients with the LQT1 genotype are at a higher risk for arrhythmic events triggered by sympathetic activation induced by exercise. Among the different types of exercise, swimming was shown to be a specific trigger for LQT1 patients [42, 43]. In contrast, patients with the LQT2 genotype experience cardiac events associated with sudden loud noise, whereas patients with the LQT3 genotype experience events during sleep or at rest without emotional arousal [42, 43, 45].

## Clinical Course of LQTS Patients

We assessed the clinical course of 3779 LQTS patients enrolled in the registry, of whom 2319 patients (60%) were females. The cumulative probability of a first cardiac event (comprising syncope, aborted cardiac arrest [ACA], and sudden cardiac death [SCD]) from age 1 to 75 years and the corresponding probability of a first life-threatening cardiac event (comprising ACA and SCD) by sex are shown in Fig. 8.5a, b, respectively. Data from previous and ongoing registry analyses regarding sex differences in the risk of the two end points, by age-groups, are shown in Tables 8.4a–c.

The results of these analyses consistently demonstrate that during childhood, male patients

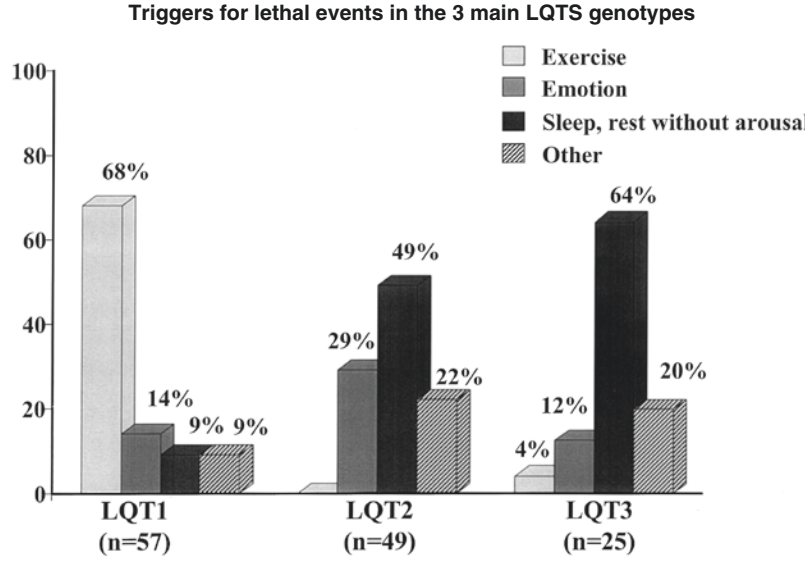



**Fig. 8.4** Triggers for lethal cardiac events according to three genotypes. Numbers in parentheses indicate number of triggers, not number of patients. (Reproduced with permission from Schwartz et al. [41])

display a higher event rate than females, whereas after this time period, risk reversal occurs and females maintain a higher risk than males during adulthood. Notably, when the combined end point of any cardiac event is considered, gender risk reversal is shown to occur during adolescence (Fig. 8.5a), whereas when the more severe end point of ACA or SCD is evaluated, the change in the male-female risk occurs later, usually during the third decade of life (Fig. 8.5b).

## Clinical Course of Pre-adolescent LQTS Patients

In a study of 479 probands (70% females) and 1041 affected family members (QTc >440 ms, 58% females) from the registry, Locati et al. [46] showed that the cumulative probability of a first cardiac event of any type from birth through age 14 years was significantly higher in males than in females, among both probands (74% vs. 51%,

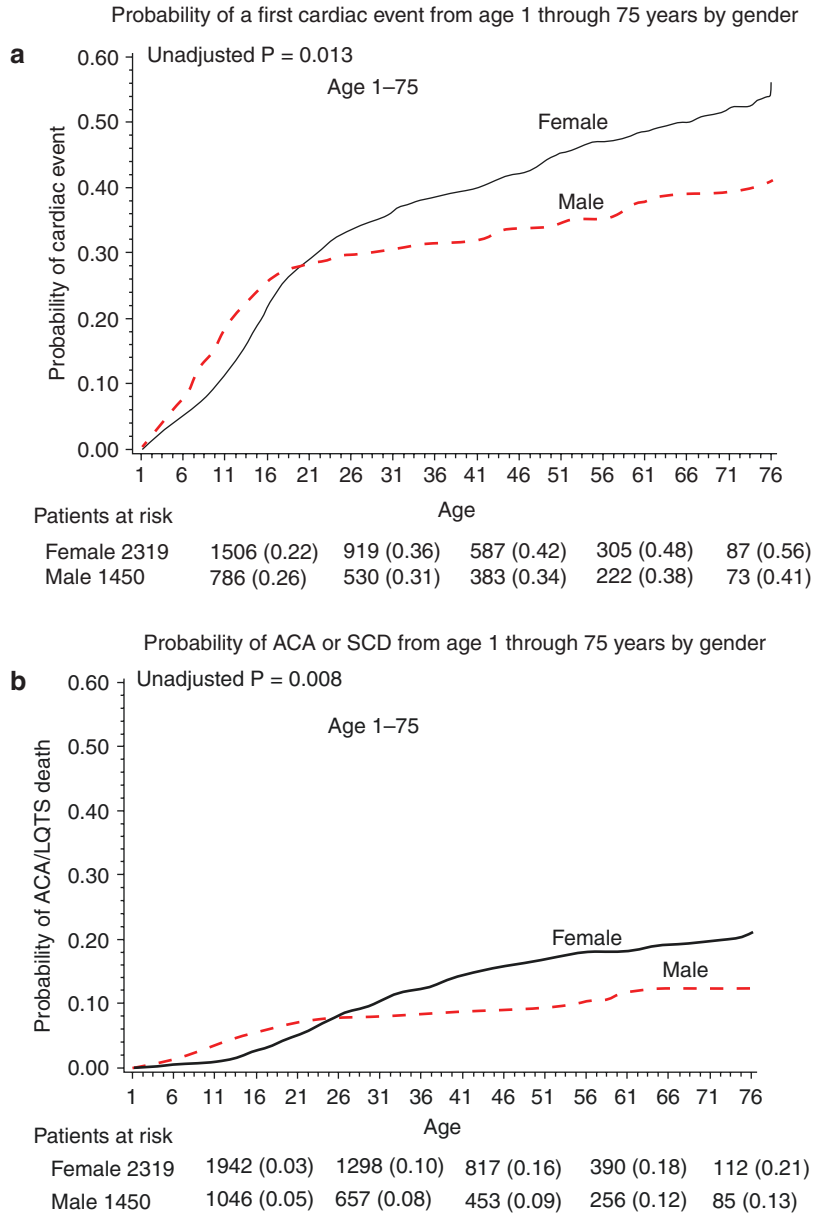



**Fig. 8.5** Probability of a first cardiac event (comprising syncope, aborted cardiac arrest, or sudden cardiac death) (A) and of a first life-threatening cardiac events (comprising aborted cardiac arrest or sudden cardiac death) (B), by sex in LQTS patients. (Unpublished data from the Rochester LQTS Registry)

respectively; $P < 0.0001$) and affected family members (20% vs. 16%, respectively; $P < 0.01$). A similar pattern was present also in 181 family members with unexplained sudden death, in whom the death rate before age 15 years was twice as high in males as in females (57% vs. 29%, respectively; $P < 0.0001$). In multivariate analysis, male gender was independently associated with a significant 85% and 72% increase in the risk of cardiac events before age 15 years among probands and affected family members, respectively (Table 8.4a).

**Table 8.4** Data from the International LQTS Registry regarding gender-related risk of cardiac events by age groups

| Population | N | Age range, (yrs) | End point | Male-female adjusted HR[b] (95% CI) | Data source |
|---|---|---|---|---|---|
| A. Pre-adolescence period | | | | | |
| Probands | 479 | 0–14 | Any cardiac event | 1.85 (1.59–2.70) | Locati et al. [46] |
| Affected family members | 1041 | 0–14 | Any cardiac event | 1.72 (1.23–2.39) | Locati et al. [46] |
| LQT1 | 247 | 0–15 | Any cardiac event | 1.72 (1.19–2.33) | Zareba et al. [6] |
| LQT2 | 209 | 0–15 | Any cardiac event | 1.37 (0.80–2.33) | Zareba et al. [6] |
| LQT3 | 81 | 0–15 | Any cardiac event | 0.54 (0.14–2.13) | Zareba et al. [6] |
| Registry pts diagnosed by QTc criteria or genetic testing | 3015 | 1–12 | ACA or SCD | 3.03 (1.80–5.12) | Goldenberg et al. [47] |
| Registry pts diagnosed by genetic testing[a] | 805 | 1–12 | ACA or SCD | 4.31 (1.14–20.50) | Goldenberg et al. [47] |
| Registry pts diagnosed by QTc criteria or genetic testing | 2772 | 10–12 | ACA or SCD | 4.0 (1.8–9.2) | Hobbs et al. [48] |
| Registry pts diagnosed by QTc criteria or genetic testing | 2772 | 10–12 | SCD | 11.6 (1.4–94.2) | Hobbs et al. [48] |
| B. Adolescence through age 40 years | | | | | |
| Probands | 479 | 15–40 | Any cardiac event | 0.53 (0.27–1.06) | Locati et al. [46] |
| Affected family members | 1041 | 15–40 | Any cardiac event | 0.31 (0.17–0.56) | Locati et al. [46] |
| LQT1 | 247 | 16–40 | Any cardiac event | 0.30 (0.12–0.71) | Zareba et al. [6] |
| LQT2 | 209 | 16–40 | Any cardiac event | 0.27 (0.09–0.73) | Zareba et al. [6] |
| LQT3 | 81 | 16–40 | Any cardiac event | 1.11 (0.35–3.45) | Zareba et al. [6] |
| Registry pts diagnosed by QTc criteria or genetic testing | 2772 | 13–20 | SCD | 1.6 (0.2–0.8) | Hobbs et al. [48] |
| Registry pts diagnosed by genetic testing | 812 | 18–40 | Any cardiac event | 3.05 (2.13–4.38) | Sauer et al. [49] |
| Registry pts diagnosed by genetic testing | 812 | 18–40 | ACA or SCD | 2.77 (1.66–4.62) | Sauer et al. [49] |
| C. After the fourth decade of life | | | | | |
| Registry pts diagnosed by QTc criteria | 540 | 41–75 | Any cardiac event | 0.54 (0.28–0.87) | LQTS registry analyses |
| Registry pts with QTc ≥470 msec. | 860 | 41–60 | All-cause mortality | 0.97 (0.52–1.81) | LQTS registry analyses |
| Registry pts with QTc ≥470 ms | 314 | 61–75 | All-cause mortality | 0.69 (0.37–1.26) | LQTS registry analyses |

*ACA* aborted cardiac arrest, *LQT1, LQT2, and LQT3* long QT syndrome types 1, 2, and 3, respectively, *SCD* sudden cardiac death

[a]Subgroup of the 3015 patients in the previous row

[b]Findings adjusted for QTc duration, time-dependent beta-blocker therapy; for the end point of ACA or SCD, findings were also adjusted for a history of time-dependent syncope

Zareba et al. [6] studied 533 registry patients who were genetically tested and found to be carriers of the LQT1 ($n = 243$), LQT2 ($n = 209$), and LQT3 ($n = 81$) genotypes. During childhood, the rate of cardiac events was significantly higher in LQT1 males than in LQT1 females (56% vs. 44%, respectively), corresponding to a 71% increase in the risk of a first cardiac event during childhood in males (Table 8.4a), whereas there was no significant gender-related difference in the risk of cardiac events among LQT2 and LQT3 carriers during the same time period.

In a comprehensive analysis from the International LQTS Registry, we assessed the end point of ACA or SCD in 3015 LQTS children (1893 males and 1122 females) from proband identified families who were diagnosed by QTc criteria [47]. In this study population, the cumulative probability of a first life-threatening cardiac event from age 1 through 12 years was 5% in males as compared with only 1% among females ($P < 0.001$). Accordingly, multivariate analysis showed that male gender was associated with a threefold increase in the risk of ACA or SCD during childhood, and with more than a fourfold increase in the risk of this end point in a subgroup of 803 genotype-positive LQTS children (Table 8.4a).

## Clinical Course of LQTS Patients from Adolescence Through Age 40 Years

In the registry analysis of 479 probands and 1041 affected family members carried out by Locati et al. [46], a sex-related risk reversal was shown to occur after age 14 years when the end point of a first cardiac event of any type was assessed. Thus, in the age range of 15–40 years, females had an 87% increase in the risk of cardiac events as compared with males among probands and a 3.3-fold increase in the risk among affected family members (Table 8.4b). Consistently, Zareba et al. [6] showed that in the age range of 16 through 40 years, the risk of cardiac events of any type was more than threefold higher among both LQT1 and LQT2 females as compared with the respective males (Table 8.4b), whereas among LQT3 genotype carriers, no significant gender difference in the risk was detected.

As noted above, when the end point of ACA or SCD is considered, sex-related risk reversal occurs at a later stage than the age shown for the less severe cardiac event end point that includes also syncope (Fig. 8.5). In a recent study of 2772 registry patients that were followed between the ages of 10 and 20 years, Hobbs et al. [48] showed that in the age range of 10–12 years, males had a fourfold increase in the risk of ACA or SCD and an 11-fold increase in the risk of SCD compared with females (Table 8.4a), whereas in the age range of 12–20 years, no significant gender difference in the risk of life-threatening cardiac events was shown (Table 8.4b). Subsequently, Sauer et al. [49], in an analysis of 812 mutation-confirmed LQTS patients from the registry who were followed up between the ages of 18 and 40 years, showed that during adulthood females have nearly a threefold increase in the risk of ACA or SCD as compared with males (Table 8.4b). In this study, the cumulative probability of ACA or SCD at age 40 years was 11% among females as compared with only 2% among males ($P < 0.001$). The two latter studies further demonstrate that gender risk reversal occurs at a later age when the end point of life-threatening cardiac events is considered.

## Clinical Course of LQTS Patients after the Fourth Decade of Life

Published studies on the clinical course of LQTS patients are limited to the first 4 decades of life due to lack of appropriate follow-up information in the older age-group. The International LQTS Registry has recently expanded the duration of follow-up in enrolled subjects, and current updated data facilitates now a comprehensive analysis of the clinical course of affected patients beyond the age of 40 years.

We assessed the end point of a first cardiac event of any type after age 40 years in 540 LQTS patients. In this analysis, the cumulative probability of LQTS-related cardiac events at age

75 years was 32% in females as compared with 18% in males ($P = 0.005$), corresponding to an 85% increase in the adjusted risk of cardiac event in females compared with males (Table 8.4c). Since in the older age-group the competing risks cardiovascular comorbidities are higher among males, and may counterbalance the higher risk of LQTS-related cardiac events in females, we also assessed the end point of all-cause mortality in affected subjects after age 40 years. In an analysis of 2759 LQTS patients, the cumulative probability of all-cause mortality from age 41 years through 75 years was 27% among females as compared with 32% in males ($P = 0.37$) [50]. Consistently, the risk associated with female sex was not significant when the end point of all-cause mortality was assessed in age ranges of 41–60 years and 61–75 years (Table 8.4c). Thus, it appears that LQTS continues to confer a high risk of arrhythmic events among females even after the fourth decade of life that counterbalances the higher risk of acquired cardiovascular morbidity and mortality of males in this age-group. These age-specific risk interactions lead to a neutral effect of gender on the end point of all-cause mortality in the older age-group.

## LQTS and Sex Hormones

The pronounced difference in the clinical course of LQTS males and females suggests that sex hormones may affect arrhythmic risk in LQTs patients.

As described above, this increased risk in cardiac events is more pronounced in LQT2 women. LQT2 women experience a significant increase in the risk of cardiac events after the onset of adolescence [48, 49]. Female sex is associated with a longer baseline QTc and is an independent risk factor for development of torsade de pointes in acquired LQTS (affecting the $I_{Kr}$ current) [51, 52]. After the onset of adolescence, women with LQTS experience hormonal changes (during menstrual cycle or associated with pregnancy and peripartum period) that may favor QT prolongation and vulnerability to arrhythmias [53]. These data suggest that hormonal factors affect the clinical course of LQTS males and females.

The possible relationship between female sex hormones and a more pronounced arrhythmic risk in $I_{Kr}$ mutation carriers is also supported by a study from our registry that showed a significant increase in the risk of cardiac events in the 9-month postpartum period, mainly among women who were LQT2 genotype carriers (Fig. 8.6) [54]. Similarly,

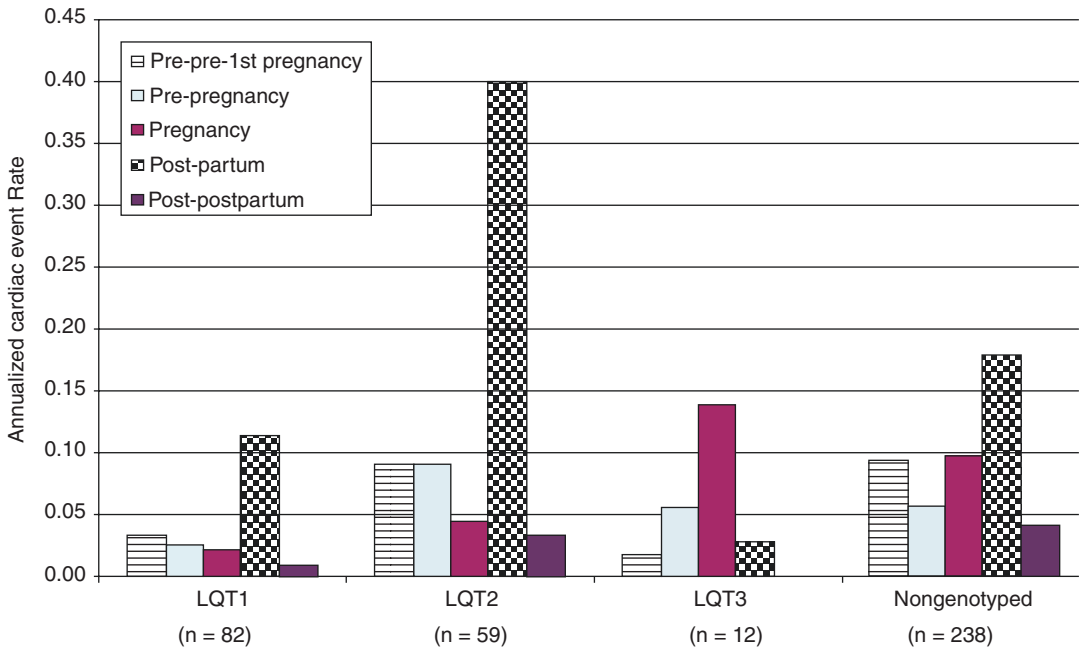



**Fig. 8.6** Rate (in patient years) of cardiac events among LQTS women by pregnancy periods. (Reproduced with permission from Seth et al. [54])

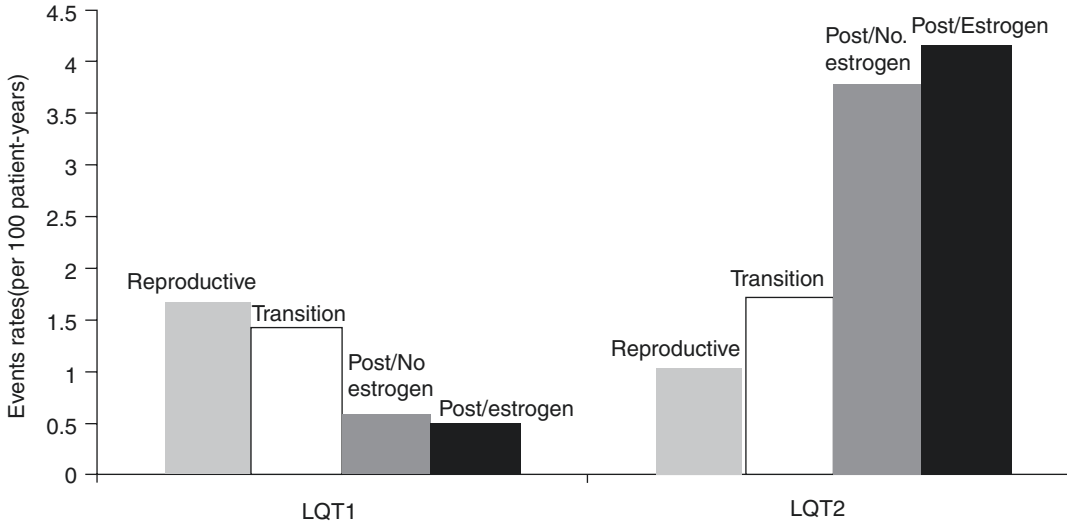



**Fig. 8.7** Rate (in patient years) of cardiac events among LQT1 and LQT2 women by menstrual stage and estrogen therapy. (Reproduced with permission from Buber et al. [55])

we have shown that LQT2 women experience a significant increase in the risk of recurrent cardiac events after the onset of menopause (Fig. 8.7) [55].

There is also an unexplained sex imbalance among patients referred to the LQTS Registry. We have shown that among the enrolled registry patients, there is a higher frequency of females (approximately 60%) than males [1–3, 5–8], consistent with data by Imboden et al. [56] who showed that LQTS alleles were transmitted more often to females than to males in 484 LQT1 and 269 LQT2 families.

## Risk Stratification for Cardiac Events in LQTS Patients

Risk stratification relies on a combined assessment of clinical information, ECG, and mutation-specific factors. Based on existing data among non-genotyped patients, LQTS patients may be classified into three main risk categories (Fig. 8.8): very high risk, high risk, and low risk (5-year Kaplan-Meier cumulative estimate rate of ACA/SCD is 14%, 3%, and 0.5%, respectively) [2]. The patients in the very-high-risk group are those with a history of ACA and/or spontaneous torsade de pointes; these patients require an ICD implantation for secondary prevention of SCD. The high-risk group includes subjects with history of prior syncope or QTc of over 500 ms, and the low-risk group includes those with QTc duration of 500 ms or less and without prior syncopal event.

## Genotype-Specific Risk Stratification

Multiple studies have shown that there are genotype-specific factors affecting the phenotypic expression in patients with LQTS; those risk factors include age, gender, postpartum time period, menopause, resting heart rate, prior syncope, mutation location, type of mutation (missense/non-missense), the biophysical function of the mutation, and response to beta-blockers. In addition cardiac events triggered by exercise, arousal, or rest/sleep are associated with distinctive clinical and genetic risk factors.

***Risk stratification in LQTS type 1:*** Our data indicate that the location, type, and biophysical function of the KCNQ1 mutation are important independent risk factors influencing the clinical course of patients with LQT1. Moss et al. [6] have demonstrated, among 600 patients with 77 different KCNQ1 mutations, that patients with

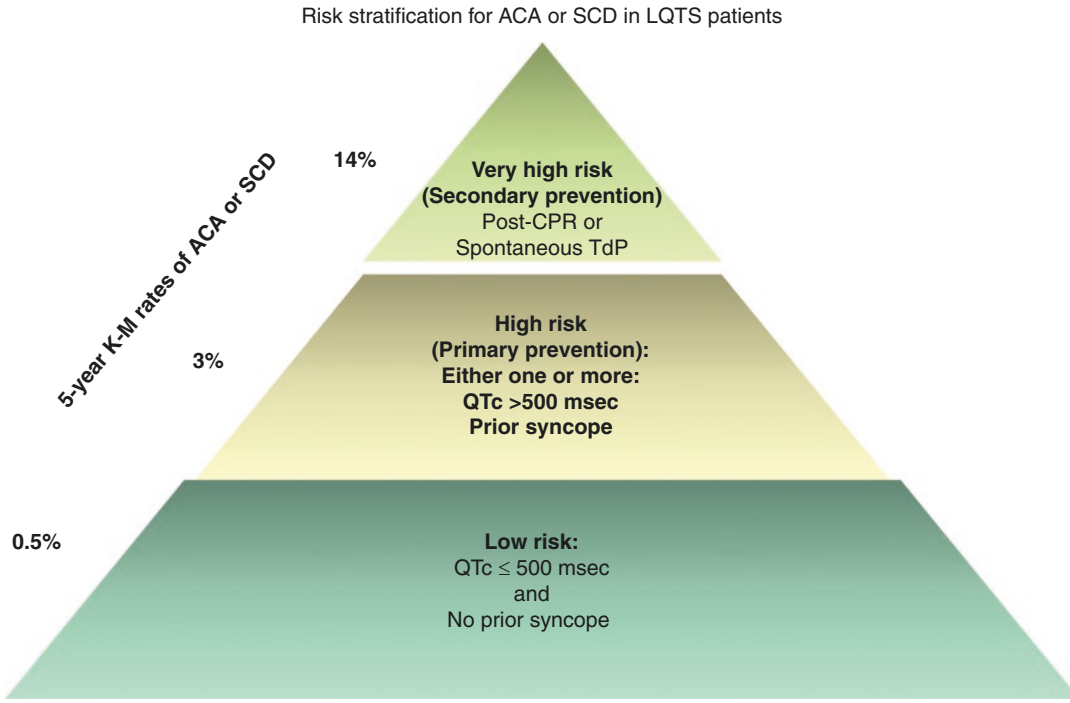
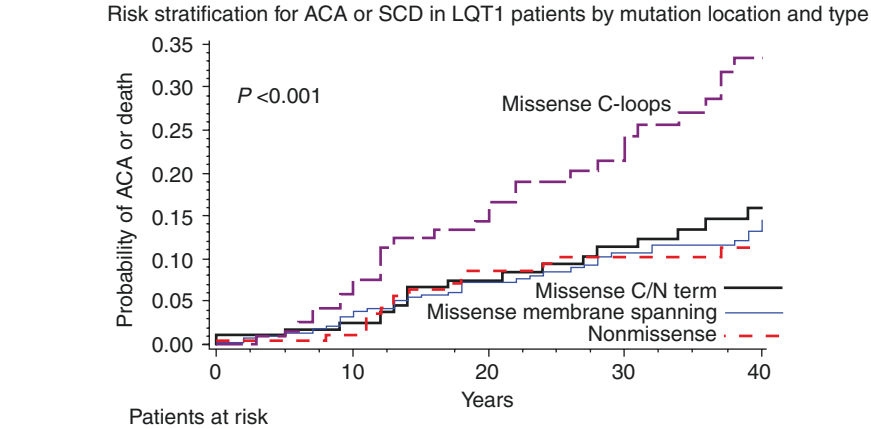



**Fig. 8.8** Risk stratification categories for LQTS patients based on published event rates. Kaplan-Meier estimates are based on a series of 869 long QT syndrome patients [2]. CPR cardiopulmonary resuscitation, TdP torsade de pointes. (Reproduced with permission from Goldenberg and Moss [2])

mutations in the transmembrane region (including the cytoplasmic loops [C-loops] domains) compared to mutations in the C-terminus of the KCNQ1 channel had a twofold ($p < 0.001$) increased risk for syncope, ACA, or SCD. Similarly, patients with dominant negative ion-current effects (>50% reduction in channel current) had a 2.2-fold increase ($p < 0.001$) in the risk of cardiac events compared with those who had mutations with haploinsufficiency effect (<=50% reduction in channel current). Furthermore, this study has shown that patients with missense mutations had a significantly higher risk of cardiac events compared to those with non-missense mutations. Subsequently, we have analyzed data on 860 patients with a KCNQ1 mutation from the International LQTS Registry [57]. Patients were categorized into carriers of missense mutations located in the S2–S3 and S4–S5 C-loops, membrane-spanning domain, C-/N-terminus, and non-missense mutations. There were 27 ACA and 78 SCD events from birth through age 40 years. The presence of C-loop mutations was associated with the longest QTc on the ECG and with highest risk for ACA or SCD (hazard ratio [HR] 2.75, $P = 0.009$ compared with non-missense mutations (Fig. 8.9) ). It is known that C-loops play an important role in the sympathetic regulation of the KCNQ1 channel [58]. Based on results from cellular expression studies [58], we have suggested that a combination of decrease in basal function and altered adrenergic regulation of the $I_{Ks}$ channel underlies the increased cardiac risk in this subgroup of patients [56].

There are accumulating data showing that the risk for cardiac events in LQT1 is affected by sex and age. Zareba et al. [6] have shown in 243 subjects with LQT1 that during childhood (age-group: 0–15 years), there was a 1.7-fold ($p = 0.005$) increased risk for cardiac events (syncope, ACA, or SCD) among males compared with females, whereas during adulthood, LQT1 females had a 3.3-fold ($p = 0.007$) higher risk for cardiac events compared with males. We have further assessed the sex-specific risk factors for




Patients at risk

| | | | | |
|---|---|---|---|---|
| C-loops 125 | 109 (0.06) | 78 (0.14) | 59 (0.22) | 39 (0.33) |
| C/N term 172 | 145 (0.02) | 108 (0.07) | 88 (0.11) | 62 (0.16) |
| Membrane spanning 376 | 328 (0.03) | 242 (0.07) | 192 (0.11) | 154 (0.13) |
| Nonmissense 186 | 157 (0.01) | 119 (0.09) | 95 (0.10) | 69 (0.11) |

**Fig. 8.9** Kaplan-Meier estimates of cumulative probability of life-threatening cardiac events by mutation location and type in LQT1 patients. The numbers in parentheses reflect the cumulative event rate at that point in time. ACA indicates aborted cardiac arrest; LQTS long QT syndrome. (Reproduced with permission from Barsheshet et al. [57])

life-threatening cardiac events (ACA/SCD) in a large population of 1051 genetically confirmed patients with LQT1 from the US portion of the International LQTS Registry [59]. We have found that during childhood (age-group: 0–13 years), males had >twofold ($P$ = 0.003) increased risk for ACA/SCD than females, whereas after the onset of adolescence, the risk for ACA/SCD was similar between men and women (HR = 0.89; $P$ = 0.64). Importantly, mutation location showed a sex-specific association with the risk for ACA or SCD. The presence of C-loop domains (S2–S3 and S4–S5) mutations was associated with a 2.7-fold ($P$ < 0.001) increased risk for ACA/SCD among women, whereas the risk for ACA/SCD among men with LQT1 was high, predominantly during the childhood period, regardless of mutation location/type. Additional risk factors for ACA or SCD among both men and women included a prolonged QTc (>500 ms) and the occurrence of time-dependent syncope [59]. Time-dependent syncope was associated with a more pronounced risk increase among men than women (HR 4.73; [$P$ < 0.001] and 2.43 [$P$ = 0.02], respectively), whereas a prolonged corrected QT interval (≥500 ms) was associated with a higher risk among women than men. The proposed risk stratification scheme for ACA or SCD in LQT1 based on these findings is presented in Fig. 8.10.

In LQT1, the majority of events are triggered by exercise activity, and a lower proportion of events are associated with acute arousal or sleep/rest. In a study carried out among 721 genetically confirmed patients with LQT1 from the US portion of the International LQTS Registry [44], we have analyzed the independent contribution of clinical and genetic risk factors to the first occurrence of trigger-specific cardiac events, categorized as exercise-induced, arousal, and nonarousal/nonexercise. This study showed that age and sex have a distinct association with exercise-, arousal-, and sleep-/rest-triggered events in patients with LQT1. During childhood (age <13 years), males had a 2.8-fold ($P$ < 0.001) increase in the risk for exercise-triggered events, whereas females >13 years showed a 3.5-fold ($P$ − 0.002) increase in the risk for sleep/rest nonarousal events. Mutations located in the C-loops portion of the KCNQ1 channel were associated with an increased risk for cardiac events triggered by both exercise- and arousal-triggered events (i.e., sympathetic stimulation,

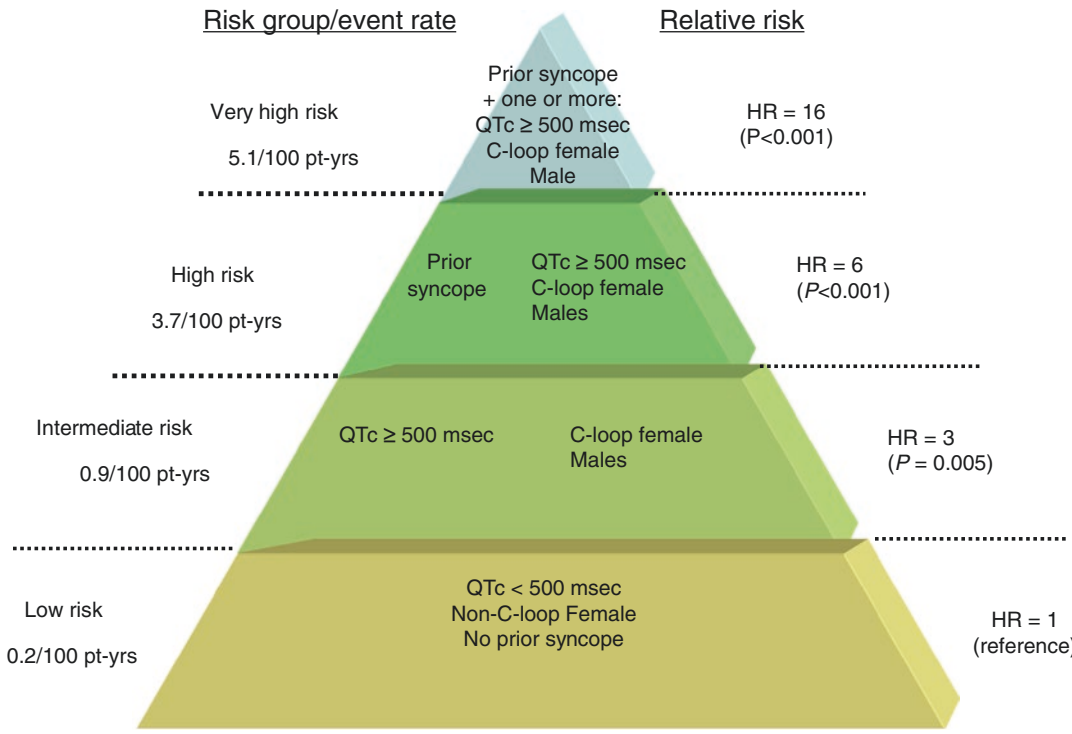



**Fig. 8.10** Proposed scheme for risk stratification for the end point of ACA or SCD in LQT1 patients by gender, mutation location, QTc, and a history of prior syncope. ∗Hazard ratios and score estimates were obtained from a multivariate Cox model that included interactions among the identified risk factors (categorized by QTc duration, time-dependent syncope, gender, and mutation location); decimal points in HRs are rounded to the nearest whole number; event rates per 100 person-years were calculated by dividing the number of life-threatening cardiac events (comprising ACA or SCD) in each risk category by the total follow-up time in the category (with follow-up censored after the occurrence of a ACA) and multiplying the result by 100. ACA aborted cardiac arrest, HR hazard ratio, LQT2 long QT syndrome type 2, SCD sudden cardiac death, QTc corrected QT. (Data based on event rates estimated provided in Ref. [59])

HR = 6.19 [$P < 0.001$] and HR = 4.99 [$P < 0.001$], respectively) but were not related to sleep/rest nonarousal events (HR = 0.72; $p = 0.46$).

Finally, it has been shown that faster resting heart rates are associated with increased risk for cardiac events in LQT1 patients and that resting heart rate provides incremental prognostic information to mere assessment of QTc in LQT1 but not in LQT2 patients [60–62]. Thus, we have suggested that risk stratification for life-threatening cardiac events in LQTS patients may be improved by incorporating a genotype-specific correction of the QT interval for heart rate with a greater degree of QT correction for heart rate in LQT1 (improved QTc = $QT/RR^{0.8}$) than in LQT2 patients (improved QTc = $QT/RR^{0.2}$) [62].

***Risk stratification in LQTS type 2:*** Similar to LQT1, the type and location of KCNH2 mutations were shown to be linked to the risk of cardiac events in patients with LQT2. In a study of 858 subjects with 162 different KCNH2 mutations, Shimizu et al. [8] have reported that patients with missense mutations located in the transmembrane pore region (S5-loop-S6 region) are associated with the greatest risk for cardiac events. Furthermore, mutations located in the alpha-helical domain of the KCNH2 channel were associated with a significantly higher risk compared with mutations in either the beta-sheet domains or other uncategorized locations.

With regard to the effect of sex and age on the phenotypic expression in LQT2 patients, Zareba

et al. [6] assessed the risk of cardiac events in 209 carriers of KCNH2 mutations and found that females >15 years old had a 3.7-fold ($p = 0.01$) increased risk for cardiac events as compared with males >15 years old, but there was no significant difference between female and male carriers <=15 years old (HR = 0.73, $p = 0.3$).

Migdalovich et al. [63] have investigated the relationship between mutation location in the KCNH2 channel and gender-specific risk for life-threatening cardiac events (ACA/SCD). This study among 1166 patients with LQT2 showed that the risk for life-threatening cardiac events from birth through age 40 years was significantly higher among women than men, with a cumulative probability of life-threatening cardiac events of 26% and 14% among women and men, respectively ($P < 0.001$). The increased risk for ACA/SCD among LQT2 females was pronounced after the age of 13 years. The risk for life-threatening cardiac events was not significantly different between women with and without pore-loop mutations (HR 1.20; $P = 0.3$). In contrast, men with pore-loop mutations displayed a significant >twofold higher risk of a first ACA/SCD as compared with those with non-pore-loop mutations (HR = 2.18; $P = 0.01$). Figure 8.11 shows a proposed risk stratification scheme for ACA or SCD in LQT2 patients.

As mentioned earlier, most of cardiac event stimuli in LQT2 patients are sudden arousal triggers, whereas a lower proportion of events are associated with exercise activity. We have

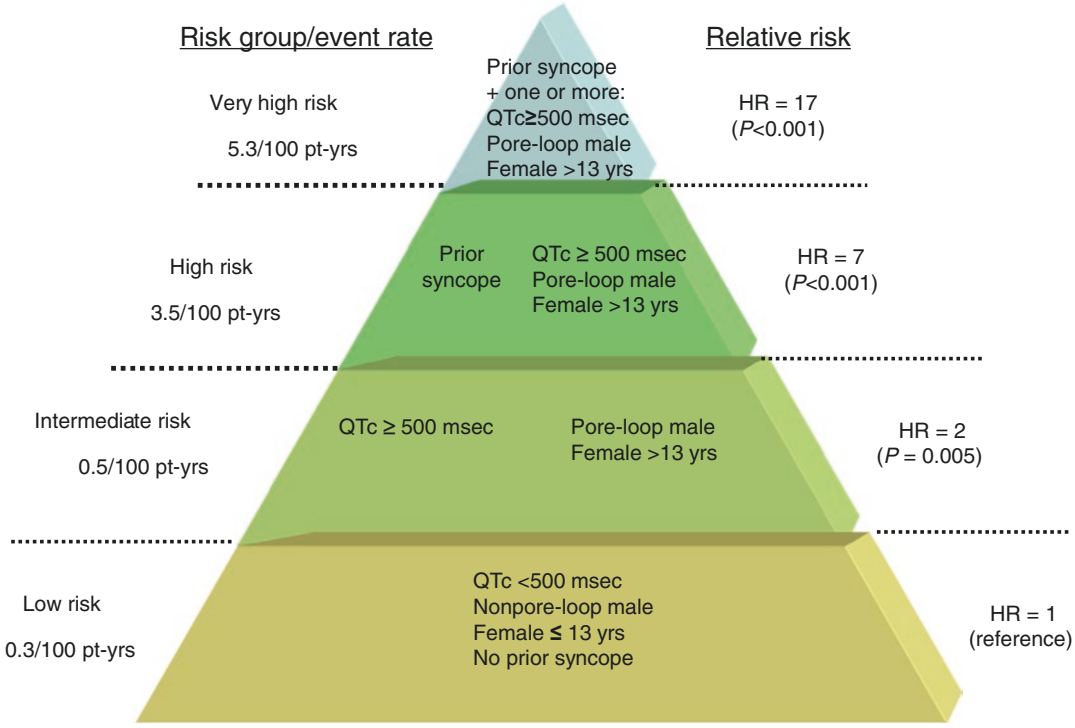



**Fig. 8.11** Proposed scheme for risk stratification for the end point of ACA or SCD in LQT2 patients by gender, mutation location, QTc, and a history of prior syncope. ∗Hazard ratios and score estimates were obtained from a multivariate Cox model that included interactions among the identified risk factors (categorized by QTc duration, time-dependent syncope, gender, and mutation location); decimal points in HRs are rounded to the nearest whole number; event rates per 100 person-years were calculated by dividing the number of life-threatening cardiac events (comprising ACA or SCD) in each risk category by the total follow-up time in the category (with follow-up censored after the occurrence of a ACA) and multiplying the result by 100. ACA aborted cardiac arrest, HR hazard ratio, LQT2 long QT syndrome type 2, SCD sudden cardiac death, QTc corrected QT. (Reproduced with permission from Migdalovich et al. [63])

recently carried out a study among 634 genetically confirmed LQT2 patients [6] investigating the clinical and genetic factors associated with trigger-specific risk for cardiac events. Females >13 years as compared with males >13 years had a ninefold ($p < 0.001$) increased risk for arousal-triggered cardiac events, and patients with pore-loop mutations had a >twofold increased risk for this end point ($P = 0.009$). In contrast, non-pore-loop transmembrane mutations were associated with a 6.8-fold ($p < 0.001$) increased risk for exercise-triggered events, whereas gender was not a significant risk factor for this end point. Risk factors for nonexercise/nonarousal events included female gender, mutation location and type, and prolonged QTc (>500 ms).

Thus, in both LQT1 and LQT2, risk factors for cardiac events showed a trigger-specific association with arrhythmic cardiac events.

***Risk stratification in LQTS type 3:*** As described above, a gain of function mutation in the cardiac sodium channel SCN5A leads to LQT3. The SCN5A channels open quickly in response to depolarization, but within a few milliseconds the channels undergo fast inactivation. The mutations leading to LQT3 produce gain of function defects by disrupting this inactivation causing late persistent sodium currents, window sodium currents, or both.

Preliminary findings from data of LQT3 patients enrolled in the US portion of the Registry show that mutations involving two functional defects (i.e., mutations leading to both late sodium currents and window sodium currents) had a 2.5-fold ($p = 0.001$) increased risk for ACA/SCD as compared with mutations involving only one functional defect. The international LQT3 research group showed no significant difference in the risk of cardiac events among patients with mutations in transmembrane versus C-terminus segments of the SCN5A channel.

Liu et al. [64] investigated the clinical course of patients with two relatively common LQT3 mutations. The study population involved 50 patients with the ΔKPQ mutation and 35 patients with the missense D1790G mutation of the SCN5A gene. Patients with the ΔKPQ mutation had a 2.4-fold ($p < 0.001$) higher risk for cardiac events from birth through age 40 years compared to patients with the D1790G mutation, demonstrating the importance of knowing the specific mutation in risk stratification of LQT3 patients (Fig. 8.12).

Zareba et al. [6] assessed the risk of cardiac events in 81 carriers of SCN5A mutations and found no trend toward age and sex dependency of the risk for cardiac events in patients with LQT3. The lethality of cardiac events in males and females with LQT3 was similar but was significantly higher than in LQT1 and LQT2 patients. In a more recent study comprising 406 LQTS patients, we have shown no statistically significant differences in the clinical course of LQT3 males and females [9]. Independent risk factors for cardiac events in this study include a history of prior syncope and baseline QTc. The rate of sudden cardiac death at 40 years in this LQT3 cohort was 14% (Fig. 8.13) [9].

## Summary

Genotype-phenotype correlation in the LQTS has been the most active line of research among the genetic diseases associated with life-threatening ventricular arrhythmias. The discovery that genes responsible for LQTS encode different ion channels involved in the control of the cardiomyocyte repolarization has led to important advancements in current knowledge regarding the relationship between basic causal mechanisms and clinical aspects of LQTS. It has been shown that life-threatening arrhythmias in LQTS patients occur under specific circumstances and in a gene-specific manner. The interplay between the genetic defect, QT duration, age, and sex can provide an algorithm for risk stratification in LQTS. Most importantly, an understanding of the genotype-phenotype relationship can lead to improved management of LQTS.

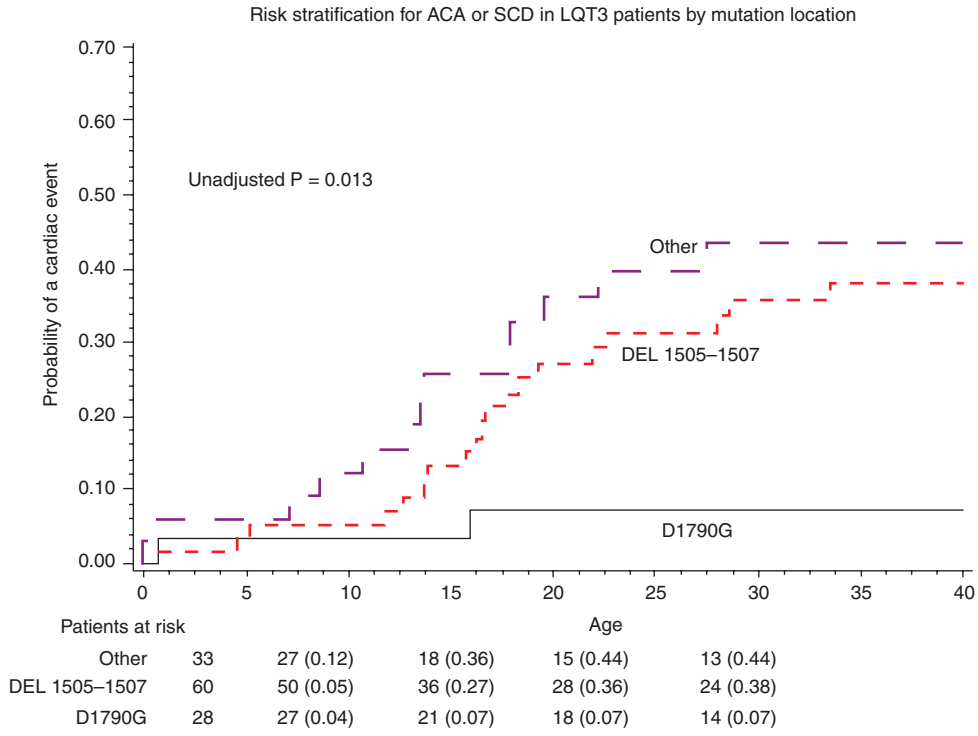



**Fig. 8.12** Kaplan-Meier estimates of cumulative probability of life-threatening cardiac events by mutation location in LQT3 patients. The numbers in parentheses reflect the cumulative event rate at that point in time. (Reproduced with permission from Liu et al. [64])

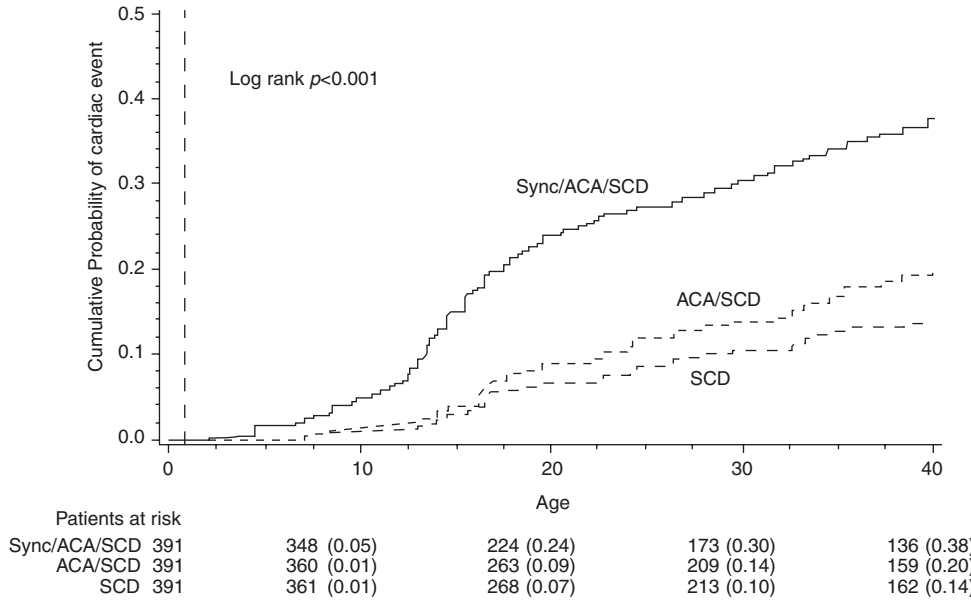



**Fig. 8.13** Kaplan-Meier cumulative probability of first LQT3-triggered cardiac event for combinations of syncope, ACA, and SCD, conditional on event-free survival to age 1 year. ACA indicates aborted cardiac arrest, LQT3 type 3 long QT syndrome, SCD sudden cardiac death. (Reproduced with permission from Wilde et al. [9])

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# 9 Clinical Management of LQTS Patients

Wojciech Zareba

## Overall Management of Patients with LQTS

The long QT syndrome (LQTS) is an inherited channelopathy characterized by QT prolongation and propensity to syncope, cardiac arrest, or sudden death in association with torsade de pointes polymorphic ventricular tachycardia or ventricular fibrillation [1–5]. Management of patients with LQTS aims to decrease the risk of arrhythmic cardiac events and sudden cardiac death in usually young, otherwise healthy individuals [6]. Goals of this therapy are to (1) minimize adrenergic response, (2) shorten QTc, (3) decrease dispersion of refractoriness, and (4) improve function of dysfunctional ion channel, all of them leading to decreasing the risk of ventricular tachyarrhythmias [4, 6]. The risk of arrhythmic events could be significantly diminished by lifestyle modifications and antiadrenergic therapy, decreasing the probability of catecholaminergic response to triggers of arrhythmias [7]. Protection in the case of the recurrent ventricular tachycardia/fibrillation is provided by implantable cardioverter-defibrillator (ICD) usually reserved for high-risk patients and left cardiac sympathetic denervation usually exercised in patients with recurrent arrhythmic events documented on ICD [8–10]. The QTc shortening is a desired treatment of choice, but the therapeutic options are limited mostly to sodium channel blockers used in patients with LQT3 including mexiletine and ranolazine [11, 12]. A better understanding of the mechanisms of ion channel dysfunction or inappropriate trafficking of channel proteins might lead to pharmacotherapy, correcting the underlying ion channel abnormalities [4]. Gene therapy is an ultimate future of treatment of inherited arrhythmia disorders, but it is not yet available for clinical trials.

## Preventive Measures in LQTS Patients

The LQTS patients frequently have their arrhythmic events triggered by stress, exercise, or sudden awakening [13–15]. Several precautions and preventive measures should be implemented in patients with LQTS (Table 9.1). Patients and their family members should be advised to remove or blunt ringing clocks and silent the phones and other devices creating sudden noise. Any conditions resulting in a startle, arousal, or stress should be avoided. Patients should avoid getting involved in competitive sports and especially water sports because swimming and sudden immersion in water might trigger ventricular tachyarrhythmias [15, 16]. The triggers of arrhythmic events are predominantly gene-specific (swimming in LQT1, loud noise in LQT2), but in general, they

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**Table 9.1** Preventive management in LQTS patients

| |
|---|
| Diminish the risk of stress and arousal situations triggering cardiac events including phone rings, alarm clocks, and other sudden startle conditions. |
| Avoid competitive sports and strenuous exercises. |
| Increase awareness of the risks associated with water activities including jumping into water and swimming. |
| Avoid QT-prolonging drugs. |
| Monitor adherence to beta-blocker treatment. |
| Use automatic external defibrillator to protect patients. |

should be avoided by all LQTS patients [15, 17, 18]. Refraining from competitive sports including swimming or jumping into water is difficult to accomplish in adolescents and young adults who are motivated to participate in competitive sport activities at schools [19]. Prospect of sport-based scholarships when considering admission to a college is a strong incentive, and LQTS patients and their parents sometimes refuse to accept advice that participation in competitive sports should be abandoned. This advice should be applied to all types of LQTS patients; however, some patients with LQT3 pursue their athletic activities since their QTc shortens with exercise and they mostly have their events during sleep at night. In the case LQTS patients still pursue athletic activities, one needs to consider extra precautions including automatic external defibrillators at trainings [20] and competitions, and in high-risk patients, an ICD could be considered on case-by-case basis [19].

LQTS patients and their family members must be informed about drugs that might prolong QT interval because a number of cardiac and noncardiac medications might cause drug-induced QT prolongation and torsade de pointes [21]. Updated list of drugs that might have QT-prolonging properties is provided at www.crediblemeds.org, and a mobile app is available to immediately access online QTdrugs database [22]. In 2018, the CredibleMeds added another list called QTFactors, describing clinical conditions/diseases associated with prolonged QTc and/or TdP ventricular tachycardia. Clinical factors predisposing to QT prolongation and TdP may include female sex, older age, hypokalemia, hypomagnesemia, bradycardia, pheochromocytoma, and Takotsubo cardiomyopathy. These two databases are maintained by the nonprofit Arizona Center for Education and Research on Therapeutics (AZCERT) and made available at no cost to researchers, healthcare providers, and patients [22].

## Beta-Blocker Therapy

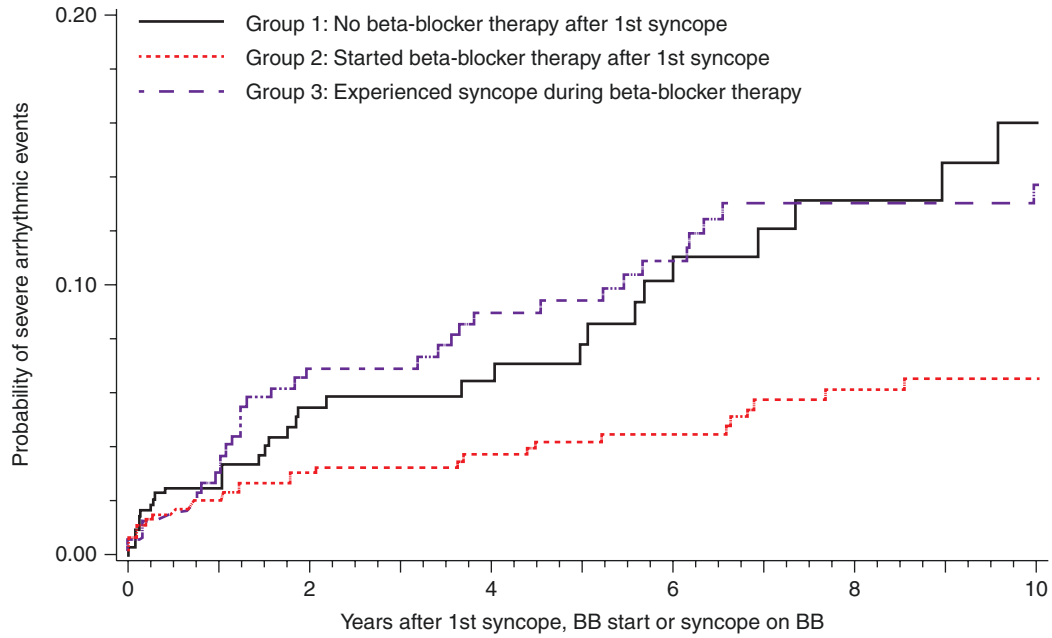
Moss et al. [23] introduced left cervicothoracic sympathetic ganglionectomy in 1971 to effectively treat high-risk patients with LQTS. From this point on, the concept of antiadrenergic therapy with left cardiac sympathetic denervation stellectomy was subsequently translated into broad use of beta-blockers in LQTS patients [24, 25]. Schwartz et al. [25] demonstrated significant effect of beta-blocker therapy in preventing cardiac events in a large cohort of over 200 patients with a 15-year follow-up. Since then beta-blockers remain the therapy of choice in patients with LQTS. In 2000, Moss et al. [26] evaluated the effectiveness of beta-blockers in 896 LQTS patients from the Rochester-Based International LQTS Registry. After initiation of beta-blockers, there was a significant ($p < 0.001$) reduction in the rate of cardiac events in probands ($0.97 \pm 1.42$ to $0.31 \pm 0.86$ events per year) and in affected family members ($0.26 \pm 0.84$ to $0.15 \pm 0.69$ events per year) during 5-year matched periods. Interestingly, when effects of beta-blockers on QTc duration was evaluated, patients with aborted cardiac arrest or death had no significant change in QTc before vs. after beta-blocker therapy, whereas patients free of events had a 20-millisecond QTc shortening after initiation of beta-blocker therapy. Cardiac event rates were more effectively reduced with beta-blockers in LQT1 than in LQT2 patients. The analysis of dose dependency of response to beta-blockers showed that lower and higher doses of beta-blockers had similar effects on cardiac event rates. Among 33 patients who died on beta-blockers, 8 stopped beta-blockers and 3 were not known whether they continued therapy with beta-blockers.

In 2004, Priori et al. [27] evaluated effects of beta-blockers in 335 LQTS patients. Cardiac

events occurred in 19 of 187 (10%) LQT1 patients, 27 of 120 (23%) LQT2 patients, and 9 of 28 (32%) LQT3 patients ($p < 0.001$). The risk of cardiac events was higher among LQT2 and LQT3 patients than among LQT1 patients, suggesting inadequate protection from beta-blocker therapy in LQT2 and LQT3 patients.

In a Rochester LQTS Registry study of high-risk adolescents with LQTS and history of syncope in the past 2 years [28], beta-blockers were associated with a 64% reduced risk (hazard ratio, 0.36; 95% CI: 0.18–0.72; $p = 0.01$). Figure 9.1 shows cumulative probability of aborted cardiac arrest in a large cohort LQTS patients from Rochester LQTS Registry who experienced syncope, which is one of the key risk factors in relationship to beta-blocker treatment [29]. Patients who started beta-blockers after first syncope had a 7% risk of life-threatening arrhythmic events at 10-year follow-up in comparison to 16% for those who did not start beta-blocker after first event and 14% for those who had syncope during beta-blocker therapy.

Vincent et al. [30] studied cardiac events in 216 LQT1 patients treated with beta-blocker and followed up for a median time of 10 years. Before beta-blockers, 157 (73%) patients had cardiac events, and after beta-blocker, 75% were asymptomatic, and cardiac events were significantly reduced ($p < 0.001$). Twelve patients suffered cardiac arrest or sudden death, but 11 of 12 were noncompliant. This study further confirmed high effectiveness of beta-blockers in LQT1 patients. However, as it was shown by the study of Barsheshet et al. from 2012 [31], LQT1 patients with mutations located in the cytoplasmic loop of the KCNQ1 channel had significantly higher risk of cardiac events than patients with other mutation




Patients at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| Group 1 | 975 | 247 (0.05) | 145 (0.06) | 103 (0.10) | 77 (0.13) | 53 (0.16) |
| Group 2 | 746 | 526 (0.03) | 410 (0.04) | 315 (0.04) | 235 (0.06) | 173 (0.07) |
| Group 3 | 314 | 250 (0.07) | 211 (0.09) | 175 (0.11) | 143 (0.13) | 119 (0.14) |

**Fig. 9.1** The cumulative risk of severe arrhythmic events and beta-blocker therapy. The solid black line represents all patients after the first syncopal event until start of beta-blocker (BB) therapy. After the start of beta-blocker therapy, patients are represented by the red dashed line. Patients with a syncopal event occurring while off beta-blocker therapy are represented by the purple dashed line. (Reproduced with permission from Jons et al. [29])

locations. Interestingly, those with cytoplasmic loop mutations had about 88% reduction in aborted cardiac arrest or sudden death with beta-blockers, whereas those with other mutations did not have benefit of beta-blockers. This important observation implies that when physicians plan treatment of LQT1 patients, they should identify location of mutation.

In 2014, Abu-Zeitone et al. [32] published data from 1530 LQTS patients from the Rochester LQTS Registry who were prescribed common beta-blockers (atenolol, metoprolol, propranolol, or nadolol). Time-dependent Cox regression analyses were used to compare the efficacy of different beta-blockers with the risk of cardiac events in LQTS. There were 346 cardiac events defined as syncope, aborted cardiac arrest, or sudden cardiac death among 1319 patients who continuously were taking beta-blockers, and in overall, any beta-blocker treatment was associated with a 37% reduction in the risk of first cardiac events (HR = 0.63; 95% CI: 0.47–0.86; $p = 0.004$). Nadolol was associated with the most pronounced reduction of these events (HR = 0.51; 95% CI: 0.35–0.74; $p < 0.001$), propranolol showed HR = 0.65 (95% CI: 0.46–0.90; $p = 0.01$), atenolol showed hazard ratio = 0.71 (95%CI: 0.50–1.01; $p = 0.06$), and metoprolol showed with hazard ratio = 0.70 (95% CI: 0.43–1.15; $p = 0.16$). When the analysis was focused on more severe life-threatening events consisting of aborted cardiac arrest or sudden cardiac death, which were observed in 73 patients, there was a 63% overall reduction with any beta-blockers, and there was no significant difference among different beta-blockers. As shown in Table 9.2 for LQT1 and LQT2 patients, the analysis of the effectiveness of beta-blockers by genotype was powered for cardiac events that included syncope. In LQT1 patients, all beta-blockers had a similar effect (although there were too few patients and too few events on metoprolol). However, in LQT2 patients, nadolol was the only beta-blocker showing significant efficacy.

Mazzanti et al. [33] evaluated efficacy of beta-blockers in reducing the risk of life-threatening arrhythmic events in 1710 LQTS patients, and they found that nadolol was associated with a significant 62% risk reduction (HR: 0.38; 95% CI: 0.15–0.93; $p = 0.03$) compared with no treatment. Propranolol was not associated with significant reduction in events (HR: 0.74; 95% CI: 0.32–1.68; $p = 0.47$). The selective beta-blockers combined (including metoprolol, atenolol, bisoprolol, carvedilol, and nebivolol) were not associated with reduction in events (HR: 0.79; 95% CI: 0.35–1.77; $p = 0.56$).

They also evaluated whether the difference in efficacy in favor of nadolol could be confirmed in the subgroup of patients treated with therapeutic

**Table 9.2** Genotype- and drug-specific first cardiac event rates on beta-blocker therapy and covariate-adjusted hazard ratios. Relative to discontinuing beta-blockers in patients with LQT1 and LQT2

| | LQT1 ($n = 379$) Total number of CE = 87 | | | LQT2 ($n = 406$) Total number of CE = 85 | | |
|---|---|---|---|---|---|---|
| Time-dependent variable | First CE | Hazard ratio (95% CI) | P value | First CE | Hazard ratio (95% CI) | P value |
| Atenolol | 21/105 (20.0%) | 0.43 (0.22–0.86) | 0.02 | 28/114 (24.6%) | 1.04 (0.48–2.27) | 0.92 |
| Metoprolol | 3/20 (15.0%) | 0.44 (0.13–1.54) | 0.2 | 10/46 (21.7%) | 0.82 (0.32–2.09) | 0.67 |
| Propranolol | 26/72 (36.1%) | 0.38 (0.19–0.73) | 0.004 | 28/100 (28.0%) | 0.65 (0.29–1.42) | 0.28 |
| Nadolol | 22/125 (17.6%) | 0.50 (0.25–0.98) | 0.04 | 10/109 (9.2%) | 0.40 (0.16–0.98) | 0.04 |
| Test of equality of 4 drug specific | | | 0.83 | | | 0.04 |

Reproduced with permission from Abu-Zeitone et al. [32]
CE denotes cardiac events (syncope or aborted cardiac arrest or death)
*CI* confidence interval

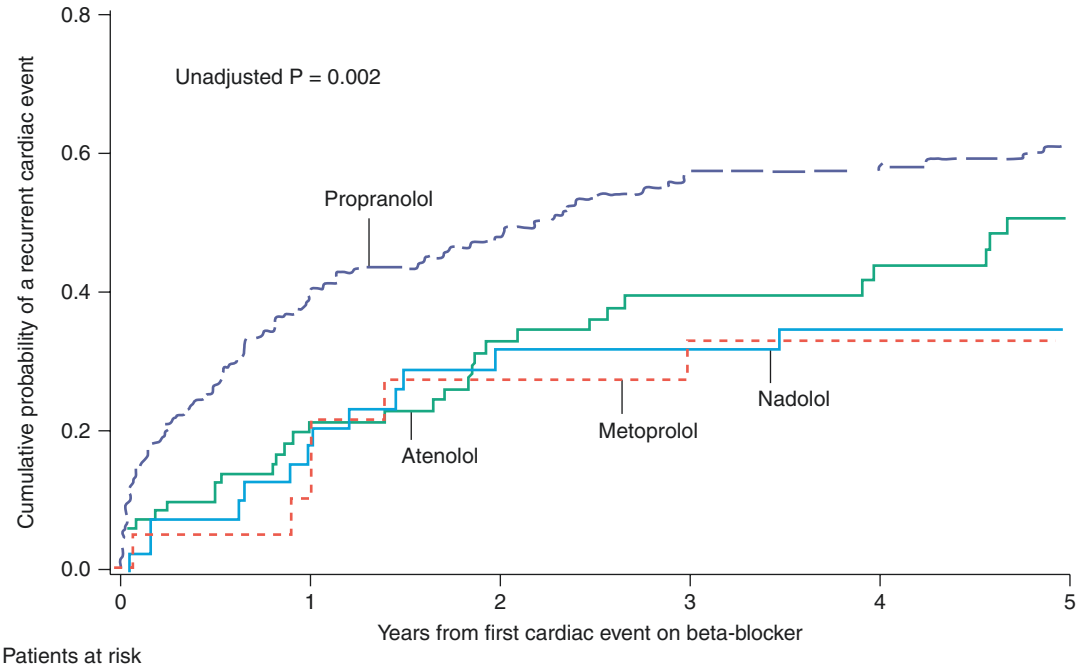
doses of nadolol, metoprolol, and propranolol, corresponding to 50% or more of the recommended daily dose (i.e., nadolol 0.5 mg/kg, metoprolol 1.0 mg/kg, and propranolol 1.5 mg/kg).

Patients treated with therapeutic doses of propranolol or metoprolol were at greater risk of events than patients treated with high doses of nadolol (HR for propranolol/metoprolol vs. nadolol: 2.77; 95% CI: 1.11–6.94; $p = 0.03$).

In the Abu-Zeitone's analysis [32], patients experiencing cardiac events while receiving beta-blocker therapy were at high risk for subsequent life-threatening cardiac events, and propranolol was the least effective agent when comparing with nadolol, metoprolol, and atenolol in preventing recurrent cardiac events in these high-risk patients (Fig. 9.2). Although nadolol remains the beta-blocker of choice based on the above data, the fact that atenolol and metoprolol might similarly reduce the risk of recurrent event is important for clinical practice when patients might not tolerate given beta-blockers.

In another study by Chockalingam et al. [34], cardiac events were analyzed in 281 previously asymptomatic patients and no differences in cardiac event occurrence was observed among patients using metoprolol, propranolol, and nadolol. They found a higher rate of cardiac events among symptomatic patients receiving metoprolol compared with those taking propranolol and nadolol.

Lower effect of propranolol and metoprolol might be related to their additional action on potassium ion channels. Kawakami et al. [35] evaluated the effect of beta-blockers on the hERG channel, and they found that the rapid component of the cardiac potassium channel (IKr) was blocked by high concentrations of propranolol, but not by atenolol or metoprolol within the therapeutic concentration range. In contrast, carvedilol directly inhibited HERG channels at clinically




Patients at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| Atenolol | 88 | 53 (0.21) | 41 (0.33) | 33 (0.39) | 26 (0.44) | 21 (0.50) |
| Metoprolol | 26 | 14 (0.22) | 13 (0.27) | 12 (0.33) | 11 (0.33) | 9 (0.33) |
| Propranolol | 147 | 83 (0.40) | 69 (0.48) | 52 (0.58) | 50 (0.58) | 45 (0.61) |
| Nadolol | 54 | 31 (0.18) | 25 (0.31) | 25 (0.31) | 24 (0.34) | 21 (0.34) |

**Fig. 9.2** Cumulative probability of a subsequent cardiac event among patients with first cardiac event while taking beta-blocker therapy. (Reproduced with permission from Abu-Zeitone et al. [32])

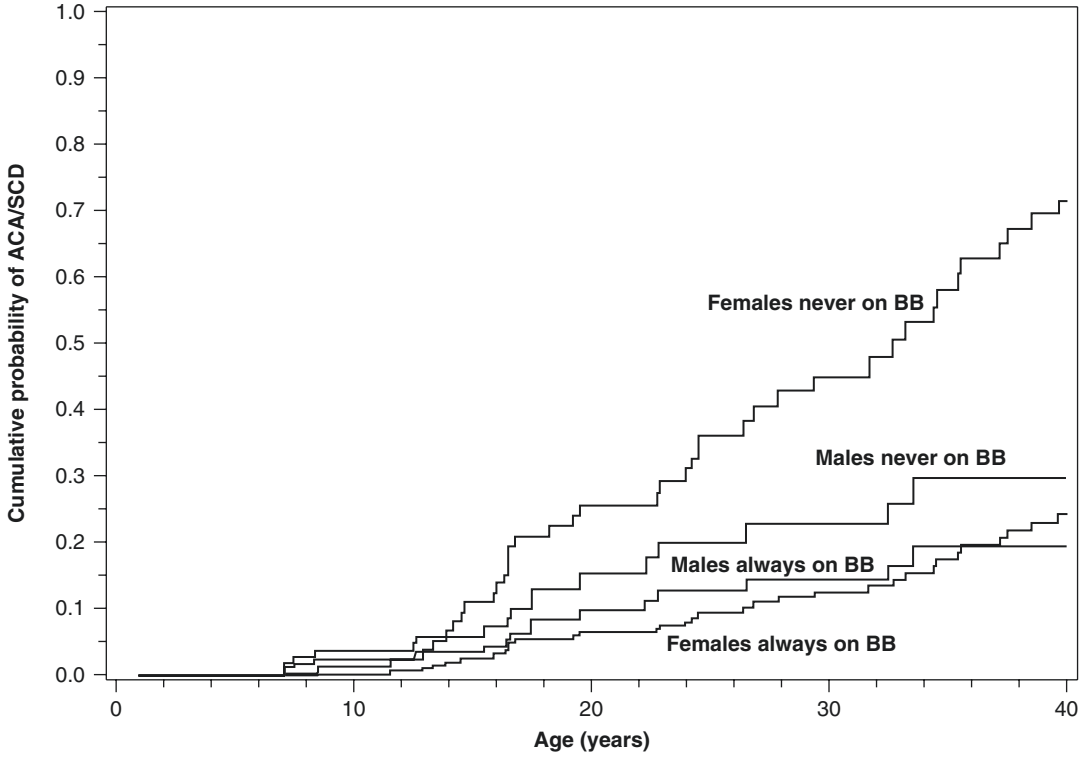



**Fig. 9.3** Cox model-based predicted distributions of the age at first ACA or SCD. The analysis is conditional on event-free survival to age 1 year, by BB and sex for asymptomatic patients (no prior syncope) born in 1971 (median) with a QTc ≥500 ms and neither lower-risk mutation (neither E1784K nor D1790G). (Reproduced with permission from Wilde et al. [38])

relevant concentrations. Thus, carvedilol might not be recommended in the treatment of LQTS patients. High-risk LQTS patients might be more sensitive to HERG-blocking action of propranolol, and possibly this is why they are less effective in LQTS patients with prior events.

The use of beta-blockers in LQT3 patients has always been controversial since these patients tend to have more pronounced QT prolongation and events during bradycardia than tachycardia [17, 27, 36]. Calvillo et al. [36] demonstrated in the LQT3 transgenic mice that propranolol was effective in reducing life-threatening arrhythmias despite prior experimental and clinical data warning against use of beta-blockers [27, 37]. In the largest to date analysis of 391 LQT3 patients by Wilde et al. [38], 118 (30%) patients had cardiac event including syncope and 24 (20%) had aborted cardiac arrest or sudden cardiac death. Females had significantly higher risk of cardiac events than males in univariate analysis, 44% vs. 28%, respectively ($p = 0.036$), and the effect of beta-blockers on reducing cardiac events was significant in females but not in males (Fig. 9.3). Cox regression analysis revealed that time-dependent beta-blocker therapy was associated with an 83% reduction in cardiac events in females (HR = 0.17; 95% CI: 0.04–0.70; $p = 0.014$) but not in males (HR = 0.94; 95% CI: 0.40–2.21; $p = 0.895$), with a significant sex-beta-blocker interaction ($p = 0.04$). When analyzing life-threatening events defined as aborted cardiac arrest or sudden cardiac death, beta-blockers reduced these events by 80% among women (HR = 0.20; 95% CI: 0.05–0.87; $p = 0.032$) and by 49% although nonsignificantly in males (HR = 0.51; 95% CI:0.13–1.88; $p = 0.308$). There is no good explanation why there is different efficacy of beta-blockers in LQT3 females than males, but these data have proven no evidence of proarrhythmia by beta-blockers even in LQT3 males.

**Table 9.3** 2017 AHA/ACC/HRS guideline for therapeutic management of patients with long QT syndrome

| Class of recommendation | Recommendations |
|---|---|
| I | In patients with LQTS and SCA, an ICD is recommended if meaningful survival of greater than 1 year is expected |
| I | In patients with LQTS with a resting QTc greater than 470 ms, a beta-blocker is recommended |
| I | In high-risk patients with symptomatic LQTS in whom a beta-blocker is ineffective or not tolerated, intensification of therapy with additional medications (guided by consideration of the particular long QT syndrome type), left cardiac sympathetic denervation, and/or an ICD is recommended |
| I | In patients with LQTS and recurrent appropriate ICD shocks despite maximum tolerated doses of a beta-blocker, intensification of medical therapy with additional medications (guided by consideration of the particular long QT syndrome type) or left cardiac sympathetic denervation is recommended |
| II a | In asymptomatic patients with LQTS and a resting QTc less than 470 ms, chronic therapy with a beta-blocker is reasonable |
| II b | In asymptomatic patients with long QT syndrome and a resting QTc greater than 500 ms while receiving a beta-blocker, intensification of therapy with medications (guided by consideration of the particular long QT syndrome type), left cardiac sympathetic denervation, or an ICD may be considered |

Based on data in Al-Khatib et al. [40]. For all these recommendations, there is a level of evidence B without data from randomized trials

As indicated by study of Vincent et al. [30] for LQT1 patients, nonadherence to prescribed beta-blockers is of major consequences. In a study from New Zealand [39], half of studied LQT1 and LQT2 patients showed suboptimal adherence indicating that significant education of LQTS patients is needed especially since a number of them are adolescents or young adults.

Table 9.3 summarizes the "2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death" specifically focusing on the LQTS patients [40]. In patients with LQTS with a resting QTc greater than 470 ms, a beta-blocker is recommended with class I indication regardless of genotype. Class IIa indication is given to asymptomatic patients with LQTS and a resting QTc less than 470 ms, in whom chronic therapy with a beta-blocker is reasonable.

## Pharmacotherapy Targeting Ion Channel Dysfunction

During the last over two decades since first identification of specific LQTS gene mutations affecting ion channel function, there were substantial advances in the understanding of the pathophysiology of inherited arrhythmia syndromes with LQTS serving as a paradigm disease [41]. These advances may open opportunities to develop gene-specific therapy for LQTS patients.

### Sodium Channel Mutations

The LQT3 is caused by an excessive inflow of sodium current during phase 3 of the action potential caused by mutations in the SCN5A gene [4, 42]. Schwartz et al. [43] published first clinical experience with Na + channel blockade with mexiletine in 6 LQT3 patients in whom a significant QTc interval shortening was observed from 535 ± 32 to 445 ± 31 ms, $p < 0.005$. Administration of mexiletine in 7 LQT2 patients did not change significantly QTc duration in that study. Benhorin et al. [44] evaluated effects of flecainide in 8 LQT3 patients with D1790G mutation carriers in whom a significant QTc shortening was observed from 517 ± 45 to 468 ± 36 ms, $p = 0.011$. These effects were observed during long-term (9–17 months) follow-up with no adverse effects. Windle et al. [45] evaluated effects of 48-hour administration of flecainide in 5 LQT3 patients with delta-KPQ mutation. After the oral dose of 50 mg twice a day, the QTc interval decreased on average by 104 ms, from a baseline value of 565 ± 60 ms to

461 ± 23 ms ($p < 0.04$) with normalization of T-wave morphology. This study was followed by a long-term crossover design (6 months on and 6 months off the drug for 2 years) with low-dose flecainide administration study, which showed an average reduction of QTc interval by 27 ms without major side effects [46]. Priori et al. [47] evaluated the effects of intravenous bolus of flecainide used for diagnosing Brugada syndrome in 13 LQT3 patients, and 6 of them developed significant ST elevation raising concerns about flecainide therapy in LQT3 patients. Flecainide has been used in LQT3 patients with atrial fibrillation, and most likely it could be safely used with a low-dose regimen [46].

The most comprehensive study regarding the use of mexiletine in LQT3 patients was published by Mazzanti et al. [11]. The study population consisted of 34 LQT3 patients, including 19 males and 15 females at the median age of 22 years and median QTc interval before therapy of 509 ms. Before starting mexiletine, 21 of 34 (62%) individuals were asymptomatic. Among the symptomatic patients, 7 had experienced cardiac arrest: 4 while on beta-blockers and 3 while off therapy. At the time of mexiletine initiation, 21 of 34 (62%) patients were on chronic beta-blocker therapy. Mexiletine was administered at the average daily dose of 8 ± 0.5 mg/kg for a median duration of 36 months. Mexiletine significantly shortened QTc by 63 ± 6 ms ($p < 0.0001$) and reduced the percentage of patients with arrhythmic events from 22% to 3% ($p = 0.031$). As shown in Fig. 9.4, the mean number of arrhythmic events per patient was reduced from 0.43 ± 0.17 to 0.03 ± 0.03 ($p = 0.027$), and the annual rate of arrhythmic events from 10.3% to 0.7% ($p = 0.0097$). This study demonstrated in a convincing way that LQT3 patients should be treated with mexiletine with therapeutic goal of reducing QTc <500 ms. Knowing high lethality of cardiac events in the LQT3 patients [3, 5], mexiletine should be considered as the first-line therapy. The question of concomitant administration of beta-blockers together with mexiletine is not fully resolved, but since data from Wilde et al. [38] showed no effectiveness of beta-blockers in LQT3 males, mexiletine should be routinely used

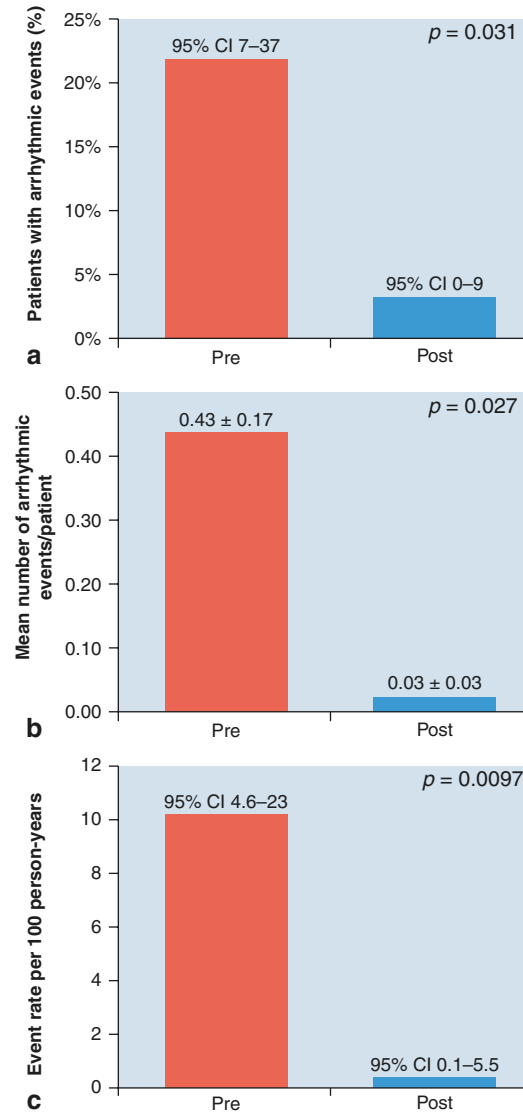



**Fig. 9.4** Reduction of arrhythmic events during treatment with mexiletine in patients with LQT3. (Reproduced with permission from Mazzanti et al. [11])

in such patients. Although Wilde et al. [38] showed very significant reduction of cardiac events in LQT3 women with beta-blockers, mexiletine should be considered too in LQT3 women especially if QTc >500 ms, who show high lethality of cardiac events [5]. Mexiletine was well tolerated in the study by Mazzanti et al. [11] without evidence for Brugada ST elevation.

Ranolazine, a late sodium current inhibitor, has been first tested by Moss et al. [48] in LQT3

patients with del-KPQ mutations showing significant reduction in QTc duration after intravenous administration. Subsequently, Chorin et al. [12] conducted a long-term clinical evaluation of ranolazine administration in eight LQT3 patients carrying the D1790G mutation in whom the QTc shortened from 509 ± 41 to 451 ± 26 ms, a mean decrease of 56 ± 52 ms ($p = 0.012$), which was maintained over a period of 22.8 ± 12.8 months of follow-up. Ranolazine reduced the QTc at all heart rates but less so during extreme nocturnal bradycardia. There was no evidence for Brugada ST elevation after ranolazine.

Flecainide blocks the open state of the channel and does not depend on channels entering the inactivated state [49, 50]. An open-state block mechanism underlies the use-dependent blocking effect of flecainide [51]. Ranolazine also is an open-state blocker that unbinds rapidly from the closed state of the sodium channel but is trapped when the channel is in the inactivated state [52]. Flecainide and ranolazine both reduce peak $I_{Na}$ and late $I_{Na}$, but in the ventricular myocardium, ranolazine more selectively blocks late $I_{Na}$. The greater selectivity for late $I_{Na}$, rather than peak $I_{Na}$, makes ranolazine a better choice than flecainide. Recent data indicates that mexiletine acts via an interaction with the Domain III of the cardiac $Na^+$ channel voltage-sensing domain, in contrary or in addition to binding effects to the channel pore [53].

The above advances in the gene-specific pharmacotherapy of the LQT3 serve as a paradigm for future of gene-specific therapies in other forms of LQTS. The LQT3 therapeutic options could rely on the existence of several sodium channel blockers, which is not the case for LQT1 and LQT2 forms of the disease since no effective drugs correcting their channel dysfunction exist.

## Potassium Channel Mutations

Experimental data shown that an increase in extracellular potassium can limit the development of an arrhythmogenic substrate under long QT conditions, due principally to its action to increase $I_{Kr}$ and $I_{K1}$ and limit the potency of $I_{Kr}$ blockers [37]. Compton et al. [54] demonstrated that administered potassium corrects repolarization abnormalities in LQT2 patients with $I_{Kr}$ defects. Potassium chloride and spironolactone were administered to 8 LQT2 patients for 4 weeks. The average daily potassium dose was 3.3 ± 1.5 mEq/kg and spironolactone dose was 3.5 ± 1.2 mg/kg, and this regimen resulted in an increase in serum potassium levels from 4.0 ± 0.3 to 5.2 ± 0.3 mEq/l. There were no serious complications associated with therapy. The increase in serum potassium resulted in a decrease in the corrected QT interval from 526 ± 94 to 423 ± 36 ms. These results were encouraging, but long-term supplementation of potassium and administration of spironolactone were proven to be difficult to achieve; therefore, this approach did not get tested in longer event-oriented trials.

Potassium channel openers were also considered based on the observation that administration of nicorandil reduces epinephrine-induced prolongation of the QT interval in LQT1 patients [55]. Oral dosing of nicorandil leads to blood levels in the range of 0.2–0.3 μmol/L, whereas intravenous injection can raise plasma levels to 4 μmol/L. Therefore, intravenously but not orally administered nicorandil may be of therapeutic value in suppressing repetitive episodes of TdP in patients with LQT1 and LQT2 syndromes; however, it will not be an option for long-term oral therapy.

LQT2 mutations might be caused by hERG-trafficking defects [4]. Lumacaftor is a drug acting on channel trafficking which was tested as safe medication and is used in cystic fibrosis patients [56]. In a study by Mehta et al. [57], induced pluripotent stem cell-derived cardiomyocytes harboring mutations from eight LQT2 patients were tested, and they showed a prolonged corrected field potential durations (equivalent of QTc interval) and increased arrhythmias. Lumacaftor significantly shortened corrected field potential duration by correcting the hERG-trafficking defect, and also it seemed to act on calcium handling by reducing RyR2S2808 phosphorylation in studied cardiomyocytes. The authors concluded that lumacaftor, a drug already in use, can rescue the pathological phenotype of

LQT2-induced pluripotent stem cell-derived cardiomyocytes and might be an option for treating select LQT2 patients, pending proofs of safety and efficacy in clinical trials.

## Left Cardiac Sympathetic Denervation (LCSD)

LQTS patients who experience cardiac events while taking beta-blockers are at increased risk for sudden cardiac death, and such patients may benefit from non-pharmacological antiadrenergic therapies such as left cardiac sympathetic denervation [10, 24, 58]. First reported case of such denervation was performed in 1970 by Moss and McDonald [23] in a 39-year-old LQTS patient with recurrent syncopal events in whom they first tried an injection of lidocaine into the region of left stellate ganglion, which resulted in dramatic QT shortening from 0.64 to 0.46 seconds. Based on this pharmacological testing 2 days later, they performed cervicothoracic sympathetic ganglionectomy of left seventh cervical through left second thoracic ganglion, and patients remained free of symptoms for decades. In 1985, Moss et al. [24] reported first data from the International LQTS Registry of 196 patients of whom 16 patients (8%) had undergone left stellate ganglionectomy for refractory ventricular arrhythmias showing a 75% reduction in the relative risk of cardiac events. Subsequently, in 1991, Schwartz et al. [10] identified 85 symptomatic LQTS patients worldwide who underwent LCSD and 55% did not have events during about 6-year follow-up after the surgery. This series was further expanded to 147 patients reported in 2004 [58], of whom 99% were symptomatic and 48% experienced prior aborted cardiac arrest, with follow-up over a mean period of 8 years after surgery. During long-term follow-up, 46% of patients became asymptomatic; syncope occurred in 31%, aborted cardiac arrest in 16%, and sudden cardiac death in 7% (Fig. 9.5). The mean yearly number of cardiac events decreased by 91% from 1.32 to 0.19 events per person per year ($p < 0.001$). There was an average QTc shortening by 39 ms after LCSD in comparison to ECGs before surgery. This study demonstrated that LCSD is associated with a significant, long-term reduction in the frequency of aborted cardiac arrest and syncope. However, the procedure is not entirely effective in preventing sudden death. This therapy should be considered in LQTS patients who experience syncope despite beta-blocker therapy and in those who have arrhythmia storms and shocks with an ICD. In 2009, Collura et al. [59] shared their experience with LCSD performed using minimally invasive video-assisted thoracic surgery in 18 LQTS patients. Subsequent long-term analyses from Mayo Clinic of 52 LQTS patients (18 with minimally invasive surgery) showed that 23% of them had cardiac events after denervation. The authors concluded that LCSD must not be viewed as curative or as an alternative in implantable cardioverter-defibrillator for high-risk patients [60].

## Cardiac Pacing

Nowadays, cardiac pacing alone without a defibrillator is rarely used in LQTS patients with exception for significant bradycardia and pauses that contributed to significant QTc prolongation and might lead to bradycardia-dependent TdP [61–63]. Moss et al. [61] demonstrated in a pre-ICD era that pacing is effective in reducing risk of cardiac events and in reducing QTc duration, especially when pacing rate exceeded intrinsic atrial rate. Very similar results were published by Eldar et al. [62]. Since development of atrioventricular blocks is infrequent in LQTS patients (with exception for infants), atrial-only pacing might be exercised. However, experimental data indicate that although atrial pacing shortens repolarization, this effect is diminished during sympathetic activation [64]. Currently, LQTS patients with bradycardia might be offered an ICD, but on individual basis, pacemaker therapy with atrial or dual-chamber pacing might be still considered. In young children with 2:1 atrioventricular block associated with extreme QT prolongation, pacing might be used to enable beta-blocker treatment [65].

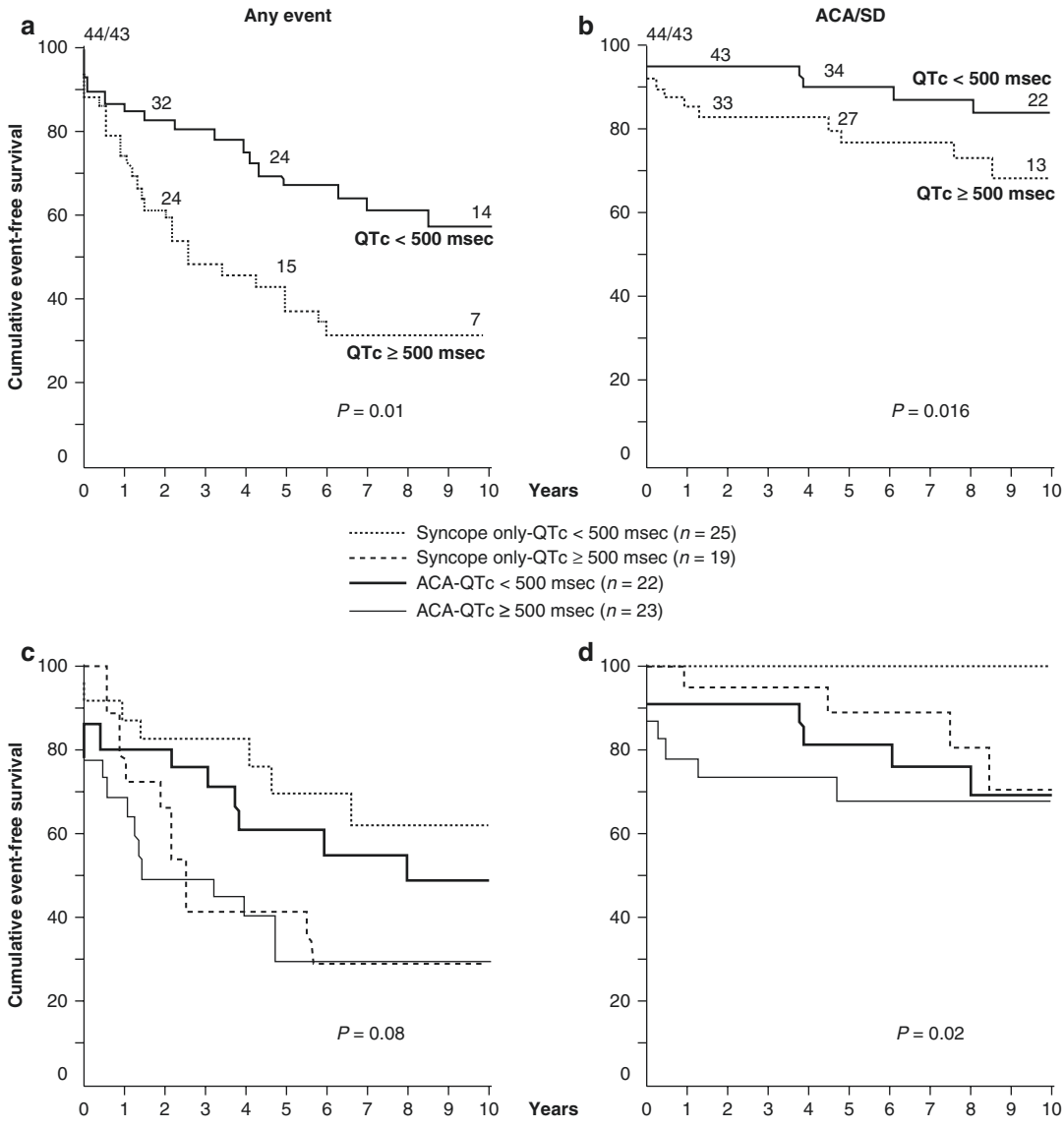



**Fig. 9.5** Kaplan-Meier curves of event-free survival and survival according to post-LCSD and QTc in entire population (**a**, **b**) and in patients with only syncope or ACA before LCSD (**c**, **d**). (Reproduced with permission from Schwartz et al. [58])

## Implantable Cardioverter-Defibrillator in LQTS

Implanted cardioverter-defibrillator (ICD) is indicated for secondary prevention in LQTS patients and for primary prevention in high-risk patients who remain symptomatic despite beta-blocker therapy [40]. Table 9.3 summarizes the "2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death" specifically focusing on the LQTS patients [40]. In patients with LQTS and sudden cardiac arrest, there is class I recommendation for an ICD if meaningful survival of greater than 1 year is expected (level of evidence B with no randomized trials). Similarly, class I indication includes high-risk patients with symptomatic LQTS in whom a

beta-blocker is ineffective (usually syncope on beta-blocker) or not tolerated, in whom intensification of therapy with additional medications (guided by consideration of the particular long QT syndrome type, which is relevant only for LQT3 patients that could be treated with mexiletine, flecainide, or ranolazine), left cardiac sympathetic denervation, and/or an ICD is recommended. There is no clear evidence whether in such high-risk cases LCSD or ICD should be exercised as the first therapy. Knowing limited worldwide accessibility to the LCSD surgery, most frequently an ICD is implanted in such cases.

Figure 9.6 further elaborates on currently proposed strategies regarding prevention of sudden death in LQTS patients [40]. In patients with cardiac events despite beta-blockers, the following risk factors would favor an ICD: QTc >500 ms, genotypes LQT2 and LQT3, females with genotype LQT2, <40 years of age, onset of symptoms at <10 years of age, and patients with recurrent syncope. Asymptomatic LQTS patients with QTc >500 on beta-blocker therapy have class IIb indication for an ICD [40]. However, there are individual asymptomatic LQT2 and LQT3 patients that might be at high 6% 5-year risk of lethal cardiac events with QTc above 500 or 550 ms [33].

The above recommendations are based on a series of observational papers showing effectiveness of ICD therapy in LQTS patients with primary and secondary prevention of sudden cardiac death. In 1996, Groh et al. [66] reported the use of the ICD in 35 patients with LQTS of whom 75% had aborted cardiac arrest, and during a mean 31-month follow-up, there was no death and 21 patients experienced one or more appropriate ICD therapies. Zareba et al. [8] described ICD therapy in 125 LQTS patients from Rochester LQTS ICD Registry of whom 73 were considered high risk due to either prior aborted cardiac arrest ($n$ = 54) or prior syncope despite

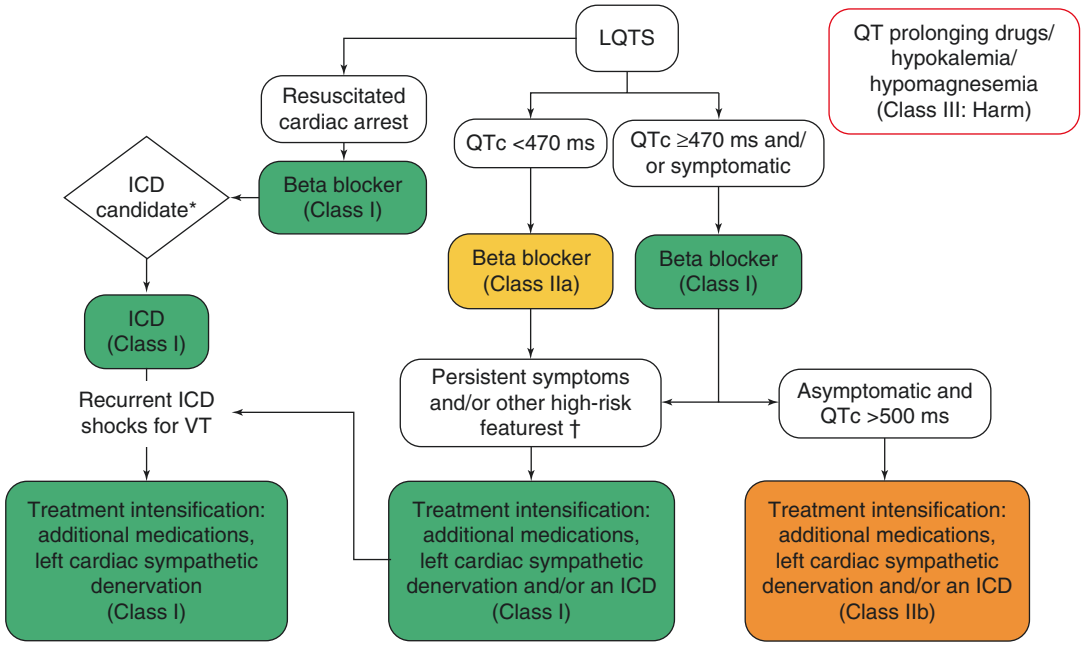
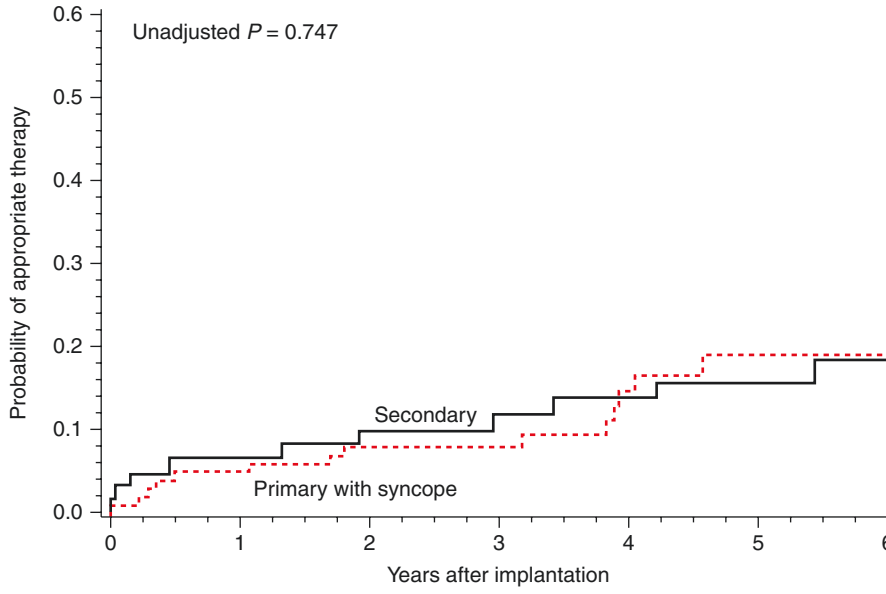



**Fig. 9.6** Prevention of SCD in patients with long QT syndrome. Colors correspond to class of recommendation in Table 9.2. (Reproduced with permission from [40])
∗ICD candidacy as determined by functional status, life expectancy, or patient preference
†High-risk patients with LQTS include those with QTc >500 ms, genotypes LQT2 and LQT3, females with genotype LQT2, <40 years of age, onset of symptoms at <10 years of age, and patients with recurrent syncope
ICD indicates implantable cardioverter-defibrillator, LQTS long QT syndrome, VT ventricular tachycardia

beta-blocker therapy ($n$ = 19). These 73 ICD patients were matched to 161 LQTS patients who met identical criteria for ICD implantation but were managed medically without an ICD because of patient or physician preference. In the 73 high-risk ICD-treated patients, one death (1.3%) occurred during an average follow-up of 3 years, whereas in the non-ICD patients, 26 deaths (16%) occurred over a follow-up of 8 years. Beta-blockers were used in nearly all the ICD patients. However, despite consistent medical therapy with beta-blockers, treated patients were shown to experience a 4% annual rate of ICD shocks [67]. Additional analyses of Rochester LQTS ICD Registry of 64 patients with secondary indications for ICD due to aborted cardiac arrest vs. 109 ICD patients with primary prevention with syncope showed a similar risk of appropriate ICD therapy at 5 years: 16% and 19%, respectively (Fig. 9.7).

Horner et al. [68] analyzed 51 LQTS patients with an ICD from the Mayo Clinic among whom 12 patients (24%) experienced an appropriate, VF-terminating therapy and 15 (29%) patients experienced an inappropriate shock with an average follow-up of 7.3 years. Secondary prevention indications, non-LQT3 genotype, QTc >/= 500 ms, documented syncope, documented torsade de pointes, and a negative family history were most predictive of an appropriate therapy. Potentially lifesaving therapies were rendered at a 5–6% per year rate among those selected for ICD therapy.

In 2010, Schwartz et al. [69] reported data on 233 patients from the European LQTS ICD Registry of whom 44% had a history of aborted cardiac arrest. During a mean 4.6-year follow-up, appropriate ICD therapy was observed in 28% of patients with a 4-year risk at about 30%, higher than risks observed in the Rochester LQTS ICD




Patients at risk

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Secondary | 64 | 57 (0.06) | 50 (0.10) | 46 (0.12) | 44 (0.14) | 36 (0.16) | 28 (0.18) |
| Primary with Syncope | 109 | 95 (0.05) | 86 (0.08) | 66 (0.08) | 46 (0.15) | 27 (0.19) | 20 (0.19) |

**Fig. 9.7** Probability of appropriate ICD therapy in LQTS patients by indications: 64 patients with secondary indications due to aborted cardiac arrest and 109 patients with primary indications with syncope. The rates of appropriate ICD therapy at 5 years are 16% in secondary prevention patients and 19% in primary prevention patients with syncope

Registry [8] despite similar mean QTc and similar proportion of patients with aborted cardiac arrest and syncope in both cohorts. In the study by Schwartz et al. [69], appropriate ICD therapies were predicted by age <20 years at implantation, a QTc >500 ms, prior cardiac arrest, and cardiac events despite therapy. These authors have developed the M-FACT score to further risk stratify LQTS patients for an ICD therapy with 1 point given for QTc >500–550 and 2 points for QTc >550 ms, 1 point for prior aborted cardiac arrest, 1 for events on therapy, and 1 point for age ≤20 years, but a negative 1 point was considered if a patient was event-free for >10 years. Cumulative score of 2–3 observed in 103 patients was associated with a 65% event-free survival, whereas score of 4–5 observed in 34 patients was associated with a 35% event-free survival indicating that these two groups were benefiting from an ICD. When analyzing 124 patients without aborted cardiac arrest, those with score of at least 2 (64 patients; 52%) had a 40% event rate, i.e., 60% event-free survival over a mean 7-year follow-up. This risk stratification approach might help in identifying patients that might benefit from an ICD therapy.

Biton et al. [70] analyzed data from Rochester LQTS ICD Registry with 212 LQTS patients that had ICD implantation for primary prevention while excluding patients with prior aborted cardiac arrest who without doubt qualify for ICD. During a median follow-up of 9.2 ± 4.9 years, 42 patients experienced at least one appropriate ICD shock with the 22% cumulative probability of appropriate shock at 8 years. QTc ≥550 ms and prior syncope on beta-blockers were significantly associated with increased risk of appropriate shocks. LQT2 mutation and multiple mutations were associated with 2–three-fold higher risk for recurrent ICD shocks as compared with LQT1 (there was not enough data to assess LQT3 patients in this cohort).

Table 9.4 shows the risk score, which was developed based on these 212 LQTS patients without aborted cardiac arrest, with 1 point given for QTc of 500–549, 2 points for QTc ≥550 ms, and 1 point given for history of syncope on beta-blocker, LQT2 mutation, and multiple mutations. As shown in Fig. 9.8, among 137 patients with available genotype data, there were 19 (14%) patients with score of 0, 47 (34%) patients with score of 1, 36 (26%) patients with score of 2, and 35 (26%) patients with score of ≥3. The cumulative probabilities of the first appropriate shock were 0%, 11%, 23%, and 46%, respectively at 6 years of follow-up where more data were available, $p$-value <0.001. Consistently, the risk score was highly predictive of the rate of appropriate shocks per 100 patient years, $p < 0.001$ (Fig. 9.9), with scores 2–3 identifying high-risk patients. We believe that this Rochester score will help identify LQTS patients without prior aborted cardiac arrest who might benefit from an ICD therapy.

**Table 9.4** Rochester ICD risk score when assessing risk of appropriate ICD shocks in LQTS patients without history of aborted cardiac arrest

| Risk variables | 0 points | 1 point | 2 points |
|---|---|---|---|
| QTc | ≤499 ms | 500–549 ms | ≥550 ms |
| Prior syncope on beta-blocker | No | Yes | |
| LQT2 | No | Yes | |
| Multiple mutations | No | Yes | |

The calculated score is the sum of the points of all four risk variables categories. Reproduced with permission from Biton et al. [70]

LQTS patients who receive an ICD are generally young and may experience complications of the device for many years [71–73]. These include inappropriate shocks, multiple shocks during a ventricular tachycardia/ventricular fibrillation storm, lead-related complications, the need for device replacement, infection, and psychological adjustment due to device therapy in general [73].

Among the 228 patients from a large study by Schwartz et al. [67], 103 patients (45%) underwent at least 1 revision of the ICD mostly due to battery depletion, and 58 patients (25%) suffered from at least 1 acute and/or chronic adverse event

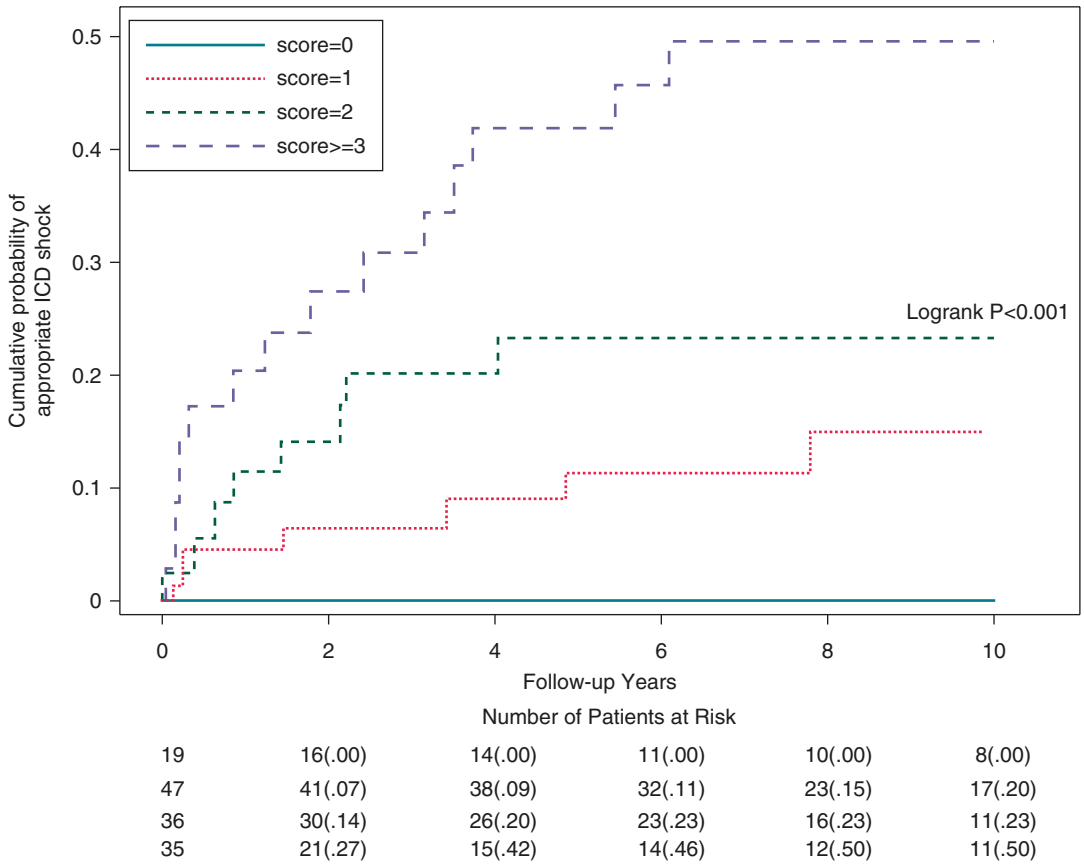



**Fig. 9.8** The Kaplan-Meier curves of appropriate ICD shocks by Rochester ICD risk score. (Reproduced with permission from Biton et al. [70])

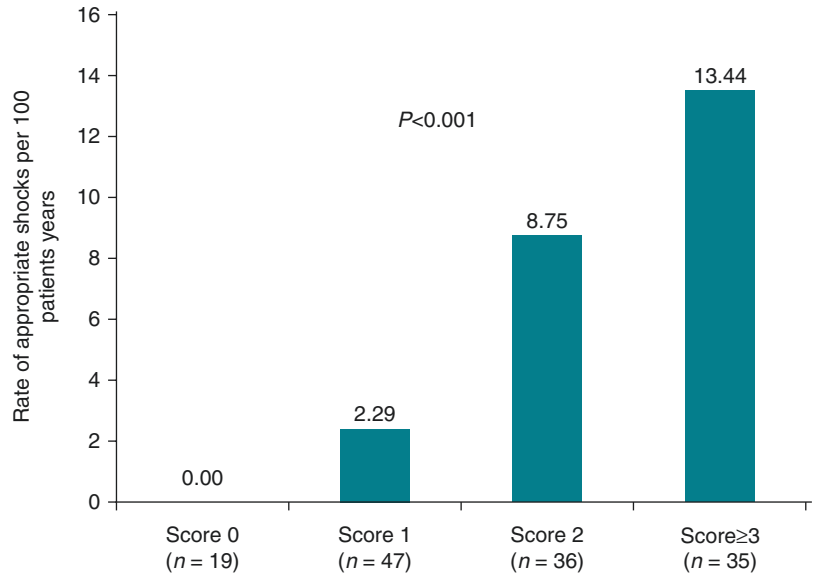



**Fig. 9.9** Event rate of appropriate ICD-rendered shocks (including recurrent) by the Rochester ICD risk score. Event rates of all the appropriate shocks (including recurrent) per 100 patients-years were calculated by dividing the total number of appropriate shocks during the period of follow-up by person-years and multiplying the results by 100. (Reproduced with permission from Biton et al. [70])

associated with the surgical procedure of ICD implantation and/or with both lead and generator functioning.

In the recent analyses of Biton et al. [68], there were 88 inappropriate ICD shocks, 41% were caused due to T-wave oversensing, 14% due to atrial flutter/fibrillation, 22% due to supraventricular tachyarrhythmias, and 20% were caused by electromagnetic interference. Most of the inappropriate shocks ended without further consequences. Two patients (2%) had appropriate shocks preceded by inappropriate shocks.

Inappropriate therapies tend to occur more often in younger patients [70], and concomitant medical therapy with beta-blockers at higher doses is recommended to minimize the incidence of shocks due to sinus tachycardia or supraventricular arrhythmias. T-wave oversensing, especially in LQT3 or LQT1 patients with larger T waves, remain a challenge requiring further innovation from device manufacturers.

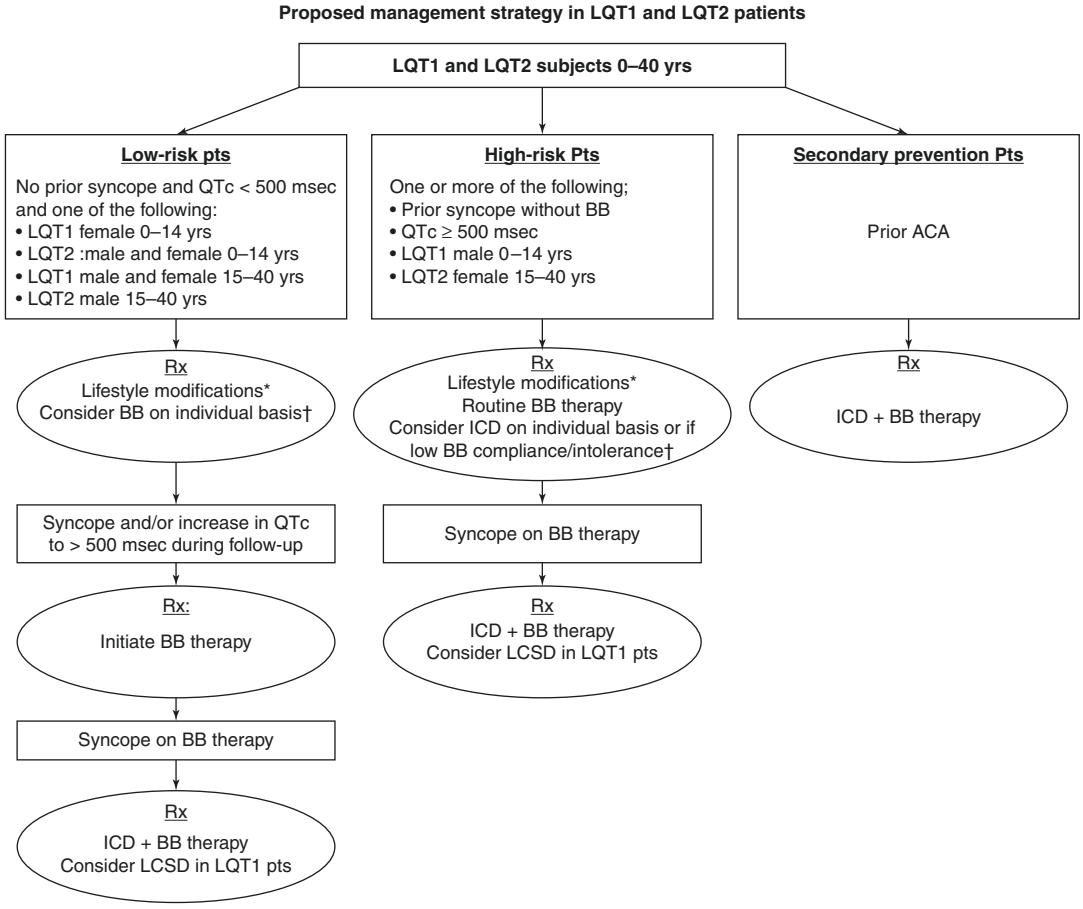



**Fig. 9.10** Proposed strategy for the management of LQT1 and LQT2 patients
BB beta-blockers, ICD implantable cardioverter-defibrillator, LCSD left cardiac sympathetic denervation, Rx treatment
∗Lifestyle modifications include restriction from competitive sports and swimming in patients with both genotypes and avoidance of unexpected auditory stimuli in the bedroom (especially during rest or sleep) and potassium supplements (to levels >4 mEq/L) in LQT2 patients. (Reproduced with permission from Goldenberg et al. [75])
†Some authorities recommend different therapeutic approaches, including routine administration of beta-blocker therapy to low-risk patients and primary ICD therapy to high-risk patients with ≥2 risk factors

Avoiding inappropriate or appropriate unnecessary ICD shocks is of major importance in LQTS patients whose catecholaminergic response to initial shock may lead to arrhythmia storm. When programming the device, the duration of detection may be set longer than usual 30 out of 40 to prevent an intervention for a nonsustained TdP that would have terminated spontaneously. VT zone rate also tends to be programmed nowadays at 200 bpm or higher up to 240–250 bpm.

Subcutaneous ICD is increasingly used, and patients with inherited arrhythmia disorders might benefit from this less invasive implantation [74]. Rudic et al. reported data on 62 patients with inherited arrhythmia disorders implanted with subcutaneous ICD for both primary and secondary prevention of sudden death. This cohort included 6 LQTS patients. A total of 20 spontaneous ventricular tachyarrhythmias requiring shock intervention occurred in 10 patients during a mean 31-month follow-up, none in LQTS patients. Averaged delay was 19 seconds, and in 55% of patients, single zone at >240 bpm was used; and in the remaining 24% dual zones, 190–240 and >240 were programmed. All episodes were terminated within the first ICD shock delivery with 80 J. Two patients had inappropriate therapies caused by oversensing. These data support the use of subcutaneous ICD in inherited arrhythmia patients.

## Conclusions

Decades of basic and clinical research focused on a better understanding of etiology, mechanisms, and clinical course of the LQTS have led to significantly improved management and therapies resulting in a better survival. Increasing availability of genetic testing provides opportunity to apply more personalized gene-specific management and therapy. LQTS treatment should begin with lifestyle modification and beta-blockers, unless there are valid contraindications. If the patient has one or more syncope despite full-dose beta-blockers, LCSD and ICD should be considered with the final decision based on the individual patient characteristics including age, sex, previous history, and genetic subgroup including sometime mutation-specific features. Figure 9.10 summarizes management of LQT1 and LQT2 patients dependent on their risk profile. Gene-specific pharmacotherapy is available for LQT3 patients with mexiletine, ranolazine, and flecainide as therapeutic options usually administered together with beta-blockers. This therapy should be administered to decrease QTc duration to values below 500 ms. In high-risk patients with QTc >500 ms despite such therapy or those with prior syncope, an ICD should be considered.

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# Drug-Induced Long QT Syndrome and Torsades de Pointes

10

Raymond L. Woosley and Peter J. Schwartz

## Recognition and Diagnosis of Drug-Induced Long QT Syndrome (diLQTS)

In the 1990s, a previously obscure adverse drug reaction, drug-induced long QT syndrome (diLQTS), achieved notoriety and set in place changes that have dramatically altered the practice of medicine [1]. This syndrome is the clinical association of (1) the administration of a drug(s) that delays cardiac repolarization, (2) resultant prolongation of the QTc (heart rate-corrected) to values ≥500 ms, and (3) the development of an unusual type of ventricular tachycardia known as torsades de pointes (TdP). The diagnosis of diLQTS requires all three features be present and should be distinguished from congenital long QT syndrome (cLQTS) [2] or "short-coupled TdP" [3–5] which have very different pathogenesis and clinical management.

Before the recognition of diLQTS as a syndrome, TdP had been recognized as a distinct type of arrhythmia and named TdP by the French cardiologist Dessertenne because of its characteristic "twisting of the points" pattern in which the ventricular complexes rotate around the electrical axis of the heart (Fig. 10.1) [2, 6, 7]. Subsequently, TdP has been subdivided into two types. The classic form has antecedent QT prolongation and is most often initiated by a long-short series of cardiac depolarizations [3]. A second form is "short-coupled" TdP, which is a polymorphic ventricular tachycardia that occurs in the absence of QT prolongation [4, 5, 8]. This chapter will focus on the classic type of TdP which is most often the result of drug-induced prolongation of repolarization [9] or is the arrhythmia naturally seen in patients with the congenital long QT syndrome (cLQTS) [2].

## International Impact of diLQTS

What initially appeared to be tragic but rare anecdotes of TdP and deaths associated with commonly prescribed drugs such as antihistamines [10] turned out to be a not uncommon clinical entity with far-reaching ramifications. Regulatory agencies responded relatively quickly to develop new guidelines for drug testing to identify the drugs with a risk of diLQTS [11]. Testing for in vitro effects of drugs on measures of cardiac repolarization and of QT prolongation in normal volunteers and/or patients has become standard for new drug development. These requirements prompted many pharmaceutical

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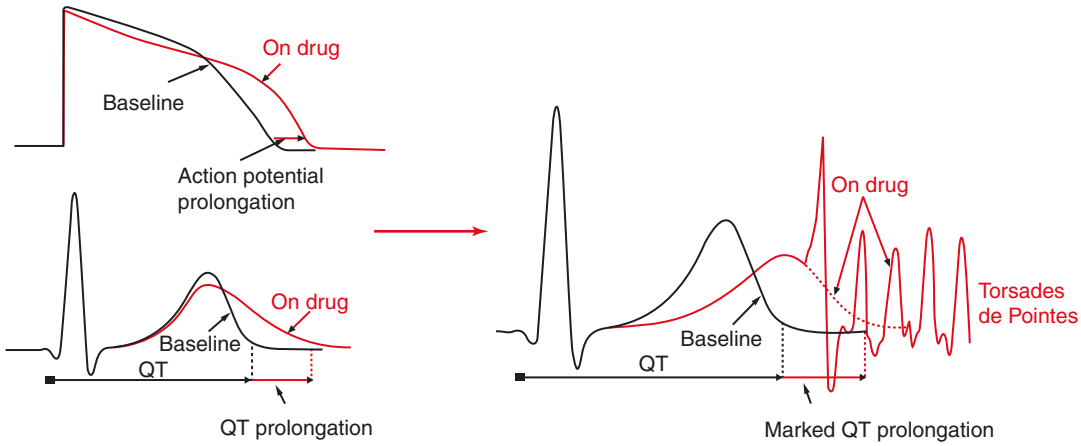


**Fig. 10.1** Pathophysiology of diLQTS. The left side of the figure shows the relationship between the surface ECG (bottom) and a typical ventricular action potential (top) in black. The "on drug" tracings in red show the concomitant prolongation of action potential (top) and the QT interval (bottom) caused by drugs that have the ability to block the hERG channel which reduces the rapid component of the delayed rectifier current, $I_{Kr}$. The right side of the figure demonstrates that as the QT prolongation becomes more marked, spontaneous early afterdepolarizations occur which can initiate the arrhythmia torsades de pointes (red)

companies to withdraw over 14 highly profitable products from the market and many to halt the development of numerous promising new agents [12]. In recent years, we have come to realize that a large number of drugs can precipitate this syndrome and an even larger number of drugs can prolong QT.

## Historical Background

In retrospect, diLQTS was most likely the culprit in cases of quinidine syncope first reported in the 1920s [13]. More than 40 years later, Dessertenne described TdP as an arrhythmia associated with QT prolongation [6] and Selzer and Wray reported cases of quinidine syncope and "paroxysmal ventricular fibrillation" with the typical TdP pattern on their ECG [14]. Subsequently cardiologists recognized that these dramatic episodes of quinidine syncope had two key features: prolongation of the QT interval and the occurrence of TdP arrhythmia. TdP and di-LQTS gained greater recognition in 1982 when Moss and Schwartz advanced the hypothesis that these drug-induced events could represent a "forme fruste" of the congenital long QT syndrome (cLQTS) [15].

Since most of the first cases of TdP reported in the 1970s and 1980s were observed in patients being treated for cardiac arrhythmias, it was initially assumed that this relatively rare form of proarrhythmic event was mainly confined to antiarrhythmic drugs that prolong the QT interval, such as quinidine [16], disopyramide [17, 18], and procainamide [19]. However, in the early 1990s, the medical community's attitude toward TdP changed dramatically when it became apparent that popular non-cardiac drugs, such as the non-sedating antihistamine terfenadine, could also cause QT prolongation, TdP, and death [10]. Shortly thereafter, reports of TdP emerged with another antihistamine, astemizole [20], followed by the gastrointestinal drug cisapride [21], the antibiotic erythromycin [22], the opiates levomethadyl [23] and methadone [24], probucol for hypercholesterolemia [25], and many other non-antiarrhythmic drugs, including antifungal agents and anticancer drugs [9]. It soon became clear that TdP could occur with drugs in any therapeutic class and, despite its uncommon occurrence, the incidence for many drugs became unacceptable, prompting their removal from the market [26]. Since 1989, 14 clinically important drugs have been removed from the market due to TdP [26], and an unknown number have been stopped

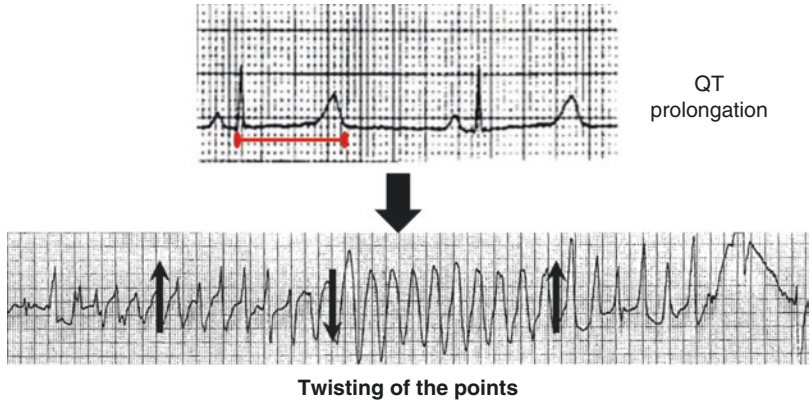


**Fig. 10.2** An example of drug-induced torsades de pointes. The top tracing shows a markedly prolonged QTc (approximately 592 ms corrected by Bazett and 620 ms by Fridericia), and the bottom is a tracing of TdP that developed subsequently in this patient shortly after initiation of sotalol therapy. The alternating black arrows show the twisting nature of the R waves during the tachycardia

in development due to evidence that they cause QT prolongation and therefore might have a risk of TdP. Although many drugs may have been discarded, many have also received FDA approval in spite of evidence that they can prolong QT and, for some, even that they can clearly cause TdP. In the last 18 years, the number of marketed drugs known to cause diLQTS has approximately doubled to total 47 and the number of drugs associated with QT prolongation alone has quadrupled from 25 to 105. The rapid increase in the number of drugs in both categories can be seen in Fig. 10.2 [27]. These drugs have reached the market because their risk of TdP is acceptably small and/or their clinical benefit is substantial, for example, drugs like arsenic trioxide which is used exclusively to treat cancer [28]. When there are no safe alternatives, clinicians, drug developers, drug regulators, and even patients, such as those with congenital long QT, must develop ways to minimize the risk of harm associated with a growing number of these medicines.

## Cellular Mechanism of diLQTS

Like many adverse drug reactions, diLQTS was initially considered to be idiosyncratic [16, 29]. As the electrophysiologic and molecular basis for diLQTS has been unraveled, it is no longer considered to be idiosyncratic [29, 30]. It has been studied extensively, and its mechanism is understood to the point that it can be replicated in animal and cellular models such as human stem cells induced to become spontaneously beating cardiac myocytes [31]. These studies are described in Chap. 4. The rich scientific basis for the mechanisms underlying TdP is well defined and described in Chap. 11 [8, 32, 33]. Much of our understanding of TdP comes from similarities found between the ion channel defects responsible for cLQTS [2] (discussed in Chap. 8) and the cellular actions of the drugs that cause diLQTS, i.e., primarily their actions that prolong repolarization by either reduction of $I_{Kr}$ [34], a major outward cardiac potassium current that is missing in patients with cLQTS type 2, or enhancement of late inward sodium currents as seen in cLQTS type 3 [35]. Other well-known causes of acquired QT prolongation include electrolyte abnormalities (hypokalemia, hypomagnesemia, and hypocalcemia), hypothyroidism, hypothermia, and extreme bradycardia, any of which can contribute to the risk of drug-induced TdP or, in extreme cases, cause TdP on their own [8]. Over 65 clinical and biochemical factors have been associated with QT prolongation and are summarized on the CredibleMeds.org website at QTFactors.org. The website includes an assessment of the quality of the evidence and the strength of the associations and is updated monthly.

There is an increased risk of TdP whenever a patient's QTc exceeds 500 ms or increases by more than 60–70 ms, especially when the increase occurs rapidly [8]. QT interval prolongation is particularly proarrhythmic when associated with increased dispersion in the recovery of excitability [36]. Although QT prolongation is an essential first step in TdP, it is not considered sufficient to induce TdP [8]. Additional electrophysiologic changes are required for TdP to occur; specifically, early afterdepolarizations that are calcium-mediated have been identified as an important element in in vitro models of TdP [33]. A common feature of drugs that induce diLQTS is the ability to block or disrupt the hERG potassium channel, thereby reducing $I_{Kr}$ and prolonging the QT interval [8]. However, Yang et al. recently reported that some $I_{Kr}$ blocking drugs known to cause TdP (dofetilide, E-4031, d-sotalol, thioridazine, and erythromycin) also increase the late sodium current which also may contribute to their proarrhythmic effect [35].

## Clinical Sequelae of diLQTS

While QT prolongation is not symptomatic, excessive prolongation above 500 ms can facilitate bursts of TdP that are often self-limited but cause symptoms of palpitations, light headedness, dizziness, presyncope, and syncope. If the arrhythmia persists, diLQTS can have a lethal outcome. Most patients survive the initial bursts of arrhythmia, especially if it is recognized and treated appropriately [8]. TdP can progress to ventricular fibrillation and death if not diagnosed as TdP and if the QT prolonging medicines are not discontinued and/or other precipitating factors such as hypokalemia or bradycardia not reversed. As an indication of the potential severity of diLQTS, many of the drugs that cause diLQTS have been associated with a higher than expected incidence of sudden cardiac death [37–41].

The fact that only a subset of patients who develop drug-induced QT prolongation experience TdP or death suggests that QT prolongation alone is not a perfect biomarker for predicting TdP [40]. To have true clinical utility, it must be combined with other known risk factors for TdP [42]. Several of these approaches to augment screening for risk of diLQTS are discussed below. Be that as it may, the prolonged QTc interval remains one of the most readily available and clinically useful early warning signals to alert for the possible occurrence of diLQTS [8]. According to the CredibleMeds website, for at least 100 drugs, the FDA-approved label includes a recommendation for monitoring the QT interval before or during therapy.

## CredibleMeds Risk Categorization of Drugs Associated with diLQTS

In the late 1990s, upon recognition that non-cardiac drugs such as terfenadine or cisapride could cause diLQTS and even death, the questions arose: *Which of the other over 3000 prescription drugs on the market might also carry this risk? How can they best be identified?* In 1999, the Agency for Healthcare Research and Quality (AHRQ) awarded funds to the University of Arizona's Center for Education and Research on Therapeutics (AZCERT), to develop a process to analyze evidence and establish criteria to identify drugs that prolong QT and have a risk of TdP [27]. This process, now known as the Adverse Drug Event Causality Analysis, has evolved and the risk categories expanded and validated by clinical investigations using large healthcare databases [43, 44]. For almost two decades, the AZCERT has conducted an independent analysis of evidence, placed drugs in risk categories, and made the information freely available to the public, healthcare providers, and research scientists [27]. In recent years, funded by a contract with the US FDA's Safe Use Initiative, AZCERT has been able to develop additional platforms such as smartphone apps in multiple languages and decision support tools to expand access to the information [45]. The lists of drugs were initially known as the QTdrugs lists, and the website can still be reached at www.QTdrugs.org [46]. More recently, they are also available on the CredibleMeds website

(www.crediblemeds.org) and as of 2018 were regularly accessed by over 112,000 registered visitors from 193 countries. AZCERT has developed an application program interface (API) that enables health information technology systems to have online, open-source access to the lists of drugs. To prevent real or perceived conflicts of interest and to assure independence in the decision-making process, AZCERT has been only supported by peer-reviewed federal awards and charitable contributions from the public and philanthropic foundations.

The methods that AZCERT uses to analyze evidence and assess TdP causality are described on the CredibleMeds website (https://www.crediblemeds.org/research-scientists/why-lists/) and in peer-reviewed publications [27, 45]. Because the available evidence frequently has limitations or gaps and is often of variable quality, AZCERT has developed a systematic, in-depth process to analyze all available data and to assess causality [27]. Since the QTdrugs list was created in 1999 with only 29 drugs in 1 category, over 194 new drugs have been added in 4 categories, and many have been moved among categories or removed. Beginning in 2012, AZCERT extended its analysis to include drugs marketed outside the United States, especially those in Europe, Australia, and Canada.

The analysis of evidence includes a thorough review of published studies (PubMed search using standardized search terms) and reports of adverse events in the FDA's Adverse Event Reporting System (AERS) or WHO's Vigibase. AZCERT uses the data-mining statistical software developed for the FDA (Empirica Signal Software, Oracle Health Sciences, Redwood Shores, California) [27]. Today, AZCERT places drugs in four categories of TdP risk (Fig. 10.3). The main category of interest, "Known Risk of TdP," are drugs found to have convincing clinical evidence that they can cause TdP, even when used as recommended in the drug's FDA-approved label. Those drugs that prolong the QT interval during routine clinical use, but do not at this time have convincing evidence of TdP causality, are placed in the "Possible Risk of TdP" category. Beginning in 2006, the "Conditional Risk" category was created and includes drugs for which there is a documented association with TdP but only under certain specific conditions, such as overdose, hypokalemia, hypomagnesemia, and bradycardia, or when there is an interaction with another drug(s). This category also

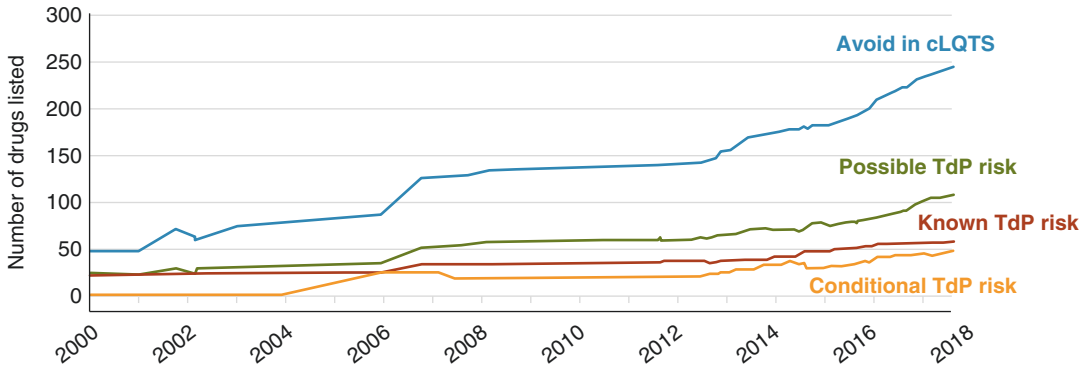


**Fig. 10.3** The number of drugs in each of the four categories of TdP risk on the CredibleMeds website from 1999 to July 2018. The graph displays the changing number of drugs in each of the four categories of TdP risk at www.CredibleMeds.org between 1999 and July 2018. The number of drugs on the list with Known Risk of TdP at any point in time is shown as a red line. The number of drugs with Possible Risk of TdP is shown in green. The number of drugs with Conditional Risk of TdP (i.e., associated with TdP under certain conditions) is shown in orange. The number of drugs on the list of Drugs to Avoid in patients with congenital long QT syndrome is shown in blue. The latter list includes all of the drugs listed in the other three risk categories plus additional drugs that do not prolong QT, per se, but have actions known to place some patients with cLQTS at increased risk of diLQTS (e.g., adrenergic drugs). cLQTS = congenital long QT syndrome, TdP = torsades de pointes

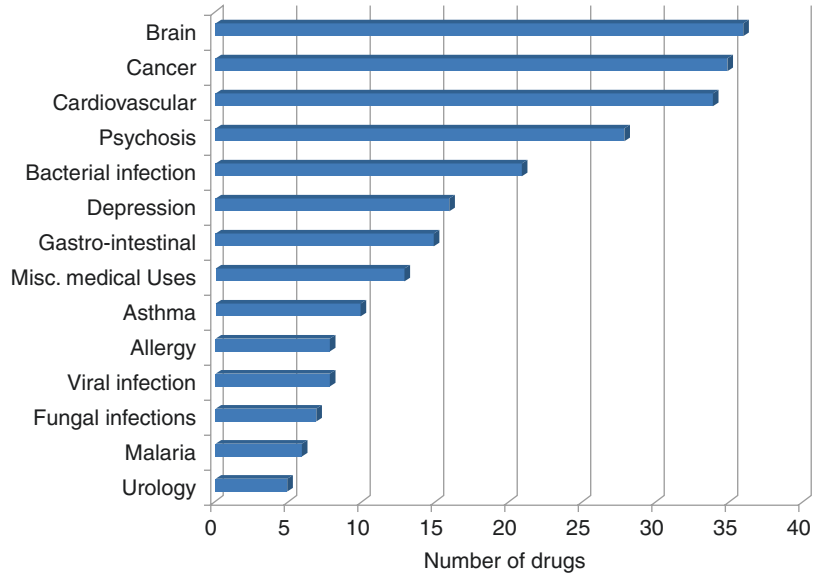


**Fig. 10.4** Spectrum of therapeutic areas for drugs with known risk of TdP, possible risk of TdP, and conditional risk of TdP. The X-axis displays the number of drugs that were in each of the therapeutic categories listed on the Y-axis as of July 2018. Each drug's therapeutic area is based on its primary clinical indication. ADHD = attention deficit hyperactivity disorder, Misc. = miscellaneous, TdP = torsades de pointes

includes drugs that are associated with TdP because they have the ability to create the conditions that enable other drugs to cause TdP (e.g., loop diuretic agents that induce hypokalemia or drugs that are metabolic inhibitors of a QT prolonging drug). The website also posts a fourth list of drugs that AZCERT recommends are "Drugs to be Avoided by patients with congenital LQTS, if clinically feasible." For maximum safety, this list includes all of the drugs in the other three risk categories plus the adrenergic drugs that are considered to place some cLQTS patients at high risk of sudden death [27]. The process for evaluating drugs and decisions regarding their inclusion on lists is overseen by an international Advisory Board of clinical and pharmacological experts. The Board's advice is sought whenever conflicting evidence must be resolved or when any Board member has a concern about placement of a drug in a category. The drugs on all four lists are monitored continuously for new evidence, and, in recent years, the lists have been revised every 4–8 weeks. As of July 2018, 58 medications are on the list of drugs known to cause TdP (including 10 removed from the US market, but which may still be available in some countries). Another 108 are on the list for possible risk of TdP and 48 are on the list for conditional TdP risk. Figure 10.4 shows the broad range of therapeutic areas impacted by the drugs on the QTdrugs lists and demonstrates their ubiquitous use in medicine.

CredibleMeds has become an invaluable tool for the clinical management of patients affected by cLQTS [2]. Experienced centers dealing with these patients refer them to the public portal of CredibleMeds where they and their families can obtain an updated list of drugs to avoid or to take only under specific monitored circumstances [2]. As most general practitioners and many cardiologists are unaware of the QT prolonging potential of many cardiac and non-cardiac drugs [47, 48], this practice has become an essential part of the proper management of patients with channelopathies [2].

## Epidemiology of diLQTS and Death Associated with QT Prolonging Drugs

For most drugs, the incidence of diLQTS is difficult to quantify due to its rarity. Estimates appear to vary greatly from drug to drug [49] and range from extremely low for drugs such as the macrolide antibiotics [50], 1.5–3% for quinidine [3, 51], and 3–9% for drugs such as ibutilide [52], sotalol [53], and azimilide [54]. Estimates for drugs such as sotalol or ibutilide are likely to be

more accurate because the FDA required more extensive premarket research on the incidence of TdP with their use. The overall incidence of TdP in a given population will depend on the relative risk of the drugs prescribed and the frequency of their use in the population. For example, a cardiac care unit (CCU) population is more likely to be prescribed antiarrhythmic drugs and will likely have a much higher frequency of TdP than would be seen in a routine hospital population treated mostly with non-cardiac drugs. A mental health population prescribed methadone, antidepressants, and/or antipsychotics may have a higher incidence than a younger healthy population such as the military.

Estimating the incidence of diLQTS in a given population is difficult, partly because the arrhythmia can be transient or may be unrecognized. An accurate diagnosis requires that an ECG be taken during an arrhythmic episode to document the presence of QT prolongation and the characteristic twisting pattern of the ventricular tachycardia. Without an ECG, diLQTS may be diagnosed as syncope, seizures, or sudden cardiac death. Reporting diLQTS is hindered by the fact that the International Classification of Diseases, tenth Revision (ICD-10), that is used to record diagnoses in medical records lacks a specific code for drug-induced TdP or excessive QT prolongation. diLQTS and TdP tend to be reported under codes for VT, VF, or SCD [55]. One study estimated that between 5% and 7% of reports of VT, VF, or SCD were, in fact, diLQTS [55]. Acknowledging the probable impact of under-reporting, European pharmacovigilance centers in Sweden [56], Germany [49], France, and Italy [57] have found an annual reporting rate for diLQTS of approximately 0.8–1.2 per million person-years [55]. In an epidemiological study, Sarganas et al. [49] reviewed patient records, looking for episodes of TdP or cases of "symptomatic QT prolongation" (defined as QTc >450 ms for men and >470 for women associated with ECG evidence of TdP, successful cardiac resuscitation, syncope, or severe dizziness) in Berlin and found the reporting rate to be 2.5 per million person-years for men and 4.0 for women. Vandael et al. found the incidence of diLQTS in the University Hospital in Leuven to be 0.16 per thousand/year, and they estimated that would extrapolate to 173 possibly lethal cases of TdP in Belgian hospitals each year [58].

The frequency of death due to diLQTS is also difficult to estimate because if a patient dies without having an ECG recorded, TdP may not be recognized as the cause of death. Perhaps this explains why reports of death are not a dominant feature in most case series of TdP [51, 59, 60]. Yet, even with these potentially confounded diagnoses, many of the drugs that prolong the QT interval have still been associated with a significantly higher risk of sudden cardiac death in some studies [37–41, 61–63]. In a population-based, case-control study in the Netherlands, Straus et al. [62] examined all-cause mortality associated with non-cardiac drugs that prolong the QT interval (drugs listed on the CredibleMeds.org website) and found an approximately threefold greater risk of death (95% CI, 1.6–47). On this basis, they estimated that the use of these non-cardiac drugs causes >15,000 deaths annually in the United States and Europe. Because many of these drugs, especially the antibiotics, have therapeutic alternatives that lack QT prolonging activity, it is possible that many of these deaths may have been preventable. Levofloxacin, erythromycin, clarithromycin, and azithromycin are among the most commonly prescribed antibiotics in the world, and all have been associated with QT prolongation, a risk of TdP, and an increased risk of sudden death [37–41, 61–65]. TdP and related deaths due to these antibiotics are a special concern, made more serious because of their widespread and often inappropriate use. In 2013, the FDA issued warnings and expressed concern regarding the overuse of antibiotics, especially citing the excess numbers of deaths associated with levofloxacin, azithromycin, and other macrolides [38, 39, 63, 64]. It was noted that in 2011, 40 million people took azithromycin and, on the basis of the study by Ray et al. [38], which found 1 excess death for every 21,000 prescriptions compared with amoxicillin, this would result in an estimated 1900 excess deaths with this drug in the United States alone. In 2014, another study from the US Veterans Health

System estimated that 4560 people die each year due to azithromycin when amoxicillin would have been a safe alternative [63]. A recent meta-analysis of 33 studies involving 20,779,963 patients taking macrolides compared outcomes with those of patients who took no macrolides and found an increased risk of developing SCD or ventricular tachyarrhythmias (RR, 2.42; 95% CI, 1.61–3.63), SCD (RR, 2.52; 95% CI, 1.91–3.31), and cardiovascular death (RR, 1.31; 95% CI, 1.06–1.62) [50]. The authors concluded that treatment with macrolides is associated with an absolute risk increase of 118.1 additional SCDs or ventricular tachyarrhythmias and 38.2 additional cardiovascular deaths per one million treatment courses. Because of the magnitude of TdP associated with these antibiotics, the FDA's Safe Use Initiative has focused on the safe use of antibiotics and funded efforts to reduce their risk of TdP [66]. It is important to note that some studies that focussed on relatively healthy populations [67] have not observed an increased mortality with these antibiotics which emphasizes the need for targeting risk mitigation strategies such as CDSS.

## Prevention of diLQTS

In 2010, a panel of experts published a thorough and thoughtful consensus paper on the prevention of diLQTS in the hospital setting and described an approach for monitoring the QT interval as part of a prevention strategy [8]. For most drugs that have demonstrated the potential for causing diLQTS, the cost and inconvenience of routine, untargeted screening for QT prolongation is difficult to justify because of the relatively low number of patients expected to develop clinically significant QT prolongation (>500 ms or >60–70 ms change) [8]. Furthermore, the number of patients who develop diLQTS is usually only a relatively small subset of those with increased QTc. On the other hand, for some drugs, such as dofetilide, the frequency of TdP can exceed 2% [68], and the FDA recommends hospital admission and ECG monitoring of the QT interval when initiating or re-initiating therapy [69]. It is important to note that, at this point in time, no strategy has been shown to prevent or reduce the incidence of TdP.

## Identifying Risk Factors for diLQTS

It has become apparent that the QT interval alone, despite its primary role, is but one of several risk factors that must be considered in order to reliably identify patients at highest risk for developing diLQTS. Numerous studies suggest that in order for a strategy for prevention to have an acceptable level of predictive accuracy and be cost-effective, it must include consideration of those risk factors in three general categories: (1) patient specific, (2) drug specific, and (3) clinical scenario specific [8].

***Patient-Specific Risk Factors:*** Patient-specific risk factors for diLQTS include the same factors that are known to be clinically associated with an increase in the QT interval, that is, female sex, bradycardia, hypokalemia, hypomagnesemia, hypocalcemia, diuretic agent use, hypothermia, and history of heart disease [42]. Additional factors associated with QT prolongation were found by Haugaa et al. [70] by extracting patient-specific information from the electronic medical record (EMR) of Mayo Clinic Health System. These factors included diabetes, cardiomyopathy, pheochromocytoma, hypoglycemia, anorexia, congenital long QT, renal dialysis, and status after conversion from atrial fibrillation. These investigators have developed a system that calculates what they have termed a "pro-QTc" risk score that is based on the presence of these risk factors and the use of drugs on the Known Risk list at the CredibleMeds.org website. They found that the pro-QTc risk score could identify hospitalized patients that have a fourfold higher mortality than that of the overall population. This system generates alerts that are part of a clinical decision support system (CDSS) that has been implemented across the institution [70, 71]. Using this CDSS, Sorita et al. were able to reduce the prescribing of many of the QT prolonging medications for high-risk patients [71].

Similarly, for a Coronary Care Unit (CCU) population, Tisdale et al. developed a CDSS and QT risk score that they found could identify patients with excessive QT interval prolongation [72]. Tisdale et al. found that their CDSS was

able to reduce the overall incidence of excessive QTc prolongation and reduce the overall prescribing of QT prolonging drugs, especially for patients who had baseline QT prolongation [73].

In Belgium, Vandael et al. have made extensive progress in developing a CDSS to help reduce the harm from QT prolonging drugs. First, they reviewed the medical literature and identified risk factors for QT prolongation [74]. They then analyzed the medical records of patients in the University Hospitals Leuven to confirm the association of risk factors in their patient population [75]. In addition to the risk factors found at Mayo Clinic and by Tisdale in the CCU, Vandael et al. found additional risk factors such as C reactive protein, body mass index, smoking, diabetes, and liver failure [75]. These investigators have used these factors and the CredibleMeds drugs lists to compute a RISQ-PATH score as part of a CDSS that has been implemented and tested in six Belgian psychiatric hospitals [58, 76]. They have used this CDDS to estimate the incidence of diLQTS in their hospital population [58] and, in a psychiatric population, to identify those patients most likely to develop excessive QT prolongation [76].

There is some overlap in the patient-specific QT risk factors used in these three CDSS, but there are also some significant differences. In addition to the risk factors utilized in the pro-QT score of Haugaa et al. [70], the Tisdale score for CCU patients identified sepsis and assigned greater weight to the patients' use of multiple QT prolonging drugs. Unlike Tisdale's score and the Mayo pro-QT score, the RISQ-PATH score included scoring for drugs on the Possible Risk and Conditional Risk lists. These differences in risk factors and in their assigned weights in these three CDSS most likely reflect the fact that they were developed using different patient populations and for slightly different purposes. This suggests that healthcare institutions will most likely have to determine which elements of QT risk scores are most appropriate for their specific clinical environment(s). The posted list of QT risk factors at CredibleMeds' website (QTFactors.org) was created to provide a resource for investigators and healthcare providers wishing to develop risk scores for evaluation and incorporation in their decision support tools.

***Drug-Specific Risk Factors:*** As discussed above, the common feature of drugs that cause TdP is their ability to block hERG channels and thereby to prolong the QT interval [77]. In theory, efforts to prevent TdP might incorporate a ranking of drugs by their in vitro hERG potency or degree of QT prolongation in humans. However, attempts to find useful correlations have not had much success [78]. Most likely, the inter-individual variability in drug sensitivity and the variable influence of factors that affect each patient's drug exposure (e.g., dose, drug metabolism, route of administration) reduce the predictive accuracy of such ranking to levels that lack clinical utility. For example, terfenadine and astemizole have very high hERG blocking potency but are extensively metabolized in most people so that it is unusual for them to attain blood levels that can cause TdP [60].

Prevention strategies must not only consider the drug's toxic potential but also its route and rate of administration and the dose and interactions with any concomitant medicines. The contribution of these factors is not always well defined for a given drug. For some drugs, like sotalol, the FDA required extensive testing during its clinical development to characterize the relationship between dose and QT prolongation and even TdP frequency. In this case, investigators found a positive dose-response relationship for sotalol-induced TdP [79]. In contrast, the risk of TdP with quinidine appears to have little relationship to dose and may be influenced more by the patient's clinical sensitivity, such as the presence of atrial fibrillation, which can cause long cardiac pauses that facilitate TdP [3]. For many drugs, like haloperidol or papaverine, the available data suggest that the risk of TdP is markedly influenced by the route of administration, with a larger proportion of cases occurring after rapid intravenous injection of haloperidol [80] or intracoronary injection of papaverine [81].

**Table 10.1** Clinical scenarios for risk of diLQTS

| |
|---|
| Concomitant therapy with two or more QT prolonging drugs |
| Concomitant therapy with a QT prolonging drug and a potassium-wasting diuretic |
| Concomitant therapy with a drug that blocks the metabolism of a QT prolonging drug |
| Development of renal failure in a patient treated with a QT prolonging drug that requires renal elimination (e.g., sotalol) |
| Development of liver failure in a patient treated with a QT prolonging drug that requires hepatic metabolism for clearance (e.g., methadone) |
| Development of vomiting or diarrhea resulting in electrolyte disturbances that potentiate the QT prolonging effects of drugs such as antiemetic agents or antibiotics |
| Taking a QT prolonging drug concomitant with extended therapy with a proton-pump inhibitor that produces hypomagnesemia and/or hypokalemia to further prolong the QT |
| Taking a QT prolonging drug concomitant with a beta-blocker or ivabradine that slows heart rate and thereby further prolongs the QT interval |
| Excessive dose titration of a QT prolonging drug due to lack of desired clinical response or development of tolerance (e.g., methadone) |
| Depression causing attempted suicide with toxic doses of QT prolonging drugs (e.g., antipsychotics, antidepressants, among others) |

***Scenario-Specific Risk:*** Analyses of case series of TdP caused by a specific drug reveal that the great majority of cases fall into certain categories of drug use that are influenced by the patients' medical condition [51, 59, 60, 82]. Many of these reports were of patients who had been successfully treated with the offending drug for extended periods of time but who developed TdP shortly after a change had occurred in their clinical condition or in the way it was being managed. For example, patients with heart failure may tolerate QT prolonging drugs until their medical condition deteriorates and a loop diuretic agent is prescribed and induces hypokalemia. Initially well-tolerated antibiotics that prolong the QT interval may later eradicate normal bowel flora, causing diarrhea, and result in electrolyte disturbances that can potentiate a patient's risk of TdP. A partial list of scenarios that, like these, can accentuate the TdP risk of a medication is in Table 10.1.

## Genetic Factors Contributing to diLQTS

The fact that the life-threatening arrhythmia in patients with diLQTS and those with cLQTS are the same (i.e., TdP) and that both conditions are associated with extreme prolongation of the QT interval (often with bizarre morphology of the T-wave) should have raised immediately the issue of a potential relationship between the two conditions [15].

A key component here was the realization that the second most frequent type of cLQTS, LQT2, is due to mutations in the hERG gene, which encodes the protein that mediates the $I_{Kr}$ current. When it became clear that a key mechanism for the development of diLQTS is the block, even partial, of $I_{Kr}$, the connection to cLQTS became obvious. In this regard, another critical step was the development of the concept of *repolarization reserve* by Roden [1]. This concept maintains that every person has a physiological *cardiac repolarization reserve*, which is genetically determined and can usually compensate for factors (e.g., drugs, hypokalemia, bradycardia, etc.) that might either decrease repolarizing or increase depolarizing currents during the action potential. People with reduced repolarization reserve are more likely to develop QT prolongation and TdP when exposed to $I_{Kr}$ blocking drugs. It follows that patients with cLQTS who, by definition, have a reduced repolarization reserve will be more likely than normal subjects to develop diLQTS. In theory, depending on the magnitude of genetically related deficiency of repolarizing currents, it should influence sensitivity to drugs, and smaller or higher doses of $I_{Kr}$ blocking drugs will trigger TdP.

In 2002, the report by Yang et al. [83] on 92 diLQTS patients suggested that cLQTS mutations could be found in 10–15% of diLQTS cases. Several studies have since followed, with mixed results [84–88]. For example, whereas the *KCNE1*-D85N polymorphism was found to be associated with an increased risk for diLQTS [85], three other studies suggested that rare variants play a prominent role [86–88]. The most recent data on 188 diLQTS patients compared

with over 1000 cLQTS patients [89] provide a different and more positive set of findings. The first is that disease-causing mutations for cLQTS were found in 28% of these patients; the second is that in baseline conditions, the QTc of diLQTS patients (453 ± 39 ms), although shorter than that of cLQTS patients (478 ± 46 ms), is significantly longer than that of control subjects (406 ± 26 ms). It is likely that some of the minor cLQTS genes [90], usually not tested, may be involved in some cases of diLQTS, thus further increasing the contribution of one syndrome to the other.

Just as genes that control expression of ion channels could directly increase risk of diLQTS, genes that control the rate of a drug's metabolism or elimination can also affect its ability to cause diLQTS by either limiting or increasing the amount of drug that reaches the heart tissue [91]. Genetic defects in drug metabolism have been shown to accentuate the ability of some drugs to cause diLQTS in affected patients [91]. Because some drugs that cause TdP, such as thioridazine or methadone [91], are strongly influenced by genetic variability in drug metabolism and others, such as the renally cleared sotalol [79], are not, the same genetic associations cannot be expected to have predictive ability for all drugs. Furthermore, genetic variability in the response receptor for drugs such as the mu receptor serves for methadone can result in reduced sensitivity to methadone and the need to titrate dosages to those that could precipitate diLQTS [92]. These examples may at least partially explain why efforts to identify gene variants associated with diLQTS have had limited success when lumping together cases caused by a diverse group of drugs that have dissimilar pharmacodynamics and pharmacokinetic disposition [86, 87].

## Opportunities for TdP Prevention: Potential Role of Genomics and CDSS

One can hope that a combination of rapid genetic screening, risk profiling, and monitoring of the QT interval during the first days of treatment with $I_{Kr}$ blocking drugs may result in a decrease of the number of patients manifesting TdP in response to a drug treatment. The massive amount of information that must be considered for the safe use of medicines has prompted the development of decision support tools to help physicians and other members of the healthcare team to reach optimal medical and therapeutic decisions. To support better prescribing of medicines, most CDSS described to date have issued alerts that warn of potential harm from a prescribing decision [73, 93, 94]. As discussed earlier, investigators at the Mayo Clinic [70], the University of Indiana [73], and Leuven, Belgium [75], have developed CDSSs to reduce the risk of QT prolongation in hospitalized patients. Unfortunately, the beneficial impact of these CDSS has been limited because in 75–95% of cases, physicians and pharmacists override or ignore the alerts [71, 73, 95] and no CDSS has yet been found to have sufficient impact on prescribing to effectively reduce the incidence of TdP. For improved acceptance of alerts and the effectiveness of CDSS overall, an expert panel has recommended that CDSS should provide clinically relevant and essential information at the time when a decision is being made, without overwhelming the decision-makers with more information than they need or can manage [96].

As an alternative to the use of interruptive alerts to signal prescribing errors, the concept of "medical autopilots" has been suggested as a potential preferred approach for CDSS [66]. Just as autopilots assist the pilot by capturing data and displaying only relevant warnings to the pilot, these programs can monitor the EMR and send signals to guide prescribers to make decisions that result in maximum benefit to patients and minimal risk of TdP. Hopefully, in the near future, a CDSS with these characteristics will be shown to prevent TdP.

A very recent attempt to identify potential genetic loci and risk factors causally associated with sudden cardiac death provides relevant suggestions. In the largest exploration of the genomics of sudden death to date, 9000 cases and 48,000 controls from 9 genome-wide association studies were analyzed by a meta-analysis in a discovery and in a replication stage [97]. Ashar et al. [97]

exploited the power of Mendelian randomization [98] by exploring causal relations underlying sudden cardiac death and relevant risk factors. Indeed, those factors which are causally associated with increased sudden death risk could represent a novel rational target for prevention. Their finding that QT interval prolongation increases risk not only after a myocardial infarction [99] but also in the general population may shift the current paradigm by which the use of $I_{Kr}$ blockers must be avoided in patients with the cLQTS [2] as it may be reasonable to consider that, whenever possible and medically appropriate, drugs without $I_{Kr}$ blocking activity should always be preferred in order to reduce TdP and sudden death [98].

## Conclusions

Most of the etiologic factors involved in diLQTS have been identified, as well as the major clinical risk factors for TdP. The relative contribution of each in specific populations is being defined. Also, for the most part, the drugs most likely to prolong the QT interval and to trigger TdP are being identified. Nonetheless, there is a considerable gap between the increased level of understanding of TdP and our current ability to effectively prevent diLQTS. This gap is being closed through the use of new technologies and standards in information management, machine learning, and system inter-operability [100].

The advances in genetics, including not only the clear evidence that genetic variants causing or favoring cLQTS play a major role in diLQTS but also the shortened turnaround time due to faster genetic testing methods, can open new options. For example, they allow the possibility of preemptive testing to screen the patients before they need to receive drugs, to rule out at least the presence of the most common variants associated with this life-threatening arrhythmia [101].

Finally, device technology such as the use of simple handheld ECG devices connected to smartphones is capable of screening for long QT and monitoring QT changes and may allow the early identification of those subjects more prone to development of a significant QT prolongation [102]. Another potentially useful approach to reduce the risk of TdP when patients cannot avoid taking an $I_{Kr}$ blocking drug has been recently described [103]. A wearable monitor can reliably assess the QT interval independently of whether it is normal, as in healthy controls, or prolonged as in patients affected by the congenital LQTS. This implies the possibility of administering $I_{kr}$ blocking drugs while remotely monitoring the QTc for 5–7 days or more to assess whether the patient manifests a prolongation of the QT interval that could predispose to TdP and, in case of risk detection, to interrupt a therapy that might become dangerous. This type of ECG patch monitors could prove useful to detect and/or track QT interval changes and could help overcome traditional barriers in addressing the mythical unpredictability of drug-induced TdP [104]. Newer technologies such as these should make diLQTS an even more rare event in the near future.

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# 11 Acquired Long QT Syndrome and Electrophysiology of Torsade de Pointes

Nabil El-Sherif, Gioia Turitto, and Mohamed Boutjdir

## Abbreviations

| | |
|---|---|
| AP-A | Anthopleurin-A |
| APD | Action potential duration |
| CL | Cycle length |
| DAD | Delayed afterdepolarization |
| DR | Dispersion of repolarization |
| EAD | Early afterdepolarization |
| ECG | Electrocardiogram |
| END | Endocardial cell |
| EPI | Epicardial cell |
| GWAS | Genome-wide association studies |
| hERG | Human Ether-à-go-go-Related Gene |
| LQTS | Long QT syndrome |
| M | Mid-myocardial cell |
| Na | Sodium |
| NST | nonself-terminating |
| QTc | Corrected QT interval |
| SCD | Sudden cardiac death |
| ST | Self-terminating |
| TdP | Torsade de pointes |
| TWA | T-wave alternans |
| VF | Ventricular fibrillation |
| VT | Ventricular tachyarrhythmia |

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## Introduction

Since its initial description by Jervell and Lange-Nielsen in 1957 [1], the congenital long QT syndrome (LQTS) has been the most investigated cardiac ion channelopathy. A prolonged QT interval on the surface electrocardiogram (ECG) is a surrogate measure of prolonged ventricular action potential duration (APD). Congenital as well as acquired alterations in certain cardiac ion channels can affect their currents in such a way as to increase the APD and hence the QT interval. The inhomogeneous lengthening of the APD across the ventricular wall results in dispersion of APD, i.e., dispersion of repolarization (DR). This, together with the tendency of prolonged APD to be associated with oscillations at the plateau level,



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termed early afterdepolarizations (EADs), provides the substrate of ventricular tachyarrhythmia (VT) associated with LQTS, usually referred to as torsade de pointes (TdP) VT [2].

Acquired LQTS is by far more prevalent than congenital LQTS. The vast majority of acquired LQTS is the result of the adverse effect of drugs [3] and/or electrolyte abnormalities [4], which, in the majority of cases, interact with the human Ether-à-go-go-Related Gene (hERG) encoding the pore-forming subunits (Kv11.1) of the rapidly activating delayed rectifier current, $I_{Kr}$. However, recent reports suggest that some drugs can also increase the late sodium current, which may contribute to their proarrhythmic effect [5].

This chapter is divided into three sections: Section "Acquired LQTS" will review the electrophysiological mechanisms, electrocardiographic (ECG) characteristics, clinical presentation, and management of acquired LQTS. Section "Comprehensive Electrophysiological Mechanisms of TdP" will provide a comprehensive review of the electrophysiological mechanisms of TdP. Section "Future Directions" is a brief discussion of future direction in the field.

## Acquired LQTS

### ECG Characteristics of TdP

In an analysis of 150 different episodes of sustained VT obtained from 62 patients with the acquired LQTS, the arrhythmia ranged in length from 3 beats (the definition of nonsustained VT) up to 117 beats (Fig. 11.1, panel a), with an average length of 16 ± 8 beats [2]. The cycle length (CL) of these episodes ranged from 193 to 364 ms, with an average of 279 ± 47 ms. The VT was frequently preceded by a variable period of bigeminal rhythm due to one or two premature ventricular beats coupled to the prolonged QT segment of the preceding basic beat (Fig. 11.1, panels a and c). This so called short-long cardiac sequence is seen in both the acquired and congenital LQTS, and the arrhythmogenic mechanism may be related to increased dispersion of repolarization (DR) [6].

Following termination of an episode of fast VT, it is not uncommon to see one or more ectopic beats of variable configuration occurring at much longer CL compared to that of the VT (see beats marked by stars in Fig. 11.2). The change in QRS configuration during VT can take several forms. During a very fast VT, periodic decrease in the amplitude of the entire QRS-T complex is seen with less distinct shifts in QRS axis (Fig. 11.1a). In VTs with slower rates, the classic twisting of the QRS axis from a predominantly positive to a predominantly negative configuration with a variable number of transitional complexes and vice versa is commonly seen as originally described by Dessertenne [7] (Fig. 11.1b). Sometimes, a polymorphic QRS configuration is seen without any of the two previously characteristic patterns (as verified in multiple simultaneous leads, Fig. 11.2c, middle recording). Different patterns can be seen in different VT episodes from the same patient (Fig. 11.1c).

### QT/T Wave Alternans and TdP

It has long been known that tachycardia-dependent T wave alternans (TWA) occurs in patients with the congenital or acquired form of the LQTS and may presage the onset of TdP (Fig. 11.2) [2, 8].

In analysis of 1103 LQTS patients with QTc interval > 0.44 seconds from the International LQTS Registry, TWA was recorded in 30 patients [8]. The frequency of occurrence of TWA was directly proportional to the length of the QTc interval on the enrollment ECG. TWA occurred in one or more occasions during an average 4-year follow-up in 21% of the patients with QTc > 0.60 seconds, but in <0.2% of the patients with QTc < 0.50 seconds. Patients with advanced forms of TWA (those with bidirectional beat-to-beat changes in T wave polarity; $n = 21$) were younger, had longer QTc values, had a higher incidence of complex VT, and were more likely to experience a cardiac event (syncope or cardiac arrest) than those with less advanced forms of TWA (those without bidirectional beat-to-beat changes in T wave polarity; $n = 9$).

In contrast to TWA in congenital LQTS, the incidence of TWA in acquired LQTS is unknown.

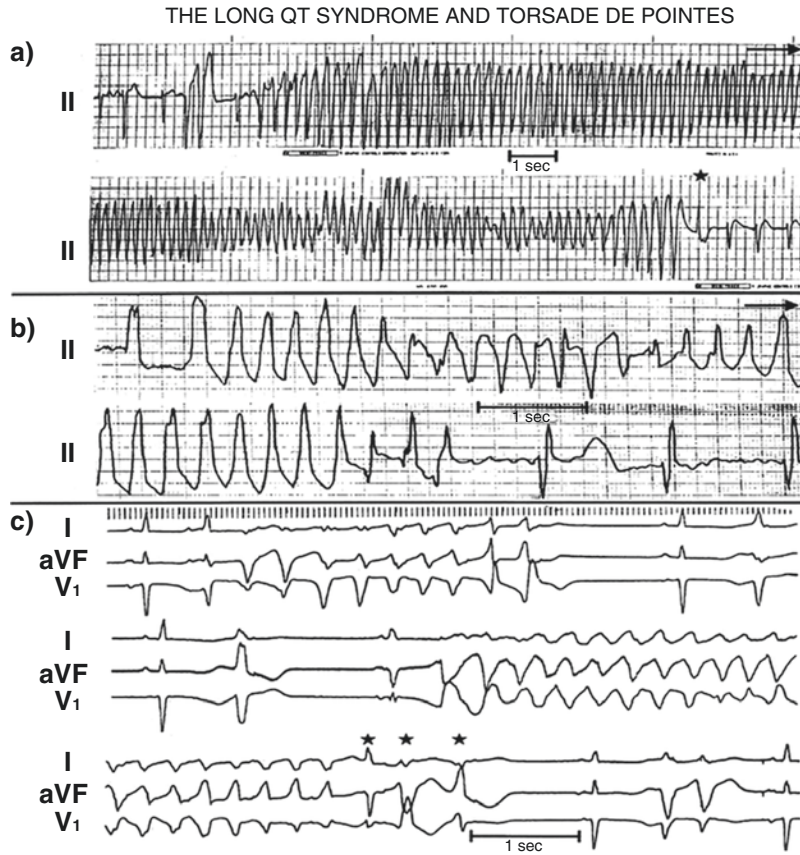


**Fig. 11.1** Three electrocardiographic examples of acquired long QT syndrome (LQTS) and torsade de pointes (TdP). (**a**) was obtained from a 23-year-old woman who was human immunodeficiency virus (HIV) positive and was receiving pentamidine. The patient was admitted with severe diarrhea and hypokalemia. (**b**) was recorded from a 62-year-old man with hypertension and chronic atrial fibrillation who was receiving digoxin and hydrochlorothiazide and had a potassium level of 3.2 mEq/L. The TdP tachycardia developed 12 hours after the patient received a total of four tablets of quinidine gluconate in an attempt to restore normal sinus rhythm. (**c**) was obtained from a 64-year-old man who was receiving procainamide for suppression of very frequent ventricular premature complexes. N.B. pentamidine, quinidine, and procainamide are considered drugs with high risk of drug-induced acquired QT syndrome and their use is currently curtailed. (Reproduced El-Sherif and Turitto [2]. Used with permission of John Wiley and Sons)

It has been reported in acquired LQTS due to hypokalemia and hypomagnesemia (Fig. 11.3) [4] as well as in association with medications that prolong the QT interval (Fig. 11.3) [9]. Patients with acquired LQTS and TWA are likely to develop TdP (Figs. 11.2 and 11.3).

Although overt TWA in the ECG is not common, in recent years, digital signal processing techniques have made it possible to detect subtle degrees of TWA [10]. This suggests that the phenomenon may be more prevalent than previously recognized and may represent an important marker of vulnerability to VT. A recent report confirms this view by showing that microvolt TWA is far more prevalent in LQTS patients than previously reported and is strongly associated with TdP history [11]. Interest in TWA is attributed to the hypothesis that it reflects a greater degree of underlying DR [12].

## Etiology of Acquired LQTS

The vast majority of acquired LQTS is the result of adverse effects of drugs that interact with the hERG gene and the $I_{Kr}$. However, although most

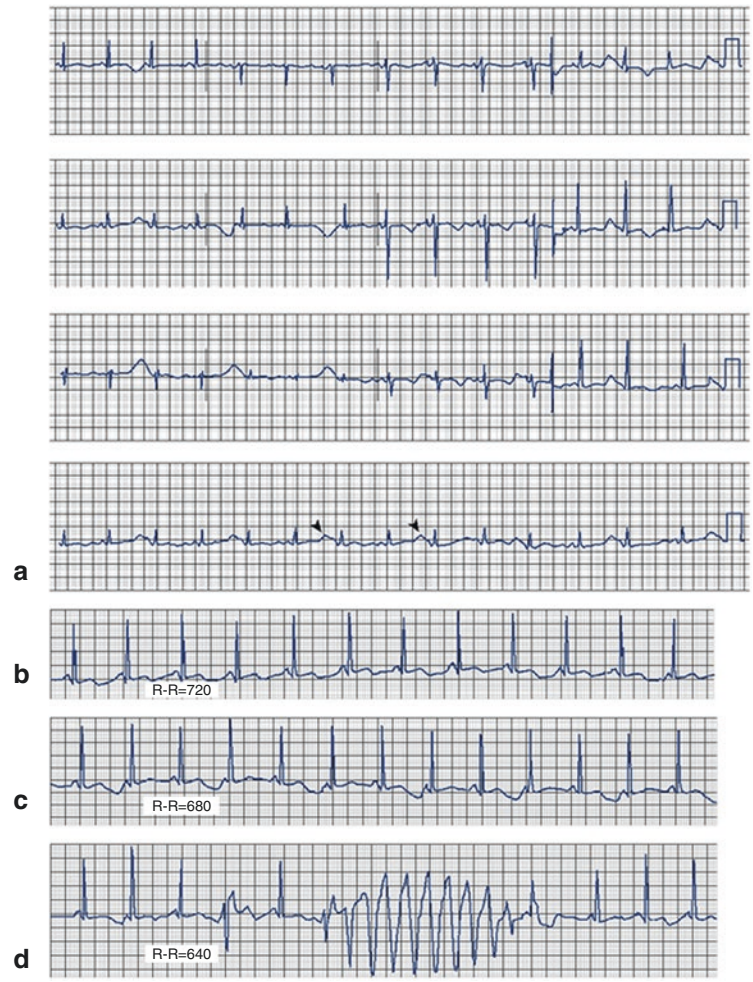


**Fig. 11.2** (**a**) Twelve-lead ECG from a patient with hypokalemia and hypomagnesemia showing marked QTU prolongation and QTU alternans (marked by arrowheads). (**b**–**d**) Representative rhythm strips from the same patient, showing tachycardia-dependent QTU alternans and torsade de pointes. (Reproduced from El-Sherif and Turitto [4], 233–245; an Open Access journal)

drugs that cause TdP do so via hERG channel blockade, TdP is not necessarily a potential consequence of all drugs blocking the hERG pathway. Milberg et al. [13] compared the TdP induction ability of two hERG blocking drugs, DL-sotalol and amiodarone. While both drugs can increase the QT interval, the former causes transmural DR and triangulation of the action potential by prolonging phase 3 and triggers both EADs and TdP. On the other hand, amiodarone does not usually cause DR, EADs, or TdP and by prolonging phase 2 results in a square-shaped action potential. While square-shaped action potentials are considered to be antiarrhythmic, triangulated action potentials are considered to be proarrhythmic [14]. Several authors have suggested that DR, attributed to preferential prolongation of the APD of midmyocardial (M) cells, is a preclinical marker of drug-induced proarrhythmia [15]. However, the existence, location, and clinical contribution of M cells have been a matter of debate [16].

Recent studies have shown that some drugs designated as arrhythmogenic $I_{Kr}$ blocker can generate arrhythmias by augmenting $I_{Na\text{-}L}$ through the PI3K pathway [5]. For example, while acute exposure of flecainide to adult mouse cardiomyocyte which lacks $I_{Kr}$ produced no change in ion currents and action potential duration, extended exposure up to 48 hours of the


**Fig. 11.3** Representative ECG recordings from a 63-year-old female with HIV who developed efavirenz-related QT prolongation and TdP arrhythmia. Panel **a** shows sinus rhythm at 95 bpm and QT alternans. Panel **b** shows sinus bradycardia, marked QT interval prolongation, and the onset of a TdP arrhythmia that required cardioversion for termination. Panel **c** shows that the arrhythmia could be suppressed by overdrive ventricular pacing at a rate of 100 bpm. For a number of days it was noted that QT prolongation and nonsustained TdP episodes developed when the pacing rate was lowered to <100 bpm. The QT returned to normal several days after discontinuation of the long-acting efavirenz. (Reproduced with permission from: Castillo et al. [9]. Used with permission of SAGE Publications)

drug generated up to 15-fold increase in $I_{Na\text{-}L}$ and resulted in arrhythmogenic EADs. However, not all $I_{Kr}$ blockers modulate $I_{Na\text{-}L}$ and this diversity of effects, in return, may contribute to the apparent difference in TdP frequency across culprit drugs [5]. A major implication of these data has to be, that relying on an assay to assess acute block of $I_{Kr}$ may not provide a comprehensive assessment of a candidate drug arrhythmogenic potential.

Other causes of acquired LQTS include electrolyte abnormalities (hypokalemia, hypomagnesemia, and hypocalcemia; see Fig. 11.3), hypothyroidism, hypothermia, and marked bradycardia (sinus bradycardia as in Fig. 11.4 or atrioventricular block [17]). Any of these factors can cause acquired LQTS or contribute to the risk of drug-induced LQTS. In addition, there is recent evidence of the high prevalence of QTc interval prolongation in patients with anti-SSA/Ro antibodies, as well as autoimmune and inflammatory diseases [18–20]. A recent report from this laboratory provided strong evidence for a pathogenic role of anti-SSA/Ro antibodies in the development of QTc prolongation. Anti-Ro antibodies from patients with autoimmune disease were shown to inhibit $I_{Kr}$ by directly cross-reacting with the hERG channel, likely at the pore region where homology between 52Ro antigen and hERG channel was demonstrated [21]. In addition, an animal model of autoimmune-associated QTc prolongation was established, for the first time, whereby induction of anti-SSA/Ro antibodies by immunization resulted in QTc prolongation on the surface ECG [21].

## Pharmacogenetics of Acquired LQTS

The susceptibility to acquired QT interval prolongation can be influenced by genetic variations [22]. This is supported by the fact that the heritability of QT interval duration in the general population (excluding congenital LQTS patients) is estimated to be around 35% [23, 24]. Further, first-degree relatives of patients with congenital LQTS have a higher risk of drug-induced QT prolongation than non-related individuals [25]. In genome-wide association studies (GWAS), a large number of genes associated with QT interval duration

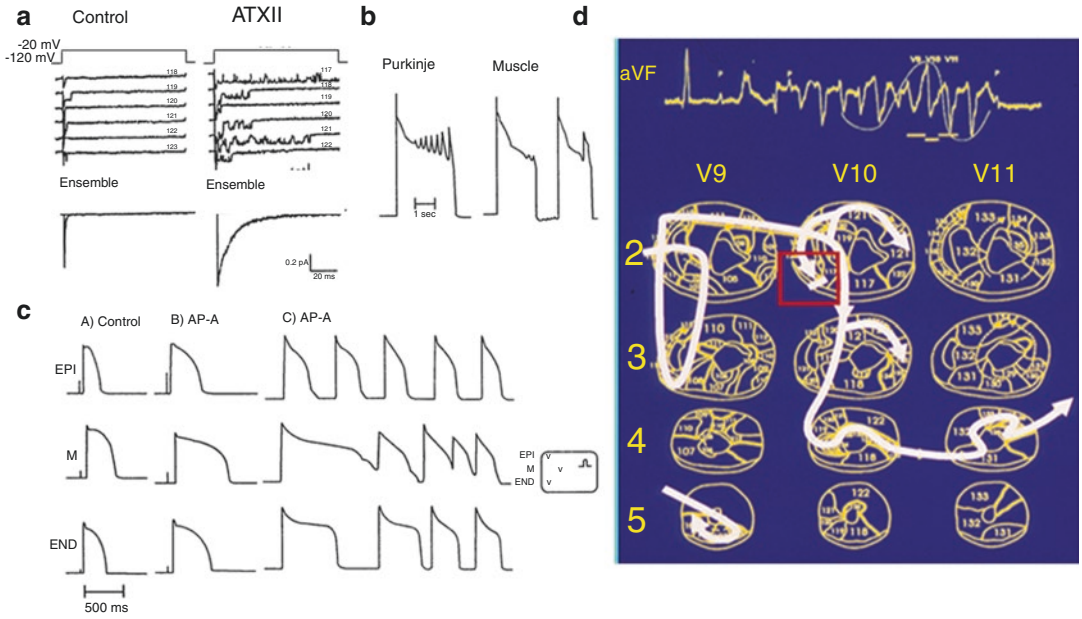


**Fig. 11.4** Composite figure that illustrates the correlation between the specific molecular changes of the Na channel in the canine surrogate model of LQT3, the electrophysiological consequence, and the final phenotype presentation of LQTS and TdP. (**a**) Cell-attached patch clamp recordings of the Na channel during control and following superfusion with sea anemone toxin ATXII. This neurotoxin faithfully reproduces the molecular changes associated with the clinical mutations of the Na channel in patients with LQT3. It illustrates sequential recordings of single Na channel current responses during depolarizing steps from −120 to −20 mV from rabbit cardiomyocytes. The left panel shows control recordings and the right panel shows recordings from a patch exposed to 100 nM of ATXII that resulted in long-lasting bursts consisting of repetitive long opening interrupted by brief closures. The ensemble current from this patch shows markedly slowed relaxation. (**b**) Action potential recordings from a Purkinje fiber (PF) and a mid-myocardial (M) cell, both isolated from a 10-week-old puppy and placed in the same chamber perfused with 50 mg/l anthopleurin-A (AP-A) and stimulated at 3000 ms. The PF shows a series of early afterdepolarizations (EADs) that increased gradually in amplitude before final repolarization. (**c**) Simultaneous recordings from a subepicardial cell (EPI), M cell, and a subendocardial cell (END) from a transmural strip isolated from the left ventricle of a 12-week-old puppy and transfused with 50 mg/l AP-A and stimulated at 4000 ms. Because of the different ionic characteristics of different myocardial fibers, AP-A resulted in marked differential prolongation of the action potential of the M cell resulting in asynchronous activation in the preparation, which is a substrate of reentrant excitation. (**d**) Tridimensional activation maps of a 12-beat run of TdP from the in vivo canine surrogate AP-A model of LQT3 which helps to summarize the final electrophysiological mechanism of TdP in the LQTS. The first beat of a TdP is due to an EAD-triggered beat from the subendocardial Purkinje network that acts on the transmural dispersion of myocardial repolarization to induce reentrant excitation in the form of circulating tridimensional wavefronts (the thick continuous line that traces the activation wavefront from one beat to the next). The twisting QRS pattern of the classic TdP is attributed in this example to transient bifurcation of a predominantly single rotating wavefront into two separate simultaneous wavefronts rotating around the left and right ventricular cavities. (Reproduced from: El-Sherif and Boutjdir [62]. Used with permission of Elsevier)

have been identified [26]. The gene with the strongest signal related to QT interval duration is the nitric oxide synthase 1 adaptor protein gene (NOS1AP), located on chromosome 1 (1q23.3) [26, 27], which inhibits the L-type calcium channel and influences impulse propagation [28]. Other findings from GWAS included polymorphisms within genes known to be mutated in congenital LQTS, genes associated with intracellular calcium handling, as well as genes previously not known to influence cardiac repolarization [29]. A recent study that

compared 188 patients with drug-induced LQTS and more than 1000 patients with congenital LQTS found disease-causing mutations in 28% of patients with drug-induced LQTS [30]. Of interest, under basal conditions, the QTc of drug-induced LQTS patients (453 ± 39 ms) was significantly longer than that of control subjects (406 ± 26 ms) [30].

It is important to consider pharmacogenetics of drug-induced LQTS as related to both pharmacokinetic and pharmacodynamic properties [22, 29]. Pharmacokinetics constitute the effect of the body on the drug, which is usually categorized into effect on absorption, distribution, metabolism, and elimination of the drug. Pharmacokinetic genetic susceptibility is mainly characterized by variation in genes encoding drug-metabolizing cytochrome P450 or drug transporter, like the P-glycoprotein. On the other hand, the pharmacodynamic component of genetic susceptibility is mainly characterized by genes known to be associated with QT prolongation in the general population and genes in which the causal mutations of congenital LQTS are located [30].

## Incidence of Drug-Induced LQTS

The overall incidence of drug-induced LQTS in a given population is difficult to estimate. One study estimated that between 5% and 7% of reports of VT, VF, or SCD were in fact drug-induced LQTS and TdP [31]. European pharmacovigilance centers in Sweden, Germany, and Italy have found an annual reporting rate of drug-induced LQTS or TdP of approximately 0.8 to 1.2 per million person-years [31]. An epidemiological study of drug-induced LQTS in Germany found the reporting rate for symptomatic acquired LQTS to be 2.5% per million person-years for men and 4.0% per million person-years for women, with 60% attributed to drugs [32].

QT prolongation is one of the most common reasons for drug withdrawal from the market, despite the fact that these drugs may be beneficial for certain patients and not harmful in every patient [29]. Since 1989, 14 clinically important drugs have been removed from the market due to TdP [33], and development of an unknown number has been stopped, due to concerns that these might pose a risk of causing QT prolongation and TdP [33]. In the 1990s, the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) began requiring routine preclinical and clinical testing to determine whether drugs have the potential to cause QT prolongation [34]. According to the CredibleMeds website, which has become the standard reference for drug-induced TdP, 38 marketed drugs are recognized for their potential to cause TdP and another 72 to cause QT prolongation [35].

In the past decade, hERG channel-mediated cardiac toxicity, manifested as QT interval prolongation, has become a major safety issue in drug development, superseding liver injury as the main cause of drug withdrawals. *In vitro* electrophysiological testing of the drug's effects on the function of the hERG channel may be cheaper, faster, and potentially more sensitive than other current surrogates for TdP risk, such as *in vivo* QT prolongation and action potential prolongation in cardiomyocytes [36].

## Ethnicity and Gender Differences in Drug-Induced LQTS

As ethnic differences ultimately reflect genetic variation, it is useful to study ethnicity with regard to susceptibility to drug-induced QT interval prolongation and TdP. However, the role of ethnic differences has not been well established in published studies on drug-induced QT interval prolongation. In 20 QT/QTc studies, only 10% of the total study population was African American and only 7% was Asian [37]. Nevertheless, the frequency of polymorphisms in genes known from congenital LQTS showed varying distribution among ethnic groups [38]. African Americans had the highest risk of prolonged QT interval after

acute overdose of QT-prolonging drugs, while Hispanics had the lowest risk compared to all other ethnic groups [39].

Females with inherited LQTS demonstrate pronounced sex difference in cardiac repolarization and arrhythmic risk. Adult women with LQT1 and LQT2 have longer QT intervals, a more pronounced transmural QT dispersion, and a higher risk of TdP and SCD than men [40, 41]. Interestingly, in female patients with LQT2, the arrhythmogenic risk remains elevated after menopause, suggesting that other gender-related factors besides sex hormones may contribute to gender difference in arrhythmogenesis [42].

Gender is a risk factor for adverse drug reactions [43, 44]. The concept of reduced repolarization reserve in females compared to males has been used to explain sex differences in arrhythmia risk in acquired LQTS [45]. The reduced repolarization reserve of the female heart is attributed to lower repolarizing K+ currents. This difference was thought to be primarily due to testosterone-mediated increase in $I_{Kr}$ and $I_{K1}$ resulting in shorter APD and QTc interval in male hearts. However, in a recent review, the effects of sex hormones go well beyond their modulation of $K^+$ currents [46]. The underlying mechanisms could be summarized as follows [47]: (1) an estradiol-induced decrease in $I_{Kr}$ as well as increase of $I_{CaL}$, sodium calcium exchange (NCX) expression and activity, RyR2 leakiness, $Ca^{2+}$ transient amplitude, and α1- and β2-adrenoreceptor responsiveness; (2) a testosterone-induced increase in $I_{Kr}$, $I_{Ks}$, and $I_{Kl}$, increased SERCA activity, and shortened $Ca^{2+}$ transient; and (3) a progesterone-induced increase in $I_{Ks}$, increased SERCA expression and activity, and increased $I_{CaL}$ current sensitivities with reduced $Ca^{2+}$ oscillations upon sympathetic stimulation. In a recent study, we have shown that modulation of voltage-$Ca^{2+}$ uncoupling [48] may provide one more attractive electrophysiological mechanism for the increased vulnerability of females to drug-induced LQTS.

## Acute and Long-Term Management of Acquired LQTS

The American College of Cardiology (ACC), American Heart Association (AHA), and European Society of Cardiology (ESC) published guidelines for management of ventricular arrhythmias, including drug-induced TdP [49], in 2006 and the key recommendations have been endorsed in a more recent ACC/AHA statement [50].

When monitoring for drug-induced prolonged QT interval, a baseline QTc should be obtained. If any one of the following conditions is observed during QT interval monitoring, the patient should be admitted to the hospital for telemetry: (1) QTc > 500 ms, (2) QTc increase > 60 ms above baseline, (3) QT prolongation accompanied by syncope, and (4) any evidence of ECG instability, specially TWA, AV block, QRS widening, or ventricular ectopy. The offending drug should be discontinued, electrolyte abnormalities corrected, and a defibrillator placed at bedside [50].

Nonself-terminating TdP with hemodynamic collapse should obviously be cardioverted with adequate post-cardioversion management. More typical TdP occurs as recurrent self-terminating episodes. In these cases, the first line of management is intravenous administration of magnesium sulfate as a single 2g (8 mmol) dose over 1–2 min followed by a second dose if necessary. Magnesium sulfate is effective in suppressing TdP without reducing the QT interval [67]. The mechanism of action may be related to suppression of late calcium influx via L-type calcium current and reduction in the amplitude of EADs [51]. If magnesium sulfate fails to suppress TdP, the next step is to increase the heart rate, typically by transvenous pacing. In the interim if necessary, isoproterenol administration could promptly increase the heart rate while waiting for insertion of pacing electrode. Increasing the heart rate is associated with shortening of the QT interval and suppression of TdP. At the same time, the culprit drug should be discontinued and acid-base and electrolytes should be corrected as necessary.

There are novel experimental drugs that can enhance the delayed rectifier conductance [52] or activate the cardiac ATP-sensitive potassium channel [53], which may have future value for the treatment of acquired LQTS.

Long-term management of acquired LQTS is important. All patients with previous drug-induced prolongation of the QT interval should be instructed about the importance of subsequent avoidance of QT-prolonging drugs and should have a list of QT-prolonging medications provided by their physicians. In patients with acquired LQTS, the risk of further episodes of TdP is reduced once the culprit drug is removed and other aggravating situations such as electrolyte abnormalities or marked bradycardia are corrected. This should result in normalization of the QTc interval. If it does not, the patient and symptomatic first-degree family members should be considered for genetic testing for the presence of LQTS-associated mutations [50, 54]. This is especially important for relatives of patients with drug-induced case fatality.

## Comprehensive Electrophysiological Mechanisms of TdP

### Experimental Models of LQTS

Both drug-induced and genetically modified animal models of various species have been generated and utilized to investigate the electrophysiological mechanisms of arrhythmogenesis in LQTS and potential pro- and antiarrhythmic agents. However, due to species differences in features of cardiac electrical function, particularly in repolarization currents, these models do not completely recapitulate all aspects of the electrophysiology of the human disease. Genetically modified animal models, such as mice and rabbits, are commonly used to investigate the arrhythmogenicity of LQTS. Current transgenic LQTS rabbit models have already been instrumental in increasing our understanding of the role of spatial and temporal dispersion of repolarization to provide an arrhythmogenic substrate, genotype differences in the mechanisms for EAD formation and arrhythmia maintenance, and mechanisms of hormonal modification of arrhythmogenesis [55].

On the other hand, two dog models of LQTS and TdP have been extensively investigated. One model is the dog with induced complete atrioventricular conduction block (AVB). Complete AVB results within few weeks in hypertrophy and remodeling of the left ventricle associated with prolongation of the QT interval, APD, as well as spatial DR [56]. When the animal is challenged with a drug that blocks the $I_{Kr}$, like dofetilide, it results in further prolongation of the QT interval and creation of a drug-induced model of acquired LQTS and TdP [57].

The other dog model of LQTS and TdP is the anthopleurin-A (AP-A) canine surrogate model of LQT3 that was developed in this laboratory [58]. The model is created by the neurotoxin anthopleurin-A or ATX II [59] that faithfully reproduces the molecular changes associated with clinical mutations of the Na channel in patients with LQT3 [60]. Of interest, the experimental surrogate model of LQT3 anticipated the first description of the clinical LQT3 by 7 years [61]. Figure 11.4 is a representative composite of the salient experimental techniques that were utilized to investigate the model and illustrate the correlation between the modulation of a cardiac ion current, its electrophysiological consequence, and the final phenotype presentation as LQTS and TdP.

### Electrophysiological Mechanisms of the Trigger of TdP in the LQTS

There is an almost complete agreement that initiating one or two beats of TdP is due to EAD-triggered focal activity from the subendocardial Purkinje network [57, 63–65]. A study by Caref and associates has confirmed beyond reasonable doubt the subendocardial origin of the trigger of TdP [66]. In this study the canine surrogate

model of LQT3 was placed on cardiopulmonary bypass and chemical ablation of the endocardial Purkinje network was obtained using Lugol's iodine following which spontaneous TdP was no longer observed. However, a properly timed premature stimulus induced reentrant ventricular arrhythmias (VA) based on the underlying marked DR. On the other hand, the perpetuation of TdP remains controversial and could be attributed to focal activity, reentrant excitation, or a combination of both mechanisms.

The ionic mechanism(s) that underlie the generation of EADs have been widely investigated. The central hypothesis on the generation of EADs suggests that a spontaneous release of calcium from the sarcoplasmic reticulum would temporarily increase cytosolic calcium concentration with a subsequent sudden activation of the NCX. This inward current could "re-depolarize" the sarcolemmal membrane to a potential from which sodium or calcium currents become reactivated, triggering an afterdepolarization [67]. Although this model has been primarily discussed to explain delayed afterdepolarizations, there is also evidence that this mechanism may be the trigger for EADs [68]. This hypothesis is also supported by studies that showed that inhibition of the NCX suppresses TdP in the intact heart model of LQTS2 and LQTS3 [69].

## Electrophysiological Mechanism(s) of Perpetuation of TdP

Contrary to the established mechanism of the trigger of TdP, the perpetuation of TdP remains controversial and could be attributed to focal activity, reentrant excitation, or a combination of both mechanisms [70, 71]. Both reentrant and focal activity are assumed to be nonstationary. For reentrant excitation, this could be a heterogeneity-induced drift of a reentrant circuit [72] or a meandering reentrant spiral wave (see Fig. 11.1, panel d) [73]. Alternatively, ectopic beats originating from different locations may explain the perpetuation of TdP. The original description of TdP by Dessertenne attributed the pattern to two variable opposing foci (deux foyers) [7]. In computational modelling as well as experimental observation of the canine chronic AVB model, both multiple competing foci and reentrant excitation could develop depending on heterogeneity of repolarization in comparison to the surrounding tissue [70]. Large heterogeneities can produce ectopic TdP, while smaller heterogeneities will produce reentrant type TdP. An experimental study in the same model reported that short-lasting episodes of TdP had a focal mechanism while long-lasting episodes were maintained by reentrant excitation [71]. However, the results were criticized because of the controversial definition of focal versus reentrant excitation [74].

However, it remains an open question why and how ectopic beats emerge and compete; and what their relationship to the observed EAD activity. EADs arising from the subendocardial Purkinje network conducted to overlying myocardium through Purkinje-muscle junctions (PMJ). Electrotonic interactions across PMJs can modulate APD locally. A recent study has proposed that, dependent on resistive properties across PMJs, large spatial gradient of APD can develop at the endocardium and the transmural plane [75]. This may provide a better explanation compared to the concept of M cells with different ionic characteristics [76].

One of the problems of sustained fast EAD-induced focal activity, is that the short cycle length will be associated with short APD that would suppress further EAD generation unless there is some form of protected islands of prolonged APD with EADs capable of conduction across PMJs to activate the ventricular myocardium. In a study, combined computational simulation and experimental observations in isolated myocytes, showed that in electrically homogeneous tissue models, chaotic EADs synchronize globally when the tissue is smaller than a critical size. However, when the tissue exceeds the critical size, electronic coupling can no longer globally synchronize EADs, resulting in regions of partial synchronization that shift in time and space. These regional, partially synchronized EADs then form

premature ventricular complexes that propagate into recovered tissue without EADs, thus creating "shifting" foci that resemble polymorphic VT [77].

## Delayed Afterdepolarization (DAD)-Triggered Activity Contributes to VA in the LQTS

The electrophysiologic mechanism of VT in LQTS is somewhat more complex than that described earlier. Figure 11.5, panel a, was obtained from one of the classic reviews of cellular mechanisms of cardiac arrhythmias by Hoffman and Rosen [78]. It shows transmembrane AP recording from a canine Purkinje fiber superfused with 20 mM cesium chloride (a surrogate experimental model for LQT2). The recording illustrates the classic bradycardia-dependent prolongation of APD associated with membrane oscillation on late phase 2/early phase 3 of the repolarization phase characteristic of EADs. But it also shows that complete repolarization of the AP is followed by a subthreshold delayed afterdepolarization (DAD). The latter is simply explained on the basis of increased intracellular $Ca^{2+}$ associated with the prolonged AP duration triggering a transient inward current. This, almost forgotten, observation strongly suggests that some VT and ectopic beats in LQTS could be secondary to DADs.

Figure 11.5, panel b, shows a corroboration of this observation from the canine surrogate model of LQT3 [79]. The top ECG tracing was obtained 10 minutes after infusion of AP-A and shows moderate prolongation of the QT interval and a run of nonsustained monomorphic VT at a rate of 150 beats/min. The VT starts with a late coupled beat that is well beyond the end of the QT interval of the preceding sinus beat. Tridimensional mapping of activation showed that the VT arose as a focal discharge (F) from the same subendocardial site. For all practical purposes, the focal discharge could be attributed to DAD-triggered activity.

The bottom ECG tracing was obtained from the same experiment 10 minutes later and shows further prolongation of the QT interval. The ectopic beats labeled F now seem to be coupled to the end of the prolonged QT interval of the preceding sinus beats. The middle of the tracing illustrates a six-beat run of polymorphic VT. Tridimensional mapping shows that the first beat arose from a

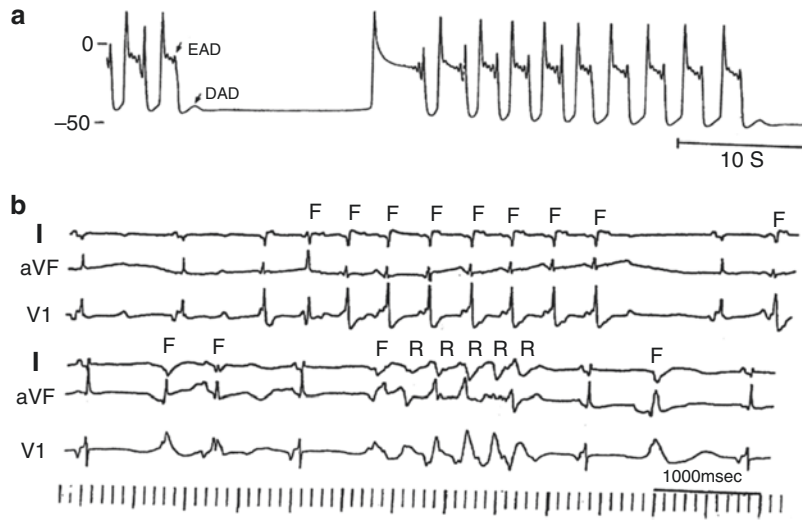


**Fig. 11.5** Transmembrane action potential recording from a Purkinje fiber superfused with 20 mM cesium chloride showing both early and delayed afterdepolarizations. (**b**) ECG recording from an *in vivo* canine anthopleurin-A surrogate model of LQT3. See text for details. F focal discharge, R reentrant excitation. (Reproduced from: El-Sherif [79]. Used with permission of John Wiley and Sons)

subendocardial focal site and could be safely attributed to EAD-triggered activity, whereas subsequent beats were due to reentrant excitation in the form of continuously varying scroll waves.

## Electrophysiological Mechanisms of Self-Terminating (ST) Versus Nonself-Terminating (NST) TdP VT

The majority of TdP episodes terminate spontaneously (self-terminating, ST) (see Fig. 11.1). However, a minority can degenerate in VF (nonself-terminating, NST). The electrophysiological mechanisms of the NST episodes of TdP have never been elucidated. Obviously, this is a more important issue than the "twisting and turning" to see if perpetuation of TdP VT is due to focal or reentrant activation [74]. Figures 11.6 and 11.7, obtained from the canine surrogate model of LQT3, provide one possible electrophysiological mechanism [79]. The lesson gained from this example is that subtle changes in underlying spatial DR and conduction characteristics can result in fractionation of activation wavefronts and VF. It also demonstrates clearly the difficulty in predicting which TdP episodes will be ST or NST.

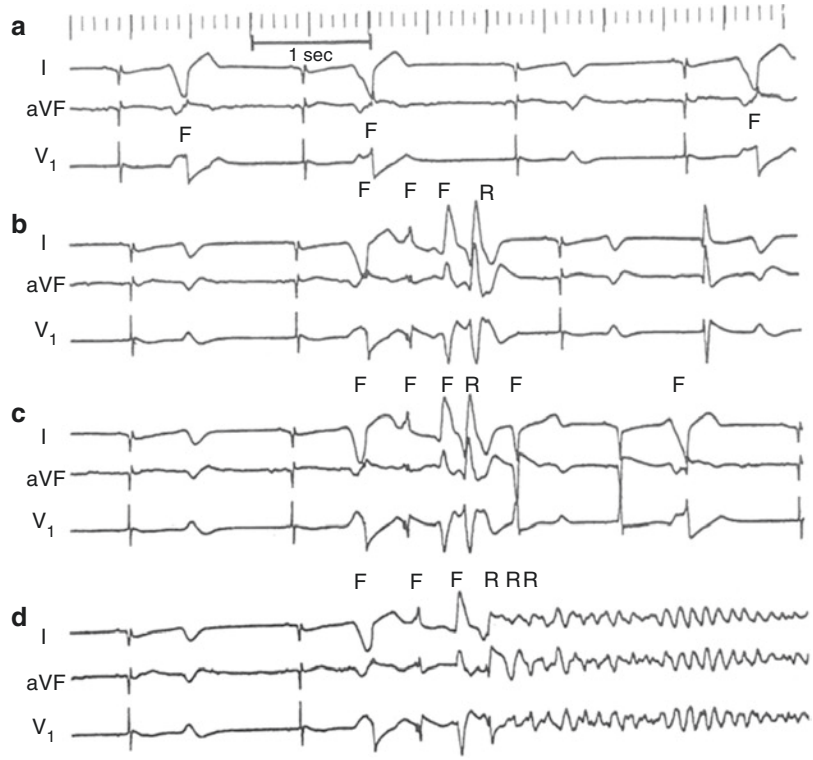


**Fig. 11.6** ECG recordings from an *in vivo* canine AP-A surrogate model of LQT3. The recordings are arranged chronologically, a few minutes apart. Panel **a** shows a stable bigeminal and trigeminal rhythm due to subendocardial discharge attributed to EAD-triggered activity from the same focus. This was followed several minutes later by runs of four- or five-beat polymorphic VT with remarkable repetition of the same QRS morphology. The first beat of each run arose from the same site of the bigeminal/trigeminal beats in panel **a**. The second and third beats of each run arose from two different subendocardial focal sites; the fourth beat was reentrant in origin. The fifth beat in a five-beat run again was focal in origin and arose well after the end of the reentrant excitation and could be attributed to DAD-triggered activity. After approximately 10 minutes of repetitive nonsustained VT, the same three initial focal beats were followed by reentrant excitation that degenerated into ventricular fibrillation (VF) (panel **d**). F = focal discharge; R = reentrant excitation. (Reproduced from: El-Sherif [79]. Used with permission of John Wiley and Sons)

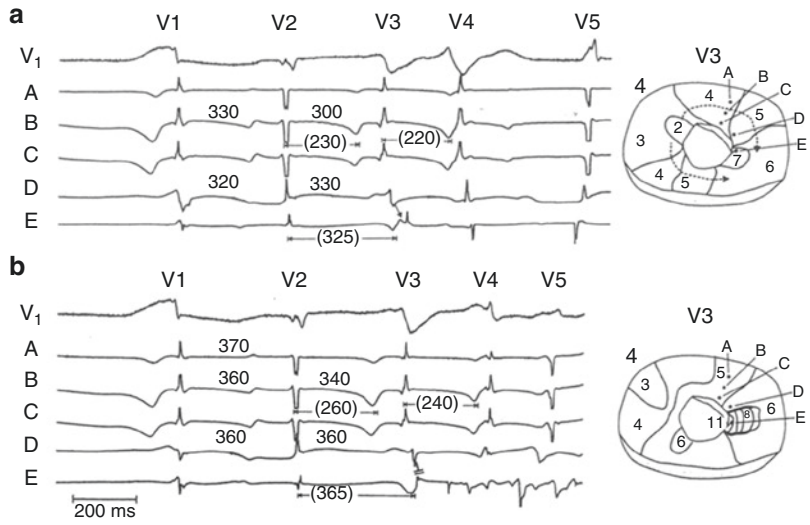


**Fig. 11.7** Selected electrograms of the five-beat nonsustained VT shown in Fig. 11.6c (V1 to V5) and the first five beats of the VT that degenerated into ventricular fibrillation shown in Fig. 11.6d (V1 to V5). Also shown is a limited activation map of both V3 beats. The figure shows the electrophysiological mechanism of the different consequences of the same V3 ectopic beat in the two episodes. It shows that the inter-ectopic intervals Vl-V2 and V2-V3 increased by approximately 30–40 msec in the second episode compared with the first episode (the equivalent of a slight slowing of the discharge of the focal activity). This resulted in lengthening of local repolarization following the V2 beat by 30–40 msec. However, the degree of lengthening of repolarization was disparate at contiguous sites, resulting in functional conduction block and the initiation of a more complex reentrant wavefront. One such site where new conduction block developed during the NST TdP is shown in the electrograms between sites D and E, as well as in the isochronal maps on the right side of the figure. The numbers without brackets represent cycle lengths in milliseconds, and the numbers in brackets represent activation-recovery intervals. (Reproduced from: El-Sherif [79]. Used with permission of John Wiley and Sons)

## Future Directions

Although congenital LQTS continues to remain the domain of cardiologists, cardiac electrophysiologists, and specialized centers, the by far more frequent acquired drug-induced LQTS is the domain of all physicians and other members of the healthcare team who are required to make therapeutic decisions. To support better prescribing of medicines, clinical decision support systems to date have issued alerts that warn of potential harm from a prescribing decision [80]. However, the impact of these systems has been limited. Moving away from the use of alerts to signal prescribing errors, the concept of "medical autopilots" has been suggested as a preferred approach [81]. These programs will monitor the electronic medical record and send signals to guide prescribers toward decisions that result in maximum benefit and minimal risk of TdP.

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# 12 Pathogenesis of Autoimmune-Associated Long QT Syndrome

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## Long QT Syndrome

Long QT syndrome (LQTS) manifests as prolongation of the QT interval on the surface electrocardiogram (ECG), predisposing to life-threatening ventricular arrhythmias particularly torsades de pointes (TdP) [1–4]. The QT interval in the ECG is a surrogate measure of the average duration of the ventricular action potential. Whenever a channel dysfunction induces an increase in the inward $Na^+$ or $Ca^{++}$ currents and/or a decrease in an outward $K^+$ current resulting in an inward shift in the balance of net current, the action potential duration prolongs and hence the QT interval. LQTS can be congenital and caused by mutations in ion channel or associated protein-coding genes, or acquired often drug-induced [1, 2]. In most cases of drug-induced QT prolongation, the target ion channel, is the hERG (human Ether-à-go-go-Related Gene) encoding the pore-forming subunits (Kv11.1) of the rapidly activating delayed $K^+$ channel conducting $I_{Kr}$ [5]. $I_{Kr}$ plays a major role during repolarization of the cardiac action potential and its reduction by drugs or genetic defects causes delayed repolarization and prolongation of the QT interval [1, 2, 6]. Regardless of the specific ion channel dysfunction involved, the prolongation of action potential duration leads to an increased susceptibility to develop oscillations at the plateau level (early afterdepolarizations). When they reach the threshold for activation of the inward $Ca^{++}$ current in the presence of a vulnerable window created by differences in action potential duration lengthening across the ventricular wall (transmural dispersion of depolarization), a triggered ectopic activity is generated that can induce reentrant arrhythmias, particularly TdP. Although frequently terminating spontaneously, TdP can degenerate into ventricular fibrillation and sudden cardiac death [1, 2].

## Anti-Ro/SS Autoantibody System and Autoimmunity

Ro/SSA antigens are intracellular proteins that associate with small cytoplasmic RNAs and form Ro-ribonucleoprotein (Ro-RNP) complex [7, 8]. Ro/SSA antigens consist of two different proteins, Ro52kD/SSA and Ro60kD/SSA. While the Ro60 kD/SSA autoantigen is a ring-shaped RNA-binding

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**Table 12.1** Prevalence of anti-Ro SSA antibodies in different autoimmune diseases and general population [26–28]

| Status | Anti-Ro/SSA antibodies (%) |
|---|---|
| Sjögren's syndrome (SS) | 70 |
| Systemic lupus erythematosus (SLE) | 24–60 |
| Scleroderma | 35.6 |
| Rheumatoid arthritis (RA) | 3–5 |
| Systemic sclerosis (SSc) | 3–11 |
| Myositis | 5–15 |
| General asymptomatic population | 2–3 |

protein [9], Ro52kD/SSA functions as an E3 ubiquitin ligase [10] and is involved in the ubiquitination of interferon regulatory factor 3 and IRF7 post toll-like receptor stimulation, suggesting an avenue for the immune system to protect the host from prolonged immune system activation [11, 12]. It is not clear why the self-body generates autoantibodies against these endogenous antigens in the first place but nevertheless, the corresponding autoantibodies are important clinical markers for several autoimmune diseases (Table 12.1). Specifically, anti-Ro52kD/SSA antibody is emerging as a unique antibody with direct pathogenic disease involvement and distinct clinical properties [13]. As such, anti-Ro52kD/SSA antibodies are being associated with clinical and laboratory markers of disease [13].

## Long-QT Syndrome and Autoimmunity

In the recent years, accumulating evidence indicates that autoimmune mechanisms are involved in the pathogenesis of cardiac arrhythmias [14, 15]. Indeed, a number of autoantibodies are able to interfere with the electrical properties of the heart by directly targeting specific molecules expressed on the cardiomyocyte surface, including β-adrenergic and muscarinic receptors, Na/K-ATPase, and ion channels [14–19]. The term "autoimmune cardiac channelopathies" was recently proposed to define a novel pathogenic mechanism for cardiac arrhythmias in patients with autoimmune disease as well as in apparently healthy subjects with idiopathic rhythm disturbances to account for a number of unexplained, molecular autopsy-negative sudden cardiac deaths (SCDs) [15]. In this regard, an acquired autoimmune-associated LQTS has been reported in patients with autoimmune diseases carrying pathogenic autoantibodies, namely, anti-Ro/SSA antibodies [20–25]. These anti-Ro/SSA antibodies are prevalent in several autoimmune diseases such as Sjögren's syndrome, systemic lupus erythematosus, scleroderma, rheumatoid arthritis, systemic sclerosis, and myositis and also in the general otherwise healthy population (Table 12.1) [26–28]. As such, autoimmunity is emerging as a novel mechanism of cardiac arrhythmias, not only in patients with autoimmune diseases but also in healthy subjects [14, 15, 29]. Different types of arrhythmogenic autoantibodies have been identified and affect cardiac ion channels by directly targeting the corresponding proteins at the myocyte surface [14, 29]. In particular, some of these autoantibodies can significantly interfere with the function of specific ion channels critically involved in determining ventricular action potential duration (*autoimmune cardiac channelopathies*), thus leading to a new acquired forms of LQTS of autoimmune origin [15]. Autoimmune cardiac channelopathies are mediated by circulating autoantibodies directly interacting with different cardiac ion channels: based on the specific channel type involved, three main groups of autoimmune cardiac channelopathies are currently recognized, i.e., $Ca^{++}$-, $K^+$-, and $Na^{++}$-channelopathies [15]. To date, the most investigated autoimmune cardiac channelopathy associated with LQTS is related to the presence of anti-Ro/SSA antibodies cross-reacting with the hERG-$K^+$ channel [30, 31], hence to focus in this book chapter. Other autoantibodies recognizing $Ca^{++}$ channels, other $K^+$ channels, and receptors have been recently reviewed elsewhere [15].

## Autoimmune Potassium Channelopathies Associated with LQTS

Several pro-arrhythmic anti-$K^+$ channel autoantibodies have been identified in patients with manifest autoimmune diseases or in otherwise healthy subjects [15]. By targeting specific $K_v$ α-subunits

such as$K_v$11.1/hERG [23, 31, 32] and $K_v$1.4 [33–35], these autoantibodies exert different electrophysiological effects resulting in LQTS.

### Autoantibodies Against Kv1.4 Channel

Although less investigated, LQTS-inducing autoimmune channelopathy is related to anti-$K_v$1.4-$K^+$ channel antibodies, detected in ~10–20% of myasthenia gravis patients [33–35]. Anti-$K_v$1.4-positive subjects frequently showed QTc prolongation and significant mortality for lethal QT-associated arrhythmias [33–35]. Even though pathogenesis studies are currently not available, LQTS may likely result from an autoantibody inhibition of the $K_v$1.4-related current, the slow transient outward current, $I_{tos}$, leading to prolongation of the ventricular repolarization.

### Anti-Ro/SSA Autoantibodies Against Kv11.1-hERG Channel

Unlike anti-$K_v$1.4 autoantibodies, the functional and molecular mechanisms of autoantibodies (anti-Ro/SSA antibodies) targeting the hERG-$K^+$ channel have been well characterized [23, 31, 32, 36] and proposed as a novel form of acquired LQTS [15]. The arrhythmogenicity of anti-Ro/SSA antibodies, including the anti-Ro52kD/SSA and anti-Ro60kD/SS subtypes, is well established from many years, specifically in the pathogenesis of autoimmune-associated congenital heart block [37–40]. More recently, patients with autoimmune connective tissue diseases tested positive for anti-Ro/SSA antibodies, commonly show QTc prolongation correlating with autoantibody levels (particularly anti-Ro52kD/SSA) and with complex ventricular arrhythmia [21, 22, 25]. In addition to patients with connective tissue diseases, anti-Ro/SSA antibodies have been also associated with QTc prolongation and TdP occurrence in the general population, in most cases independent of the presence of a concomitant autoimmune diseases [23]. Below, we present and discuss the functional and molecular basis of anti-Ro/SSA-associated LQTS.

## Functional and Molecular Basis of Autoimmune-Associated LQTS

An increasing body of experimental data points to hERG-$K^+$ channel as the molecular target responsible for anti-Ro/SSA-associated LQTS [23, 31, 32, 36].

### In Vivo Experiments Addressing Reproducibility of the Clinical LQTS Phenotype

To demonstrate the pathogenic role of anti-Ro/SSA antibodies in the development of QTc prolongation in the absence of other confounding risk factors usually present in patients with autoimmune diseases, healthy guinea pigs were actively immunized with Ro/SSA antigen [31]. ECGs were measured and anti-Ro/SSA antibody titers were quantified by enzyme-linked immunosorbent assay (ELISA) at regular intervals to insure proper immune response (Table 12.2). Indeed, QT interval and corrected QT (QTc) were significantly prolonged in immunized guinea pigs compared to nonimmunized guinea pigs (Fig. 12.1a, b) without any measurable changes in heart rate, PR interval, and QRS duration (Table 12.2). The observed QTc prolongation was explained by anti-Ro/SSA antibodies' lengthening of guinea pig ventricular action potential (Fig. 12.2c) resulting from the inhibition of the native $I_{Kr}$ (Fig. 12.2d) as well as $I_{Kr}$

**Table 12.2** Anti-Ro/SSA antibodies Levels and ECG parameters before and after immunization

| | Anti-Ro/SSA antibodies range (OD) | QTc (ms) | Heart rate | PR interval (ms) | QRS duration (ms) | Anti-Ro Antibody Range (OD) |
|---|---|---|---|---|---|---|
| Baseline | 0.02–0.04 | 248.3 ± 30 | 294.4 ± 12 | 50.5 ± 2.9 | 19.1 ± 1.0 | 0.02–0.04 |
| Immunized | 1.14–2.16* | 272.5 ± 23* | 280.2 ± 42 | 56.5 ± 1.4 | 19.2 ± 2.0 | 1.4–2.16* |

OD indicates optical density and $^*P < 0.05$

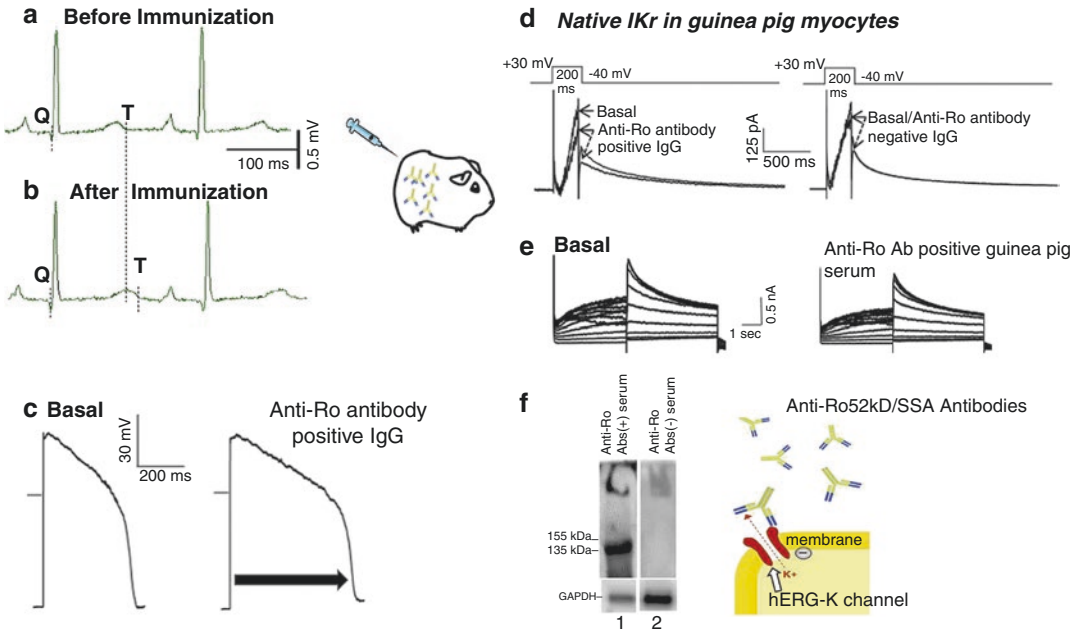


**Fig. 12.1** Immunization of guinea pigs with Ro52kD/SSA antigen causes delay in repolarization, prolongation of action potential, and inhibition of hERG-$K^+$ channel. Representative ECGs from a guinea pig (**a**) before immunization and (**b**) after immunization with Ro52kD/SSA antigen. Vertical dashed lines indicate QT interval. (**c**) Effects of anti-Ro/SSA antibody-positive IgG from serum of a patient with connective tissue disease and QTc prolongation on action potential duration and (**d**) on $I_{Kr}$ current recorded from guinea pig ventricular myocytes. (**e**) Representative $I_{Kr}$ tracings from HEK293 cells stably expressing the hERG channel, at basal (left) and after the application of anti-Ro/SSA antibody-positive serum from an immunized guinea pig (right). (**f**) Western blot of guinea pig ventricles probed with anti-Ro/SSA antibody-positive (lane 1) and anti-Ro/SSA antibody-negative (lane 2) guinea pig sera. A schematic representation of direct anti-Ro/SSA antibody block of hERG-$K^+$ channel is shown on the right of panel F. (Based on data from Yue et al. [31])

recorded from HEK293 cells stably expressing the hERG channel (Fig. 12.2e). This inhibition occurred likely by direct binding of anti-Ro/SSA antibodies from immunized guinea pig sera with guinea pig ERG channel proteins (Fig. 12.2f, left panel) as proposed in the schematic of Fig. 12.2f, right panel. Collectively, these findings are consistent and support the clinical observation that the sole presence of anti-Ro/SSA antibodies is associated with QTc prolongation and constitute a nonconventional risk factor for the development of ventricular arrhythmias such as TdP [23, 32].

## In Vitro Experiments Demonstrating the Pathogenesis of anti-Ro/SSA Antibodies

To directly address whether the actual anti-Ro/SSA antibodies from patients with autoimmune diseases are pathogenic, sera, purified IgG, and affinity purified, anti-Ro/SSA antibodies from patients with connective tissue disease with a documented long QTc, were tested on $I_{Kr}$ recorded from HEK293 cells stably expressing hERG channel. Indeed, not only sera and purified IgG but also affinity purified IgG containing anti-Ro/SSA antibodies from these patients inhibited $I_{Kr}$ in a time- and dose-dependent manner without affecting the $I_{Kr}$ kinetics suggesting a direct channel block (Fig. 12.2a–h). This premise is further supported by the Western blot data, demonstrating the direct interaction of these anti-Ro/SSA antibodies from connective tissue disease patients with the hERG channel proteins by Western blots (Fig. 12.2i). Similar findings were first reported by Nakamura et al. [32], demonstrating that anti-Ro/SSA antibodies from serum and purified IgG of a female patient

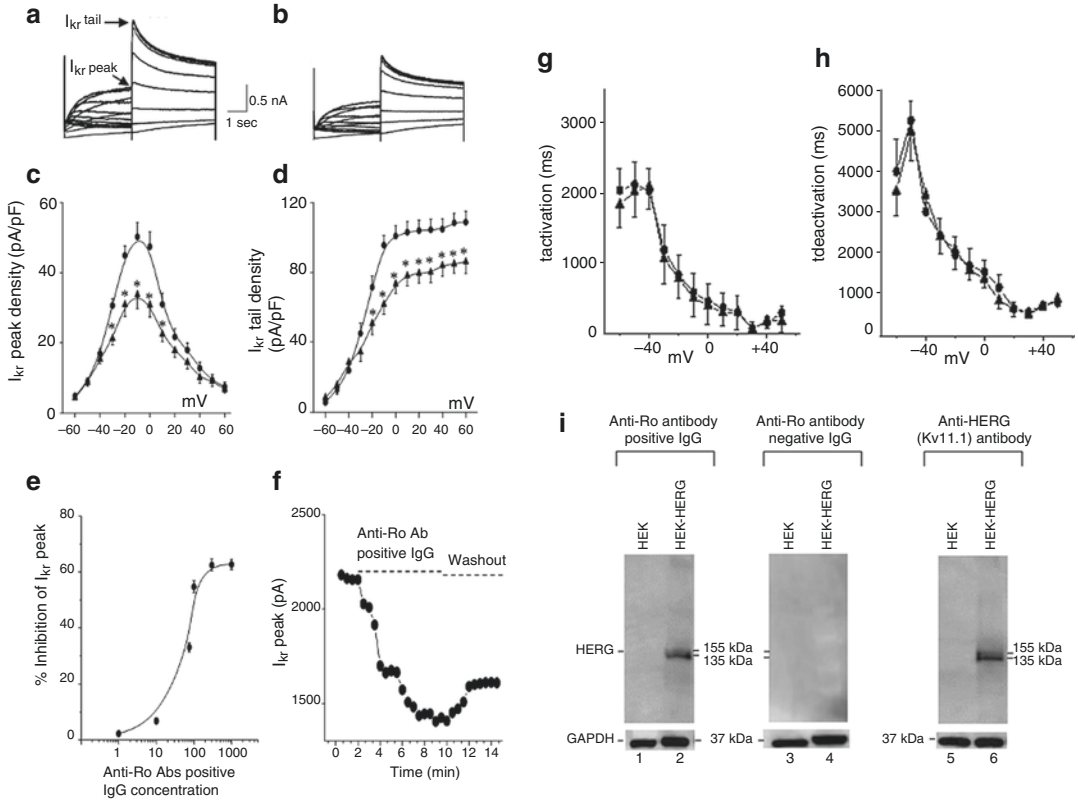


**Fig. 12.2** Electrophysiologic and biochemical effects of anti-Ro/SSA antibodies on hERG-$K^+$ channel. Representative currents ($I_{Kr}$) were recorded from HEK293 cells stably expressing hERG channels under basal (**a**) conditions and (**b**) in the presence of 75 μg/ml anti-Ro/SSA antibody-positive IgG from a patient with connective tissue disease and QTc prolongation. I-V relationships of $I_{Kr}$ peak (**c**) and tail (**d**) current densities during basal (circles) and anti-Ro/SSA antibody-positive IgG application (triangles). (**e**) Dose-response curve of anti-Ro/SSA antibody-positive IgG resulted in an EC50 = 87.3 μg/ml. (**f**) Time course of anti-Ro/SSA antibody-positive IgG (75 μg/ml) on $I_{Kr}$ peak. The dashed lines indicate the time of IgG application and washout, as indicated. The Boltzmann fit of $I_{Kr}$ activation (**g**) and deactivation (**h**) before (circle) and after anti-Ro/SSA antibody-positive IgG application (triangle). (**i**) Proteins from un-transfected (lane 1) and transfected HEK293 cells with hERG channels (lane 2) were probed with anti-Ro/SSA antibody-positive IgG from a patient with connective tissue disease and QTc prolongation. Bands at 155 kD and 135 kD correspond to glycosylated and endoplasmic reticulum-retained hERG channels in lane 2. Proteins from un-transfected (lane 3) and transfected HEK293 cells with hERG channels (lane 4) where probed with anti-Ro/SSA antibody-negative IgG from a control patient with connective tissue disease but normal QTc. Proteins from un-transfected (lane 5) and transfected HEK293 cells with hERG channels (lane 6) were probed with a commercial anti-hERG channel antibodies. Bands at 155 kD and 135 kD were seen in lane 6 only as in lane 2 with anti-Ro/SSA antibody-positive IgG. The 37 kD band represents GAPDH. (∗) indicates statistical significance at $p < 0.05$. (Based on data from Yue et al. [31])

whose QTc was excessively prolonged (700 ms) with episodes of TdP, functionally inhibited and biochemically interacted with the hERG channel proteins in a dose-dependent manner. However, these effects were observed only when HEK293 cells in culture were incubated with anti-Ro/SSA antibodies for 1–5 days but no acute effects (5 min) were observed. It is possible that longer period of time than the 5 min exposure, is necessary to see an acute effect as reported above. Nevertheless, both studies agree that anti-Ro/SSA antibodies are pathogenic.

## In Silico Experiments Demonstrating Epitope Mimics on hERG Channel

As mentioned earlier, the native target of anti-Ro/SSA antibodies is SSA/Ro antigen, which is

intracellularly located and as thus not accessible to the circulating antibodies in the normal myocyte (Fig. 12.3a). As such it seems reasonable to hypothesize that anti-Ro/SSA antibodies must then recognize an epitope mimic on the extracellular side of the hERG channel which shares homology with Ro52kD/SSA antigen. To test this hypothesis linear homology analysis revealed 44% homology between Ro52kD/SSA antigen (aa302-aa321) and the hERG α1 subunit (aa574-aa598) at the pore region of which 25% are identical (Fig. 12.3b) [31]. The presence of this homology at the pore region may be sufficient for anti-Ro52kD/SSA antibodies' binding to the hERG channel at this epitope mimic, especially in the tetrameric conformation of the channel where the four pore extracellular loops come together and are accessible to the antibodies (Fig. 12.3c). To test this, a 31 aa peptide (hERG E-pore peptide) corresponding to a por-

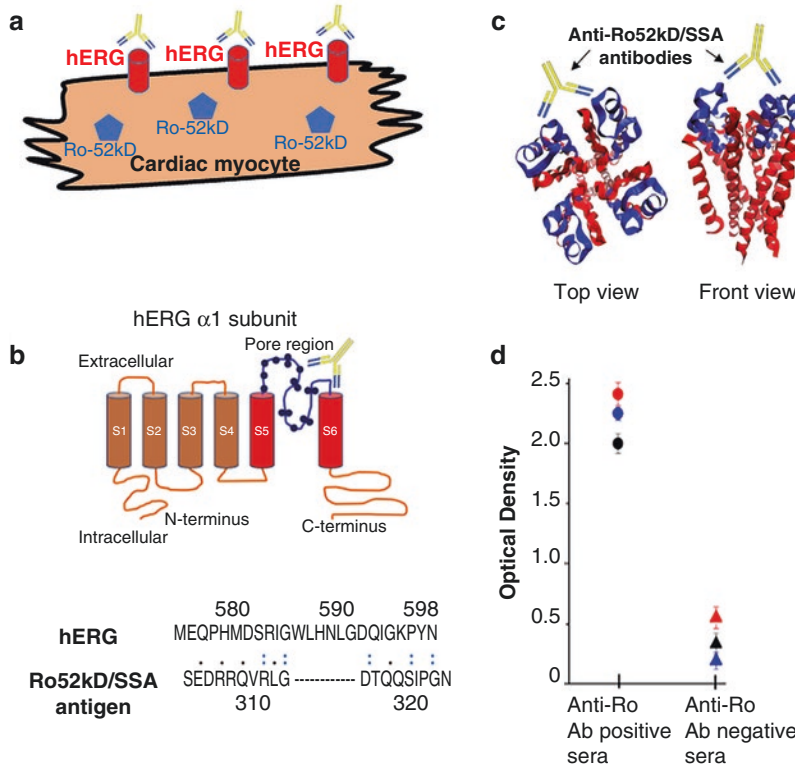


**Fig. 12.3** Secondary and tetrameric structure of hERG-$K^+$ channel pore region and reactivity of the corresponding peptide to sera from patients with connective tissue diseases. (**a**) Cartoon depiction of a cardiac myocyte showing the inaccessible intracellularly located Ro/SSA antigen and the accessible hERG-$K^+$ channels to anti-Ro/SSA antibodies. (**b**) The upper panel is a schematic representation of the secondary structure of a single hERG channel α1 subunit. The six segments (S1–S6) are shown, along with the intracellularly located N and C termini. The pore-forming extracellular loop is located between S5 and S6, where single and double circles illustrate similar and identical amino acids (aa) between the 52-kDa Ro (52Ro) protein and hERG channel, respectively. The lower panel shows the linear homology analysis between the Ro52kD/SSA protein and hERG channel at the pore region. (**c**) Tetrameric structure of the hERG channel model at the pore region based on KcsA crystal structure. Left, top view, and (right) front view of the pore region. Red represents segment S5/S6 helixes, and blue indicates the predicted anti-Ro/SSA antibody binding sites in the extracellular loop between S5/S6 based on the homology shown in (**b**). (**d**) Reactivity of connective tissue disease patient's sera to the peptide corresponding to the pore-forming region of hERG. (Based on data from Yue et al. [31])

tion of the hERG extracellular loop at the pore region between S5 and S6 was designed, synthesized, and tested against sera from anti-Ro/SSA antibody-positive and anti-Ro/SSA antibody-negative connective tissue disease patients. Significant reactivity to the peptide was observed in only anti-Ro/SSA antibody-positive patient's sera [31], thus implicating this pore region as a potential binding epitope for the autoantibodies. Further support of this view are data from guinea pig immunization with this hERG E-pore peptide (but not with a scrambled peptide) demonstrating significant QTc prolongation correlating with the antibody titers [36]. Sera from hERG E-pore peptide-immunized animals inhibited $I_{Kr}$ and prolonged action potential, without any evidence of structural heart modifications [36].

### Anti-Ro/SSA-Positive Sera from Patients with Connective Tissue Diseases or TdP Shows High Reactivity to the hERG E-pore Peptide

If the hERG E-pore region is the target of anti-Ro/SSA antibodies, then reactivity with patients' sera containing these autoantibodies is expected. In this regard, serum samples from patients with connective tissue diseases, anti-Ro/SSA positive, and with documented QTc prolongation [20, 22] demonstrate high reactivity to the hERG E-pore peptide [36]. No reactivity was observed with sera from patients with connective tissue diseases that were anti-Ro/SSA negative and had a normal QTc (control group) [36].

As mentioned above, anti-Ro/SSA antibodies are present in up to ~3% of the general population (Table 12.1) [26], where they could significantly contribute to SCD risk [23]. Indeed, anti-Ro52kD/SSA antibodies exerting hERG-blocking properties were frequently found (60%) in unselected TdP patients, mainly without manifest autoimmune diseases [23]. The reactivity of these patients' sera with the hERG E-pore peptide and its scrambled form were tested by ELISA. Interestingly, anti-Ro/SSA-positive sera showed significant reactivity to the hERG E-pore peptide compared with the scrambled peptide and with anti-Ro/SSA-negative sera suggesting the hERG E-pore peptide bound to the anti-Ro/SSA antibodies [23]. Altogether, the sera reactivity findings open new avenues in peptide-based diagnostic and/or therapeutic approaches to pathogenic anti-Ro/SSA antibodies and associated arrhythmogenesis (Fig. 12.4).

## Clinical Significance and Conclusions

Emerging and recent evidence indicates that autoimmunity is involved in the pathogenesis of cardiac arrhythmias, not only those occurring in patients with overt autoimmune diseases but also in a number of rhythm disorders currently classified as "idiopathic." Because arrhythmogenic anti-Ro/SSA antibodies are prevalent in several autoimmune diseases and also in healthy individuals, they should be considered as a risk factor for the development of QTc prolongation, TdP, and SCD. Thus, it is warranted that such individuals be screened for these antibodies, considering performing routine ECGs and counselling for those who may have congenital or medication-related QTc prolongation. In fact, although anti-Ro/SSA antibodies are in most cases probably not per se able to induce a QTc prolongation as critical as to induce TdP (but actually this is true for all recognized causes of LQTS, when present alone), nevertheless they can reduce the ventricular repolarization reserve, thereby significantly increasing the risk of life-threatening arrhythmias in the presence of other classical QT-prolonging factors (drugs, electrolyte imbalances, genetic polymorphisms, etc.). While it is well conceivable that these events may take place in patients with autoimmune chronic inflammatory diseases, thus putatively contributing to explain the increased risk of sudden death observed in the course of connective tissue diseases [41], nevertheless asymptomatic circulating

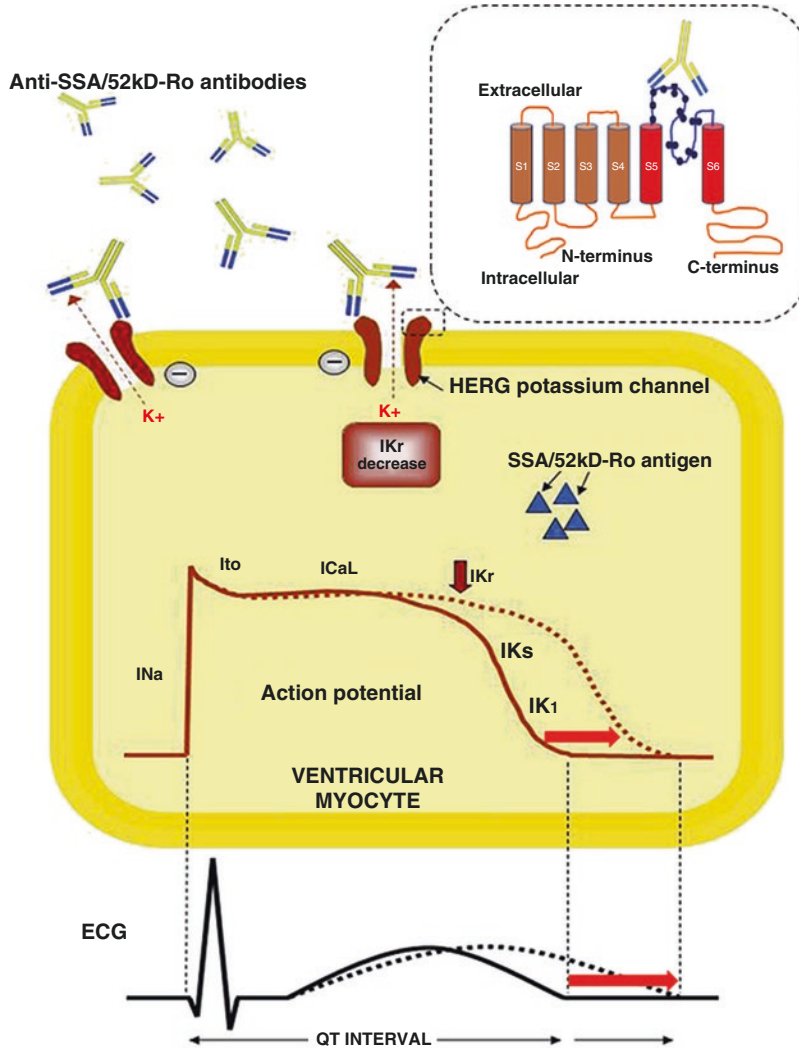


**Fig. 12.4** The molecular and electrophysiologic basis of anti-Ro/SSA-associated QTc prolongation, from the cell to the surface ECG. Circulating anti-Ro52/SSA antibodies cross-react with the extracellular loop of the pore-forming region of the hERG $K^+$ channel (inset) as a result of a molecular mimicry mechanism with Ro52kD/SSA antigen (blue triangles) that are located inside the cardiac myocyte but are not accessible to autoantibodies. The autoantibody-hERG channel interaction results in the inhibition of the related $I_{Kr}$ current, thus leading to an increase in the cardiomyocyte action potential duration (dotted lines). The clinical correlate of these electrophysiologic changes is a prolongation of the QT interval on the surface ECG (dotted lines). (Reprinted from Boutjdir et al. [30], with permission from Elsevier)

anti-Ro/SSA may be also silently involved, as a predisposing factor, in a number of unexpected life-threatening arrhythmias, including drug-induced TdP, and sudden deaths occurring in the general population.

From a therapeutic point of view, the evidence that pathogenic activities of anti-Ro/SSA autoantibodies are functional, thus potentially reversible, suggests that treatments targeting the immune-inflammatory system might effectively reduce arrhythmic risk in these subjects. In addition, since these autoantibodies induce channelopathies by directly cross-reacting with specific amino acid sequences on ion channels, an innovative therapeutic approach could be proposed based on the use of short decoy peptides

distracting the pathogenic antibodies from channel binding sites.

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# 13 The Role of Inflammation and Autoimmunity in Long QT Syndrome

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The long QT syndrome (LQTS) is a cardiac electric disorder characterized by the presence of a prolonged heart rate-corrected QT interval (QTc) on the electrocardiogram (ECG) and an increased susceptibility to life-threatening ventricular arrhythmias, particularly torsades de pointes (TdP) [1, 2]. LQTS can be the result of a wide number of etiologic factors, both congenital and acquired, often variously associated in a single patient and synergistically operating to promote TdP [2–5]. Indeed, different QT-prolonging factors interfere with the function of one or more cardiac ion channels determining the duration of ventricular repolarization, leading to an inward shift in the balance of current as a result of an increase in the inward sodium ($Na^+$) or calcium ($Ca^{++}$) currents and/or a decrease in an outward potassium ($K^+$) current [2, 3].

While congenital LQTS is a rare disease with an estimated prevalence of ~1:2000 apparently healthy live births [6], acquired forms are quite common occurring in up to 1 every 4 hospitalized patients [7–9]. Drugs, electrolyte imbalances, and structural heart diseases are the most common acquired QT-prolonging factors involved, although also bradyarrhythmias, endocrine diseases, liver disorders, nervous system injuries, HIV infection, starvation, hypothermia, and toxins are recognized causes of acquired LQTS in the general population [1–3, 5, 10–12].

However, "classical" risk factors cannot fully explain LQTS/TdP occurrence in a fraction of subjects, thereby pointing to the existence of a number of "nonclassical" risk factors, currently unrecognized and largely overlooked. In this view, accumulating evidence identified inflammation and autoimmunity as novel pathogenic mechanisms of acquired LQTS, actively cooperating with classical risk factors to increase the risk of TdP, and sudden cardiac death (SCD) [13]. By integrating basic and clinical research, in this chapter we provide an updated overview of this subject, also discussing practical implications and future therapeutic perspectives.

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## The Role of Inflammation in Long QT Syndrome

### Clinical Evidence

The risk of developing acquired LQTS and TdP is significantly increased in several pathologic conditions characterized by cardiac and/or systemic inflammatory activation.

#### Inflammatory Heart Diseases

In inflammatory heart disease, particularly myocarditis, QTc prolongation is a rather common finding, sometimes complicated by TdP. QTc lengthening was found to be the most frequent ECG abnormality in biopsy-proven myocarditis [14], and a significant association between QTc prolongation and the occurrence of complex ventricular arrhythmias during 24-hour electrocardiographic recordings was demonstrated in patients with myocarditis [15]. These findings were confirmed in a large cohort of almost 200 myocarditis patients, where a QTc $\geq$ 440 occurred in ~25% of the cases, also representing a predictor of poor clinical outcome, including cardiac death [16]. Increasing evidence indicates that TdP risk is particularly high when the QT interval is prolonged as a result of an increase in the terminal portion of the T wave, from the peak of the T wave to its end (Tpeak-Tend interval, Tp-Te) [2, 17]. Recently, Gunes et al. reported that also Tp-Te duration and Tp-Te/QTc ratio were significantly increased in myocarditis patients when compared to healthy controls (Table 13.1) [18]. In addition, 14 cases of TdP in patients with acute myo-/endocarditis have been reported in the literature, regardless of the specific etiologic agent implicated [19–32]. Moreover, in a prospective cohort of 40 consecutive, unselected TdP patients from the general population, 2 subjects (5% incidence) were found to be affected with acute infective endocarditis [33].

Further evidence supporting a significant prolonging effect of cardiac inflammation on ventricular repolarization derives from patients with specific forms of inflammatory heart diseases, such as Chagas disease (ChD) and acute rheu-

**Table 13.1** Clinical studies showing an association between QTc prolongation and inflammatory heart diseases

| Authors | Study population | Subjects *n* | Controls *n* | Main results |
|---|---|---|---|---|
| Ramamurthy et al. [14] | Myocarditis | 20 | – | QTc prolongation occurred in 70% of the patients |
| Karjalainen et al. [15] | Myocarditis | 26 | – | QTc prolongation was associated with complex ventricular arrhythmias |
| Ukena et al. [16] | Myocarditis | 186 | – | QTc prolongation occurred in 25% of the patients and predicted cardiac death |
| Gunes et al. [18] | Myocarditis | 30 | 25 | Tp-e and Tp-e/QT were higher in myocarditis patients than in controls |
| Williams-Blangero et al. [37] | Chagas disease | 722 | 667 | *T. Cruzi*-seropositive subjects showed a longer QTc when compared to controls |
| Sabino et al. [38] | Chagas disease | 546 | 483 | Chagas disease/*T. Cruzi* PCR-positive subjects showed a longer QTc when compared to controls |
| Salles et al. [36] | Chagas disease | 738 | – | QTc max independently predicted SCD |
| Bradfield et al. [39] | Chagas disease | 31 | 31 | Tp-e was longer in Chagas disease patients than controls and associated with increased mortality |
| Alp et al. [45] | Acute rheumatic carditis | 64 | 41 | QTc was significantly longer in patients with acute rheumatic fever than in controls and predicted the presence of carditis |
| Balli et al. [48] | Acute rheumatic carditis | 73 | – | QTc prolongation occurred in 2/3 of patients with carditis and strongly correlated with levels of acute phase reactants |

*QTc* corrected QT interval, *Tp-e* interval from the peak to the end of the T wave, *Tp-e/QT* interval from the peak to the end of the T wave/QT interval ratio, *PCR* polymerase chain reaction

matic carditis. ChD is a diffuse myocarditis triggered by the protozoan parasite *Trypanosoma cruzi* and then maintained by immune-mediated mechanisms [34]. The disease is endemic in Latin America and is burdened by a high risk of life-threatening arrhythmias and SCD [35]. In ChD, QTc prolongation is common, and maximal QTc independently predicts SCD [36–38]. Also Tp-Te duration was found to be increased in these patients and associated with the risk of death (Table 13.1) [39]. QT prolongation has been reproduced in murine models of the disease [40–42], where an increased propensity to drug-induced TdP and ventricular fibrillation (VF) was demonstrated [43]. Moreover, in these animals QT length and the extent of cardiac inflammation at the histological examination significantly correlated [41]. Acute rheumatic carditis is an immune-mediated inflammatory heart disease frequently presenting with QTc prolongation [44, 45], occasionally complicated with TdP [46, 47]. Notably, the presence of carditis and high levels of inflammatory markers (C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) were found to be significant predictors of a prolonged QTc in patients with acute rheumatic fever (Table 13.1) [48].

### Systemic Inflammatory Diseases

A growing body of evidence, mostly derived from patients with chronic autoimmune diseases such as rheumatoid arthritis (RA) and connective tissue diseases (CTD), demonstrated that systemic inflammatory activation is associated with an increased incidence of acquired LQTS (Table 13.2).

RA is a chronic joint disease characterized by persistent high-grade systemic inflammation [49], which is burdened by a ~2-fold higher risk of SCD [50] and cardiac arrest [51] when compared to the general population. RA patients frequently show QTc prolongation, correlating with disease activity and inflammatory markers, also predicting mortality [52]. Indeed, after we demonstrated for the first time that in RA patients QTc was longer than in spondyloarthritis and control subjects, also correlating with CRP levels [53], several other authors confirmed this finding. In a larger retrospective study, Chauhan et al. [54] found that the cumulative incidence of QTc prolongation at 20 years after RA incidence was significantly higher among RA than non-RA subjects and ESR values associated with risk of idiopathic QTc prolongation. Moreover, any QTc prolongation independently predicted all-cause mortality [54]. Accordingly, in a prospective cohort of 357 RA patients, Panoulas et al. [55] found that a prolonged QTc was a strong predictor of death, with a doubling of the all-cause mortality risk for each 50 ms increase in QTc. In these patients, QTc prolongation independently correlated with CRP levels, and the value of QTc in predicting mortality was lost after the adjustment for CRP [55]. More recently, Geraldino-Pardilla et al. [56] reported that in RA, CRP levels and disease activity score in 28 joints (DAS-28) were associated with an increased QTc length, while Acar et al. [57] demonstrated that also Tp-Te and Tp-Te/QT ratio were increased in these patients and significantly correlated with CRP, ESR, and DAS-28 score. These data are further supported by the evidence that in RA patients circulating levels of inflammatory cytokines (TNFα, IL-1β, IL-6, IL-10) associated with QTc duration [58] and that anti-IL6 therapy with tocilizumab (TCZ) led to a rapid and significant QTc shortening, which correlated with both the decrease in CRP and TNFα levels [59]. In addition, although no data are currently available on TdP prevalence in the RA population, cases of marked QTc prolongation and TdP have been reported in RA patients with elevated CRP and IL-6 levels [60]. Notably, these subjects accounted for 5% of the cases in a cohort of 40 consecutive TdP patients [33], an incidence 5–10 times higher than expected in the general population (0.5–1%) [49].

QTc prolongation is common also in CTD. Several studies in patients with different CTD reported a high prevalence of QTc prolongation [61–65], also being independently predicted by IL-1β serum level [65]. Consistently, systemic lupus erythematosus (SLE) patients show a longer QTc than controls [66–69], with QTc prolongation occurring in 7–17% of the patients [70–72]. QTc duration correlated with

**Table 13.2** Clinical studies showing an association between QTc prolongation and systemic inflammatory diseases

| Authors | Study population | Subjects *n* | Controls *n* | Main results |
|---|---|---|---|---|
| Lazzerini et al. [53] | RA | 25 | 20 | Mean QTc longer in RA patients than healthy controls and significantly correlated with CRP levels |
| Chauhan et al. [54] | RA | 518 | 499 | Cumulative incidence of QTc prolongation higher in RA than non-RA patients; any QTc prolongation independently associated with all-cause mortality; risk of idiopathic QTc prolongation correlated with ESR (HR 1.14 per 10 mm/h increase; 95% CI, 1.03–1.27) |
| Panoulas et al. [55] | RA | 357 | – | QTc prolongation was independently associated with CRP levels and predicted all-cause mortality |
| Geraldino-Pardilla et al. [56] | RA | 139 | – | QTc length correlated with CRP levels and disease activity (each unit higher DAS28-CRP score associated with a 1.5- and 1.6-fold higher odds of having a QTc ≥ 460 ms and ≥ 480 ms, respectively |
| Acar et al. [57] | RA | 96 | 50 | Tp-e was higher in RA patients than controls and correlated with CRP, ESR, and DAS-28 |
| Adlan et al. [58] | RA | 112 | – | QTc prolongation correlated with circulating levels of inflammatory cytokines |
| Lazzerini et al. [59] | RA | 17 | – | Anti-IL-6 therapy (TCZ) was associated with a rapid (within 12 weeks) QTc shortening, which correlated with the decrease in both CRP and TNFα levels |
| Acar et al. [92] | AS | 62 | 50 | Tp-e and Tp-e/QT were higher in AS patients than controls and correlated with CRP levels |
| Senel et al. [99] | AS | 21 | – | Anti-TNF therapy (IFX) significantly reduced QTc duration, along with disease activity scores, ESR, and CRP |
| Lazzerini et al. [61] | CTD | 57 | – | QTc prolongation in 31% of patients |
| Costedoat-Ch et al. [62] | CTD | 89 | – | QTc prolongation in 12% of patients |
| Lazzerini et al. [63] | CTD | 46 | – | QTc prolongation (28% of patients) correlated with complex ventricular arrhythmias |
| Lazzerini et al. [64] | CTD | 49 | – | QTc prolongation in 32% of patients |
| Pisoni et al. [65] | CTD | 73 | – | QTc prolongation (15% of patients) was independently predicted by circulating IL-1β levels |
| Cardoso et al. [66] | SLE | 140 | 37 | Mean QTc longer in patients than healthy controls |
| Milovanović et al. [67] | SLE | 52 | 41 | Mean QTc longer in patients than healthy controls |
| Rivera-Lopez et al. [68] | SLE | 93 | 109 | Mean QTc longer in patients than healthy controls |
| Bourrè-Tessier et al. [70] | SLE (two studies) | 150<br>278 | –<br>– | QTc prolongation (7% of SLE patients) was independently associated with SDI |
| Bourrè-Tessier et al. [71] | SLE | 779 | – | QTc prolongation (15% of SLE patients) was independently associated with SDI |
| Myung et al. [72] | SLE | 235 | – | QTc prolongation in 17% of patients |
| Teixera et al. [74] | SLE | 317 | – | Marked QTc prolongation (>500 ms) in 3% of SLE patients |
| Sham et al. [69] | SLE | 100 | 100 | Mean QTc was longer in SLE patients than healthy controls and correlated with disease activity |
| Avci et al. [73] | SLE | 69 | 57 | Tp-e and Tp-e/QT were higher in SLE patients than controls and correlated with disease duration |
| Sgreccia et al. [85] | SSc | 38 | 17 | Mean QTc longer in SSc patients than healthy controls |

**Table 13.2** (continued)

| Authors | Study population | Subjects *n* | Controls *n* | Main results |
|---|---|---|---|---|
| Rosato et al. [86] | SSc | 20 | NA | Mean QTc longer in SSc patients than healthy controls; QTc associated with disease severity |
| Massie et al. [88] | SSc | 689 | – | QTc prolongation (25% of SSc patients) independently associated with disease duration and severity |
| De Luca et al. [90] | SSc | 100 | – | QTc prolongation in 11% of patients |
| Foocharoen et al. [89] | SSc | 103 | – | QTc prolongation (14.6% of SSc patients) associated with disease severity |
| Okutucu et al. [87] | SSc | 107 | 100 | Tp-e and Tp-e/QT were higher in SSc patients than controls and correlated with ESR and CRP levels |
| Diederichsen et al. [91] | PM/DM | 76 | 48 | Mean QTc longer in PM/DM patients than healthy controls; QTc prolongation occurred in 5% of patients |
| Pattanshetty et al. [94] | IBD | 142 | – | QTc prolongation in 46% of patients |
| Simsek et al. [95] | Psoriasis | 94 | 51 | QTc max was longer in psoriasis patients than healthy controls |
| Poorzand et al. [97] | Psoriasis | 30 | 30 | QTc was longer in psoriasis patients than healthy controls |
| Arisoy et al. [98] | Psoriasis | 74 | 74 | QTc, Tp-e, and Tp-e/QT were higher in psoriasis patients than controls; Tp-e/QT correlated with CRP |
| Soylu et al. [96] | Psoriasis | 71 | 70 | Tp-e and Tp-e/QT were higher in psoriasis patients than controls and correlated with disease severity |
| Tisdale et al. [101] | ICU patients | 900 | – | Sepsis was a strong and independent predictor of QTc prolongation (OR, 2.7; 95% CI, 1.5–4.8) |
| Wasserstrum et al. [101] | Sepsis | 257 | – | QTc duration independently predicts mortality |
| Ozdemir et al. [102] | Sepsis | 93 | 103 | Tp-e and Tp-e/QT were higher in patients with sepsis than controls; Tp-e/QT independently predicted mortality |
| Lazzerini et al. [33] | Inflammatory diseases[a] | 46 | – | QTc prolongation in 26% of patients; CRP reduction was associated with significant QTc shortening, which correlated with IL-6 level decrease |

*RA* rheumatoid arthritis, *AS* ankylosing spondylitis, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *TCZ* tocilizumab, *CTD* connective tissue disease, *SLE* systemic lupus erythematosus, *SSc* systemic sclerosis, *PM/DM* polymyositis/dermatomyositis, *IBD* inflammatory bowel disease, *ICU* intensive care unit, *QTc* corrected QT interval, *Tp-e* interval from the peak to the end of the T wave, *Tp-e/QT ratio* interval from the peak to the end of the T wave/QT interval ratio, *ESR* erythrocyte sedimentation rate, *TNFα* tumor necrosis factor alpha, *IL-1β* interleukin-1 beta, *IL-6* interleukin-6, *DAS28* disease activity score in 28 joints, *OR* odds ratio, *HR* hazard ratio, *NA* not available
[a]Inflammatory diseases of different origin (infective, immuno-inflammatory, others)

disease activity, increasing during severe flares [69], and with overall inflammatory burden [70, 71]. Moreover, also Tp-Te and Tp-Te/QT ratio significantly increase in SLE, positively associating with disease duration [73]. A marked QTc prolongation (>500 ms) is demonstrated in ~3% of patients [74], and 10 reports of TdP in SLE have been described in the literature [75–84]. In systemic sclerosis (SSc) patients, where values of QTc, Tp-e, and Tp-e/QT are significantly higher in comparison to controls [85–87], QTc prolongation has a prevalence ranging from 11% to 25%, also correlating with disease duration and severity [88–90]. Notably, Tp-Te and Tp-Te/QT showed also a specific association with ESR and CRP levels [87]. In addition, recent studies provided evidence that QTc and Tp-Te are also increased in polymyositis/dermatomyositis [91], as well as other autoimmune inflammatory diseases, such as ankylosing spondylitis [92], inflammatory bowel disease [93, 94], and psoriasis [95–98], also correlating with disease severity and CRP [92, 96, 98]. Finally, in ankylosing spondylitis

patients a 6-month anti-TNF treatment with infliximab significantly reduced both inflammatory markers and QTc duration [99], and occurrence of TdP or premonitory ECG signs of TdP (marked QTc prolongation with macroscopic T-wave alternans) have been recently reported in both polymyalgia rheumatica and psoriatic arthritis patients with elevated CRP and IL-6 levels [60, 100].

Besides immune-mediated inflammatory activation, increasing evidence suggests that also infections, particularly severe forms such as sepsis, can be associated with QTc prolongation and increased TdP risk. Indeed, in patients with sepsis QTc is frequently prolonged, and Tp-Te and Tp-Te/QT values are significantly higher than in controls, all independently predicting short-term mortality [101, 102]. Moreover, by studying 900 consecutive patients admitted to cardiac care units, Tisdale et al. [103] identified sepsis as one of strongest independent predictors of QTc prolongation. Several cases of TdP in patients with sepsis have been reported [104–110], and in a recent study performed in our Institution we found that an acute infection (more commonly a sepsis) was demonstrable in 30% of cases in a cohort of 40 consecutive TdP patients [33].

Altogether, these data strongly suggest that systemic inflammation per se represents a risk factor for QTc prolongation and TdP, regardless of its origin and pathogenesis. Accordingly, in the above cited study on unselected TdP patients, elevated CRP and IL-6 levels were present in most cases (80%), including several patients in whom a specific inflammatory disease was not detected [33]. In support of this view, we demonstrated that in subjects with elevated CRP levels from different inflammatory conditions (infective, immune-mediated, others), QTc prolongation was common, but rapidly and significantly shorten as soon as CRP and IL-6 decreased following specific treatments (Fig. 13.1) [33].

## Noninflammatory Heart Diseases and Congenital Long QT Syndrome

Recent evidence suggests that systemic inflammation may also play a role in modulating QTc duration/arrhythmia development in patients with noninflammatory heart diseases (Table 13.3) and congenital LQTS.

By studying 466 hypertensive patients, Chang et al. [111] found that low-grade chronic systemic inflammation as assessed by high-sensitivity CRP (hsCRP) levels was strictly correlated with QTc length, also independently predicting the presence of a prolonged QTc. Consistent findings were reported in patients with coronary artery disease (CAD), where a significant association between QTc duration and circulating hsCRP was demonstrated [112]. Moreover, in CAD patients short-term exposure to ambient particulate air pollution leading to hsCRP increase is associated with a significant prolongation of QTc duration [113]. In Takotsubo cardiomyopathy, QTc prolongation is frequently observed, also predicting occurrence of ventricular arrhythmias, TdP, and death [114, 115]. Among these patients, those developing a prolonged QTc showed ~3 times higher hsCRP levels when compared to subjects with a normal QTc [116]. In addition, we demonstrated that in patients with cardiovascular diseases who developed marked QTc prolongation and TdP, CRP and IL-6 levels were markedly higher than in those who did not (~5–15 times, respectively), in about a half of cases in the presence of a definite, concomitant inflammatory disease [33].

Finally, recent data point to a role for inflammatory activation also in increasing electrical instability in patients with congenital LQTS. In fact, Rizzo et al. [117] provided evidence that in subjects who underwent left cardiac sympathetic denervation for malignant intractable arrhythmias, inflammatory infiltrates including activated T lymphocytes and macrophages, suggestive of a chronic T-cell-mediated stellate ganglionitis, were demonstrable. Consistent findings, already described in early reports in single cases/case series of LQTS patients who died suddenly [118–120], were also recently found in stellate ganglia from patients with cardiomyopathy and refractory ventricular arrhythmias [121]. These data suggest that locally released inflammatory mediators may be arrhythmogenic by enhancing efferent sympathetic tone on the heart, in turn representing a

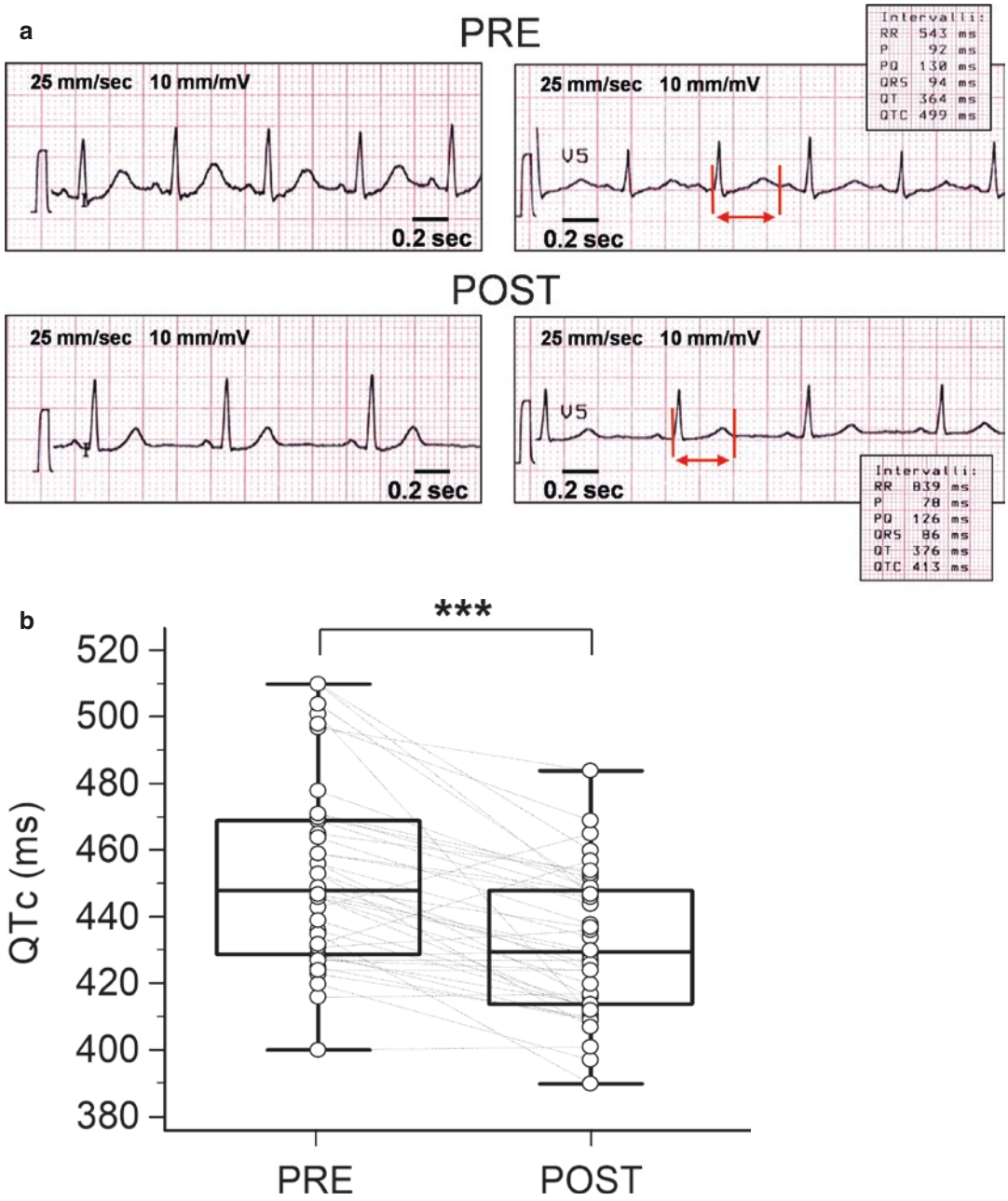


**Fig. 13.1** Changes in the corrected QT (QTc) interval duration in patients with active inflammatory diseases during active disease (PRE) and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST). (**a**) Representative ECG strips of a patient with acute pancreatitis, during active disease (PRE; CRP 27.0 mg/dl) and after a 13-day treatment with gabesate mesilate (POST; CRP 1.64 mg/dl). Red vertical lines in lead V5 show QT interval. (**b**) QTc changes observed before (PRE) and after (POST) treatment in the entire study population. Patients, $n = 46$. Two-tail Student's paired "t" test, $***p < 0.0001$. (Reproduced from Lazzerini et al. [33], with permission from BMJ Publishing Group)

**Table 13.3** Clinical studies showing an association between QTc duration/TdP occurrence and inflammatory activation in patients with noninflammatory heart diseases or apparently healthy subjects of the general population

| Authors | Study population | Subjects *n* | Controls *n* | Main results |
|---|---|---|---|---|
| Chang et al. [111] | Arterial hypertension | 466 | – | CRP levels correlated with QTc duration and independently predicted QTc prolongation |
| Yue et al. [112] | Coronary artery disease | 52 | – | CRP levels correlated with QTc duration |
| Song et al. [116] | Takotsubo cardiomyopathy | 105 | – | Patients with QTc prolongation had higher CRP levels than those with normal QTc |
| Lazzerini et al. [33] | Torsades de pointes | 40 | 30 | Increased CRP levels in most subjects (80%); IL-6 levels were elevated and comparable with those observed in patients with severe active RA and markedly higher than controls |
| Kazumi et al. [128] | Healthy subjects | 179 | – | QTc length independently correlated with CRP |
| Kim et al. [129] | Healthy subjects | 4758 | – | QTc prolongation independently associated with elevated CRP |
| Medenwald et al. [130] | Healthy subjects | 1716 | – | Soluble TNF-receptor 1 levels independently correlated with QTc duration in women |

*TdP* torsades de pointes, *QTc* corrected QT interval, *Tp-e* interval from the peak to the end of the T wave, *Tp-e/QT* ratio interval from the peak to the end of the T wave/QT interval ratio, *CRP* C-reactive protein

well-recognized trigger for malignant arrhythmias in LQTS [122]. Accordingly, Wang et al. [123] demonstrated that IL-1β injection into the left stellate ganglion increased ganglion activity, sympathetic indices of heart rate variability, and VA occurrence in canine hearts. Indeed, acute inflammatory processes are increasingly recognized as possible precipitant factors of malignant arrhythmias/electrical storms in congenital LQTS subjects [124, 125]. Although fever can be per se arrhythmogenic by altering temperature-sensitive biophysical properties of mutant channels [126, 127], it is conceivable that in these patients high levels of cytokines may further contribute to arrhythmia susceptibility by directly affecting cardiomyocyte APD (see below, *Molecular basis and pathophysiology*), as well as by indirectly increasing sympathetic output in the heart.

### General Population

Subclinical low-grade chronic inflammation may be associated with an increased risk of QTc prolongation also in apparently healthy subjects (Table 13.3). Based on preliminary evidence by Kazumi et al. [128] who demonstrated that in male college students QTc duration independently correlated with hsCRP, two large community-based studies supported the existence of a strict link between inflammatory markers and QTc in both middle-aged and elderly individuals of the general population. The Korean Health and Genome Study found that hsCRP levels independently predicted the presence of QTc prolongation among 4758 subjects aged 40–69 years [129]. Consistently, in the Cardiovascular Diseases, Living and Ageing in Halle (CARLA) Study analyzing 1716 subjects aged 45–83 years, Medenwald et al. [130] demonstrated that circulating markers of inflammation correlated with QTc length, particularly the TNFα system, as reflected by soluble TNF-receptor 1 levels (sTNF-R1), in women.

These findings, together with the evidence from large prospective population studies that hsCRP and IL-6 are strong independent predictors of SCD in apparently healthy persons [131–133], intriguingly suggest that low-grade chronic systemic inflammation may increase the susceptibility of long QT-associated malignant arrhythmias also in the general population, specifically TdP rapidly degenerating to ventricular fibrillation.

## Molecular Basis and Pathophysiology

Accumulating evidence points to inflammatory cytokines, particularly TNFα, IL-1, and IL-6, as the key mediators linking inflammatory activation

**Table 13.4** Effects of inflammatory cytokines on cardiac ion channels, ventricular APD, and QT interval: evidence from in vitro and animal studies

| Cytokine | Ion currents | Molecular mechanisms | APD | QT/QTc interval |
|---|---|---|---|---|
| TNFα | $I_{to}$ decrease [137–140] | Reduced expression of $K_v4.2/K_v4.3$ potassium channels [137, 139, 140], mediated by iNOS induction [140], ROS generation [140], and KChIP-2 inhibition [139] | Prolongation [136, 140, 141] | Prolongation [134, 135, 144] |
| | $I_{Kr}$ decrease [141] | Functional impairment of hERG potassium channel, mediated by TNF-receptor 1 engagement and ROS production [141] | | |
| | $I_{Ks}$ decrease [142] | Mediated by sphingosine-1-phosphate generation and cyclic AMP decrease [142] | | |
| | $I_{Kur}$ decrease [137, 138] | Reduced expression of $K_v1.5$ potassium channel [137] | | |
| IL-1 | $I_{to}$ decrease [145] | NA | Prolongation [145, 146] | Prolongation [145] |
| | $I_{CaL}$ increase [146] | Mediated by activation of cyclooxygenase and lipooxygenase pathways [146] | | |
| IL-6 | $I_{CaL}$ increase [152] | Enhancement of $Ca_v1.2$ calcium channel function [150, 152], dependent on a SHP2/ERK-mediated phosphorylation of the serine residue at the position 1829 [151] | Prolongation [152] | NA |

*Modified from*: Lazzerini et al. [13]
*TNFα* tumor necrosis factor alpha, *IL-1* interleukin-1, *IL-6* interleukin-6, *ROS* reactive oxygen species, *hERG* human Ether-a-go-go-Related Gene $K^+$ channel, $I_{to}$ transient $K^+$ outward current, $I_{Kur}$ ultrarapidly activating component of the delayed outward rectifying current, $I_{Kr}$ rapidly activating component of the delayed outward rectifying current, *IKs* slowly activating component of the delayed outward rectifying current, *iNOS* inducible nitric oxide synthase, *KChIP-2* K(+) channel-interacting protein, *SHP/ERK* Src homology 2 domain-containing phosphatase/extracellular signal-regulated kinase, *APD* action potential duration, *QTc* corrected QT interval, *NA* data not available

to QTc prolongation, via direct electrophysiological effects on the myocardium (Table 13.4) [13]. In fact, several experimental studies provided evidence that such molecules can prolong ventricular APD by modulating the expression and/or function of several ion channels of the cardiomyocyte (*inflammatory channelopathies*), resulting in an inward shift in the balance of currents [13, 52]. The best documented are the inhibitory effects exerted by TNFα on outward potassium currents. In mouse models, cardiac-specific overexpression of TNFα is associated with QT/QTc interval prolongation [134, 135]. Ex vivo experiments demonstrated that perfused hearts from transgenic mice overexpressing TNFα showed APD prolongation and increased propensity to reentrant ventricular arrhythmias, in the presence of a significant reduction of the transient outward potassium current ($I_{to}$) and the rapidly activating, slowly inactivating delayed rectifier currents ($I_{Kslow1}$,$I_{Kslow2}$) in left ventricular myocytes [136, 137]. Consistent inhibitory effects on these currents were also found by Grandy et al. [138] in cardiomyocytes from ventricles of mice chronically injected with TNFα, resulting in elevated circulating levels of this cytokine. Notably, such changes were associated with a parallel expression decrease of related channel proteins ($K_v4.2$, $K_v4.3$, and $K_v1.5$) in the TNFα-overexpressing model only [137, 138], possibly depending on the higher cardiac tissue TNFα concentrations reached in these animals [138]. Moreover, two further studies reported significant $I_{to}$ and $K_v4.2$ downregulation in rat ventricular myocytes cultured in vitro with TNFα, also demonstrating the involvement of an intracellular pathway including overexpression of nitric oxide synthase, generation of reactive oxygen species (ROS), and inhibition of potassium channel-interacting protein 2 [139, 140]. Evidence indicates that also the rapid and the slow components of the delayed rectifier potassium current ($I_{Kr}$ and $I_{Ks}$, respectively) are significantly reduced by TNFα. By using both HEK293 cells and canine cardiomyocytes, Wang et al. [141] found that TNFα prolonged APD and decreased $I_{Kr}$ by impairing human Ether-a-go-go-Related Gene (hERG) potassium channel function, via TNF-receptor 1 engagement and ROS

production [141]. Moreover, in guinea pig ventriculocytes Hatada et al. [142] proved the inhibitory effect of TNFα on isoproterenol-activated $I_{Ks}$, possibly mediated by sphingosine-1-phosphate generation and cyclic AMP decrease. The in vivo relevance of all these electrophysiological effects has been recently confirmed by the group of Kollias in two TNFα-overexpressing mouse models developing polyarthritis, where QTc prolongation (*Tg197* model) [143] and premature mortality associated with increased incidence of fatal arrhythmic events ($Tnf^{\Delta ARE}$ model) [144] have been demonstrated.

Although less extensively investigated, emerging experimental data support a significant QT-prolonging potential also for IL-1 and IL-6, able to increase ventricular APD by inhibiting outward $K^+$ and/or enhancing inward $Ca^{++}$ currents. A recent study by Monnerat et al. [145] showed that IL-1β incubation increases APD in ventricular rat cardiomyocytes as well as field potential duration (FPD) in human induced pluripotent stem cell-derived cardiomyocytes, along with a ~35% reduction of $I_{to}$. Moreover, the same authors demonstrated that in diabetic rats showing QTc prolongation and increased susceptibility to arrhythmias, the genetic deletion of the IL-1-receptor or the treatment with the IL-1-receptor antagonist anakinra was sufficient to prevent both electric abnormalities in these animals [145]. A further possible mechanism by which IL-1β could increase QTc length is the enhancement of L-type $Ca^{++}$ current ($I_{CaL}$), although available data are conflicting. In fact, while Li et al. [146] found that IL-1β significantly augmented $I_{CaL}$ density and prolonged APD via cyclooxygenase and lipooxygenase pathways in guinea pig papillary myocytes, other authors reported opposite effects on such current (despite no concomitant data on APD were provided) [147–149]. Regarding IL-6, several studies provided evidence that it can increase $I_{CaL}$ in cardiomyocytes [150–152], a mechanism probably accounting for the ventricular APD prolongation induced by such cytokine [152]. In particular, IL-6 activates a SHP2/ERK (Src homology 2 domain-containing phosphatase/extracellular signal-regulated kinase)-mediated signaling cascade leading to $I_{CaL}$ enhancement as a result of the phosphorylation of the serine residue at the position 1829 of the Cav1.2 calcium channel subunit [151]. In addition, IL-6 also inhibits $I_{Kr}$, by activating Janus kinase signaling cascade [unpublished data].

Altogether, these data provide robust mechanistic support to the hypothesis that TNFα, IL-1β, and IL-6 represent key mediators of inflammation-induced QTc prolongation. The previously reported evidence that circulating levels of these cytokines correlated with QTc length in patients with inflammatory diseases [33, 58, 59, 65] as well as apparently healthy subjects [130], strongly indicates that these pathophysiological mechanisms are also crucially involved in vivo, a conclusion also supported by several animal models of cardiac or systemic inflammation. In mice with experimental autoimmune myocarditis (EAM), APD is significantly prolonged along with increased tissue expression of IL-6 and TNFα, reduced $I_{to}$ current density, and enhanced susceptibility to triggered ventricular arrhythmias [153, 154]. Moreover, in a murine model of myocardial infarction (MI), lipopolysaccharide (LPS)-induced systemic inflammation markedly increases APD and ventricular arrhythmia propensity, when compared to non-LPS-injected MI animals [155]. Such electric changes, showing an association with the degree of macrophage activity and IL-1β expression in the myocardium [155], were significantly attenuated by anti-IL1 treatment with anakinra [156]. Other authors demonstrated the key role of IL-1β in increasing APD and QTc interval, as well as ventricular arrhythmia vulnerability in diabetic mice [145]. Moreover, in cardiac-specific liver kinase B1 knockout mice, a model of spontaneous cardiac and systemic inflammation, QTc prolongs as much as circulating levels of CRP, TNFα, and IL-6 increase [157]. Notably, in these animals SCD was a common finding, frequently resulting from ventricular arrhythmias, including TdP degenerating to ventricular fibrillation [157].

Finally, increasing evidence suggests that inflammation could promote QTc prolongation also in an indirect manner, by inducing cytokine-mediated cardiac autonomic dysfunction via

central (*inflammatory reflex*) and peripheral mechanisms (left stellate ganglia activation) [13]. These changes could intriguingly have an important role in precipitating life-threatening arrhythmias during inflammatory activation, given that cardiac sympathetic overactivity is a well-recognized trigger for arrhythmic events in patients with LQTS [122].

## The Role of Autoimmunity in Long QT Syndrome

Autoimmunity is recently emerging as a novel mechanism of cardiac arrhythmias, not only in patients with autoimmune diseases (AD) but also in otherwise healthy subjects with "idiopathic" rhythm disorders [158]. Different types of arrhythmogenic autoantibodies affecting cardiac electrophysiologic by directly targeting proteins expressed on the myocyte surface have been identified [158, 159]. In particular, some of these autoantibodies can significantly interfere with the function of specific ion channels critically involved in determining ventricular APD (*autoimmune channelopathies*), thus leading to acquired forms of LQTS of autoimmune origin [160]. To date, the most investigated autoimmune channelopathy associated with LQTS is related to the presence of anti-Ro/SSA antibodies (anti-Ro/SSA) cross-reacting with the hERG-$K^+$ channel [161, 162]. Recent data suggest that other autoantibodies recognizing $K^+$ or $Ca^{++}$ channels, i.e., anti-voltage-gated potassium channel $K_v1.4$ antibodies and agonist-like anti-L-type $Ca^{++}$ antibodies, might also promote life-threatening arrhythmias by prolonging APD/QTc interval [160].

### Clinical Evidence

Anti-Ro/SSA, including the two main subtypes anti-Ro/SSA-52kD and anti-Ro/SSA-60kD, primarily recognize the Ro/SSA antigen, an intracellular ribonucleoprotein constituted by two subunits of different molecular weight, named Ro52/E3 ubiquitin-protein ligase and Ro60, respectively [163]. Maternal anti-Ro/SSA, particularly anti-Ro/SSA-52kD, play a key pathogenic role in autoimmune congenital heart block (ACHB) [164], at least in part mediated by an inhibitory cross-reaction with L-type $Ca^{++}$ channels in the cardiac conduction system of the fetus [165, 166]. Anti-Ro/SSA are frequently detected in patients with AD, particularly Sjögren's syndrome and SLE [163], but also in a non-negligible percentage of apparently healthy subjects (0.5–2.7%) [166–169]. Notably, although techniques such as immunoenzymatic tests (ELISA, FEIA) and line blot immunoassay (LIA) are routinely used, evidence points to immuno-Western blot (IWB), as the most sensitive method to detect arrhythmogenic autoantibodies [170].

Besides conduction system, accumulating clinical data indicate that ventricular repolarization represents another potential target of anti-Ro/SSA pathogenic activity, both in fetal and in adult hearts. In fact, several studies in the last two decades provided evidence that circulating anti-Ro/SSA are associated with a higher risk of developing acquired LQTS [161], as well as the occurrence of ventricular arrhythmias [63, 171], including TdP (Tables 13.5 and 13.6) [172, 173].

The first data were obtained in newborns in early 2000s, when Cimaz et al. [174] reported how infants from anti-Ro/SSA-positive mothers presented a QTc interval significantly prolonged when compared to those born from negative mothers, despite no development of ACHB. Notably, in a prospective follow-up study the same authors demonstrated that such abnormality was transient, as it spontaneously recovered during the first year of life in parallel with the disappearance of acquired maternal anti-Ro/SSA in the blood [175]. Both such findings were later confirmed by two independent groups, one from England showing that QTc was significantly longer in children born from anti-Ro/SSA-positive vs. anti-Ro/SSA-negative mothers [176] and the other one from Canada reporting that among 116 newborns without ACHB, 17 (15%) had transient QTc prolongation [177]. Although none of these studies reported concomitant occurrence of life-threatening arrhythmias/SCD, a case of QTc prolongation complicated with pre-

**Table 13.5** Clinical studies on anti-Ro/SSA antibodies and QTc in newborns

| Authors | Study population | Anti-Ro/SSA+ patients (*n*) | Anti-Ro/SSA- patients (*n*) | Main results |
|---|---|---|---|---|
| Cimaz et al. [174] | Children of CTD mothers | 21 | 7 | Mean QTc significantly longer in anti-Ro/SSA-positive subjects |
| Gordon et al. [176] | Children of CTD mothers | 38 | 7 | Mean QTc significantly longer in children of anti-Ro/SSA-positive mothers |
| Cimaz et al. [175] | Children of anti-Ro/SSA-positive mothers | 21 | – | Concomitant disappearance of QTc prolongation and acquired maternal antibodies at 1-year follow-up |
| Costedoat-Chalumeau et al. [182] | Children of CTD mothers | 58 | 85 | No differences in mean QTc duration or in QTc prolongation prevalence between groups |
| Duke et al. [171] | Child of an anti-Ro/SSA-positive mother | 1 | – | QTc prolongation and ventricular tachycardia |
| Motta et al. [179] | Children of CTD mothers | 51 | 50 | Mean QTc slightly longer in children of anti-Ro/SSA-positive mothers ($p = 0.06$) |
| Gerosa et al. [183] | Children of AD mothers | 60 | 30 | No difference in the prevalence of QTc prolongation between the groups |
| Jaeggi et al. [177] | Children of anti-Ro/SSA-positive mothers | 116 | – | Transient QTc prolongation in 14.6% of cases |

*CTD* connective tissue disease, *AD* autoimmune disease, *QTc* corrected QT interval

natal ventricular tachycardia was described in a ACHB fetus born from an anti-Ro/SSA-positive mother [171].

The existence of a link between anti-Ro/SSA, QTc prolongation, and ventricular arrhythmias was confirmed and further characterized by several other studies performed in adults. In our Institution, we first provide evidence that anti-Ro/SSA-positive CTD patients showed a high prevalence of QTc prolongation, with a mean QTc length significantly increased when compared to negative patients [61]. In a following 24-hour ECG monitoring study, we also demonstrated that such abnormality persisted throughout the day-night time and correlated with an increased incidence of complex ventricular arrhythmias [63]. Consistent data were later reported by Bourre-Tessier et al. [70] in two large consecutive studies involving SLE patients, where the presence of anti-Ro/SSA positivity was associated with a 5–12 times higher risk of presenting QTc prolongation. Moreover, the authors found that such a risk increased as much as autoantibody levels were high and that the association between anti-Ro/SSA and QTc prolongation strongly persisted over a 17-month follow-up [70]. The key pathogenic role of circulating anti-Ro/SSA concentrations was also confirmed in our own CTD cohort, where the existence of a direct correlation between antibody levels and QTc length was demonstrated. In addition, we provided evidence that anti-Ro/SSA specificity is crucial, as when considered separately only anti-Ro/SSA-52kD subtype levels remained significantly associated with QTc duration [64]. More recently, other supportive studies have been published. Pisoni et al. [65] showed that QTc prolongation was more common in anti-Ro/SSA-positive (20%) vs. anti-Ro/SSA-negative CTD patients (0%), and Sham et al. [69] found that anti-Ro/SSA positivity was associated with a significantly prolonged QTc in a SLE cohort. Besides reporting that anti-Ro/SSA-52kD-positive CTD patients had higher QTc maximum values when compared to both negative subjects and healthy controls, Tufan et al. [178] demonstrated that also Tp-Te and Tp-Te/QT were significantly increased in these patients, with a strong direct correlation between Tp-Te duration and anti-Ro/SSA-52kD titer. In addition, evidence exists that, in adult patients, anti-Ro/SSA positivity is associated with TdP, regardless of the presence of a manifest AD, thus possibly representing a silent risk factor for life-

**Table 13.6** Clinical studies on anti-Ro/SSA antibodies and QTc/TdP in adults

| Author, year | Study population | Anti-Ro/SSA+ patients (*n*) | Anti-Ro/SSA- patients (*n*) | Main results |
|---|---|---|---|---|
| Gordon et al. [180] | AD patients | 49 | 62 | Mean QTc slightly longer in anti-Ro/SSA-positive patients ($p = 0.06$) |
| Lazzerini et al. [61] | CTD patients | 31 | 26 | Mean QTc significantly longer and prevalence of QTc prolongation significantly higher in anti-Ro/SSA-positive subjects |
| Costedoat-Chalumeau et al. [62] | CTD patients | 32 | 57 | No differences in mean QTc duration or in QTc prolongation prevalence between groups |
| Lazzerini et al. [63] | CTD patients | 26 | 20 | Mean QTc significantly longer and prevalence of QTc prolongation significantly higher in anti-Ro/SSA-positive subjects; QTc prolongation significantly associated with the presence of complex ventricular arrhythmias |
| Bourrè-Tessier et al. [70] | SLE patients (two studies) | 57<br>113 | 93<br>165 | 5.1–12.6 times higher risk of QTc prolongation in anti-Ro/SSA-positive vs. anti-Ro/SSA-negative group |
| Lazzerini et al. [64] | CTD patients | 25 | 24 | Mean QTc significantly longer and prevalence of QTc prolongation significantly higher in anti-Ro/SSA-positive subjects; significant correlation between anti-Ro/SSA-52kD concentration and QTc duration |
| Nomura et al. [181] | SLE patients | 43 | 47 | Anti-Ro/SSA positivity slightly more frequent among SLE patients with QTc prolongation ($p = 0.08$) |
| Teixeira et al. [74] | SLE patients | 111 | 206 | No difference in the prevalence of marked QTc prolongation (>500 msec) between groups |
| Massie et al. [88] | SSc patients | 148 | 541 | No difference in the prevalence of QTc prolongation between groups |
| Bourrè-Tessier et al. [71] | SLE patients | 283 | 314 | Prevalence of QTc prolongation slightly higher in anti-Ro/SSA-positive subjects, but not significantly for wide confidence intervals |
| Pisoni et al. [65] | AD patients | 55 | 18 | Anti-Ro/SSA positivity significantly more frequent among CTD patients with QTc prolongation (all patients with QTc prolongation were anti-Ro/SSA positive) |
| Sham et al. [69] | SLE patients | 47 | 53 | Mean QTc significantly longer in anti-Ro/SSA-positive subjects |
| Nakamura et al. [172] | TdP patient | 1 | – | Marked QTc prolongation and TdP in an anti-Ro/SSA-positive woman without AD |
| Lazzerini et al. [173] | TdP patients | 25 | – | High prevalence of anti-Ro/SSA-52kD in unselected TdP patients (60%) |
| Geraldino-Pardilla et al. [56] | SLE patients | 28 | 22 | No association between anti-Ro/SSA positivity and QTc length |
| Tufan et al. [178] | CTD patients | 15 | 39 | QTc maximum, Tp-e, and Tp-e/QT ratio higher in anti-Ro/SSA-52kD-positive vs. anti-Ro/SSA-52kD-negative CTD patients (and healthy controls, $n = 22$); Tp-Te duration strongly correlated with anti-Ro/SSA-52kD titer |

*CTD* connective tissue disease, *AD* autoimmune disease, *SLE* systemic lupus erythematosus, *SSc* systemic sclerosis, *QTc* corrected QT interval, *TdP* torsades de pointes, *Tp-e* interval from the peak to the end of the T wave, *Tp-e/QT ratio* interval from the peak to the end of the T wave/QT interval ratio

threatening arrhythmias/SCD in the apparently healthy population. Nakamura et al. [172] reported for the first time the case of an otherwise healthy woman developing TdP, despite intensive search excluded genetic or acquired known causes of QT prolongation. In this patient, asymptomatic for AD, only specific testing revealed high circulating levels of anti-Ro/

SSA. These data were confirmed and expanded in a cohort of 25 consecutive TdP patients, where anti-Ro/SSA-52kD positivity was detected in 60% of cases, almost always in the absence of a concomitant AD (Fig. 13.2) [173]. Notably, likewise in ACHB [170] and CTD patients [64], also in these subjects IWB was the most sensitive technique in detecting arrhythmogenic autoantibodies. Finally, it must be mentioned that a number of other studies found near statistically significant differences in QTc duration and/or QTc prevalence between anti-Ro/SSA-positive and anti-Ro/SSA-negative patients, possibly due to relatively small sample sizes. Motta et al. [179] showed that mean QTc of infants born from anti-Ro/SSA-positive mothers was slightly longer than controls ($p$ = 0.06), and consistent findings were reported by Gordon et al. [180] by

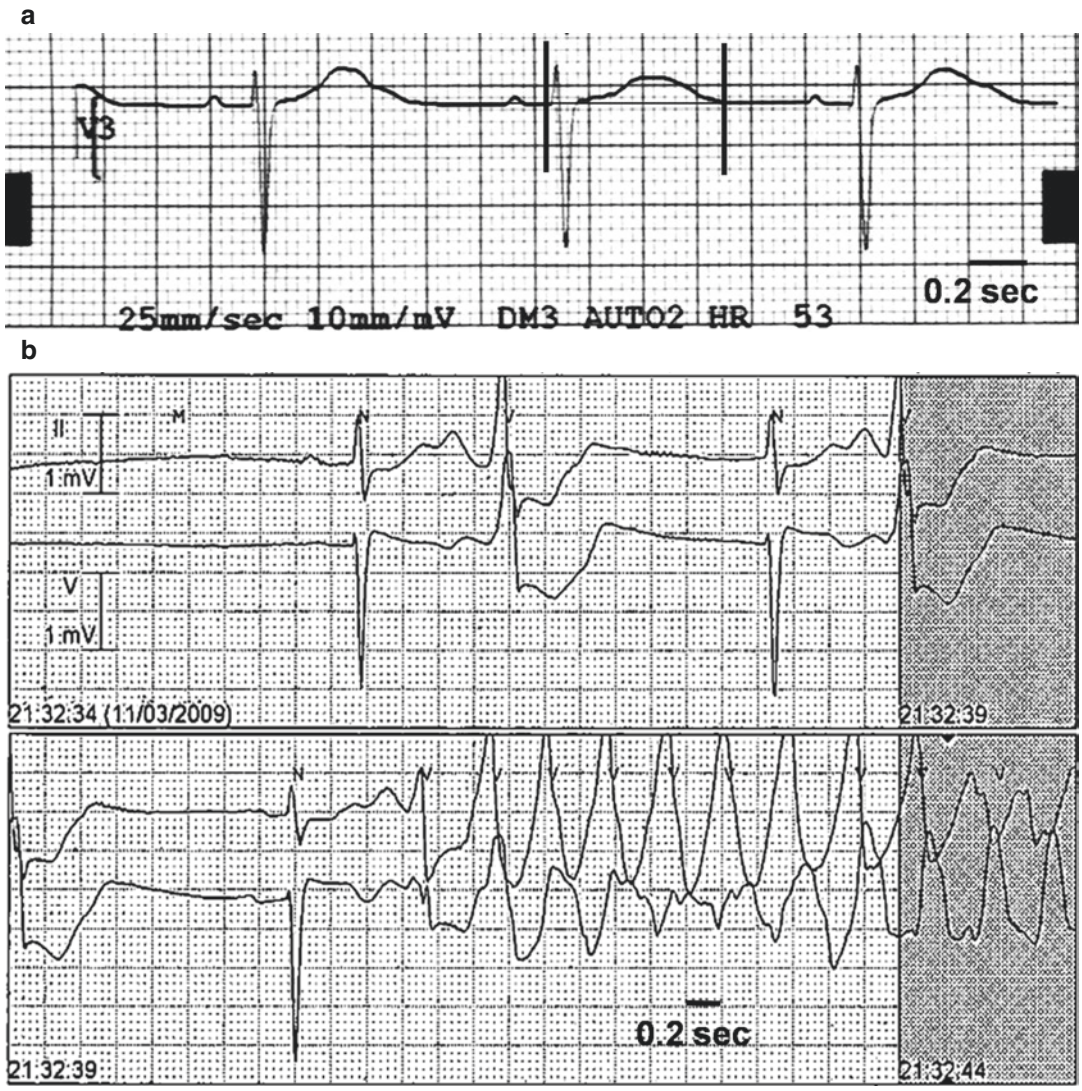


**Fig. 13.2** QTc prolongation and torsades de pointes in a patient with circulating anti-Ro/SSA antibodies. ECG strip in sinus rhythm (**a**) and during torsades de pointes (**b**) from a patient who is anti-Ro/SSA positive and has a QTc of 620 ms. Vertical lines in lead V3 show QT interval. (Reproduced/adapted from Lazzerini et al. [173], with permission from Wolters Kluwer Health)

comparing anti-Ro/SSA-positive vs. anti-Ro/SSA-negative adults with CTD ($p$ = 0.06). Moreover, two studies performed in adult SLE patients [71, 181] found that anti-Ro/SSA positivity was slightly more frequent among subjects with QTc prolongation ($p$ = 0.08) [181] and vice versa the percentage of patients presenting with a prolonged QTc was slightly higher in the anti-Ro/SSA-52kD-positive group, but not significantly for wide confidence intervals (Tables 13.5 and 13.6) [71].

Altogether, these data strongly support the existence of a clinical association between anti-Ro/SSA and LQTS. Nevertheless, it should be remarked that some authors found conflicting results, both in newborns [182, 183] and in adults [56, 62, 74, 88] (Tables 13.5 and 13.6), and, even in positive studies, prevalence of QTc prolongation varied significantly, from 10% to 60% [64, 161]. Several factors may account for such discrepancies throughout the studies, including disease-specific differences in autoantibody titers (specifically the anti-Ro/SSA-52kD subtype [184], by most authors not separately evaluated), as well as QTc measurement methods and QTc prolongation cutoffs used. Moreover, electrophysiological considerations related to the aptitude of anti-Ro/SSA to concomitantly interact with multiple ion channels on the cardiomyocyte could also contribute to explain discrepancies [160, 185] (see below *Pathogenesis and pathophysiology* and chapter titled "Pathogenesis of Autoimmune-Associated LQTS").

Recent clinical evidence preliminary suggests that anti-ion channel autoantibodies other than anti-Ro/SSA might be also responsible for acquired LQTS and life-threatening arrhythmias [160] (Table 13.7). Anti-$K_v$1.4 antibodies, recognizing the cardiac channel that by conducting $I_{to}$ critically determines the early repolarization phase of the AP [186], are found in up to 20% of patients with myasthenia gravis (MG) [187–189]. The group of Suzuki demonstrated that anti-$K_v$1.4-positive MG subjects frequently showed QTc prolongation (15–35%) [188, 189], also providing evidence of an association between such electrocardiographic abnormality and TdP/SCD [189]. Agonist-like anti-L-type $Ca^{++}$ antibodies exerting APD-prolonging effects were detected in a significant percentage of patients with idiopathic and ischemic cardiomyopathies, where represented independent predictors of ventricular tachycardia and SCD [190, 191]. However, although electrophysiological considerations predict that circulating anti-L-type $Ca^{++}$ antibodies may be associated with a LQTS electrocardiographic phenotype [190,

**Table 13.7** Clinical studies demonstrating an association between anti-Kv1.4 or agonist-like anti-L-type $Ca^{++}$ channel antibodies and QTc prolongation/ventricular arrhythmias/SCD

| Authors | Study population | Antibody+ patients (*n*) | Antibody- patients (*n*) | Main results |
|---|---|---|---|---|
| Suzuki et al. [188] | MG patients | 11 | 45 | QTc prolongation prevalence significantly higher in anti-Kv1.4-positive patients (36% vs. 0%) |
| Suzuki et al. [189] | MG patients | 70 | – | QTc prolongation in 14% of anti-Kv1.4-positive patients, who presented high prevalence (20%) of fatal QT-associated arrhythmias |
| Xiao et al. [190] | DCM patients | 39 | 41 | Increased incidence of ventricular tachycardia and SCD in anti-L-type $Ca^{++}$ channel-positive patients; anti-L-type $Ca^{++}$ channel positivity was the strongest independent predictor of SCD |
| Yu et al. [191] | DCM patients | 43 | 689 | Increased incidence of SCD in anti-L-type $Ca^{++}$ channel-positive patients; anti-L-type $Ca^{++}$ channel positivity was an independent predictor of SCD |
| | ICM patients | 51 | 1048 | |

*MG* myasthenia gravis, *QTc* corrected QT interval, *DCM* dilated cardiomyopathy, *ICM* ischemic cardiomyopathy, *SCD* sudden cardiac death

192], studies specifically focused at determining occurrence and prevalence of QTc prolongation in these patients are currently lacking.

## Molecular Basis and Pathophysiology

An increasing body of experimental data point to hERG-$K^+$ channel as the molecular target responsible for anti-Ro/SSA-associated LQTS (Table 13.8) [160–162, 172, 173, 193]. The chapter titled "Pathogenesis of Autoimmune-Associated LQTS" covers the basic aspects of the pathogenesis. Notably, it is well demonstrated that anti-Ro/SSA can also block cardiac $Ca^{++}$ channels [165, 194–197], and this multichannel electrophysiological interference could contribute to explain the above-reported discrepancies in clinical studies on QTc prolongation. In fact, simulation studies support the hypothesis that a concomitant inhibitory effect of anti-Ro/SSA on $I_{CaL}$ during the plateau phase can partially counteract the $I_{Kr}$ inhibition-dependent prolongation of APD and as a result determines the net duration of QTc in vivo [193]. Nevertheless, since $I_{Kr}$ is activated after the peak of T wave [186, 198], specific measurement of $T_p$-$T_{end}$ interval on the electrocardiogram should in any case reveal if anti-Ro/SSA are interfering on the ventricular repolarization of a patient. Accordingly, the recent demonstration by Tufan et al. [178] that Tp-Te is significantly prolonged in anti-Ro/SSA-52kD-positive CTD subjects, also when the QTc was found normal, confirms this view and points to this parameter as a better reflection of the anti-Ro/SSA-mediated $I_{Kr}$ inhibition in vivo.

Regarding the other anti-ion channel autoantibodies possibly associated with LQTS, much less information on the potential underlying mechanisms is currently available (Table 13.8). Although it is proposed that anti-Kv1.4 antibodies can prolong QTc by inhibiting $I_{to}$ via direct binding with the channel [187], at the moment no mechanistic studies specifically tested such a hypothesis. The evidence that some anti-Kv1.4-positive MG patients developed signs of myocarditis suggests that besides functional interference, also inflammatory heart changes may contribute to LQTS and TdP occurrence [188, 189]. More investigated are the molecular arrhythmogenic mechanisms of agonist-like anti-L-type $Ca^{++}$ channel antibodies, probably triggered by an increased $Ca^{++}$ influx during the AP plateau phase. Indeed, experimental evidence indicates that such autoantibodies can directly activate the channel by recognizing an intracellular epitope at the N-terminus of the $Ca_v1.2$ subunit [190, 192]. Xiao et al. demonstrated that affinity-purified autoantibodies from dilated cardiomyopathy patients enhance $I_{CaL}$ in oocytes, prolong APD, and promote early after-

**Table 13.8** Effects of anti-ion channel autoantibodies on cardiac ion currents, ventricular APD, and QT interval: evidence from in vitro and animal studies

| Autoantibody | Functional effect | Ion current | Binding site | APD | QTc interval |
|---|---|---|---|---|---|
| Anti-hERG-$K^+$ channel (anti-Ro/SSA) | Inhibiting [162, 172, 173, 193] | $I_{Kr}$ decrease [162, 172, 173, 193] | Pore region (extracellular loop between S5-S6 of D1) [162, 173, 193] | Prolongation [162, 193] | Prolongation [162, 193] |
| Anti-L-type -$Ca^{++}$ channel | Agonist-like [190, 192] | $I_{CaL}$ decrease [190, 192] | Intracellular N-terminus of D1 [190, 192] | Prolongation (with EAD occurrence and VT induction) [190] | NA |
| Anti-$K_v1.4$ channel | Inhibiting[a] | $I_{to}$ decrease[a] | NA | NA | NA |

*hERG* human Ether-a-go-go-Related Gene $K^+$ channel, $I_{Kr}$ rapidly activating component of the delayed outward rectifying current, $I_{CaL}$ L-type calcium current, $I_{to}$ transient $K^+$ outward current, S5–S6 5th–6th transmembrane segments, *D1* domain 1 of the channel $\alpha_1$ subunit, *AP* action potential, *APD* action potential duration, *EADs* early afterdepolarizations, *VT* ventricular tachycardia, *QTc* corrected QT interval, *NA* data not available

[a]Proposed, as no direct evidence is currently available

depolarizations in rat ventricular myocytes, also inducing ventricular tachycardia in perfused hearts [190, 192].

## Conclusions and Clinical Perspectives

Inflammation and autoimmunity are increasingly recognized as causative factors of LQTS and TdP in patients with immuno-inflammatory diseases, but also in apparently healthy subjects, where asymptomatic low-grade chronic inflammation and/or circulating anti-ion channel autoantibodies (particularly anti-Ro/SSA) might represent occult predisposing factors for unexpected life-threatening arrhythmias/SCD [13, 160]. In fact, likewise other better recognized determinants of LQTS, both genetic [199] and acquired [200], inflammatory and autoimmune mechanisms alone are not able in most cases to prolong QTc as critically as to precipitate TdP development. Nevertheless, by reducing the ventricular repolarization reserve [201], they could significantly enhance the risk of malignant arrhythmias when other classical QT-prolonging factors (drugs, electrolyte imbalances, genetic mutations/polymorphisms, etc.) are concomitantly present [33, 173, 202–205]. However, the potential impact of inflammatory and autoimmune channelopathies on ventricular repolarization is to date largely overlooked in the clinical practice and therefore poorly considered in terms of diagnostic and therapeutic implications in LQTS, even when an immuno-inflammatory disease is clinically evident. In this view, increasing awareness to the existence of these forms is essential to identify and adequately manage those patients. Indeed, systemic inflammatory activation, also subclinical, may be easily revealed by routine markers, particularly CRP. Anti-Ro/SSA testing is largely available in the clinical practice worldwide, although IWB technique is recommended for detecting arrhythmogenic subtypes [64, 173].

Given that arrhythmogenic effects of cytokines and autoantibodies are due to a functional and thereby potentially reversible electrophysiological interference with the channel function [13, 52, 160], anti-inflammatory and immunomodulating interventions might represent an innovative anti-arrhythmic approach in a number of subjects. Accordingly, preliminary data suggest a rapid effectiveness of immunosuppressive therapies in reducing QTc duration in patients with inflammatory or anti-Ro/SSA-associated LQTS [33, 59, 99, 206]. Moreover, based on ex vivo studies with sera from anti-Ro/SSA-positive TdP subjects [173] or affinity-purified anti-L-type $Ca^{++}$ channel autoantibodies from DCM patients [190, 192], showing how competing peptides can prevent autoantibody recognition of the channel binding site, as well as electrophysiological changes and VA occurrence, short decoy peptide-based therapy distracting the pathogenic antibodies could be also intriguingly proposed in autoimmune channelopathies.

Finally, induction of functional anti-ion channel antibodies by peptide vaccination might represent an original anti-arrhythmic approach in inherited channelopathies, specifically in congenital LQTS patients refractory to conventional treatments, as recently hypothesized by Li et al. [207]. Indeed, these authors demonstrated that immunization against $K_v1.7$ channel peptide accelerates cardiac repolarization and ameliorates drug-induced QTc prolongation in a rabbit model via induction of agonist-like anti-$K_v1.7$ antibodies increasing $I_{Ks}$. Based on such theoretical premises, the evidence that guinea pig vaccination with a hERG channel pore-derived peptide generates $I_{Kr}$-inhibiting antibodies which slow ventricular repolarization [193] might also lead to speculate on a therapeutic potential for hERG channel immunization in selected patients with congenital short QT syndrome.

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# Part III

# Pathophysiology, Molecular Biology, and Clinical Aspects of Early Repolarization Syndromes

# 14 Genetics, Molecular Biology, and Emerging Concepts of Early Repolarization Syndrome

Charles Antzelevitch and Gregory Dendramis

## Abbreviations and Acronyms

| | |
|---|---|
| AP | action potential |
| ECG | electrocardiogram |
| EG | electrogram |
| ERS | early repolarization syndrome |
| BrS | Brugada syndrome |
| JWS | J wave syndromes |
| LV | left ventricle |
| P2R | phase-2-reentry |
| RFA | radiofrequency ablation |
| RVOT | right ventricular outflow tract |
| VF | ventricular fibrillation |
| VT | ventricular tachycardia |

## Introduction

An early repolarization pattern (ERP) in the electrocardiogram (ECG), characterized by a distinct J wave or J point elevation (when part of the J wave is "buried" inside the QRS), a notch or slur of the terminal part of the QRS (with or without ST segment elevation), has traditionally been viewed as benign [1, 2]. When accompanied by arrhythmic symptoms, ERP is referred to as early repolarization syndrome (ERS) and is considered to be one of the J wave syndromes.

The appearance of prominent J waves in the ECG and their association with sudden cardiac death (SCD) due to life-threatening cardiac arrhythmias has long been recognized and reported in clinical cases of hypothermia [3–5], hypercalcemia [6, 7], and ischemia [8]. Accentuated J waves have recently been associated more with inherited life-threatening cardiac arrhythmia disorders including Brugada syndrome (BrS) and ERS, the so-called J wave syndromes (JWS).

The risk for SCD in patients afflicted with ERS was not fully appreciated until recently. Its benign nature was challenged in 2000 [9] on the basis of experimental data obtained using canine ventricular wedge preparations [9–12] showing that an ERP was associated with imminent development of polymorphic ventricular tachycardia and fibrillation (VT/VF). Clinical evidence in support of this hypothesis was provided 8 years later by Haïssaguerre et al. [13], Nam et al. [14], and Rosso et al. [15].

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## Diagnosis of ERS

*ERP* is most often encountered in apparently healthy individuals, particularly in young males, black individuals, and athletes. Early repolarization is recognized with the appearance of: (1) an *end QRS notch (J wave)* or *slur* on the downslope of a prominent R wave (with or without ST segment elevation). The onset of the J wave is referred to as $J_0$, (2) a distinct J wave with a peak (designated as $J_P$) ≥ 0.1 mV in two or more contiguous ECG leads, excluding leads V1–V3; and (3) a QRS duration (measured in leads in which a notch or slur is absent) must be <120 ms. The end of the *J wave* or that of the *QRS slur* or *notch* is designated as $J_T$ (Fig. 14.1) [16].

Electrocardiographically, three distinct forms of ERP are recognized. ER is characterized by distinct J waves, $J_0$ elevation, notch or slur of the terminal part of the QRS and ST segment, or $J_T$ elevation in the lateral (*Type I*), in infero-lateral (*Type II*), or in infero-lateral + (anterior) right precordial leads (*Type III*) [10].

Unlike in BrS, sodium channel blockers generally do not unmask or accentuate the J wave manifestation on the surface ECG of patients with ERS. These agents may indeed reduce the amplitude of the J wave in individuals with ERS [17]. This has been suggested to mean that the pathophysiological basis for ERS and BrS is fundamentally different, but this is not necessarily the case. A recent study by Nakagawa et al. reported that J waves recorded using unipolar LV epicardial leads in ERS patients are augmented following provocative drug testing with a sodium channel blocker, while the J waves concurrently recorded in the lateral precordial leads are diminished, demonstrating that the J waves in the surface leads are reduced because they are engulfed by the widening QRS [17, 18]. Support for the hypothesis that the pathophysiological basis for ERS and BrS is closely related also comes from reports of cases in which ERS transitions into ERS plus BrS phenotypes [19, 20].

Table 14.1 shows the Shanghai Score System recently proposed for the diagnosis of ERS [21].

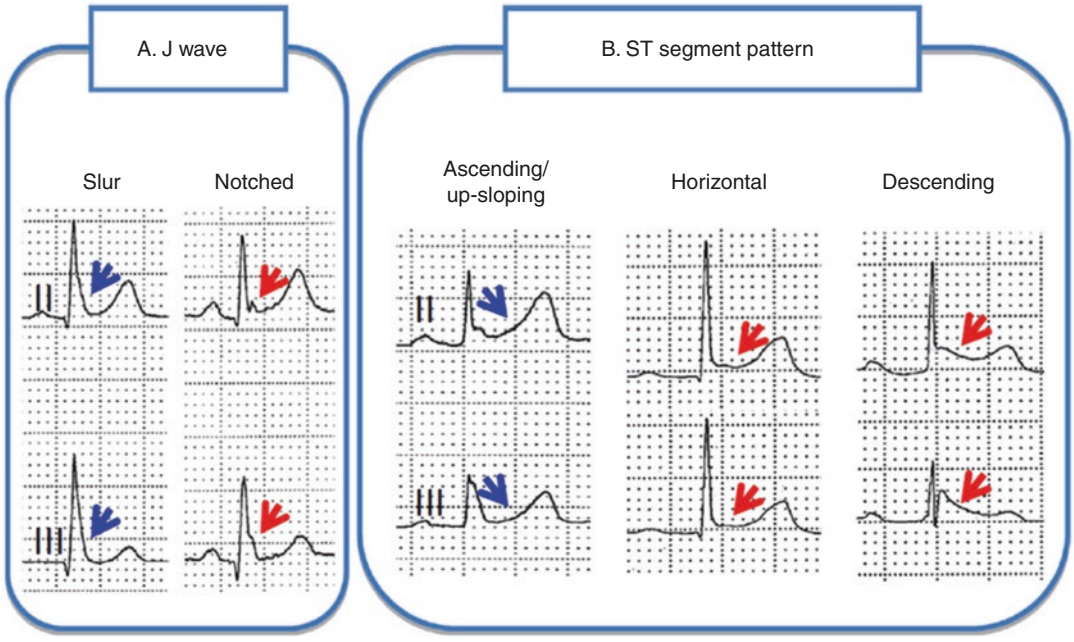


**Fig. 14.1** Different manifestations of early repolarization. (**a**) The J wave may be distinct or appear as a slur. In the latter case, part of the J wave is buried inside the QRS, resulting in an elevation of $J_0$. Patients with a **very prominent J waves have a worse prognosis**. (**b**) The ST segment may be upsloping, horizontal, or descending. **Horizontal and descending ST segments** are associated with a **worse prognosis**. (Reproduced from Antzelevitch et al., Copyright 2016 [21], with permission from Elsevier)

**Table 14.1** Shanghai Score System for diagnosis of early repolarization syndrome

| | Points |
|---|---|
| I. Clinical history | |
| A. Unexplained cardiac arrest, documented VF, or polymorphic VT | 3 |
| B. Suspected arrhythmic syncope | 2 |
| C. Syncope of unclear mechanism/unclear etiology<br>*∗Only award points once for highest score within this category* | 1 |
| II. Twelve-lead ECG | |
| A. ER ≥ 0.2 mV in ≥2 inferior and/or lateral ECG leads with horizontal/descending ST segment | 2 |
| B. Dynamic changes in J point elevation (≥ 0.1 mV) in ≥ inferior and/or lateral ECG leads | 1.5 |
| C. ≥ 0.1 mV J point elevation in at least 2 inferior and/or lateral ECG leads<br>*∗Only award points once for highest score within this category* | 1 |
| III. Ambulatory ECG monitoring | |
| A. Short-coupled PVCs with R on ascending limb or peak of T wave | 2 |
| IV. Family history | |
| A. Relative with definite ERS | 2 |
| B. ≥ 2 first-degree relatives with a II.A. ECG pattern | 2 |
| C. First-degree relative with a II.A. ECG pattern | 1 |
| D. Unexplained sudden cardiac death <45 years in a first- or second-degree relative<br>*∗Only award points once for highest score within this category* | 0.5 |
| V. Genetic test result | |
| A. Probably pathogenic ERS susceptibility mutation | 0.5 |
| **Score (requires at least 1 ECG finding)** | |
| ≥ 5 points: Probable/definite ERS | |
| 3–4.5 points: Possible ERS | |
| < 3 points: Nondiagnostic | |

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*ER* early repolarization, *ERS* early repolarization syndrome, *PVC* premature ventricular contraction, *VF* ventricular fibrillation, *VT* ventricular tachycardia

A similar scoring system was proposed for BrS and recently validated by Kawada and co-workers [22]. Clinical validation of the ERS scoring system has not as yet been reported.

## Prevalence of ERP and Arrhythmic Risk

It is important to recognize that the discovery of a J wave on a routine ECG should not be routinely interpreted to be a marker of risk for SCD. Rosso et al. reported that the presence of a J wave on the ECG increases the probability of VF from 3.4:100,000 to 11:100,000 [15, 23]. However, careful attention needs to be paid to subjects with "high-risk" ER or J waves.

J waves in the lateral ECG leads have a high prevalence but are generally associated with a very low arrhythmic risk. Patients displaying a rapidly ascending ST segment elevation also have a high prevalence and low risk. In contrast, J waves appearing in the inferior leads or inferolateral leads are associated with a much higher risk, particularly when displaying a flat or descending ST segment [24]. J waves appearing globally in the ECG have a very low prevalence, but are thought to be associated with a relatively high level of arrhythmic risk as are subjects resuscitated from cardiac arrest [21].

The prevalence of an ER pattern in the inferior and/or lateral leads with a J point ($J_0$) elevation of ≥0.1 mV ranges between 1% and 24% and for $J_0$ elevation of ≥0.2 mV ranges between 0.6% and 6.4% [25–27]. Unlike BrS, where prevalence is high in individuals of Asian or Southeast Asian origin, no significant regional differences are reported in the prevalence of an ER pattern [28].

## Similarities and Difference Between BrS and ERS

ERS and BrS have several clinical similarities, which once again suggest a closely related pathophysiology [11, 19, 29–31]. Males predominate in both syndromes [32, 33]. In general, the occurrence of accentuated J waves and ST segment elevation is concurrent with bradycardia or pauses [34, 35], and thus VF often occurs during sleep or at a low level of physical activities [17, 36]. They may be totally asymptomatic

until presenting with cardiac arrest. The highest incidence of VF or SCD occurs in the third decade of life in both syndromes [37].

ERS and BrS also share similarities with respect to the response to pharmacological therapy. Electrical storms (and the associated accentuated J waves) can be suppressed with β-adrenergic agonists [38–41]. Chronic oral administration of quinidine [42, 43], bepridil [40], denopamine [38, 44], and cilostazol [38, 40, 44–48] is reported to prevent VT/VF in both syndromes, probably secondary to inhibition of $I_{to}$ and/or augmentation of $I_{Ca}$ [14, 45, 49].

There are a number of differences between the two syndromes. These include: (1) the region of the heart most affected (RVOT in BrS vs. inferior LV in ERS); (2) the incidence of late potentials in signal-averaged ECGs (SAECG), 60% in BrS/7% in ERS [17]; (3) greater elevation of $J_O$, $J_P$, or $J_T$ (ST segment elevation) in response to sodium channel blockers in BrS vs. ERS; (4) higher prevalence of atrial fibrillation in BrS vs. ERS [50]; and (5) the presence of subtle structural abnormalities in BrS, which as yet have not been reported in ERS [51].

Steep and localized repolarization gradients in the inferior and lateral regions of the LV have been reported in ERS patients in conjunction with normal ventricular activation [52]. In contrast, fractionated electrogram activity was recorded in the RVOT of BrS patients in addition to a steep dispersion of repolarization gradients [53].

Several studies indicate that ERS patients are at greater risk of VF during hypothermia [54–58] as well as in the event of an acute myocardial infarction [59]. BrS patients are known to be at greater risk of VF in febrile states [60, 61] as well as when accompanied by an ER pattern in the infero-lateral leads [62].

## Genetics

ERS has been associated with variants in seven different genes (Table 14.2).

An ER pattern in the ECG is known to be familial [63–65]. Consistent with experimental findings that $I_{K\text{-}ATP}$ activation can generate an ER pattern in canine ventricular wedge preparations, variants in *KCNJ8* and *ABCC9*, responsible for the pore-forming and ATP-sensing subunits of the $I_{K\text{-}ATP}$ channel, have been reported in patients with ERS [66–68]. Loss-of-function variations in the α1, β2, and α2δ subunits of the cardiac L-type calcium channel (*CACNA1C*, *CACNB2*, and *CACNA2D1*) and the α subunit of Nav1.5 and $Na_V1.8$ (*SCN5A* and *SCN10A*) have also been reported in patients with ERS [69–71].

**Table 14.2** Gene defects associated with the early repolarization and Brugada syndromes

| | Locus | Gene/protein | Ion channel |
|---|---|---|---|
| ERS1 | 12p11.23 | *KCNJ8, Kir6.1* | $\uparrow I_{K\text{-}ATP}$ |
| ERS2 | 12p13.3 | *CACNA1C, $Ca_v1.2$* | $\downarrow I_{Ca}$ |
| ERS3 | 10p12.33 | *CACNB2b, $Ca_vβ2b$* | $\downarrow I_{Ca}$ |
| ERS4 | 7q21.11 | *CACNA2D1, $Ca_vα2δ1$* | $\downarrow I_{Ca}$ |
| ERS5 | 12p12.1 | *ABCC9, SUR2A* | $\uparrow I_{K\text{-}ATP}$ |
| ERS6 | 3p21 | *SCN5A, $Na_v1.5$* | $\downarrow I_{Na}$ |
| ERS7 | 3p22.2 | *SCN10A, $Na_v1.8$* | $\downarrow I_{Na}$ |

Listed in chronological order of discovery

It is important to point out that only a small fraction of identified genetic variants in the genes associated with BrS and ERS have been examined using functional expression studies to ascertain causality and establish a plausible contribution to pathogenesis. The lack of functional or biological validation of mutation effects remains the most severe limitation of genetic test interpretation, as highlighted by Schwartz et al. [72].

To date a large number of variants remain in "genetic purgatory" and this number will only increase as the use of exome/genome sequencing becomes more utilized. This then demands the development and utilization of a uniform variant repository that would include clinical assertions and evidence for variant classification. Even with these issues, the emergence of exome/genome sequencing holds promise for the opportunity to study genetic variation like never before, holding the promise of improvements in diagnostic, prognostic, and therapeutics for the JWSs and the other heritable cardiac channelopathies.

Recent efforts have led to exhaustive studies using American College of Medical Genetics

and Genomics and Association for Molecular Pathology guidelines of genes and variants currently catalogued as deleterious and causative of inherited syndromes. This has been performed for BrS and the result is that 20 of the 21 genes identified as being causative of the disease have been reclassified as *disputed* with regard to any assertions of disease causality. Such a rigorous study has yet to be performed for ERS. Accordingly, we recommend that great care be taken with these previously reported genes and variants for ERS until their clinical validity can be further tested.

## Ionic and Cellular Mechanisms of ERS

The first studies to explore the basis of J point elevation in the ECG appeared in the early 1950s. Osborn reported the appearance of J point elevation secondary to severe hypothermia and acidosis in a canine model. Although it was unclear which of the two factors caused the ECG change, he speculated that the appearance of J waves in lead II of the surface ECG represented a "current of injury" J point elevation was consistently associated with VF in the hypothermic dogs [73].

The J wave in the ECG is due to the presence of a prominent $I_{to}$-mediated action potential (AP) notch in ventricular epicardium but not endocardium [31, 74, 75]. The transmural gradient and associated J wave are much greater in the right vs. left ventricle, especially in the region of the RVOT [76]. This is the basis for why BrS is a right ventricular disease. An end of QRS notch, resembling a J wave, may also be due to an intramural conduction delay and these two possibilities could be discerned on the basis of the response to increase in heart rate, with the latter showing accentuation at faster rates [16, 77].

Koncz et al. [78] provided evidence that in ERS, as in BrS, accentuation of transmural gradient of the $I_{to}$-mediated action potential notch in the LV wall is responsible for the repolarization abnormalities underlying the ECG phenotype. This and subsequent studies provided evidence for the hypothesis that an outward shift in the balance of current contributing to the early phase of the left ventricular (LV) epicardial action potential (AP) either via an increase in ATP-sensitive potassium current ($I_{K\text{-}ATP}$) or transient outward current ($I_{to}$) or via a decrease in calcium inward current ($I_{Ca}$) in LV wedge preparations can recapitulate the ECG and arrhythmic manifestations of ERS (Fig. 14.2). Pharmacologic modeling of the genetic defects responsible for ERS, using pinacidil and NS5806 to increase $I_{K\text{-}ATP}$ and $I_{to}$, respectively, and verapamil and acetylcholine (ACh) to reduce $I_{Ca}$, was shown to give rise to a substrate capable of inducing phase 2 reentry and polymorphic VT/VF [78].

Vagal activity has long been implicated in the development of an ER pattern in the ECG [79, 80] and recent clinical observations suggest that parasympathetic tone contributes to the electrocardiographic and arrhythmic manifestations of ERS [81, 82]. Sleep is commonly associated with spontaneous VF in patients with ERS [17].

Koncz et al. also provided evidence in support of the hypothesis that higher levels of $I_{to}$ in the inferior wall can explain why an ER pattern in the inferior ECG leads is associated with a higher risk for sudden cardiac arrest [78].

Rudy and colleagues provided additional evidence for repolarization abnormalities in patients with ERS using noninvasive ECGI techniques. They demonstrated short activation-recovery intervals and marked dispersion of repolarization in the inferior and lateral regions of the LV in ERS patients [52].

## Approaches to Therapy of ERS

### Implantable Cardioverter Defibrillator

The approach to therapy of ERS as recommended in the 2016 HRS/APHRS/EHRA/SOLAECE Consensus Statement is presented in Fig. 14.3. The most effective therapy for the prevention of SCD in high-risk ERS patients is an implantable cardioverter defibrillator (ICD) [83, 84]. However, ICDs are associated with complications, especially in young active individuals [85,

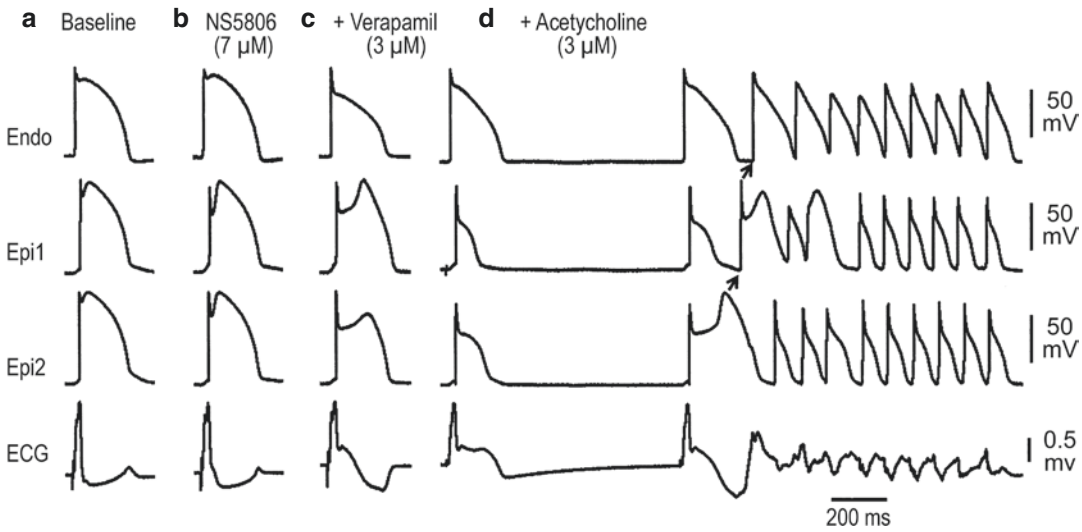


**Fig. 14.2** Cellular mechanism underlying the development of polymorphic VT in ERS. Each panel shows simultaneous recordings from one endocardial (Endo) and two epicardial (Epi) sites together with a pseudo-ECG recorded from a canine left ventricular coronary wedge preparation. (**a**) Control. (**b** and **c**) The combination of calcium channel block using verapamil (3 μM) and accentuation of the transient outward current ($I_{to}$) using (NS5806, 7 μM) caused an ER pattern to develop in the left ventricular wedge preparation, but no arrhythmogenic substrate. (**d**) The addition of ACh, used to mimic increased vagal tone (ACh, 3 μM), led to the development of phase 2 reentry and polymorphic VT/ventricular fibrillation. Basic cycle length = 1000 ms. (Reprinted from Koncz et al., Copyright 2014 [78], with permission from Elsevier)

86], and, over time, inappropriate shocks and lead failure are not uncommon. Remote monitoring can identify lead failure and prevent inappropriate shocks [87]. The advent of subcutaneous ICDs is a welcome innovation associated with fewer complications over a lifetime [88].

## Pacemaker Therapy

Although life-threatening arrhythmias and SCD in JWS are generally associated with slow heart rates, the potential therapeutic benefit for cardiac pacing at relatively fast rates remains largely unexplored [89–91].

## Radiofrequency Ablation (RFA) Therapy

Few clinical data are available with respect to the effectiveness of RFA in patients with ERS. RFA has been shown to suppress arrhythmogenesis by eliminating the extrasystoles precipitating VT/VF in patients with ERS [92–95]. Low-voltage fractionated electrogram activity and high-frequency LP are observed in the LV epicardium of patients with ERS [18]. Nademanee et al. [96] in their seminal studies targeted such activity in the RVOT for RFA in patients with BrS, demonstrating a significantly reduced arrhythmia risk [97]. Additional evidence in support of the effectiveness of epicardial RVOT ablation in BrS patients was provided by Sacher et al., Shah et al., and Brugada and co-workers [98–100]. RF ablation is also an option in BrS patients with frequent appropriate ICD shocks due to repeated electrical storms [101].

Although no clinical data are available with respect to epicardial RFA in cases of ERS, recent experimental evidence of its effectiveness has been reported [102]. Yoon and co-workers [102] showed that in experimental models of ERS, low-voltage fractionated electrical activity and high-frequency late potentials recorded from the epicardial surface of the left ventricle can identify regions of abnormal repolarization responsible for ventricular tachycardia/ventricular fibrillation

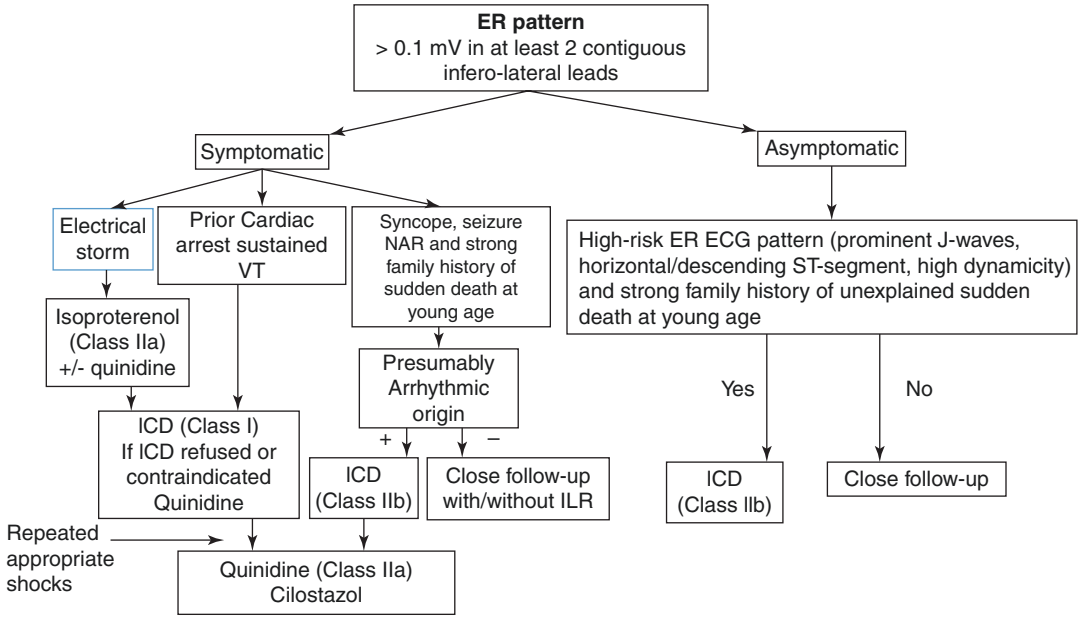


**Fig. 14.3** Indications for therapy of patients with early repolarization syndrome. Recommendations with class designations are taken from the 2013 HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. Heart Rhythm [147] and the 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death [148]. Recommendations without class designations are derived from unanimous consensus of the authors of the 2016 HRS/APHRS/EHRA/SOLAECE J wave syndrome expert consensus report [21]. (Reproduced from Antzelevitch et al., Copyright 2016 [21], with permission from Elsevier. ER early repolarization, ICD implantable cardioverter defibrillator, ILR implantable loop recorder, NAR nocturnal agonal respiration, VT ventricular tachycardia)

and that radiofrequency ablation of these regions in left ventricular epicardium can suppress VT/VF by destroying regions of ER.

## Pharmacologic Therapy

ICD implantation often leads to complications, particularly in children and active young adults, and is unaffordable in many regions of the world. A pharmacologic approach to therapy is therefore desirable. Because the presence of a prominent $I_{to}$ is believed to be a prerequisite for the development of both *ERS* and *BrS*, partial inhibition of this current is thought to be effective regardless of the underlying ionic or genetic basis for these syndromes. Unfortunately, ion channel-specific and cardio-selective $I_{to}$ blockers are not available at present. The best drug currently available in the clinic capable of blocking $I_{to}$ is *quinidine*. Quinidine was first recommended as therapy for BrS by our group in 1999 based on experimental evidence obtained using coronary-perfused RV wedge models of BrS [11, 103–107]. A large volume of clinical data point to the effectiveness of quinidine in BrS [40, 43, 47, 108–120]. Belhassen, Viskin, and colleagues, who pioneered the use of quinidine in VF [121], reported a 90% efficacy in prevention of VF induction following treatment with quinidine, despite the use of very aggressive protocols of extrastimulation [121]. Much less information is available relative to the effectiveness of quinidine in ERS. The effectiveness of quinidine to suppress arrhythmogenesis in a case of ERS was reported by Sacher and co-workers in 2014 [122].

Agents that increase the L-type calcium channel current, such as isoproterenol or orciprenaline, are useful as well in treating patients with JWS [11, 40, 44, 117, 123, 124]. Augmentation of $I_{Ca}$

prevents arrhythmogenesis associated with *JWS* by opposing early repolarization forces during phase 1 of the action potential, thus restoring the epicardial AP dome in both ERS [125] and BrS [126]. Isoproterenol, sometimes in combination with quinidine, has been used to successfully control VF storms and normalizing ST elevation [38–41, 101, 109, 110, 115, 127–137].

Another promising pharmacologic approach for JWS is *cilostazol*, a phosphodiesterase (PDE) III inhibitor [40, 44, 46] which normalizes the ST segment by augmenting $I_{Ca}$ as well as by reducing $I_{to}$ secondary to an increase in cAMP and heart rate [138]. Iguchi and co-workers [45] reported a case in which cilostazol was effective in suppressing recurrent VT in a patient with ERS.

*Milrinone*, another PDE III inhibitor, has been identified as a more potent alternative to cilostazol in suppressing ST segment elevation and arrhythmogenesis in an experimental models of ERS [125, 139] and BrS [106, 126]. No clinical data are available as yet.

*Wenxin Keli*, a traditional Chinese medicine, has recently been shown to inhibit $I_{to}$ and suppress arrhythmogenesis in experimental models of *BrS* when combined with low concentrations of quinidine (5 μM) [105]. No experimental or clinical data are available concerning its efficacy in ERS.

Agents that increase peak and late $I_{Na}$, including *bepridil* and *dimethyl lithospermate B* (dmLSB), have been suggested to be of value in both ERS and BrS. Bepridil has been reported to suppress VT/VF in several studies of patients with ERS or BrS [40, 48, 137, 140–143]. The drug's action is thought to be mediated by: (1) inhibition of $I_{to}$, (2) augmentation of $I_{Na}$ via upregulation of the sodium channels [144], and (3) prolongation of QT interval at slow rates, thus increasing the QT/RR slope [140, 142].

*Dimethyl lithospermate B,* an extract of Danshen, a traditional Chinese herbal remedy, has been reported to slow inactivation of $I_{Na}$, thus increasing $I_{Na}$ during the early phases of the AP, thereby reducing the AP notch and restoring the epicardial AP dome and, in the process, suppressing arrhythmogenesis in experimental models of BrS [145]. No clinical data are available as yet.

*Acacetin,* a natural flavone produced by several plants including snow lotus, damiana, and black locust, has proved useful for multiple purposes, including treatment of rheumatoid arthritis, impotence, irregular menses, asthma, bronchitis, cough, and altitude sickness [146]. *Acacetin* has been shown to inhibit $I_{to}$ in human atrial myocytes in a frequency-dependent manner [146]. The potential ameliorative effect of this agent in experimental models of BrS and ERS is currently under study by our group and holds promise as a safe and effective therapy for JWS.

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# 15 Electrocardiographic J Wave and Cardiovascular Risk in the General Population

Heikki V. Huikuri

## Preface

The electrocardiographic (ECG) pattern of early repolarization (ER) has historically been regarded as a benign ECG variant, but during the past few years, this concept has been challenged based on multiple reports linking the ER pattern in the inferior and/or lateral leads of the standard 12-lead ECG with an increased risk of sudden cardiac death. Case-control studies have unanimously shown that patients resuscitated from cardiac arrest without a known etiology have higher prevalence of ECG pattern of ER in the inferior and/or lateral leads (=ER syndrome) than matched controls. Epidemiological follow-up studies have also shown that ER pattern carries an increased risk of future arrhythmic death. Although the prevalence of ER pattern is relatively high especially in young asymptomatic males, there is increasing information about the ECG and phenotype characteristics, which can help in separating ER syndrome from benign patterns of ER. This chapter presents the current knowledge of the prevalence and significance of ECG pattern of ER in general population.

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## History of Early Repolarization ECG Pattern

Early repolarization (ER) is a common electrocardiographic (ECG) pattern characterized by *J* point and ST segment elevation in 2 or more contiguous leads. The presence of ER pattern in the precordial leads (V1–V3) is still considered a benign phenomenon and it has been generally viewed as a marker of good health. Most recommendations for standardization and interpretation of the ECG include a statement that the term ER is used to describe a normal QRS-T variant with ST segment elevation especially in the left precordial leads [1], and most clinicians have considered ER as nonspecific ST elevation.

Shipley and Hallaran referred to QRS notching and slurring in the electrocardiogram (ECG) of 200 apparently healthy subjects in 1936 using four unconventional ECG leads [2]. Myers et al. reported in 1947 that an elevation of the RS-T of 0.05–0.2 mV may be considered a normal variant, provided that the ST segment begins to rise above the RS-T junction in an arc with upward concavity to end in a tall upright T wave [3]. Later, Goldman noted in 25 subjects that there was elevation of 0.2–0.4 mV of the RS-T segment in leads V4–V6, which was regarded as a normal variant and occurred more commonly in young black males [4]. In 1961, Wasserburger et al. also described the normal RS-T segment elevation variant, which was termed "early repolarization" [5].



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Osborn described the classic *J* wave in experimental hypothermia in 1953 [1]. Dogs subjected to hypothermia developed spontaneous ventricular fibrillation (VF) that was preceded by the development of *J* waves [6]. The *J* wave, which was attributed to a current of injury (hence the term "J"), was later coined the Osborn wave. Later experiments have demonstrated that *J* waves are the ECG reflection of increased dispersion of repolarization [7–9]. After some case reports pointing to an arrhythmogenic potential of ER, a series of case-control studies have described an apparent over-presentation of *J* waves, i.e., terminal QRS notching and/or slurring in infero-lateral leads with and without ST segment elevation, in patients with idiopathic VF [10–15]. Thereafter infero-lateral QRS notching and slurring, referred to as ER ECG pattern, have also been demonstrated to carry an increased risk of sudden cardiac death (SCD) and mortality in general population [16–18].

There are conflicting data regarding the prognosis of subjects with ECG pattern of ER, but as discussed later, there has been a major lack of consistence between these studies in the pattern definition as well the duration of the follow-up [16–23]. There seems to be variability in the magnitude of risk between different ECG patterns. Especially *J* wave distribution, *J* wave amplitude, and dynamicity and morphology of the ST segment modify the risk. This article summarizes the current knowledge of the prevalence of ER and of arrhythmic risk associated with various *J* wave patterns and intentionally elides any debate on the underlying pathophysiology as this has been discussed in several articles [7–9].

## Terminology and Definitions

A recent consensus paper was aimed at harmonizing the terminology and definitions of *J* waves and ER patterns from standard 12-lead ECG [24]. Notched *J* was defined as notching in the final 50% of the downslope of the R wave occurring in the final segment of the QRS complex (Fig. 15.1a). It should be distinguished from a notch in the middle part of the downslope of R wave, which should be classified as the fragmentation of the QRS complex. Slurred *J* wave is the apparent slowing of the waveform at the end of the QRS complex and it merges with the ST segment (Fig. 15.1b). A slur should also occur in the final 50% of the downslope of the R wave. The consensus paper recommends the following terminology: (i) *J* onset (Jo) should represent the onset of a notch, (ii) *J* peak (Jp) should represent the peak of a notch or onset of a slur, and (iii) *J* termination (Jt) should represent the end of a notch or slur (Fig. 15.1a, b). In the case of a slur, Jo and Jp are the same points. The amplitude of early repolarization or *J* point elevation equates with the Jp amplitude. The ST segment slope should be measured from Jt. It should be regarded as horizontal or downsloping if the amplitude of the ST segment 100 ms after Jt is the same or smaller than the amplitude of Jt (Fig. 15.2). The ST segment should be regarded as upward sloping if the amplitude of the ST segment 100 ms after Jt is higher than the amplitude of Jt (Fig. 15.1a, b).

The consensus report proposed new definitions of early repolarization as follows: (i) there is an end QRS notch or slur on the downslope of a prominent R wave. If there is a notch, it should lie entirely above the baseline. The onset of a slur must also be above the baseline, and (ii) Jp $\geq$ 0.1 mV in two or more contiguous leads of the 12-lead ECG, excluding leads V1–V3. If the ST segment is upward sloping and followed by a tall symmetrical upright T wave, the pattern should be described as early repolarization with ascending ST segment. If the segment is horizontal or downward sloping, the pattern should be described as early repolarization with horizontal or descending ST segment.

## Prevalence of Early Repolarization in the Inferior and/or Lateral ECG Leads

ER ECG pattern in the inferior/lateral leads occurs in 1–24% of the middle-aged general population [16–26] (see Table 15.1) and in 15–70% of idiopathic VF cases [3–11]. Male sex is

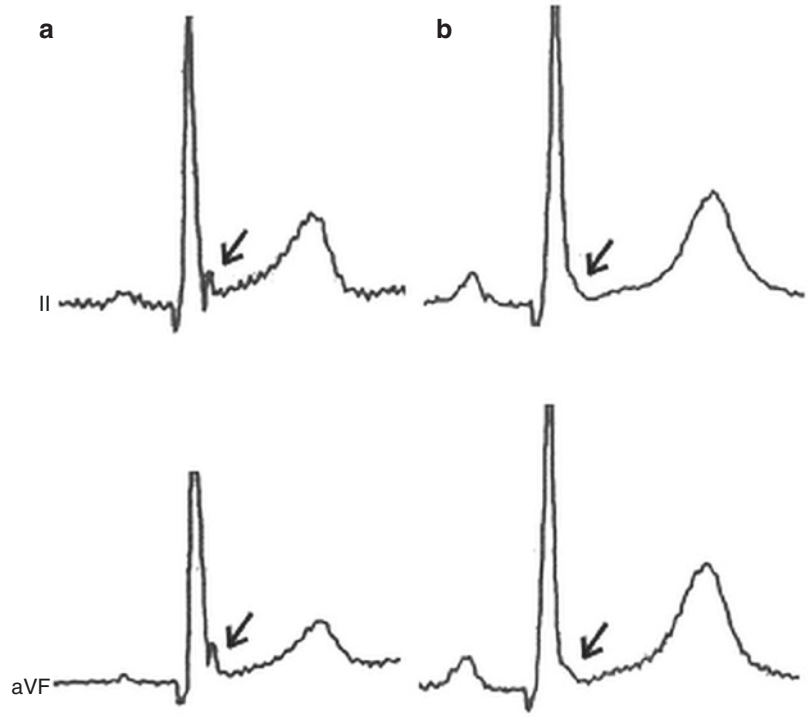


**Fig. 15.1** Example *J* wave patterns in two young and healthy athletes illustrating terminal QRS notching (a) and slurring (b) with rapidly ascending ST segments. (Reprinted with permission from Wolters Kluwer from: Tikkanen et al. [29])


**Fig. 15.2** Example *J* wave patterns in two middle-aged individuals with QRS changes and horizontal ST segments. (Reprinted with permission from Wolters Kluwer from: Tikkanen et al. [29])

strongly associated with ER ECG pattern, since over 70% of subjects with ER are males. The prevalence of ER ECG pattern declines from early adulthood until middle age in males, and gender difference diminishes with age, suggesting a hormonal influence, probably testosterone, on the presence of ER [27]. The ER pattern is more common in young physically active individuals, athletes (up to 40%), and African Americans [28]. There is an increased prevalence of ER reported in Southeast Asians (27%) [18]. ER is associated with high vagal tone, as well as hypothermia. ECG features of bradycardia, prolonged QRS duration, short QT interval, and left ventricular hypertrophy assessed by the Sokolow-Lyon index are also associated with ER [25].

**Table 15.1** Prevalence of early repolarization in inferior and/or lateral leads in different ethnic and age groups

| Study | Number of subjects | Prevalence if ER (%) | ER with horizontal/ descending ST segment (%) | Origin | Age (years) |
|---|---|---|---|---|---|
| Tikkanen et al. [29] | 10,288 | 5,6 | 4,0 | Whites/Finland | 44 ± 8 |
| Noseworthy et al. [25] | 3955 | 6,1 | – | Blacks and Whites/ USA | 40 ± 8 |
| Noseworthy et al. [25] | 5489 | 3,3 | – | Whites/Finland | 49 ± 11 |
| Sinner et al. [17] | 6213 | 13,1 | – | Whites/Germany | |
| Haruta et al. [18] | 5976 | 23,9 | – | Whites/Japan | 43 |
| Olson et al. [19] | 15,141 | 12,3 | – | Blacks and Whites/ USA | 54 ± 6 |
| Tikkanen et al. [29] | 503 | 31,0 | 3,1 | Athletes/USA | 17–24 |
| Sager et al. [26] | 719 | 17,0 | 3,0 | Blacks and Whites/ USA | 8–12 |
| Sager et al. [26] | 755 | 16,0 | 3,0 | Blacks and Whites/ USA | 21–25 |
| Aagaard et al. [20] | 211,920 | 2,2 | – | African Americans/ USA | 18–75 |
| Aagaard et al. [20] | | 1,5 | – | Hispanics/USA | 18–75 |
| Aagaard et al. [20] | | 0,9 | – | Non-Hispanics, Whites/USA | 18–75 |

The ER pattern with rapidly ascending ST segment and tall peaked T waves is commonly observed in healthy, young athletes and is considered as a benign normal variant [29]. Its prevalence declines rapidly with aging. The pattern with horizontal/downsloping ST segment following the *J* wave is not age dependent and its prevalence is relatively consistent (3–4%) in different populations and in different age groups.

## Prognostic Significance of Early Repolarization in General Population

Table 15.2 summarizes the results of studies assessing the prognostic significance of ER pattern in the inferior and/or lateral ECG leads in various populations. It should be emphasized that the ER pattern is more predictive of sudden arrhythmic death than overall cardiovascular mortality or all-cause mortality. Majority of the studies using the criteria by Haissaguerre et al. for diagnosing the ER pattern have shown that the ER, especially in the inferior ECG leads, predicts cardiac and arrhythmic death. The negative studies in this field have usually used different diagnostic criteria for ER pattern, such as ST segment elevation or no ST segment elevation, or

**Table 15.2** Prognosis of patients with early repolarization in inferior and/or lateral ECG leads

| Study | Number of subjects | All-cause mortality (adjusted relative risk) | Arrhythmic death/sudden cardiac death[a] (adjusted relative risk) |
|---|---|---|---|
| Tikkanen et al. [16] | 10,288 | 1.14 /1.03–1.26) | 1.30 (1.00–1.70) |
| Olson et al. [19] | 15,141 | 1.35 (1.24–1.48) | 2.20 (1.63–2.97) |
| Haruta et al. [18] | 5976 | 0.88 (0.80–0.96) | 1.75 (1.10–2.80) |
| Sinner et al. [17] | 6213 | 1.08 (0.95–1.24) | 1.40 (1.13–1.74)[a] |
| Rollin A et al. [23] | 1161 | 2.45 (1.44–1.45) | 5.60 (2.27–11.8)[a] |
| Wu et al. (meta-analysis) [22] | 126,583 | 1.06 (0.85–1.31) | 1.70 (1.19–2.42) |
| Aagaard et al. [20] | 211,920 | 0.98 (0.89–1.07) | – |

included ER in the precordial leads in their analysis. Furthermore, negative studies have usually had a relatively short follow-up and/or have not included sudden cardiac death or arrhythmic death as a major endpoint [21].

The lack of consistency in the diagnostic criteria of ECG pattern of ER may partly explain the large heterogeneity in the prevalence and prognostic significance of this ECG pattern between the different studies. A consensus group has now created criteria for diagnostics, which will hopefully result in more homogeneous interpretations of the ER patterns [23]. Importantly, fragmented QRS complex of the downward sloping R wave should clearly be separated from the ER pattern, which should be linked to the ST segment and occur clearly in the terminal portion of the QRS complex.

The seminal study by Tikkanen et al. showed that ER pattern with a rapidly ascending ST segment is not associated with an increased risk of arrhythmic death, but the pattern with horizontal/downsloping ST segment with a high amplitude of *J* wave (>0.2 mV) is a strong predictor of arrhythmic death [29]. An interesting discovery of this study was that the survival curves for cardiac mortality started to diverge 15 years after the baseline recordings (in the early 1980s) and continued to diverge at a constant rate throughout the follow-up period, even though the treatment and prognosis for patients with cardiac disease overall have improved during recent years [16, 29]. The arrhythmic deaths seemed to occur at a relatively high age (>55 years), in which age group the SCD has been most commonly caused by an acute ischemic coronary event in the Finnish population. This led to the hypothesis that ER pattern may be a trigger of fatal events during myocardial ischemia. Later, in a case-control layout, it was found that there was a significant and independent association in the general population sample between ER, documented in a random ECG recording prior and remote to the event and the risk of sudden cardiac death at the time of an ischemic event [30]. The risk for sudden death was doubled even after multivariate adjustments, with the highest risk being observed for ER with horizontal or descending ST segments, which supported the hypothesis of reduced ischemia tolerance. Although there were demographic and clinical differences between the victims and controls, the association between ER and sudden cardiac death was independent of those factors suggesting, although not confirming, the vulnerability of individuals with ER to suffer malignant arrhythmias during ischemia. Furthermore, the victims of sudden cardiac death had a lower prevalence of previously diagnosed cardiac disease in support to the concept that ER increases the risk of SCD in those subjects who suffer sudden death as their first manifestation of cardiac disease. Furthermore, the risk for ventricular fibrillation during the first 48 hours after myocardial infarction has been shown to be higher in patients who exhibited ER with horizontal/descending ST segments in the premorbid ECG [31, 32]. These studies provide one plausible mechanistic link between ER and risk of SCD: the presence of ER might act as a factor facilitating the occurrence of fatal arrhythmia during an acute ischemic event. In patients with ST elevation myocardial infarction (STEMI), previous ER was more common in patients with subsequent ventricular tachyarrhythmias (26% vs 4%), especially when present in the inferior leads [33]. A recent study has suggested that ER ECG pattern increases the risk of mortality also in chronic heart failure [34]. When these observations are combined, it can be speculated that subjects with ER ECG pattern suffer an increased risk for ventricular fibrillation during events such as those present in myocardial ischemia or heart failure that further shorten the action potential and increase the repolarization dispersion.

The amplitude and dynamicity of *J* waves are major modifiers of risk. In the general population, the risk of arrhythmic death increases significantly if *J* wave amplitude exceeds 0.2 mV, and this observation seems to hold true in a larger scale [16]. Although higher amplitude has been associated with a higher risk, the dynamic nature of *J* waves should also be acknowledged. In some patients both major high amplitude *J* waves and completely normal ECGs can be observed.

Especially in those with the ER syndrome, *J* waves are dynamic and increase in amplitude prior to VF episodes [35–37], and thus the *J* wave amplitude at random ECG does not always accurately define the possible underlying risk. However, when dynamicity of *J* waves is observed, it might be a significant marker of risk as pause-dependent augmentation of *J* waves has been reported to have a 100% specificity and high positive predictive value for idiopathic VF in ER syndrome patients [38–41]. These observations together suggest that higher amplitude and transient augmentation of *J* wave patterns should be recognized as signs of a high risk of fatal arrhythmias. However, it should also be noted that the benign forms of ER followed by rapidly ascending ST segment observed often in young athletes are also rate dependent, i.e., their amplitude increases at slow heart rates [42].

Third major mediator of risk in those with *J* waves seems to be the following ST segment morphology. First observed in unselected general population individuals [29], ER pattern with horizontal or descending ST segment has been shown to possess the highest risk of arrhythmic events in IVF patient samples [43], patients with myocardial ischemia, as well as in other general population samples [30, 31]. The definition of calling an ST segment non-ascending has been that it has to "stay flat" (under 0.1 mV) at least 100 ms after the preceding *J* wave. However, this concept lacks both the sensitivity and specificity as in several IVF patients the *J* waves are followed by ascending ST segments, and horizontal ST segments are observed quite commonly also in healthy individuals without any events on follow-up. However, for example, in patients with IVF and early repolarization those with ascending ST segments usually have other pathological co-findings, such as short QT interval or Brugada-type ECG pattern. Overall, evidence is accumulating in support of the finding that terminal QRS changes in the absence of ST elevation confer the highest risk of fatal arrhythmias.

There is also some evidence of the prognostic significance of *J* wave morphology, as the presence of QRS notching over terminal QRS slurring has been proposed to confer an increased risk of sudden death. Terminal QRS notching has been commonly observed in some IVF patients, and in meta-analysis it has been associated with significant risk of sudden death [22]. This concept however has not uniformly distinguished the risk in all studies and its sensitivity and specificity to predict fatal arrhythmias is weak.

## Characteristics of a Benign ECG Pattern of ER

Overall, not all manifestations of *J* wave patterns across the ECG leads seem to be equally proarrhythmic. In asymptomatic healthy individuals, *J* point elevation with high takeoff ST elevation is most prominently seen in mid-precordial leads, and terminal QRS notching accompanied by ST elevation in left precordial leads is likewise a common finding among young healthy individuals. These classical types of ER have for long been demonstrated to possess a benign prognosis, also in the current era. ER is commonly observed in the young, especially in fit and highly trained athletes, and therefore it is not surprising that our information of the ECG characteristics found in healthy individuals comes mainly from athlete populations, in which the prognosis of ER has also been found to be benign. Prevalence of *J* waves also in the inferior and lateral leads in athletes ranges up to 40%, but in >80% of cases the following ST segments are rapidly ascending [29]. *J* waves with rapidly ascending ST elevations have not predicted a high risk of arrhythmic death in the general population [29] or in IVF patients [43]. Therefore, this ER pattern can generally be considered as a benign phenomenon similar to ER patterns in the precordial leads in asymptomatic subjects. In conclusion, the ECG characteristics of benign ER patterns include *J* wave distribution mainly in the anterior, lateral, or inferior leads, tall peaked R waves, and the hallmark of athlete ER: rapidly ascending ST elevations. These patterns found especially in young and bradycardic athletic males, presumably with high testosterone levels and high Sokolow score, are with the current knowledge most likely normal variants.

## Conclusions

The terminology and the criteria of *J* point elevations, *J* waves, and ER have been under debate in recent years. In addition, it is not completely resolved, whether the ER ECG pattern reflects depolarization or repolarization abnormality. Regardless of terminology and electrophysiological background, the ER pattern has the potential for arrhythmia prediction that can be seen in ECG tracing several years or decades before the actual risk for sudden death.

With the present knowledge, any screening for ER in completely asymptomatic individuals is not legitimate. The identification of individuals with ER patterns at high risk of ventricular arrhythmias and sudden death continues to be a challenge as the various ECG phenotypes are fairly common in the general population, but only a small subset experiences an arrhythmic event. It should be acknowledged that part of the data for risk stratification has been collected from heterogeneous populations, and observations from general population might not be straightforward applicable to those patients with ER syndrome, as the mechanisms of the onset of VF might vary. Given these limitations, on the ECG interpretation, emphasis should be put to the distribution and magnitude of *J* waves, most likely also to the following ST segments, but probably also to the dynamicity of *J* waves in order to unveil those with the highest risk for fatal arrhythmias. Finally, young athletes with a benign ECG pattern of ER should not be alerted to be at any risk for life-threatening arrhythmias.

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# 16 Benign Versus Malignant Early Repolarization Patterns

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Distinguishing the "benign" from the "malignant" patterns of early repolarization is of outmost importance [1–3]. While the former electrocardiogram (ECG) pattern is frequently observed in healthy individuals (particularly young, male [4–6], athletic [7, 8], and of African American origin [5, 9]), the latter is clearly associated with idiopathic ventricular fibrillation (VF) in case-control studies [10–13]. In simple terms, the goal is to identify the isolated individual at increased risk from the "silent majority" who has an excellent prognosis [14].

There have been serious attempts to precisely define and even quantitate each component of the early repolarization pattern in the ECG [15, 16]. However, we believe it is important to keep the approach to the patients with early repolarization as simple as possible [1], and we have called for a separate analysis of the two components of the "early repolarization pattern," namely, the J-wave (or J-point elevation) and the ST-segment [2]. More recently, the importance of a third ECG component, which is the amplitude of the T-wave, has been emphasized [17, 18]. All three components will be first analyzed for the early repolarization related to hypothermia, accepting that hypothermia represents a prototype of highly arrhythmogenic early repolarization [19].

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## The Arrhythmogenic Early Repolarization of Hypothermia

The ECG pattern recorded during hypothermia can be regarded as a classic example of highly arrhythmogenic early repolarization because of the following: (1) As originally described by Osborn in animal studies [20], dogs exposed to hypothermia consistently develop prominent J-waves in their ECG. Moreover, with deeper degrees of hypothermia, the J-waves increase in amplitude (Fig. 16.1a) and virtually all the hypothermic animals develop spontaneous VF [21]. (2) Experiments involving differential cooling of cardiac-wedge preparations [22] or cooling of preparations mimicking early repolarization [23] demonstrate that hypothermia-related J-waves are the ECG reflection of increased dispersion of repolarization caused by disproportionate abbreviation of the epicardial action potential, eventually leading to phase 2 reentry and VF (Fig. 16.1b) [19]. (3) Therapeutic and accidental hypothermia are well-described causes of spontaneous VF in humans [24–26]. Importantly, although the prominent J-wave (also known as "Osborn wave") has been traditionally viewed as the hallmark ECG marker of hypothermia, careful analysis of the ECG recorded during hypothermia reveals that the "arrhythmogenic early repolarization" actually has three characteristics: tall J-waves, a flat ST-segment *without* ST-segment elevation and low-amplitude T-waves (Fig. 16.1). The value of each of these features for telling



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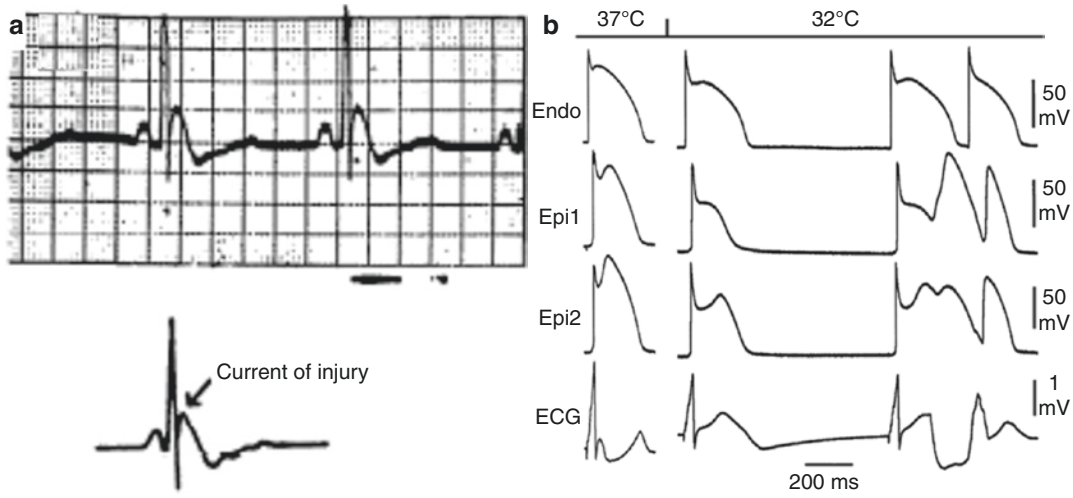


**Fig. 16.1** Prominent J-waves in response to hypothermia in the classic study by Osborn (panel **a**). The prominent J-wave observed during deep hypothermia was labelled by Osborn as "current of injury" because it preceded the onset of spontaneous ventricular fibrillation in studied dogs. Note also the absence of ST-segment elevation and the low-amplitude T-wave. (Reprinted with permission from Osborn [10]). Arrhythmogenic effect of hypothermia in the setting of early repolarization (panel **b**). Traces recorded at 2 epicardial (Epi) and 1 endocardial (Endo) sites, together with a pseudo-electrocardiogram in a model of "early repolarization" created with infusion of NS5806, verapamil, and acetylcholine to the coronary perfusate at 37 °C. The second grouping was recorded 10 minutes after lowering of the perfusate to 32 °C. *Hypothermia in the setting of early repolarization leads to the development of phase 2 reentry and polymorphic ventricular tachycardia*. (Reproduced with permission from Gurabi et al. [23])

apart the benign from the malignant patterns of early repolarization will now be reviewed.

## The Amplitude of the J-Wave

In a case-controlled study comparing 206 patients with idiopathic VF to 412 healthy subjects matched for age, sex, race, and level of physical activity, Haissaguerre et al. demonstrated, for the first time, that early repolarization is more frequently observed in the ECG of idiopathic VF survivors than in control subjects (31% vs. 5%, $P < 0.001$) [10]. In the same study, the amplitude of the J-waves (i.e., the height of the J-point elevation) was greater in idiopathic VF patients with early repolarization than in controls (2.0 ± 0.9 mm vs. 1.2 ± 0.4 mm; $P < 0.001$) [10]. In our own case-controlled study, published the same year [11], we also found that idiopathic VF survivors had taller J-waves than matched controls (0.14 vs. 0.09 mV), but the difference did not reach statistical significance because of the smaller patient population in our study [11]. Thus, the amplitude of the J-wave does matter. However, there is significant overlap between the amplitude of "malignant" vs. "benign" J-waves. Moreover, in a carefully conducted large population study where 20% of ostensibly healthy adults displayed early repolarization in their ECG [14], 8.2% of adults had J-waves taller than and 1.5 mV and 5% had J-waves taller than 2 mV (representing a J-point elevation of 2 mm or more at standard gain) without adverse prognostic events. In a more recent publication, 7% of athletes had J-waves taller than 2 mV [27]. This suggests that except for rare cases with "monstrous J-waves" (as in the examples shown in Fig. 16.2), the amplitude of the observed J-waves will not have sufficient deterministic value for clinical decision making in patients with asymptomatic early repolarization. One small study suggests that for J-waves of comparable height, those of idiopathic VF patients are wider and therefore have a wider angle from the QRS [27]. Finally, data from Japan suggest that the J-waves of patients with

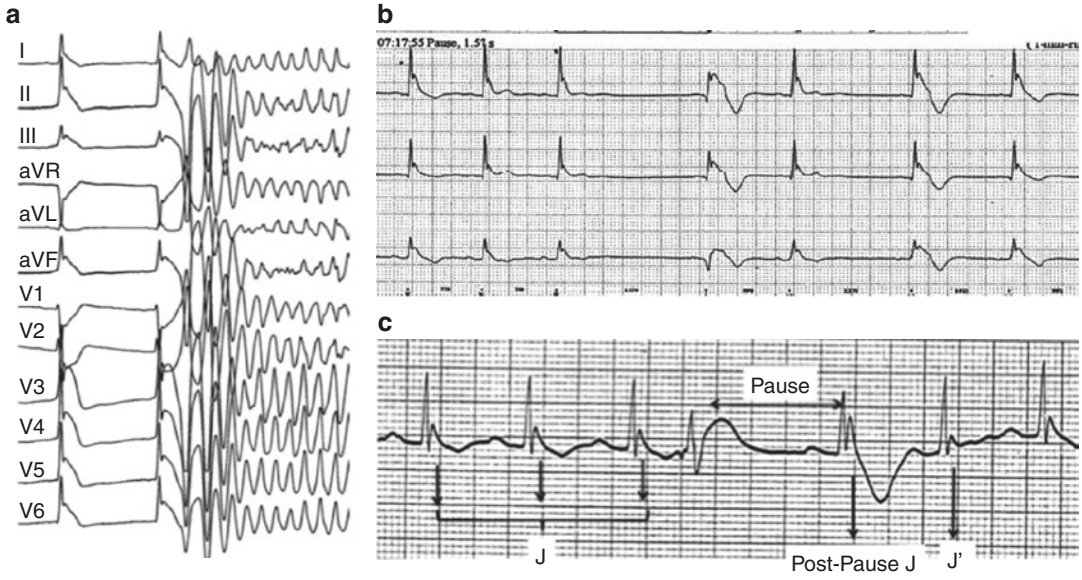


**Fig. 16.2** Examples of monstrous J-waves. The examples here are from two unrelated cases with idiopathic ventricular fibrillation. *Panels* (**a**, **b**) are electrocardiograms from a 14-year-old female with arrhythmic storm. Coronary spasm was excluded by coronary angiography performed when this bizarre electrocardiogram was recorded. (Reproduced with permission from Haissaguerre et al. [32]). *Panel* (**c**) demonstrates the dynamicity of the J-waves as a function of the heart rate. (Reprinted from Aizawa et al. [33], with permission from Elsevier). Note that the very tall J-waves are followed by flat (horizontal and not elevated) S-segments and by flat or even inverted T-waves. Also, note that in both cases, the sudden decrease in heart rate leads to further augmentation of the J-wave amplitude and deeper negative T-waves (panels **b**, **c**)

idiopathic VF *increase* when the heart rate suddenly *decreases* (Fig. 16.2), while the opposite is true for healthy individuals with J-waves [28].

## The Contour of the ST-Segment

Tikkanen et al. were the first to notice in a large population study (including over 10,000 subjects) that healthy athletes who have early repolarization almost invariably have their J-waves followed by a "rapidly ascending ST-segment" (defined as a 0.1 mV elevation [1 mm elevation at standard gain recordings]) of the ST-segment within 100 ms after the J point as it approaches the T-wave, or as a persistently elevated ST-segment of 0.1 mV (1 mm) throughout the ST-segment] [7]. They then speculated that this "rapidly ascending ST-segment pattern" would prove to be benign, whereas the other pattern observed in their population study, namely, the "horizontal or descending ST-segment" pattern, is the one associated with increased risk during long-term follow-up [7]. Indeed, among individuals with early repolarization at their baseline ECG, only the horizontal/descending ST variant was associated with an increased risk of arrhythmic death (relative risk 1.43; 95% confidence interval 1.05–1.94). Furthermore, when limited to high-amplitude J-waves (J-waves of 0.2 mV [2 mm] or more) in the inferior leads, the presence of the horizontal/descending ST-segment variant was associated with a threefold increased risk of arrhythmic death (95% confidence interval 1.56–6.30). In contrast, in subjects with early repolarization who had the "benign" rapidly ascending ST variant, the relative risk for arrhythmic death was not increased (0.89; 95% confidence interval 0.52–1.55) [7].

Following this important observation by Tikkanen [7], we revisited our original case-controlled study of idiopathic VF [11] and noted that the presence of J-waves was associated with a history of idiopathic VF with an odds ratio of

4.0 (95% confidence intervals 2.0–7.9), but having *both* J-waves *and* horizontal ST-segment yielded an odds ratio of 14 (95% confidence intervals 5.1–37.2) for having idiopathic VF [29]. Recognizing the importance of this observation, numerous studies focused on the contour of the ST-segment in early repolarization in further attempts to define its diagnostic/prognostic value. In 2013 we summarized all studies on early repolarization reporting on the type of ST-segment pattern by that date [3]. As clearly seen in Fig. 16.3, reproduced from our 2013 publication [3], as one moves from cohorts with the best prognosis (asymptomatic athletes on the far left of the figure followed by asymptomatic adults) to patient groups at higher risk (patients with acute myocardial infarction complicated by VF on the right) and highest risk (idiopathic VF survivors on the extreme right), the partition of individuals who have early repolarization into individuals with J-waves and rapidly ascending pattern (blue columns) and patients with J-waves followed by horizontal or descending ST-segment (red columns) changes accordingly. Thus, among athletes [except for the study by Cappato et al. [30], which was a case-controlled study of athletes with and without cardiac arrest], virtually all individuals with early repolarization have J-waves followed by rapidly ascending ST-segment, whereas the opposite pattern (horizontal/descending) is far more prevalent among idiopathic VF survivors [3]. Clearly then, the pattern of the ST-segment in early repolarization matters. However, as discussed earlier for the value of the J-wave amplitude, the diagnostic and prognostic value of the ST-segment contour are too limited for clinical decision making for the asymptomatic individual. This is because (as also seen in Fig. 16.3), among healthy adults with early repolarization, the horizontal/descending ST-segment pattern is observed as commonly as the rapidly ascending pattern [3]. Furthermore, in a more recent population study including more than 20,000 individuals who had their baseline ECG carefully evaluated by experts in electrocardiography [14], the presence of J-waves followed by a horizontal/descending ST-segment did not correlate with cardiovascular death during 17 years of follow-up [14]. Regrettably, information on arrhythmic death was not available for this cohort [14]. Note, however, that the very rare cases of monstrous J-waves also demonstrate flat ST-segments (Fig. 16.2).

## The Amplitude of the T-Waves

Pursuing the idea that the other ECG components of early repolarization (and not only the J-wave) are important for risk stratification, Roten et al. (Haissaguerre's group) looked at the value of T-wave amplitude in a case-controlled study of 92 idiopathic VF survivors and 247 matched controls [17]. Importantly, both groups had early repolarization on their electrocardiogram (defined as J-waves of ≥0.1 mV in the inferior or inferolateral leads). "Low-amplitude T-waves" were defined as any T-wave in leads I, II, or V4–V6 that were either inverted, biphasic, or had an amplitude that was both ≤0.1 mV and ≤10% of its respective R-wave [17]. Using this definition, "low-amplitude T-waves" were observed in 29% of idiopathic VF survivors but in only 3% of controls ($p < 0.001$) [17]. Moreover, although both the presence of large-amplitude J-waves and flat ST-segment correlated with a history of VF, the best correlation between a history of idiopathic VF and an ECG parameter of early repolarization was with low T/R-wave ratio [17]. This study has not been reproduced but it agrees with our (unpublished) impression. Since this is a relatively new finding, there is no data from large population-based studies on the frequency of "low-amplitude T-waves" in other unselected populations. Consequently, caution should be exercised when analyzing the ECG of an asymptomatic patient.

## Unmet Needs and Future Directions

Although important advances in our understanding of the phenomenon of early repolarization have been made during the last decade, our ability for risk stratification of patients with early repolarization in the absence of warning

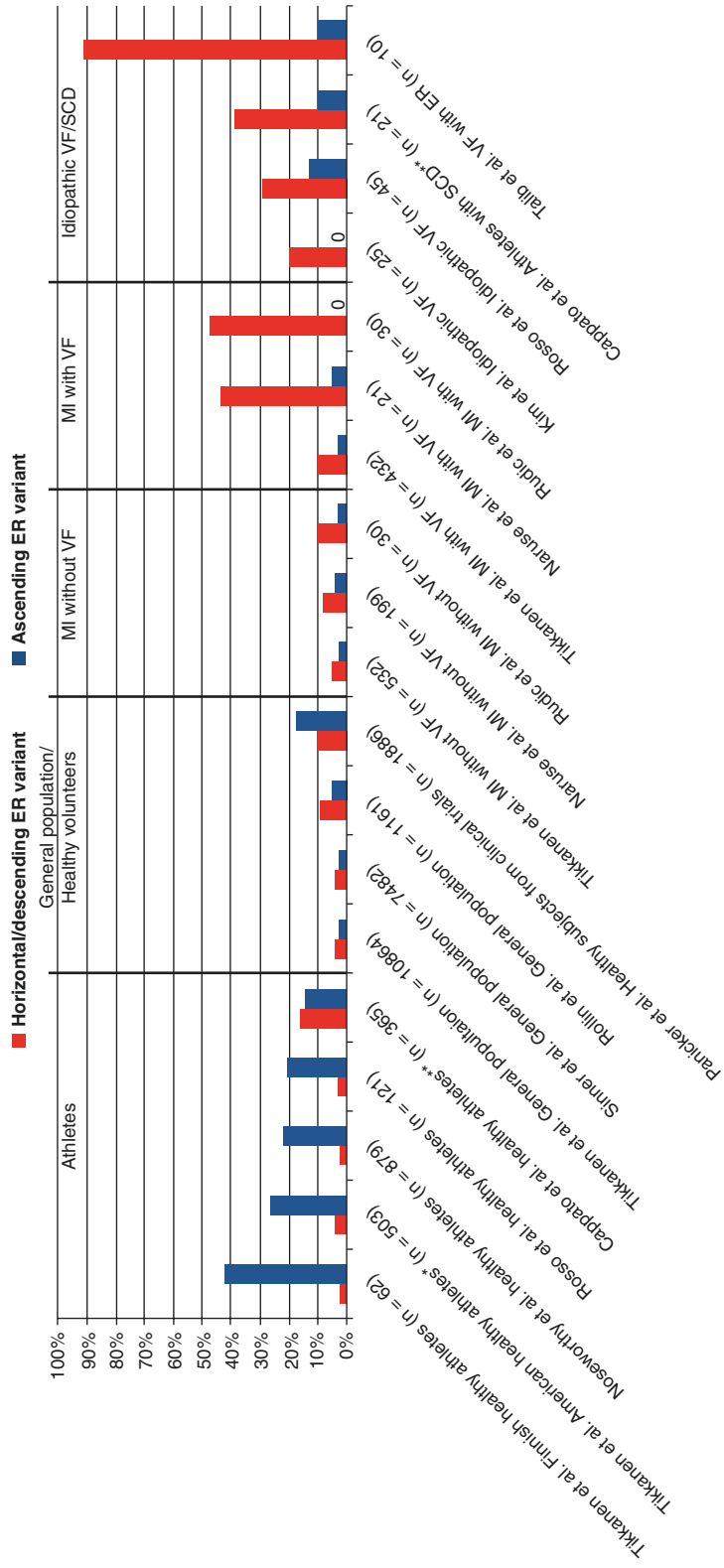


**Fig. 16.3** Incidence of early repolarization with rapidly ascending ST-segment (blue bars) and with horizontal ST-segment (red bars) in different populations. The numbers in parentheses represent the number of patients included in each study. (Reprinted from Adler et al. [3], with permission from Elsevier)

symptoms is still very limited. J-waves of large amplitude followed by a flat ST-segment and flat or inverted T-waves are signs of relatively increased risk. However, except for very rare cases with monstrous J-waves (as those shown in Fig. 16.2), the majority of asymptomatic patients with these features ought to be treated conservatively. Recently, noninvasive mapping of the epicardial substrate of patients with early repolarization with ECGI (electrocardiographic imaging techniques) has demonstrated that the epicardial depolarization of patients with idiopathic VF with J-waves is normal, while their epicardial surface has an area with steep repolarization gradients caused by areas with very short action potentials in the areas correlating with the presence of J-waves in their surface ECG [31]. Unfortunately, the healthy controls used in this study did not have early repolarization on their ECG. More studies are needed to define if ECGI techniques can reliably be used to tell apart individuals with benign vs. malignant early repolarization.

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# 17 Genetic Architecture, Pathophysiology, and Clinical Management of Brugada Syndrome

John R. Giudicessi and Michael J. Ackerman

## Abbreviations

| | |
|---|---|
| ACC | American College of Cardiology |
| AHA | American Heart Association |
| BrS | Brugada syndrome |
| ClinGen | Clinical Genome Resource |
| EHRA | European Heart Rhythm Association |
| EPS | Electrophysiology study |
| ESC | European Society of Cardiology |
| ExAC | Exome Aggregation Consortium |
| gnomAD | Genome Aggregation Database |
| GUS | Gene of uncertain significance |
| GWAS | Genome-Wide Association Study |
| HRS | Heart Rhythm Society |
| ICD | Implantable cardioverter-defibrillator |
| PVC | Premature ventricular contraction |
| RVOT | Right ventricular outflow tract |
| SCD | Sudden cardiac death |
| VF | Ventricular fibrillation |
| VT | Ventricular tachycardia |
| VUS | Variant of uncertain significance |

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## Introduction

Brugada syndrome (BrS) is considered classically to be a primary electrical disorder characterized clinically by spontaneous or class I antiarrhythmic-provoked coved ST-segment elevation ≥2 mm in ≥1 right precordial leads ($V_1$ or $V_2$; Fig. 17.1a) and an increased risk of sudden cardiac death (SCD), often during rest, sleep, or febrile episodes, in the absence of overt structural heart disease [1–3]. However, a growing body of evidence suggests BrS may represent a subtle cardiomyopathy that arises from both electroanatomic and structural abnormalities located within and around the right ventricular outflow tract (RVOT) [4–6]. Additional clinical manifestations observed commonly in BrS include conduction delay (i.e., slight PR prolongation and QRS widening) and supraventricular tachyarrhythmias (i.e., atrial fibrillation) [7], which suggest the pathogenic substrate(s) that underlie BrS may extend beyond the RVOT in some cases. However, whether the supraventricular and ventricular arrhythmias observed in BrS arise secondary to impaired conduction, abnormal repolarization, both, or neither remains the subject of lively debate and ongoing investigation.

Classically, BrS is considered a monogenic (i.e., single gene/variant) disorder that follows an autosomal-dominant pattern of inheritance. Whereas ~20–30% of BrS cases are attributed to pathogenic loss-of-function variants in the *SCN5A*-encoded $Na_v1.5$ cardiac sodium channel



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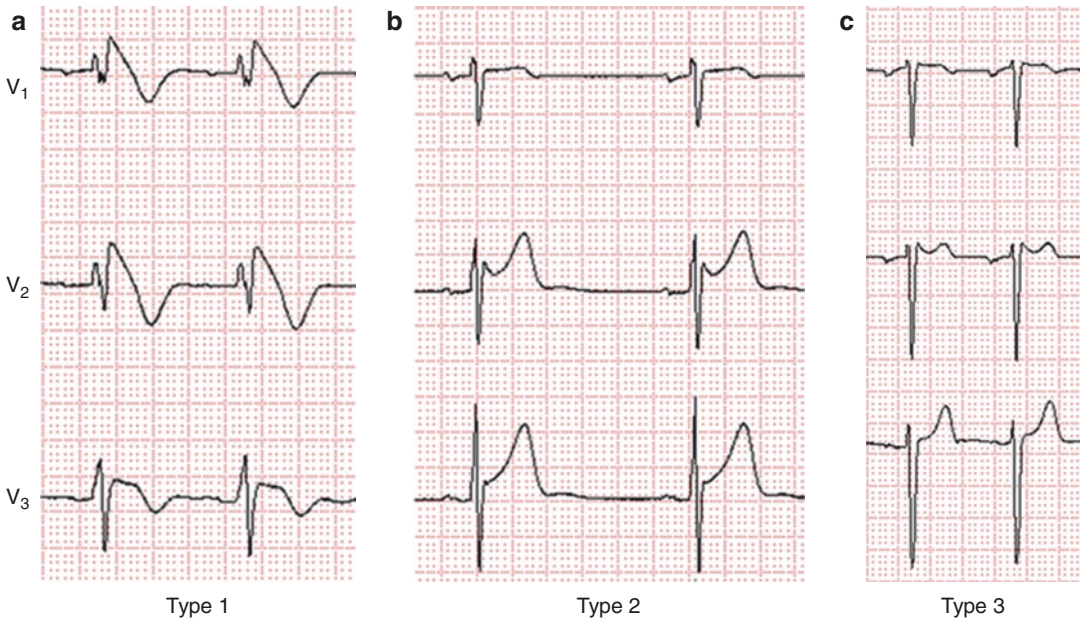


**Fig. 17.1** The three types of ST-segment elevation associated with Brugada syndrome. (**a**) Type 1 BrS ECG pattern. The only pattern that is considered diagnostic for the disease. (**b**) Type 2 BrS ECG pattern. (**c**) Type 3 BrS ECG pattern. Abbreviations: BrS, Brugada syndrome, and ECG, electrocardiogram. (Adapted from Anztelevitch et al. [17] with permission from Elsevier)

[8], at least 20 additional minor BrS-susceptibility genes have been reported to date. However, the majority of these genes were discovered using hypothesis-driven, mutational analysis of biologically plausible candidate genes in an era before the true burden of background amino acid-altering genetic variation was known [9]. As a result, the gene-disease association strength of most minor (i.e., non-*SCN5A*) BrS-susceptibility genes is rightfully in question [10].

Furthermore, even among families with a BrS-causative pathogenic *SCN5A* variant, disease penetrance is relatively low (~16%). Although this phenomenon may be explained, in part, by i) a strong male predominance, ii) late disease onset (third and fourth decades of life), and iii) genetic background (BrS is more common among individuals of Asian descent), mounting evidence suggests that a larger number of BrS cases may have an oligogenic/polygenic (i.e., ≥2 genes/variants) basis than anticipated previously.

In this chapter, we review succinctly the current paradigms surrounding the genetic basis and electrophysiological mechanism(s) underlying BrS, ongoing efforts to redefine BrS genetic architecture, and recent advances in the diagnosis, risk stratification, and clinical management of BrS.

## Genetics and Pathophysiology of BrS

Classically, BrS is considered a Mendelian disorder that follows an autosomal-dominant pattern of inheritance, albeit with low/incomplete penetrance and variable expressivity (Fig. 17.2) [11, 12]. As a result, family members with the same putative BrS-causative variant frequently assume vastly different clinical courses ranging from manifest electrocardiographic abnormalities (i.e., spontaneous coved-type ST elevation in $V_1$–$V_2$) with a history of ventricular arrhythmia-triggered cardiac events (i.e., syncope, seizures, or SCD) to the complete absence of spontaneous or provoked electrocardiographic features and a lifelong asymptomatic state (Fig. 17.2) [11]. Furthermore, many cases of BrS arise sporadically and efforts

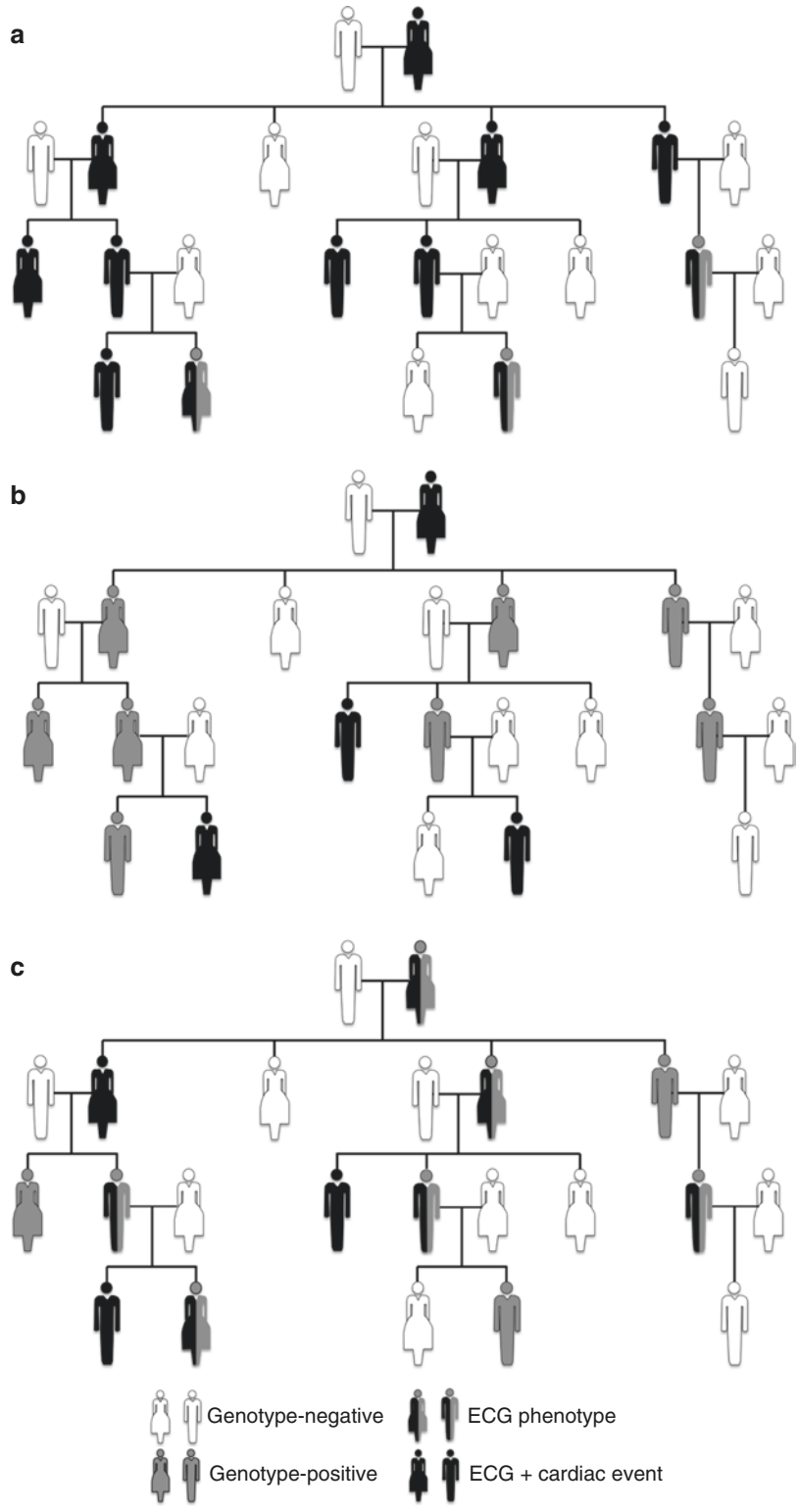


**Fig. 17.2** Penetrance and expressivity in Brugada syndrome. (**a**) Representative multigenerational pedigree displaying complete penetrance (100%) for electrocardiographic hallmarks (i.e., coved-type ST-segment elevation in leads $V_1$ and $V_2$ for BrS) and disease-related symptomatology (i.e., arrhythmic syncope, SCA, or SCD). (**b**) Representative multigenerational pedigree displaying incomplete penetrance (33%) for the electrocardiographic hallmarks and disease-related symptomatology. (**c**) Representative multigenerational pedigree displaying incomplete penetrance (66%) and variable expressivity as some individuals display the electrocardiographic hallmarks of the disease without symptomatology. Abbreviations: BrS, Brugada syndrome; SCA, sudden cardiac arrest; and SCD, sudden cardiac death. (Adapted from Giudicessi and Ackerman [11] with permission from Elsevier)

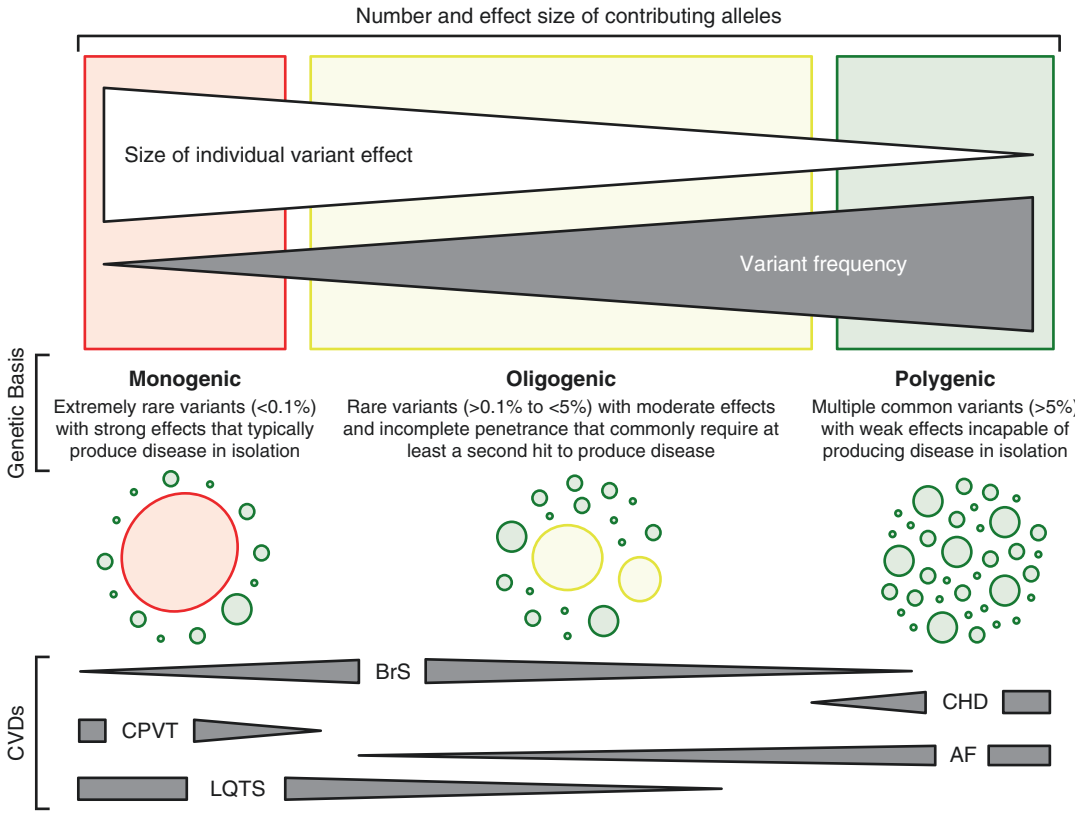


**Fig. 17.3** The spectrum of genetic variation underlying the heritable component of commonly encountered cardiovascular disorders (CVDs). At the severe (red) end of the spectrum are extremely rare disease-causative mutations with strong effects on gene function that typically result in monogenic disorders such as catecholaminergic polymorphic ventricular tachycardia (CPVT), long QT syndrome (LQTS), and some cases of Brugada syndrome (BrS). In the middle of the spectrum (yellow) are rare variants with moderate effects on gene function that rarely produce disease in isolation, but in the presence of one or more second hits result in disease as seen in some instances of BrS with a high burden of relatively common *HEY2* and *SCN10A* variants. Lastly, at the benign (green) end of the spectrum are common variants with weak effects on gene function that are incapable of producing disease in isolation, but may confer disease risk when multiple risk-associated common variants are present within the genome of an individual with environmental risk factors for disorders such as coronary heart disease (CHD) and atrial fibrillation (AF). In recognition that the genetic basis of most heritable CVDs is variable, dark gray triangles denote the spectrum of genetic variation underlying each CVD or class of CVDs. (Adapted from Giudicessi [14] with permission from Elsevier)

to utilize linkage analysis to identify novel monogenic (i.e., single gene; Fig. 17.3) causes of BrS have largely proven unsuccessful [13–15]. As such, a growing body of evidence, discussed later in this chapter, suggests that many cases of BrS may follow a complex inheritance model (i.e., oligogenic/polygenic disease) akin to that observed for most atrial fibrillation and atherosclerotic coronary heart disease cases (Fig. 17.3) [13, 14]. Nevertheless, in the ensuing sections, we review briefly the classic Mendelian genetic basis of *SCN5A*- and non-*SCN5A*-mediated BrS as well as their suspected underlying electrophysiological mechanism(s).

## *SCN5A*-Mediated BrS

As discussed previously, 20–30% of BrS arises secondary to loss-of-function genetic variants in the *SCN5A*-encoded $Na_v1.5$ cardiac sodium channel that either i) decrease *SCN5A* expression (i.e.,

haploinsufficiency secondary to nonsense variants) or ii) encode defective $Na_v1.5$ channels that reach the sarcolemma, but fail to conduct $I_{Na}$ current or have abnormal gating properties (i.e., delayed activation or faster inactivation). Each of these mechanisms in turn decrease peak $I_{Na}$ current during phase 0 of the cardiac action potential and slow cardiac conduction. However, the precise electrophysiological mechanism(s) that link decreased $I_{Na}$ current to right precordial ST-segment elevation and ventricular arrhythmia susceptibility remains the subject of great debate.

At present, two distinct hypotheses have been advanced. The "repolarization" hypothesis, advanced largely on the basis that the heterogeneous distribution of the transient outward potassium current ($Kv4.3/I_{to}$) results in an epicardial > endocardial transmural voltage gradient in canine RV wedge preparations [16], postulates that decreased $I_{Na}$ outwardly shifts the balance of currents in the RV epicardium predisposing to phase 2 reentry and the generation of closely coupled premature ventricular contractions (PVCs) capable of ventricular arrhythmias [17]. In contrast, the "depolarization" hypothesis, advanced largely on data obtained from diagnostic electrophysiology studies (EPS) and more recently concomitant RVOT endomyocardial biopsies, suggests that decreased $I_{Na}$ current [6, 18–20], perhaps mediated or exacerbated by myocardial inflammation [6], predisposes to pathological remodeling in the epicardial RVOT and anterior RV wall (i.e., focal myocardial fibrosis/connexin 43 downregulation) that leads to conduction slowing and ultimately electrical instability [17].

Although the "repolarization" and "depolarization" hypotheses are not necessarily mutually exclusive, evidence from recent imaging [21, 22], postmortem necropsy [23], and concomitant electroanatomic mapping/targeted endomyocardial biopsy [6] studies all suggest structural alterations in the RVOT/anterior RV free wall play a prominent role in the pathogenesis of BrS. Interestingly, endomyocardial biopsies obtained from living patients with in vitro loss-of-function BrS-causative *SCN5A* pathogenic variants display increased rates of myocyte apoptosis and cardiomyopathy-like changes such as myocyte hypertrophy, vacuolization, and cytoplasmic degeneration [24]. This has led to speculation that perturbations in cellular sodium homeostasis (i.e., decreased $I_{Na}$), which influences cellular pH, calcium homeostasis, and excitation-contraction coupling, may lead to increased rates of myocyte apoptosis/necrosis and trigger a reactive immune-mediated process that ultimately leads to focal myocardial inflammation and fibrosis within the RVOT/anterior RV [6, 24]. As such, the pathophysiological mechanisms underlying BrS appear to be more in line with arrhythmogenic cardiomyopathy than primary electrical disorders such as long QT syndrome.

## Non-*SCN5A*-Mediated BrS

Following the sentinel discovery that pathogenic variants in *SCN5A* underlie a subset of BrS cases [25], more than 20 additional BrS-susceptibility genes have been reported in the literature (Table 17.1). Not surprisingly, many of the "minor" BrS-susceptibility genes encode components of the $Na_v1.5$ macromolecular complex and have been shown, in vitro, to confer a similar $I_{Na}$ loss of function to that observed in *SCN5A*-mediated BrS (Table 17.1). In addition, variants in genes known to encode pore-forming α- and accessory β-subunits that impart either a loss of function to the depolarizing L-type calcium current ($I_{Ca,L}$) or a gain of function to repolarizing $Kv.4.3/I_{to}$ and ATP-sensitive potassium ($I_{KATP}$) currents have been implicated in BrS (Table 17.1).

However, with advances in sequencing technology, it has become increasingly clear that *SCN5A* is the only BrS-causative gene identified to date where a greater burden of rare variants is observed in BrS cases versus controls [26]. As such, great caution must be taken when interpreting rare variants identified in any of the alleged minor BrS-susceptibility genes. Lastly, even if the 20+ BrS-susceptibility genes are included, after over 20 years of genetic investigations, nearly two-thirds of BrS cases still remain genetically elusive. When coupled with

**Table 17.1** Reported Brugada syndrome-susceptibility genes

| Gene | Historical convention | OMIM | Protein | Functional effect | Reported frequency | Key reference |
|---|---|---|---|---|---|---|
| **ClinGen definitive evidence** | | | | | | |
| *SCN5A* | BrS1 | 601,144 | Cardiac sodium channel alpha subunit ($Na_v1.5$) | ↓ $I_{Na}$ | 20%–30% | [25] |
| **ClinGen disputed evidence** | | | | | | |
| *ABCC9* | BrS13 | N/A | ATP-binding cassette, subfamily C member 9 | ↑ $I_{KATP}$ | 2% | [50] |
| *CACNA1C* | BrS3 | 611,875 | Voltage-gated L-type calcium channel ($Ca_V1.2$) | ↓ $I_{Ca,L}$ | 6.6% | [51] |
| *CACNA2D1* | BrS9 | N/A | Voltage-gated L-type calcium channel 2 delta 1 subunit | ↓ $I_{Ca,L}$ | 1.8% | [52] |
| *CACNB2* | BrS4 | 611,876 | Voltage-gated L-type calcium channel beta 2 subunit | ↓ $I_{Ca,L}$ | 4.8% | [51] |
| *FGF12* | BrS16 | N/A | Fibroblast growth factor 12 | ↓ $I_{Na}$ | Rare | [53] |
| *GPD1L* | BrS2 | 611,777 | Glycerol-3-phosphate dehydrogenase 1-like | ↓ $I_{Na}$ | Rare | [30] |
| *HCN4* | N/A | N/A | Hyperpolarization-activated cyclic nucleotide-gated channel 4 | ↓ $I_f$ | Rare | [54] |
| *KCND3* | BrS10 | 616,399 | Voltage-gated potassium channel alpha subunit ($K_V4.3$) | ↑ $I_{to}$ | Rare | [31] |
| *KCNE3* | BrS6 | 613,119 | Potassium channel beta subunit 3 (MiRP2) | ↑ $I_{to}$ | Rare | [55] |
| *KCNE5* | N/A | N/A | Potassium channel beta subunit 5 | ↑ $I_{to}$ | Rare | [56] |
| *KCNH2* | N/A | N/A | Voltage-gated potassium channel alpha subunit (hERG; $K_V11.1$) | ↑ $I_{Kr}$ | Rare | [57] |
| *KCNJ8* | BrS8 | 613,601 | Inwardly rectifying potassium channel alpha subunit ($K_{ir}6.1$) | ↑ $I_{KATP}$ | 2% | [58] |
| *RANGRF* | BrS11 | N/A | RAN guanine nucleotide release factor 1 | ↓ $I_{Na}$ | Rare | [59] |
| *PKP2* | BrS15 | N/A | Plakophilin-2 | ↓ $I_{Na}$ | Rare | [60] |
| *SCN10A* | BrS17 | N/A | Voltage-gated sodium channel alpha subunit ($Na_V1.8$) | ↓ $I_{Na}$ | 5%–16.7% | [35] |
| *SCN1B* | BrS5 | 612,838 | Sodium channel beta 1 | ↓ $I_{Na}$ | 1.1% | [61] |
| *SCN2B* | BrS14 | N/A | Sodium channel beta 2 | ↓ $I_{Na}$ | Rare | [62] |
| *SCN3B* | BrS7 | 613,120 | Sodium channel beta 3 | ↓ $I_{Na}$ | Rare | [63] |
| *SEMA3A* | N/A | N/A | Semaphorin 3A | ↑ $I_{to}$ | Rare | [64] |
| *SLMAP* | BrS12 | N/A | Sarcolemma associated protein | ↓ $I_{Na}$ | Rare | [65] |
| *TRPM4* | N/A | N/A | Transient receptor potential cation channel, subfamily M, member 4 | ↓ $I_{Na}$ | Rare | [66] |

Abbreviations: BrS, Brugada syndrome; ClinGen; Clinical Genome Resource; and OMIM, Online Mendelian Inheritance in Man

the large number of sporadic/nonfamilial BrS cases, substantial genetic heterogeneity, and low penetrance/variable expressivity observed even in familial *SCN5A*-mediated BrS, the number of BrS cases that remain genetically elusive suggests strongly that the genetic architecture of BrS may be substantially more complex than anticipated initially.

## Reappraising the Nature of Non-*SCN5A*/Minor BrS Gene-Disease Associations

Following the completion of the human genome, rapid advances in sequencing technology have allowed for the discovery of novel disease-susceptibility genes in situations (i.e., unrelated singletons, small kindreds, etc.) not amenable to classic linkage analysis. As a result, nearly half of all gene-disease associations in existence today [27], including the majority of the 20+ BrS-susceptibility genes (Table 17.1), were discovered over the last ~10 years using largely hypothesis-driven, mutational analysis of biologically plausible genes and their gene-encoded proteins. Unfortunately, many of these genes were discovered before large-scale next-generation sequencing projects such as the Exome Aggregation Consortium (ExAC)/Genome Aggregation Database (gnomAD) illuminated the true burden of amino acid-altering genetic variation in the human genome. Furthermore, the level of clinical, genetic, and experimental evidence to support existing gene-disease associations, including those in BrS, is widely variable. In the ensuing sections, we examine efforts to systematically reclassify the minor BrS-susceptibility genes and the impact of these efforts on clinical BrS genetic testing.

## Systematic Efforts to Reappraise Minor BrS-Susceptibility Gene-Disease Associations

The clinical utility of genetic testing, for any disease, is predicated on the assembly of a sufficiently evidence-based gene panel. Unfortunately, from the onset of clinical genetic testing for BrS and other SCD-predisposing genetic disorders, the prevailing mindset has been that "bigger is better" in terms of the number of genes included on a given gene panel. As a result, current clinical genetic tests for a myriad of Mendelian/monogenic disorders, including BrS, are littered with so-called genes of uncertain significance (GUS) with a paucity of genetic and experimental evidence to definitively confirm or deny their role in disease pathogenesis.

In an effort to improve the validity of genes included on clinical genetic tests, the National Institutes of Health-funded Clinical Genome Resource (ClinGen) consortium developed a semiquantitative, evidence-based framework designed to standardize how the strength of genetic and functional evidence supporting a given gene-disease association is assessed and reported [28]. With the assistance of three independent gene curation teams and a panel of genetic heart disease experts, the ClinGen framework was used recently to reappraise the gene-disease association strength of 21 genes associated previously with BrS. The results were sobering as 20/21 (95%) or BrS-susceptibility genes assessed received a "disputed" evidence classification (Table 17.1) [10].

Not surprisingly, the primary justification for these reclassifications were (i) insufficient co-segregation data, (ii) excess prevalence of putative disease-causative variants in public exome/genome databases such as ExAC/gnomAD, and (iii) an overreliance on experimental evidence obtained exclusively from in vitro heterologous expression systems [10]. Interestingly, even *GPD1L*, a minor BrS-susceptibility gene discovered initially using classical linkage analysis and characterized functionally in vitro [29, 30], was demoted to "disputed" status due to the excess frequency of the sentinel p.Ala280Val-GPD1L variant in public exomes/genomes and concern that alternative causes in the large genomic region containing *GPD1L* discovered by genetic linkage were not fully explored [10].

## Impact of ClinGen Reclassifications on Clinical BrS Genetic Testing

One might expect that the recent demotion of ~95% of BrS-susceptibility genes included currently on commercial gene panels to "disputed" status would impact negatively the clinical utility of genetic testing in BrS [10]. However, genetic testing in BrS, unlike long QT syndrome and catecholaminergic polymorphic ventricular tachycardia, has never been shown to play a substantive role in the diagnosis, prognostication, or

clinical management of patients with BrS [31]. As such, current American Heart Association (AHA)/American College of Cardiology (ACC) [3], European Society of Cardiology (ESC) [2], and Heart Rhythm Society (HRS)/European Heart Rhythm Association (EHRA) [1, 31] expert consensus guidelines, which largely suggest that *SCN5A*-specific genetic testing of BrS index cases may be helpful in facilitating the screening of first-degree relatives, are not affected by the recent ClinGen BrS gene reclassification efforts (Fig. 17.4).

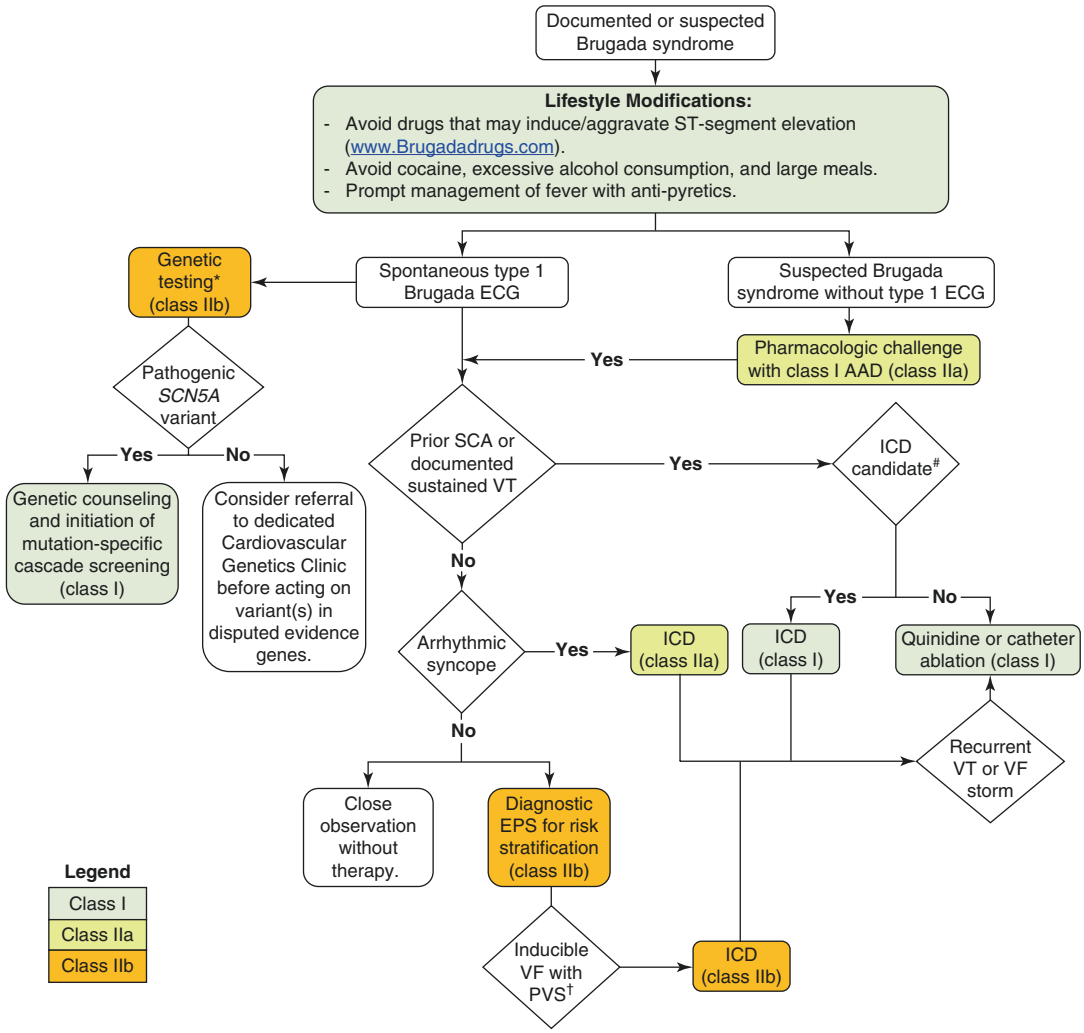


**Fig. 17.4** Prevention of ventricular arrhythmias and SCD in Brugada syndrome. Colored boxes indicate strength of recommendation [class I (green/strong), recommended; class IIa (yellow/moderate), reasonable; and class IIb (orange/weak), may be reasonable]. ∗At present, the majority of commercially available genetic tests contain a large number of weak/disputed evidence genes. In the absence of an ACMG pathogenic/likely pathogenic variant in *SCN5A*, the decision to pursue cascade screening of first-degree relatives based on a variant of uncertain significance (VUS) in a weak/limited evidence gene is best accomplished in the setting of a dedicated Cardiovascular Genetics Clinic. #ICD candidacy determined by functional status, life expectancy, feasibility, and patient preference. †ICD implantation may be considered in patients with a diagnosis of Brugada syndrome with inducible VF during diagnostic EPS with PVS (one or two extrastimuli at ≥2 sites). This recommendation was endorsed by the current ESC and HRS/EHRA guidelines, but not the AHA/ACC guidelines. Abbreviations: AAD antiarrhythmic drug, ECG electrocardiogram, EPS electrophysiological study, ICD implantable cardioverter-defibrillator, SCA sudden cardiac arrest, VF ventricular fibrillation, VT ventricular tachycardia. (Adapted from Al-Khatib et al. [3, 67] with permission from Wolters Kluwer Health, Inc.)

However, from a practical perspective, most commercially available BrS genetic tests are likely to include a number of "disputed" evidence genes on their respective panels for the foreseeable future. Given the interpretative nightmare the discovery of a novel variant of uncertain significance (VUS) in a weak/disputed evidence gene (i.e., GUS) poses and the elevated potential that misinterpretation of such variants could lead to potentially harmful diagnostic miscues, referral of patients with ambiguous BrS genetic testing findings to dedicated Cardiovascular Genetics Clinics represents a reasonable and prudent approach (Fig. 17.4).

## Genetic Architecture of BrS: Monogenic, Polygenic, or Both?

In light of the large number of BrS cases that remain genetically elusive [32], low penetrance and variable expressivity of BrS-causative *SCN5A* pathogenic variants [12], and questionable validity of many putative BrS-susceptibility minor genes [10], it is becoming increasingly evident that BrS is not a predominantly Mendelian/monogenic disorder. Instead, if the rest of BrS is genetically driven, then an oligenic/polygenic basis must be considered.

To this end, a genome-wide association study (GWAS) identified and validated the association of three genetic loci located within or near the *SCN5A* (rs11708996), *SCN10A* (rs10428132), and *HEY2* (rs9388451) genes with BrS [13]. Interestingly, the risk of developing BrS rose substantially as individuals accumulated risk alleles within these genetic loci (i.e., 2 risk alleles, odds ratio of 1.9 vs >4 risk alleles, odds ratio of 21.5) [13]. However, as ~1.5% of individuals of European descent are expected to possess >4 risk alleles in the identified *SCN5A*, *SCN10A*, and *HEY2* BrS-associated genetic loci, it is unlikely that common genetic variation in these three genetic loci alone is sufficient to cause BrS, a disorder with an estimated prevalence of no more than 1:2000 (0.05%) [13, 17].

Unlike most novel genetic loci discovered through GWAS, potential mechanisms underlying the BrS-associated GWAS genetic loci in *SCN10A* (rs10428132) and *HEY2* (rs9388451) have both been elucidated [33]. Although the *SCN10A* rs10428132 BrS risk allele has not been studied directly, it is known to be in tight linkage disequilibrium with a functional variant (rs6801957) that affects T-box transcription factor (TBX3 and TBX5) binding and thereby the activity of an intronic cardiac enhancer region in *SCN10A* [34]. Through a complicated series of in vivo murine and ex vivo human studies, van den Boogaard demonstrated subsequently that *SCN10A* rs6801957 likely weakens the affinity of the *SCN10A* intronic cardiac enhancer for the *SCN5A* promoter [33]. This results in a modest overall reduction in *SCN5A* expression and may alter the pattern/gradient of *SCN5A* expression [33]. As such, *SCN10A* rs6801957 and by virtue of linkage disequilibrium *SCN10A* rs10428132 appear to confer BrS risk through genomic dysregulation of *SCN5A* expression rather than through the controversial role of *SCN10A*-encoded $Na_v1.8$ sodium channel in cardiac electrophysiology [33, 35, 36].

Similarly, data derived from human and murine left ventricular transcriptome studies suggests that the *HEY2* rs9388451 BrS risk allele results in increased expression of a *HEY2*-encoded basic helix-loop-helix transcriptional repressor, predominantly expressed in the ventricular subepicardium, which regulates transmural ion channel patterning in the heart [37]. Interestingly, *HEY2* haploinsufficiency in mice (i.e., $HEY2^{+/-}$) flattens the epicardial-to-endocardial transmural electrophysiological gradient in the RV via effects on both depolarizing $I_{Na}$ and repolarizing $I_{to}$ currents and results in less prominent J-waves [37]. Although the direct effect of increased *HEY2* expression has yet to be assessed directly in vivo, accentuation of the $I_{Na}$- and $I_{to}$-mediated epicardial-to-endocardial transmural electrophysiological gradient in the RVOT is consistent with existing models of BrS pathogenesis.

Collectively, the aforementioned BrS GWAS and subsequent elucidation of potential mechanisms underlying the BrS risk associated with *SCN10A* rs10428132 and *HEY2* rs9388451 risk alleles provide compelling evidence that common genetic variation that influences the

transmural expression/patterning of cardiac ion channels may contribute to the pathogenesis of BrS. However, it remains to be seen if (i) these GWAS-identified BrS risk alleles underlie the marked incomplete penetrance and variable expressivity observed in *SCN5A*-mediated BrS and (ii) larger/better powered multicenter GWAS will unearth additional novel BrS-associated genetic loci. Furthermore, how common and rare genetic risk factors interact with environmental risk factors such as gender (i.e., sex hormones), age (fibrosis/biophysical changes in $I_{Na}$), and comorbid conditions (i.e., hypertension) in BrS pathogenesis requires further investigation. Hopefully, ongoing studies in these areas will help refine/redefine our current understanding of the genetic architecture(s) underlying BrS.

## Advances in the Diagnosis, Risk Stratification, and Clinical Management of BrS

As discussed previously, according to current AHA/ACC (2017) [3], ESC (2015) [2], and HRS/EHRA (2013) [1] consensus guidelines for the management of patients with ventricular arrhythmias/SCD, BrS is diagnosed in a patient with either spontaneous or class I antiarrhythmic-induced coved-type ST-segment elevations ≥2 mm in ≥1 right precordial leads (V1 or V2; Fig. 17.1a) positioned in either the 2nd, 3rd, or fourth intercostal position. This specific ECG morphology is often referred to as a type 1 Brugada ECG pattern. However, due to concerns that the inclusion of a class I antiarrhythmic-provoked type I Brugada ECG pattern could result in overdiagnosis, the recent HRS/EHRA J-wave syndrome task force recommended that a diagnosis of BrS in this circumstance also require at least one of the following: documented ventricular fibrillation (VF)/polymorphic ventricular tachycardia (VT), arrhythmic syncope, family history of autopsy-negative SCD, type I Brugada ECG pattern in relatives, or nocturnal agonal respirations [17].

As a result of the complexities surrounding the diagnosis of BrS, particularly the role of a drug-induced type 1 Brugada ECG pattern and nonspecific type 2 (saddle-back)/type 3 ST-segment elevations (Fig. 17.1b, c), the J-wave syndrome task force proposed a weighted diagnostic BrS diagnostic scorecard termed the "Shanghai Score System" (Table 17.2) [17)]. Although the Shanghai Score is based entirely on expert opinion and not directly derived from large-scale BrS outcome/risk stratification data, its recent independent validation [38] supports its ongoing use, especially by non-experts, when considering a potential BrS diagnosis.

In the following sections, we detail current expert consensus approaches to the risk stratification and clinical management of patients with a clinical diagnosis of BrS.

## Risk Stratification Approaches in BrS

Numerous prior studies have indicated that the single strongest predictor of subsequent cardiac events in patients diagnosed ultimately with BrS is initial clinical presentation (i.e., asymptomatic < arrhythmic syncope < aborted sudden cardiac arrest) [39, 40]. Furthermore, the presence of a *spontaneous* type 1 Brugada ECG pattern is a widely accepted, independent predictor of risk [40, 41].

A number of additional electrocardiographic markers (i.e., QRS fragmentation, concomitant inferolateral early repolarization pattern) [42, 43] appear to signify increased risk as does VF inducibility with programmed electrical stimulation during diagnostic EPS using a nonaggressive protocol (i.e., ≤2 extrastimuli from the RV apex) [44]. However, it remains to be seen whether these additional ECG parameters and/or the results of a diagnostic EPS with programmed electrical stimulation are robust enough to alter clinical decision-making, particularly in asymptomatic patients, and remain the subject of ongoing study and expert debate.

Fortunately, the majority of patients with a newly rendered diagnosis of BrS are asymptomatic

**Table 17.2** Proposed "Shanghai Score" Brugada syndrome diagnostic scorecard

| Clinical criteria | Points |
|---|---|
| *Electrocardiographic criteria (requires ≥ 1 finding on 12-lead ECG or ambulatory Holter monitoring)* | |
| Spontaneous type 1 Brugada ECG pattern at nominal or high leads | 3.5 |
| Fever-induced type 1 Brugada ECG pattern at nominal or high leads | 3 |
| Type 2 or 3 Brugada ECG pattern that converts with provocative drug challenge | 2 |
| ∗*Only award points for highest score in this category* | |
| *Clinical history* | |
| Unexplained cardiac arrest or documented VF/ polymorphic VT | 3 |
| Nocturnal agonal respirations | 2 |
| Suspected arrhythmic syncope | 2 |
| Syncope of unclear mechanism/unclear etiology | 1 |
| Atrial flutter/fibrillation in patients <30 years without alternative etiology | 0.5 |
| ∗*Only award points for highest score in this category* | |
| *Family history* | |
| First- or second-degree relative with definite BrS | 2 |
| Suspicious SCD (fever, nocturnal, Brugada aggravating drugs) in a first- or second-degree relative | 1 |
| Unexplained SCD <45 years in first- or second- degree relative with negative autopsy | 0.5 |
| ∗*Only award points for highest score in this category* | |
| Genetic test result[a] | |
| ACMG pathogenic or likely pathogenic variant in BrS-susceptibility gene | 0.5 |
| *Shanghai BrS score (requires at least 1 electrocardiographic criteria)*<br>≥3.5 points: High pretest probability of BrS (probable/ definite BrS; ≥ 90% likelihood)<br>2–3 points: Intermediate pretest probability of BrS (possible BrS; ~50% likelihood)<br><2 points: Low pretest probability of BrS (nondiagnostic) | |

Adapted from Anztelevitch et al. [17] with permission from Elsevier

Abbreviations: *ACMG* American College of Medical Genetics and Genomics, *BrS* Brugada syndrome, *ECG* electrocardiogram, *SCD* sudden cardiac death, *VF* ventricular fibrillation, *VT* ventricular tachycardia

[a]Only *SCN5A* is definitively associated with BrS. Extreme caution should be exercised when adjudicating/interpreting variants identified in the 20+ weak/disputed evidence minor BrS-susceptibility genes

(~63%) and are therefore associated with relatively low annual risk (~0.5%) [39, 40]. In these patients, it is important to remember that neither a family history of SCD nor the detection of a pathogenic *SCN5A* variant influences prognosis [39, 40]. As such, family history and genetic testing results have no bearing on clinical decision-making, including the decision to pursue implantable cardioverter-defibrillator (ICD) implantation.

## Invasive Management of Previously Symptomatic BrS

At present, the only proven SCD prevention strategy in high-risk BrS patients (i.e., those with a history of SCA, documented VF/sustained VT, or arrhythmic syncope) is an ICD. As outlined in Fig. 17.4, current AHA/ACC, ESC, and HRS/EHRA guidelines all recommend an ICD as first-line therapy for all BrS patients with a life expectancy >1 year that either i) presented with aborted SCD or ii) have documented evidence of sustained VT/VF (class I recommendation) [1–3].

In addition, current ESC and HRS/EHRA, but not AHA/ACC, guidelines recommend consideration of an ICD in i) symptomatic BrS patients with a clinical history strongly suggestive of arrhythmic syncope (class IIa recommendation) and ii) asymptomatic BrS patients with a spontaneous type I Brugada ECG and inducible VT/VF during a diagnostic EPS with programmed ventricular stimulation (PVS) limited to ≤2 extrastimuli (Fig. 17.4) [1–3].

Lastly, although the use of diagnostic EPS with PVS is reemerging as a risk stratification strategy (currently a class IIb recommendation in the AHA/ACC, ESC, and HRS/EHRA guidelines), the role of therapeutic radiofrequency ablation in BrS is currently limited to previously symptomatic patients who (i) are not candidates for or refuse an ICD, (ii) have experienced frequent appropriate ICD therapies, and (iii) have a history of electrical storm (Fig. 17.4) [1–3]. As our understanding of the pathophysiology of BrS continues to evolve, particularly as it relates to the role of structural alterations in the RVOT/

anterior RV free wall, and better powered outcome studies with longer follow-up duration emerge, radiofrequency ablation may assume a larger role in the treatment of BrS in the future [18, 19, 45]. However, until such data emerge, a prophylactic epicardial RVOT ablation in asymptomatic patients with BrS should be discouraged [20].

## Pharmacologic Management of Previously Symptomatic BrS

Unfortunately, in some patients (i.e., infants/young children, individuals from developing nations), an ICD may be refused or not physically/financially feasible. In these cases, quinidine, a class Ia antiarrhythmic that blocks $I_{to}$ nonspecifically, suppresses spontaneous ventricular arrhythmias in BrS [46–49]. As such, the AHA/ACC, ESC, and HRS/EHRA guidelines on the management of patients with ventricular arrhythmias/SCD all support the use of quinidine in BrS patients with i) symptomatic ventricular arrhythmias who either are not candidates for or decline an ICD, ii) a history of recurrent polymorphic VT shocks or VF storm, and iii) supraventricular arrhythmias requiring pharmacologic management (Fig. 17.4) [1–3].

## Future Directions

As illustrated in this chapter, our understanding of the genetic architecture and pathophysiological mechanisms underlying BrS is currently in a state of active flux. Although BrS was once considered a primary electrical disorder, emerging electroanatomic and pathologic evidence suggest BrS may, mechanistically, have more in common with the arrhythmogenic cardiomyopathies than its long presumed cousin long QT syndrome. Furthermore, in light of the substantial rate of amino acid-altering genetic variation within most genes in the human genome and techniques employed in the discovery of most BrS-susceptibility genes, it comes as little surprise that many of the genes/variants associated previously with BrS may represent background genetic noise and deserve rightfully their current ClinGen "disputed" evidence label.

However, as illustrated by the discovery of BrS-associated common variants in *HEY2* and *SCN10A* by GWAS, the genetic architecture of BrS is likely more complicated than envisioned previously. As such, some of the genes/variants in "disputed" evidence minor BrS-susceptibility genes may, in reality, represent strong contributors to an oligogenic/polygenic disease. In this light, developing a better understanding of the mechanistic link between decreased $I_{Na}$ current and/or disruptions in transmural ion channel gradients conferred by rare and common genetic variants in *SCN5A*, *HEY2*, *SCN10A*, and perhaps some "disputed" evidence genes and subtle structural alterations in the RVOT/anterior RV is of utmost important.

Furthermore, developing a better mechanistic understanding of the complex interplay between established genetic and environmental risk factors such as gender, age, inflammation, and other comorbid conditions is sorely needed. With an enhanced understanding of how potentially modifiable environmental risk factors interact with genetic predisposition, development of approaches to slow or prevent the formation of a high-risk BrS substrate in the RVOT/anterior RV may one day be feasible.

Lastly, the ICD, with its inherent risk of inappropriate therapies and other nontrivial complications, remains the only widely accepted SCD prevention strategy in high-risk BrS patients. As such, ongoing assessment of the role of diagnostic EPS with programmed ventricular stimulation in risk stratification, durability of epicardial ablation, and the development of additional therapeutic approaches promises to provide physicians with better therapeutic options that will hopefully improve substantially the lives of patients with this potentially lethal condition.

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# Part IV

# Pathophysiology and Clinical Aspects of Special Cardiac Repolarization Syndromes

# The Short QT Syndrome

18

Chiara Scrocco, Fiorenzo Gaita, and Carla Giustetto

## Introduction

The short QT syndrome (SQTS) is a rare familial ion channel disease characterized by shortened cardiac repolarization and increased susceptibility to life-threatening arrhythmias, in the absence of structural heart disease.

The first speculation about the potential malignant effects of a short QT interval dates back to 1993, when a retrospective analysis of 24-hour Holter recordings in 6693 subjects highlighted that both prolonged (>440 ms) and shortened (<400 ms) mean QTc interval were associated with a doubled risk of sudden death (SD) as compared with QTc values comprised between 400 and 440 ms [1]. The first report of a short QT interval at ECG associated with cardiac symptoms is from Gussak et al. [2], who reported a family of three (one of whom aged 17 suffered from paroxysmal atrial fibrillation (AF)) and an unrelated subject who had died suddenly. All of them showed QT and QTc intervals <300 ms. In 2003 the inherited nature of the condition and its role in familial SD was highlighted by Gaita et al. [3] with the description of two unrelated families in which QT intervals between 210 and 280 ms (with QTc always <300 ms) were associated with palpitations (in some case with evidence of AF), syncope, and SD across several generations.

## Diagnosis

### QT and QTc Interval

The hallmark of SQTS is a constantly short QT interval on ECG (Fig. 18.1). As the QT interval reflects the duration of the repolarization, it is influenced by fluctuations in heart rate and vagal tone, as well as by hormonal factors linked to age, race, and sex and also by electrolyte abnormalities and certain drugs [4, 5]. To overcome the rate dependence of the QT interval, different correction formulas have been introduced. The most commonly used is the one proposed by Bazett ($QTc = QT/\sqrt{RR}$) [6], despite its tendency to underestimate the QT interval at low frequencies and to overestimate it at high heart rates. In 1992 a formula by which the expected QT interval (predicted QT, QTp) can be calculated for a specific heart rate was developed [7], but this is seldom used in the clinical practice. Although alternative formulas have been developed (Fridericia's formula, Framingham's formula), it has become apparent that a universal correction model may not

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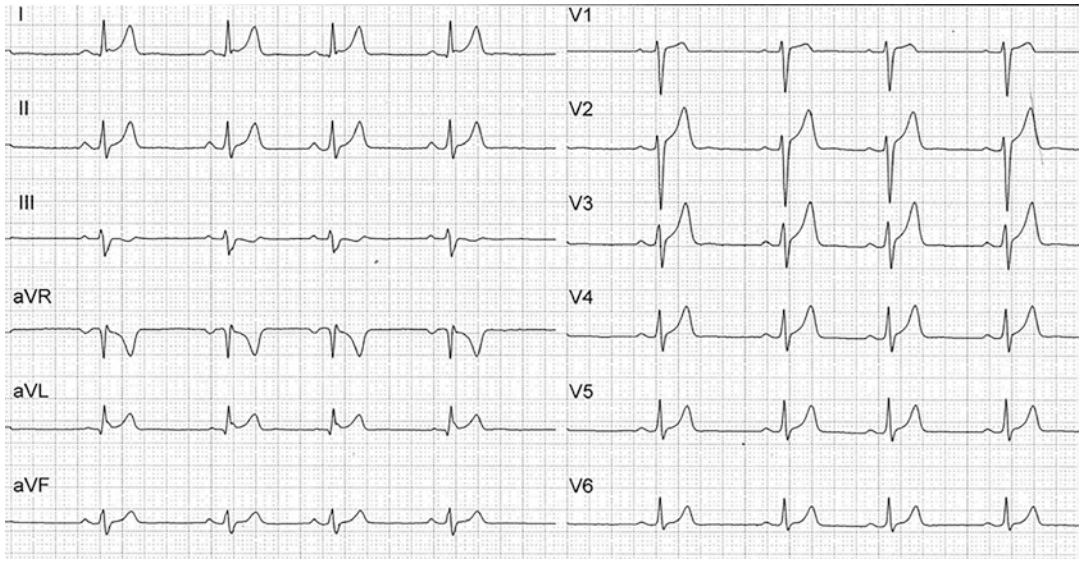


**Fig. 18.1** A 18-year-old male with unknown mutation. HR 57 bpm, QT 340 ms, QTc 330 ms

be feasible, and therefore it is advisable that QT interval should be measured as close as possible to 60 beats/min.

Based on the observation that the QT values follow a Gaussian distribution in the general population [8–12], the lower limit of normality for the QT interval can be set at 2 standard deviations from the mean, that is, 360 ms in men and 370 ms in women [2, 13]. On the contrary, the precise cut-off value for the diagnosis of SQTS is controversial. In the *2013 HRS/EHRA/APHRS Expert Consensus Statement on the Diagnosis and Management of Patients with Inherited Primary Arrhythmia Syndromes*, a cut-off value ≤330 ms was recommended for the diagnosis, while in the *2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death*, the threshold is set to 340 ms [14, 15]. Both documents agree that borderline values (up to 360 ms) may be considered for the diagnosis only in the presence of a confirmed pathogenic mutation, a family history of SQTS or of SD at age <40 years, and personal history of VT/VF episode in the absence of heart disease.

In the aforementioned epidemiological studies, the presence of shorter than normal QT-QTc intervals was not associated with an increased risk of SD. Due to this overlap between SQTS patients and general population, it appears that a single QTc value cannot distinguish all affected from healthy individuals.

## J-Tpeak Interval

In the first described SQTS patients, very impressive ECGs with tall, peaked, and symmetrical T waves together with a "virtual" ST segment had been observed [3]. In 2009, Anttonen et al. analyzed the differences in ECG between SQTS patients, subjects with short QT intervals from the general population, and subjects with normal QTc values. The main finding of this study was that SQTS subjects exhibited shorter QT and QTc values, together with shorter J-Tpeak intervals (between 80 and 120 ms) [16]. This observation was confirmed in a larger population of SQTS patients in 2011 [17].

## PQ Segment Depression

PQ segment depression (PQD) is a well-defined ECG marker of acute pericarditis. It is also associated with extensive myocardial damage and atrial fibrillation. In a paper by Tulumen et al., it emerged that 52/74 (81%) of SQTS patients displayed PQD at ECG, defined as >0.05 mV

(0.5 mm) depression from the isoelectric line. This finding, though present also in 24% of healthy control subjects, has been interpreted as a consequence of the heterogeneous abbreviation of atrial repolarization [18].

## QT/HR Relationship

A relevant feature in patients with SQTS is the lack of adaptation of the QT interval to the variations in the HR, with relatively little shortening in response to an increasing HR [19]. In 2015, the usefulness of exercise test in the diagnosis of SQTS was evaluated comparing the QT/HR relationship during exercise in 21 SQTS patients and 20 control subjects with short QT intervals [20]. SQTS patients showed a steeper β slope of the HR/QT linear relationship (≤ −0.90 ms/beat/min), together with a significantly lower mean QT variation from rest to peak exercise (48 + 14 ms vs.120 + 20 ms) (Fig. 18.2).

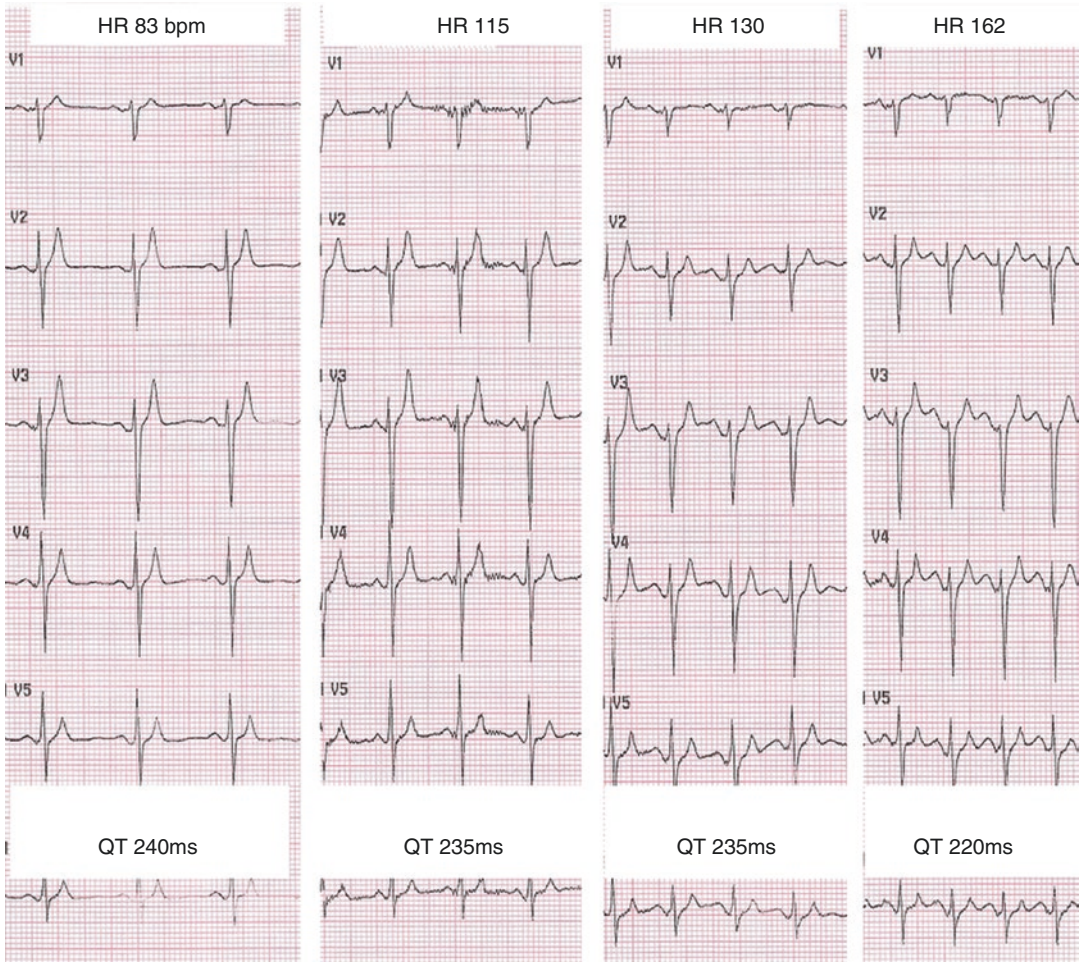


**Fig. 18.2** A 31-year-old female patient with KCNH2 (N588K) mutation. During the stress test a normal increase in heart rate was observed with only a slight further decrease of the QT interval duration. HR, heart rate

### Ventricular Effective Refractory Periods (VERPs)

Electrophysiological study (EPS) has a role in confirming the diagnosis of SQTS. In fact, both atrial and ventricular effective refractory periods (VERPs) have been shown to be shorter in SQTS subjects as compared to controls (140–200 ms at a cycle length between 500 and 600 ms), both at baseline and after premature extrastimuli [3, 17, 21].

In 2011 a diagnostic score system in the style of that used for long QT syndrome, defining the probability of having SQTS as "high," "intermediate," and "low," has been proposed [22]. The analyzed variables comprised clinical manifestations, family history, ECG parameters, and genetic analysis. The data were not drawn from a population of SQTS patients, but from several reports in worldwide literature, which included mainly symptomatic subjects. This score has not been validated in an independent population of SQTS patients and is not currently recommended for the diagnosis of SQTS.

## Genetics and Molecular Basis

SQTS is considered to have an autosomal dominant inheritance, with high penetrance in families and variable expressivity. To date, the molecular basis of the disease has been associated with mutations in six genes, which encode the potassium (SQTS1–3) and the L-type calcium (SQTS4–6) channel [23–27]:

- KCNH2 (rapidly activating delayed rectifier potassium channel [IKr], SQTS1)
- KCNQ1 (slowly activating delayed rectifier potassium channel [IKs], SQTS2)
- KCNJ2 (inward rectifier potassium channel [IK1], SQTS3)
- CACNA1C [ICa] (SQTS4)
- CACNB2b [ICa] (SQTS5)
- CACNA2D1[ICa] (SQTS6)

These mutations determine either an increase in the outward potassium currents or a decrease in the inward calcium current, which eventually results in acceleration of the repolarization process and an abnormal abbreviation of the QT interval at surface ECG.

Several genetic variants in *KCNH2* gene have been reported in the literature [28, 29], even though not in all cases a clear role in pathogenesis has been proved [30, 31]. It has also been speculated that some modifier gene variants may affect the QT interval duration and the potassium currents and possibly the response to therapy in some affected patients [32]. Mutations in the L-type calcium channel have been detected in a high percentage of probands with J-wave syndromes, Brugada syndrome, and early repolarization syndrome.

## Clinical Manifestations

Since its first description, less than 200 subjects with SQTS have been described in the literature worldwide. Few case series have been published so far: results from the Euroshort Registry, including 29 and 53 subjects, respectively, were published in 2006 and 2011 [17, 19]. Other registries included a cohort of 73 adults [33] and 25 pediatric patients [34].

SQTS affects predominantly males (above 75% of cases), with a mean age at observation below 25 years. More than a half (53–62%) of patients had experienced symptoms at the time of first clinical observation, of which cardiac arrest is the first in up to one-third of cases (26–32%). SD can be observed in individuals of all ages and its distribution shows two peaks: one in the first year of life and another between 20 and 40 years. It has been estimated that the probability of experiencing CA between birth and 40 years of age is between 40% and 50% [33]. With regard to the circumstances in which cardiac arrest or SD occurs, there are no clear triggers for arrhythmias, although a higher number of events have occurred during sleep or at rest. Syncope of presumed arrhythmic origin is the first clinical presentation in less than 20% of cases (13–16%), and short episodes of fast nsVT can be detected in both previously symptomatic and asymptomatic subjects

(Fig. 18.3). The presence of AF or atrial flutter at a young age has been documented in 11% of subjects in the Euroshort series [17] (Fig. 18.4), while in other series its prevalence has not been reported.

The incidence of events at follow-up in the available series ranges from 3.2% to 4.9% per year, with an estimated annual CA rate of 10.6% in SD survivors [33].

Due to the limited number of patients reported so far, and to the low yield of genetic testing, precise data on genotype-phenotype correlation in the setting of SQTS are not available.

Data from the EuroShort Registry showed that SQTS1 patients exhibit shorter QT, QTc, and J-Tpeak intervals at ECG (Fig. 18.5), shorter VERPs at electrophysiological study, and steeper slope of the QT/HR relationship during exercise [17, 20]. A recent review on 64 mutation-positive SQTS patients from the literature reported that 34 SQTS1 patients showed a later onset of symptoms as compared with other SQTS types, with a mean age at clinical manifestation of 35 ± 19 years [35]. However, several reports highlighted the high incidence of SD in children and newborns in SQTS1 families [3, 17, 19, 28], and although a definitive diagnosis could not be made due to the absence of available ECGs or molecular analysis on autoptic tissue, it is likely that these events represent a manifestation of the disease. Another observation from the study by Harrell and colleagues is that SSS or bradycardia were significantly more prevalent in SQT2 patients (6/8, 75%) than non-SQT2 patients (5/57, 9%; $p < 0.001$). In their study no significant differences in the QTc values between different genotypes emerged.

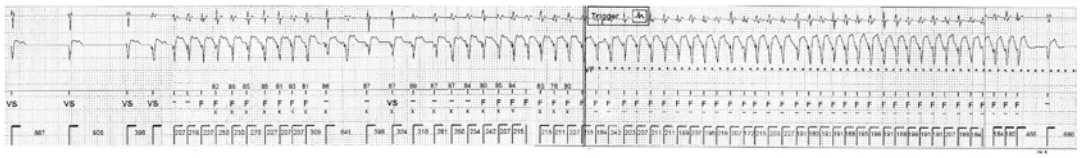

**Fig. 18.3** Episode of nsVT recorded by the ICD in a 43-year-old asymptomatic female with KCNH2 (N588K) mutation and family history of SD. The ICD had been implanted in primary prevention 11 years before

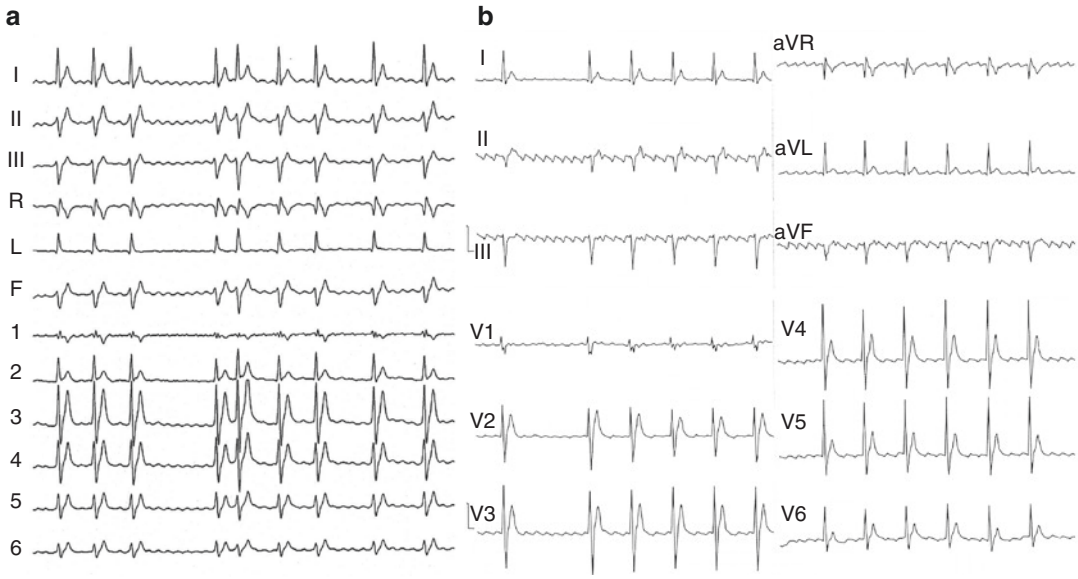


**Fig. 18.4** 33-year-old male with N588K mutation in KCNH2. (**a**) Atrial fibrillation with coarse and regular f waves. (**b**), common atrial flutter with very short FF intervals, suggesting very short atrial refractory periods

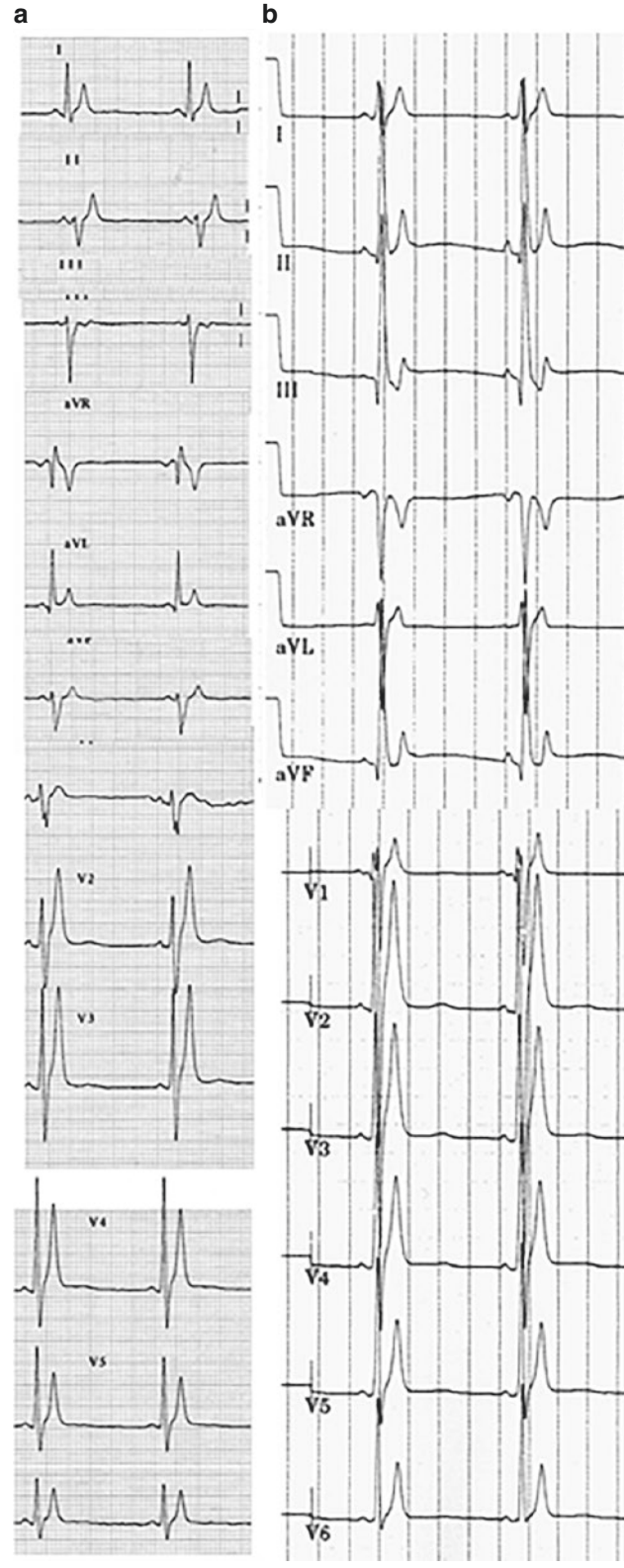


**Fig. 18.5** ECG from two male patients with KCNH2 (N588K) mutation. Panel **a**: HR 52 bpm, QT 260 ms, QTc 280 ms. Panel **b**: HR 62 bpm, QT 248 ms, QTc 252 ms. (Courtesy of Prof. M. Borggrefe, Department of Medicine–Cardiology, University Hospital Mannheim, first Department of Medicine–Cardiology, Mannheim, Germany)

## Risk Stratification and Management

Precise data on the risk stratification in SQTS are limited by the small number of subjects reported. In the three largest available series, neither sex nor age were significantly different in subjects with or without major arrhythmic events. Similarly, several ECG parameters, including QTc and JTp values, failed to demonstrate a clear correlation with the occurrence of symptoms [17, 33, 34]. Also the induction of sustained ventricular arrhythmias at EPS is not helpful in predicting CA, having a sensitivity below 40% [17, 33]. In a univariate analysis including the abovementioned parameters and others such as familial versus sporadic disease, family history of CA, mutation-positive status, and prior symptoms, only a personal history of aCA resulted to be associated with an increased risk of events at follow-up. A modified Gollob's diagnostic score had been proposed as a valuable tool to predict events in a pediatric cohort of SQTS patients [34] but failed to reproduce similar results in a cohort of adult patients [33].

Given the lack of precise risk stratification tools and the high incidence of SD, an implantable cardioverter-defibrillator (ICD) represents the treatment of choice for high-risk individuals, those with aborted CA or syncope [14]. Particular considerations with regard to the programming of the device must be made to reduce the risk of inappropriate shocks due to T wave oversensing in SQTS subjects; in fact, the high-amplitude T waves occurring shortly after the R wave may lead to double counting and inappropriate therapies [36, 37]. The intravenous ICD experience in the SQTS is burdened by a high incidence of complications (inappropriate shocks, lead fractures, infections requiring extraction of the device, and psychological distress) with rates up to 51% in adults [17] and 80% in children [34], not to mention the technical challenges of the implant in pediatric patients related to the small body and heart size, the difficult vascular access, and the need for modifications of the implant system during growth. Conversely, data on the use of subcutaneous ICD are still limited [38].

Concerning the pharmacological approach to the electrophysiological abnormalities underlying the SQTS, the first study with several antiarrhythmic drugs has been carried out in 2004 [39]. Selective IKr blocking agents were tested (sotalol and ibutilide) but failed to produce an increase in the QT interval in patients with SQT1, and subsequent genetic studies showed that the N588K mutation in *KCNH2* reduced the sensitivity of the channel to sotalol [40]. More recently, in vitro studies demonstrated that the T618I-*KCNH2* mutation is associated with a smaller loss of the inhibitory effect of sotalol on IKr channel, due to a less profound effect on inactivation of HERG current [27]. However, these findings were not confirmed in a SQTS1 family with the T618I mutation and the K897 T polymorphism in KCNH2 gene [32].

A robust evidence on the efficacy of hydroquinidine (HQ) in SQTS exists. After the first report highlighting the prolongation of QT intervals and VERPs, and the non-inducibility of arrhythmias at EPS in treated subjects [39], its long-term efficacy in reducing life-threatening arrhythmias has been confirmed by the results of the EuroShort Registry cohort follow-up in 2011 [17]. Twelve patients had been receiving HQ for a mean period of 76 ± 30 months. HQ induced normalization of the QT interval and of the VERPs in patients with *KCNH2* mutations, both those with the N588K and T618I mutations. A lower and less homogeneous QTc increment was observed in patients with different or unknown genotype; however, HQ prevented both induction of VF at EPS and arrhythmic events at follow-up regardless of genotype. Similar results have been reported in 2017 in a cohort of 15 subjects, with a reduction in the annual rate of late arrhythmic events from 12% while off-HQ to 0 while on-HQ [41].

Among the other drugs, flecainide caused only a slight QT prolongation (mainly due to an increase in the QRS duration) in 4 patients [39]. Isolated reports on the efficacy of dofetilide (QT prolongation and AF suppression), amiodarone (polymorphic VT recurrences prevention), and isoproterenol (management of electrical storm) have been published [17, 34, 42].

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# 19 Microvolt T-Wave Alternans: Pathophysiology and Clinical Aspects

Richard L. Verrier

## Abbreviations

| | |
|---|---|
| ABCD | Alternans Before Cardioverter Defibrillator (NCT00187291) |
| AECG | Ambulatory electrocardiogram |
| CAD | Coronary artery disease |
| ECG | Electrocardiogram |
| EU-CERT-ICD | Comparative Effectiveness Research to Assess the Use of Primary ProphylacTic Implantable Cardioverter Defibrillators in Europe (NCT02064192) |
| FINCAVAS | Finnish Cardiovascular Study |
| HRT | Heart rate turbulence |
| ICD | Implantable cardioverter-defibrillator |
| LQTS | Long QT syndrome |
| LVEF | Left ventricular ejection fraction |
| MADIT II | Multicenter Automatic Defibrillator Implantation Trial II |
| MASTER | Microvolt T Wave Alternans Testing for Risk Stratification of Post-Myocardial Infarction Patients (NCT00305240) |
| MERLIN TIMI 36 | Metabolic Efficiency with Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndrome-Thrombolysis in Myocardial Infarction – TIMI 36 (NCT00099788) |
| MMA | Modified Moving Average |
| NPV | Negative predictive value |
| PPV | Positive predictive value |
| REFINE-ICD | Risk Estimation Following Infarction Noninvasive Evaluation – ICD (NCT00673842) |
| SCD | Sudden cardiac death |
| SCD-HeFT | Sudden Cardiac Death in Heart Failure Trial (NCT00000609) |
| SWORD | Survival With Oral *d*-Sotalol |
| TdP | Torsade de pointes |
| TWA | T-wave alternans |
| VF | Ventricular fibrillation |
| VT | Ventricular tachycardia |

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**Fig. 19.1** Precordial ($V_4$) electrocardiogram rhythm strip (left panel) and high-resolution template of QRS-aligned complexes (right panel) during routine exercise tolerance testing from a patient with coronary artery disease who experienced cardiovascular death at 12 months following the recording. The template illustrates T-wave alternans (TWA) as a separation between ST-T segments in A and B beats. mV, millivolt. Sec, second. (Reproduced with permission from Elsevier from Verrier et al. [1])

## Introduction

Fascination with T-wave alternans (TWA), a beat-to-beat fluctuation in ST-segment or T-wave morphology (Fig. 19.1) [1], can be traced to the dawn of electrocardiology. In 1910, less than a decade after Einthoven's invention of the electrocardiograph, Sir Thomas Lewis noted, when TWA was visible in the electrocardiogram (ECG) of his patients with angina pectoris, that there was elevated risk for premature death [2]. For decades, macroscopic, visible TWA, observed episodically in cases of long QT syndrome (LQTS), acute coronary syndrome, and adverse drug responses, was regarded as a relatively rare but ominous sign. It was not until the late 1980s, when systematic attempts were made to apply advanced signal processing techniques including frequency-domain spectral analysis and time-domain recursive averaging to quantifying TWA, that it was realized that this phenomenon is prevalent in diverse pathologic conditions.

This review addresses the following questions:

1. What are the main pathologic conditions responsible for sudden cardiac death? And what is the evidence that TWA might be useful in risk assessment in these main arrhythmogenic states?
2. What are the electrophysiologic mechanisms underlying TWA's predictive capacity?
3. What analysis methods are approved for clinical use by the United States Food and Drug Administration? And what clinical studies support their utility?
4. What is the rationale for quantitative analysis of TWA?

## Pathophysiology of Sudden Cardiac Death and Rationale for T-Wave Alternans Testing

Identification of people at risk for sudden cardiac death (SCD) continues to present a major challenge in contemporary cardiology. SCD is responsible for 1.5 million deaths annually worldwide, including 512,000 in the United States [3]. In 45–50% of cases, death is the first expression of heart disease [4, 5]. Epidemiological findings reveal that coronary artery disease (CAD) is the main culprit in 80% of life-threatening arrhythmias [6] (Fig. 19.2). Dilated and hypertrophic cardiomyopathies are the second most frequent cause of SCDs, in the range of 10–15%. Other disorders, such as valvular or congenital heart diseases, acquired infiltrative disorders, primary electrophysiological disorders, and genetically determined ion channel abnormalities, are responsible for a small proportion, ~1%, of SCDs that occur in the general population. Two main processes leading to malignant arrhythmias have been observed in patients with ischemic heart dis-

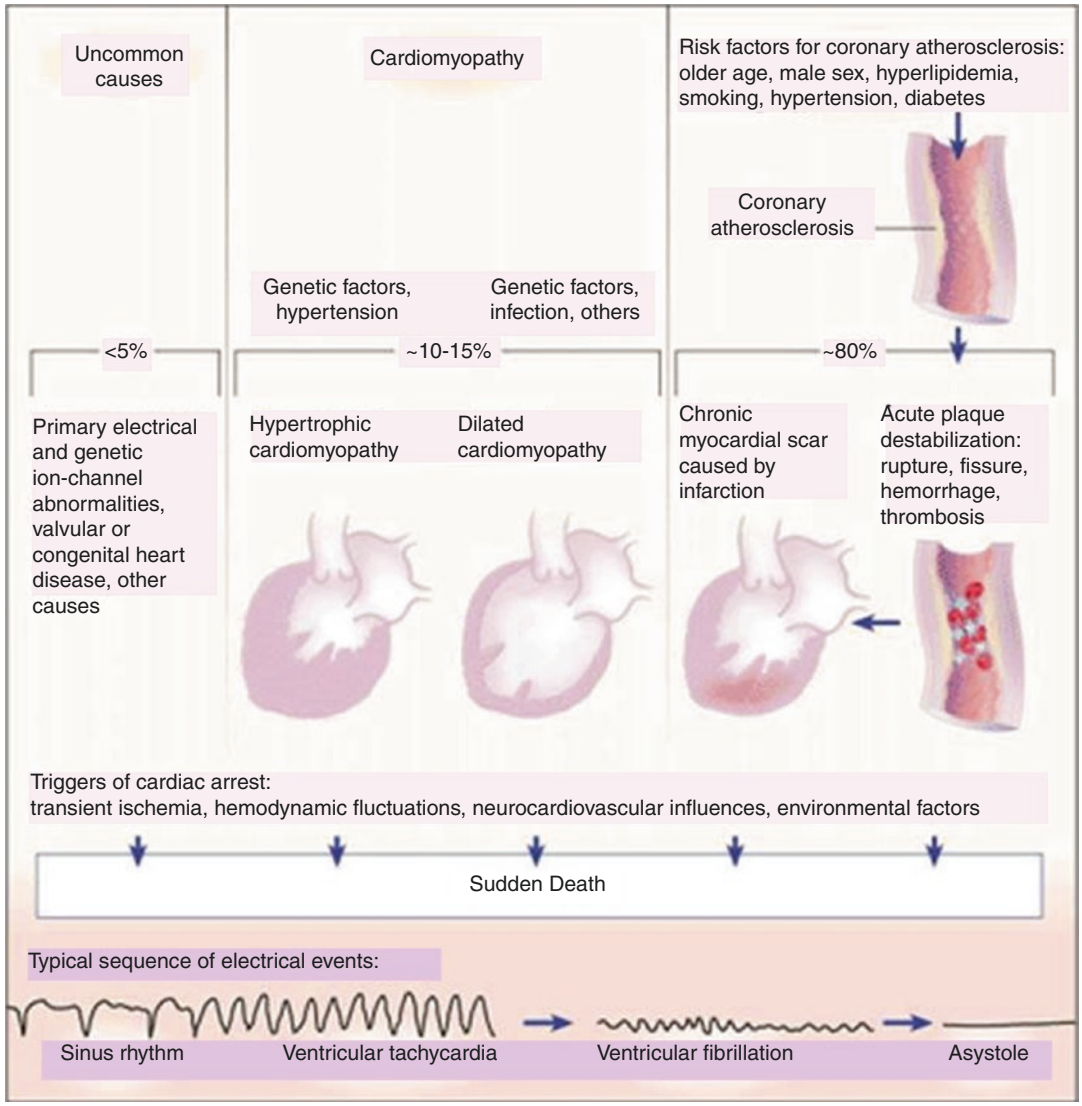


**Fig. 19.2** Pathophysiology and epidemiology of sudden death from cardiac causes. (Published with permission from Massachusetts Medical Society from Huikuri et al. [6])

ease. These include ventricular tachyarrhythmia triggered by acute myocardial ischemia in individuals with or without preexisting myocardial scar and ventricular tachyarrhythmia attributable to an anatomical substrate, usually scarring from a previous infarction, without active or identifiable myocardial ischemia. Acute myocardial ischemia is considered to be the primary factor that triggers lethal arrhythmias. Additional factors have been implicated, specifically, systemic metabolic and hemodynamic alterations, neurochemical and neurophysiological influences, and exogenous toxic or proarrhythmic drugs. These precipitating factors can interact with ischemia or cardiac structural abnormalities culminating in SCD due to ventricular arrhythmia [6].

While conventional cardiovascular risk factors including smoking, hypertension, and hyperlipidemia correlate with cardiovascular disease, they are not adequate markers of risk for SCD. The primary contemporary risk factor is depressed left ventricular ejection fraction (LVEF). Its value in

identifying patients who would benefit from placement of implantable cardioverter defibrillators (ICDs) is based on the randomized, controlled Multicenter Automatic Defibrillator Implantation Trial (MADIT) II trial, which demonstrated that ICDs confer improved survival over conventional pharmacologic therapy in patients with LVEF ≤30% [7]. However, in the general population, LVEF does not have adequate sensitivity and specificity to guide ICD implantation, as the majority of individuals who succumb to SCD have relatively preserved LVEF, and only 1 of 8 patients who receive ICDs based on MADIT II criteria experience appropriate device discharge to terminate ventricular tachycardia/fibrillation (VT/VF) across the lifetime of the ICD [8]. The findings from the large Israeli National ICD Registry suggest that the number of patients needed to treat in a "real-world setting" may be even less favorable, specifically 1 of 20 [9].

Extensive data showing that TWA is useful in risk assessment in most of these pathophysiologic conditions has been reported in >13,000 individuals. An overview of the clinical studies supporting this assertion is provided in Table 19.1 [10–32].

## Synopsis of Electrophysiologic Mechanisms and Physiologic Influences

TWA's utility for evaluating risk for cardiovascular mortality and SCD is based on the extent of heterogeneity of repolarization [33] and of derangements in intracellular calcium cycling (Fig. 19.3) [34–36]. In turn, these abnormalities result in a beat-to-beat alternation of action potential duration culminating in alternating changes in the size and shape of the T wave. TWA can be either spatially concordant, when action potentials in adjoining cell regions alternate in phase, or discordant, when they are out of phase [34, 37]. The transition from concordant to discordant TWA is an established indicator of increased risk for serious arrhythmias [33].

Investigations of TWA in integrative physiology studies with regard to clinical correlates and pharmacologic influences are summarized in Table 19.2 [32, 38–65]. Surges in heart rate, adrenergic activity, and myocardial ischemia and reperfusion increase TWA level while also enhancing arrhythmia risk. Conversely, vagus nerve stimulation, beta-adrenergic receptor

**Table 19.1** Clinical studies of prediction of sudden cardiac death or life-threatening arrhythmias by quantitative T-wave alternans with the time-domain modified moving average method

| First author, year (trial) | Patient population (enrollment, disease, mean age) | Mean LVEF | TWA hazard ratios (95% CI), AUC, NVP, PPV for SCD, or life-threatening arrhythmias |
|---|---|---|---|
| Verrier et al. 2003 (ATRAMI) [10] | Acute post-MI; case-control analysis (15 cases, 29 controls) from 1284 ATRAMI patients; 60–62 years | Moderately depressed (42 ± 3%) | 4.2 (1.1–16.3, $p < 0.04$) to 7.9 (1.9–33.1, $p < 0.005$) for cardiac arrest or arrhythmic death at 21 ± 8 months for a priori 75th percentile cut point (47 μV); patients were monitored at 15 ± 10 days post-MI |
| Slawnych et al. 2009 (REFINE and FINCAVAS) [11] | 322 post-MI patients; 62 (interquartile range 53–70) years | Moderately depressed (38%–48%) | 3.7 (1.4–9.7, $p = 0.008$) monitored during postexercise recovery at 10–14 weeks after event for CV death at 47 months for TWA ≥60 μV; NPV = 95%; PPV = 14%; sensitivity = 20%; specificity = 92%; 2.6 (1.0–6.6, p = 0.05) for SCD. AUC = 0.69 |
| Stein et al. 2008 (EPHESUS) [12] | Acute post-MI, LVEF ≤40%, and heart failure; Case-control analysis (46 cases, 92 controls) from 493 EPHESUS patients in AECG substudy; 68 ± 11 years | Depressed (34 ± 5%) | 5.5 (2.2–13.8, $p < 0.001$) for SCD at 16.4 months for 47 μV; patients were monitored at 2–10 days post-MI. For SCD, AUC = 0.73 for lead $V_1$ and 0.70 for lead $V_3$ ($p < 0.001$) |
| Sakaki et al. 2009 [13] | 295 consecutive patients with ischemic or nonischemic cardiomyopathy and left ventricular dysfunction; 66 ± 16 years | Depressed (34 ± 6%) | 17.1 (6.3–46.6, $p < 0.001$) for CV death, 22.6 (2.6–193.7, $p < 0.005$) for witnessed SCD at 1.1 year for TWA ≥65 μV; NPV for CV death = 97%; PPV = 37%; sensitivity = 74%; specificity = 87% |

(continued)

**Table 19.1** (continued)

| First author, year (trial) | Patient population (enrollment, disease, mean age) | Mean LVEF | TWA hazard ratios (95% CI), AUC, NVP, PPV for SCD, or life-threatening arrhythmias |
|---|---|---|---|
| Maeda et al. 2009 [14] | 63 consecutive patients including 21 controls, 21 post-MI patients without VT, and 21 post-MI patients with VT; 65 ± 11 years | Depressed (36–43%) for post-MI group | 6.1 (1.1–34.0, $p < 0.041$) for sustained VT or VF at 6 years for TWA ≥65 μV; patients were monitored at 1–3 months after event |
| Stein et al. 2010 (CHS) [15] | General population patients ≥65 years old; case-control analysis (49 cases, 97 controls) from 1649 CHS patients; 73 ± 5 years | Not tested, assumed preserved | 4.84 (1.48–15.81, $p < 0.009$) for SCD across 4.7 ± 3.0 (range 0.2–10.4) years for TWA ≥37 μV |
| Leino et al. 2011 [16–19] | 3598 consecutive patients referred for exercise tolerance testing, 56 ± 13 years | Mostly preserved | 1.55 (1.150–2.108, $p < 0.004$) for CV death; 1.58 (1.041–2.412, $p < 0.033$) for SCD per 20 μV TWA in lead $V_5$ |
| Hoshida et al. 2012 [20] | 313 consecutive post-MI patients, 70 ± 12 years | 48% | 3.6 (1.3–10.4, $p < 0.0174$) for cardiac mortality; 5.8 (1.6–20.8, $p < 0.0072$) for SCD for TWA ≥65 μV at 3.3 years; patients were monitored >2 weeks after event |
| Ren et al. 2012 [21] | 173 consecutive post-MI patients with and without diabetes mellitus; 66 years | 46% | AUC = 0.708 for cardiac mortality at 1.6 years; patients were monitored at 1–3 weeks after event |
| Shimada et al. 2012 [22] | 40 consecutive patients with vasospastic angina, 59 years | 66% | 15.5 (1.7–142.0, $p = 0.009$) for VT at 2.9 years for TWA ≥65 μV during asymptomatic periods |
| Sulimov et al. 2012 [23] | 111 post-MI patients, 64.1 years | 46.6% | 5.01 (1.5–17.0, $p < 0.005$) for SCD at 1 year for TWA >53.5 μV at heart rate of 100 bpm, NPV = 93.9%; PPV = 24.4%; sensitivity = 73.3%, specificity = 64.6%; patients were monitored at 2 months to 36 years after event |
| Hou et al. 2013 [24] | 219 consecutive acute post-MI patients, 56 years | >35% in 201; ≤35% in 18 | 15.07 (2.88–78.68, $p < 0.0031$) for SCD within 16 ± 7 months for TWA ≥47 μV; patients were monitored at 7 ± 4 days after event |
| Verrier et al. 2013 [25] | 48 acute STEMI patients; 61 years | 51% | AUC = 0.87; NPV = 79%; PPV = 71% for NSVT during or after PCI |
| Arisha et al. 2013 [26] | 199 consecutive post-MI patients, 61.7 years | 45% | ROC = 0.64 for SCD or life-threatening ventricular arrhythmias within 6 months; ROC = 0.80 for TWA combined with HRT onset; ROC = 0.86 for TWA combined with HRT onset in patients with LVEF <40% |
| Nieminen et al. 2014 (MERLIN) [27] | 210 ACS patients, 68 years | <40% | 2.35 (1.03–5.37, $p = 0.04$) for 1 year mortality for TWA ≥47 μV; patients were monitored in-hospital at onset of ACS |
| Uchimura et al. 2014 [28] | 45 Brugada syndrome patients, 45 ± 15 years | [not stated] | 7.217 (2.503–35.504, $p = 0.002$) for VT history |
| Takasugi et al. 2016 [29] | 32 consecutive LQTS patients, 13 (range: 11–17.5) years | [not stated] | 100% sensitivity for VT history at ≥42 μV cut point |
| Chung et al. 2016 [30] | 63 ARVD/C patients, 44.7 ± 14.8 years | Preserved | 1.06 (1.03–1.10, $p < 0.001$) |
| Yamada et al. 2018 [31] | 215 hospitalized decompensated heart failure patients, 62 ± 14.5 years | Both depressed and preserved | 1.015 (1.003–1.027, $p = 0.016$) |
| *Summary* | 5884 patients in full-cohort prospective studies 391 patients in case-control studies | Both depressed and preserved | |

Expanded from Verrier and Ikeda [32]

Key: *ACS* acute coronary syndrome, *AUC* area under the curve, *CAD* coronary artery disease, *EPS* electrophysiologic study, *ICD* implantable cardioverter-defibrillator, *LQTS* long QT syndrome, *MI* myocardial infarction, *NSVT* nonsustained ventricular tachycardia, *PCI* percutaneous coronary intervention, *SCD* sudden cardiac death, *STEMI* ST-segment elevation myocardial infarction, *SUDEP* sudden unexplained death in epilepsy patients, *TdP* torsade de pointes, *TWA* T-wave alternans, *VF* ventricular fibrillation, *VNS* vagus nerve stimulation, *VT* ventricular tachycardia

**Table 19.2** Physiologic and pharmacologic influences on T-wave alternans: experimental and clinical correlates

| Experimental intervention | Clinical correlates |
|---|---|
| *Increase TWA* | |
| Elevated heart rate and rapid atrial pacing [38] | Right atrial pacing or exercise increases TWA [39] |
| Adrenergic stimuli and behavioral stress [40] | Anger recall and mental arithmetic increase TWA in ICD patients [41]<br>Anger-induced TWA predicts ICD discharge [42] |
| Myocardial ischemia [38, 43–45] | TWA predicts intraprocedural VT during PCI in STEMI patients (AUC = 0.87) [25]<br>TWA stratifies risk for 1-year mortality in ACS patients [27] |
| Reperfusion (initial minutes) [38, 44, 45] | Surge in TWA prior to VT/VF during PCI [46] |
| $I_{Kr}$ blockade [47] | d-Sotalol increased arrhythmia in CAD patients [48]; penicillin provoked TWA and TdP [49] |
| Flecainide [50] | Flecainide provoked proarrhythmia in patients with CAD [51]; clinical effects on TWA have not been reported |
| *Decrease TWA* | |
| Stellectomy [44] | Reduced SCD in LQTS [52] and post-MI patients [53] |
| VNS [54] | VNS reduced SUDEP [55] and TWA [56, 57] in patients with epilepsy |
| Beta-blockade [40] | Metoprolol reduced SCD in post-MI patients [58]<br>Beta-blockade during fixed-rate EPS [59–61] or graded exercise [62] decreased TWA |
| Calcium channel blockade [63] | Decreased NSVT and TWA in patients with vasospastic angina [22] |
| Late $I_{Na}$ blockade [47, 50] | Decreased VT [64, 65] and TWA [27, 65] |

Key: *ACS* acute coronary syndrome, *AUC* area under the curve, *CAD* coronary artery disease, *EPS* electrophysiologic study, *ICD* implantable cardioverter-defibrillator, *LQTS* long QT syndrome, *MI* myocardial infarction, *NSVT* nonsustained ventricular tachycardia, *PCI* percutaneous coronary intervention, *SCD* sudden cardiac death, *STEMI* ST-segment elevation myocardial infarction, *SUDEP* sudden unexplained death in epilepsy patients, *TdP* torsade de pointes, *TWA* T-wave alternans, *VF* ventricular fibrillation, *VNS* vagus nerve stimulation, *VT* ventricular tachycardia

blockade, calcium channel antagonism, inhibition of late $I_{Na}$, and sympathetic denervation, which reduce susceptibility to ventricular tachyarrhythmias, also diminish TWA level. Experimentally induced myocardial ischemia increases TWA magnitude in parallel with vulnerability to ischemia-induced VF [38, 43, 44]. Abnormalities in both calcium cycling and conduction are linked to the development of TWA during myocardial ischemia. Studies with luminescent dye have demonstrated both concordant and discordant alternation in calcium transients during myocardial ischemia [35]. Heart failure augments TWA level in parallel with vulnerability to arrhythmias and reduction in the heart rate threshold for TWA as a consequence of impaired intracellular calcium cycling [66]. Abnormal calcium handling and action potential alternans have been documented in patients with systolic dysfunction and VT and/or VF but not in normal individuals [67]. TWA level has been found to be correlated to extent of scar tissue [68, 69].

## Clinical Methodologies

Two methods have been cleared by the United States Food and Drug Administration to determine microvolt levels of TWA for risk stratification for arrhythmic death, namely, the Spectral and Modified Moving Average (MMA) Methods. Other implementations have been used in a variety of clinical and preclinical studies.

The spectral method employs an exercise-based system that was formerly commercialized by Cambridge Heart, Inc. (Tewksbury MA, USA). This method is based on a fast Fourier transform to measure the amplitude of the spectrum at 0.5 cycle/beat (Fig. 19.4) [70, 71]. The average alternans level across the ST-T wave is determined. The spectral method requires increasing and maintaining the subject's heart rate at 105–110 beats per minute and use of specialized electrodes. Tests with TWA levels $\geq$1.9 μV with signal-to-noise ratio $K$ = 3 sustained for 2 minutes are classified as positive

tests; tests with TWA values below this level are considered negative. Due to the relatively high incidence (20–40%) of tests with excessive ectopy, inability to raise heart rate to 105–110 bpm, or transient TWA (Fig. 19.4, lower left panel), the classification "abnormal due to patient factors" is used [72] and these tests are considered "nonnegative" and combined with positive tests in clinical studies of risk assessment. Recordings with muscle, respiration, or other movement artifacts or electrode noise are regarded as "technically indeterminate."

The MMA method, commercialized by GE Healthcare, Inc. (Milwaukee WI, USA), operates on two platforms: ambulatory ECGs (AECGs) employing a MARS or Getemed (Berlin, Germany) reader and the CASE 8000 treadmill system for routine, symptom-limited exercise tolerance testing. MMA is a time-domain analytical approach that involves the noise-rejection principle of recursive averaging (Fig. 19.4, right panel) [73]. The algorithm continuously transfers odd and even beats into separate bins and generates median complexes for each bin. The maximum separation between the A and B beats within the ST-T wave is demarcated with a vertical line in the template of superimposed beats; this separation indicates the patient's TWA level and is used for risk assessment. This method does not require elevation or stabilization of heart rate or use of specialized electrodes. A TWA level of ≥47 μV is classified as abnormal and ≥60 μV as severely abnormal in terms of elevated risk for SCD and/or cardiovascular mortality [1]. Indeterminate results are few (3–5%) with the MMA method because reaching a target heart rate is not required.

MMA-based TWA can be analyzed during routine exercise tolerance testing, permitting patients to be monitored in the flow of clinical evaluation. AECG-based TWA reflects the influences of daily activity, behavioral stress, and sleep, trigger factors that cannot be duplicated by exercise tolerance tests. The peak TWA value during the entire 24-hour period has proved to be more informative than brief recordings. Nocturnal TWA monitoring is important with regard to the fact that 20–30% of SCDs in individuals with

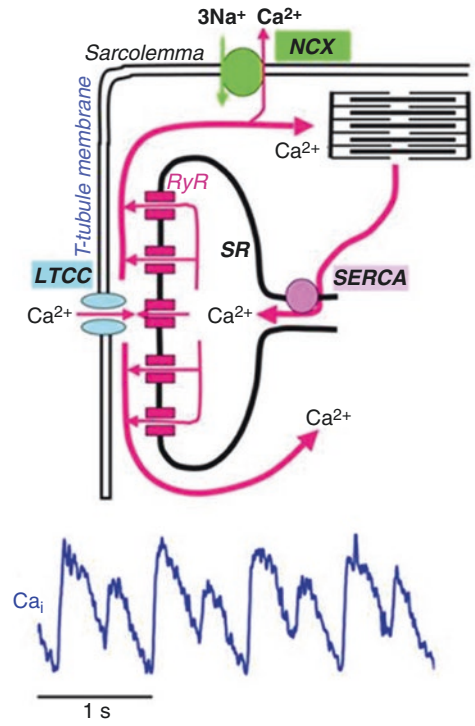


**Fig. 19.3** Intracellular calcium cycling dynamics in cardiac myocytes. Upper panel: Schematic of calcium ($Ca^{2+}$) cycling, illustrating that a small amount of $Ca^{2+}$ entering the cell through L-type $Ca^{2+}$ channels (LTCC) triggers release of a large amount of $Ca^{2+}$ from internal stores (sarcoplasmic reticulum, SR) by activating SR $Ca^{2+}$ release channels (ryanodine receptors, RyR). $Ca^{2+}$ is then pumped back into the SR by sarcoplasmic reticulum calcium ATPase (SERCA) pumps or removed from the cell by sodium ($Na^+$)-calcium exchange (NCX). Lower panel: Calcium transient ($Ca_i$) alternans is under the dual influences of membrane voltage and the dynamics of intracellular $Ca^{2+}$ handling by the SR. Action potential duration (APD) alternans occurs when perturbations in membrane voltage arise and/or when elevated heart rate or other physiologic or pathophysiologic factors disrupt the myocytes' capacity to cycle calcium. (Adapted with permission from Wolters Kluwer from Weiss et al. [34])

CAD and/or heart failure occur during nighttime [74]. Nocturnal precipitating factors include REM sleep-associated bursts in sympathetic nerve activity and disturbed nighttime breathing due to apneas, which occur in 50% of heart failure patients. In specific disease states such as the Brugada syndrome, TWA is rate-suppressed, and thus TWA should not be measured during exercise tolerance testing [75]. Monitoring these patients at rest or during nighttime, when their

peak TWA levels occur [28], is preferable. Moreover, AECG-based monitoring offers the distinct advantage of permitting TWA measurement in patients who cannot exercise.

The four- to ten-fold difference in TWA values reported by the two methods is largely a consequence of differences in the extent of signal averaging and filtering [1]. Another basis for the lower values reported by the spectral method as compared to the MMA method is that with the former, the average TWA value across the ST-T wave is calculated, while with the latter, the peak TWA level is provided.

## Clinical Evidence of Prediction

An AHA/ACC/ESC guideline committee [76] assigned Class I Level of Evidence A to TWA monitoring on AECG to assess risk or to evaluate therapy and Class IIa Level of Evidence A for TWA monitoring on AECG or exercise tolerance test recordings to improve diagnosis and risk assessment in individuals with ventricular arrhythmias. A subsequent consensus guideline statement sponsored by the International Society for Holter and Noninvasive Electrocardiology recommended that TWA analysis be considered "whenever there is suspicion of vulnerability to lethal cardiac arrhythmias" [1]. However, the document also recognized the unmet need to provide evidence of the potential of TWA to guide device and medical therapy.

TWA testing with the spectral method has demonstrated its predictive capacity in prospective studies enrolling 7200 patients with diverse cardiovascular diseases, including myocardial infarction, congestive heart failure, ischemic cardiomyopathy, and nonischemic dilated cardiomyopathy [1]. No further studies with the spectral method have been announced.

Using routine exercise tolerance testing protocols, the MMA method has stratified risk for cardiovascular death and SCD in ~3600 patients with generally preserved LVEF enrolled in the Finnish Cardiovascular Study [16–19]. This investigation is the largest TWA study conducted with any method. TWA analysis on AECG using the MMA method has proven to predict cardiovascular mortality and SCD in nearly 2300 patients with CAD, acute myocardial infarction, acute coronary syndrome, heart failure, LQTS, Brugada syndrome, vasospastic angina, ischemic and nonischemic cardiomyopathy with differing degrees of left ventricular dysfunction, and arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) as well as in the general population (Table 19.1). In all MMA studies, TWA was measured while patients continued chronic medications, including beta-adrenergic blocking agents. All studies in which the commercialized MMA method was accurately performed have been predictive.

An important recent discovery is that microvolt TWA is far more prevalent in LQTS patients than previously reported, 69%, and above the cut point of 42 μV is strongly associated with TdP history [29]. The authors recommended that TWA should be monitored from precordial leads in LQTS patients, as they surmised that use of a limited set of ECG leads in conventional monitoring led to underestimation of the prevalence of TWA and its association with TdP. The reader is referred to several chapters in this book for discussion of the cellular, genetic, and molecular bases for arrhythmogenesis in LQTS.

Odds ratios generated by the spectral and MMA methods for all studies are comparable based on multivariate analyses adjusted for standard clinical variables for cardiovascular disease, namely, demographic factors (e.g., age, sex, and race) and traditional cardiovascular risk markers (e.g., smoking, blood pressure, history, medications, and depressed LVEF) [1]. Specifically, for predictive studies using the Spectral Method, the odds ratios ranged from 2.1 to ∞ for SCD/arrhythmic death in patients with depressed LVEF and from 5.9 to 23.5 for SCD/arrhythmic death in patients with better preserved LVEF. For the MMA method, odds ratios ranged from 2.94 to 17.1 for cardiovascular death and from 4.8 to 22.6 for SCD in patients with both depressed and preserved LVEF. A head-to-head comparison of the spectral and MMA methods in 322 post-myocardial infarction patients reported that the odds ratios for estimating cardiovascular mortal-

ity and SCD were equivalent, at 2.75 for the spectral method and at 2.94 for the MMA method [11, 77]. Across all studies, the negative predictive value (NPV) averages 97.2% for the spectral method and ranges from 94 to 99% for the MMA method. For both techniques, the positive predictive value (PPV) is variable, consistent with the fact that PPV is based on the prevalence of disease in the study population.

## Importance of Quantitative Analysis of TWA to Enhance Clinical Prediction

In light of the fact that TWA reflects a spectrum of cardiac electrical instability, its quantification can enhance the potential for diagnosing degrees of risk and for improving prognostic capacity, because treating a continuous variable as binary reduces its predictive power by 33–50% [78]. Analysis of the FINCAVAS database demonstrated that risk for cardiovascular death and SCD increased by 55% and 58%, respectively, for each 20-μV increase in MMA-based TWA [19] (Fig. 19.4, right panel). Quantitative TWA analysis may permit dynamic tracking of changes in risk across time. This consideration is relevant to post-MI remodeling, changing status of cardiac disease or heart failure, or response to medical therapy or cardiac exercise rehabilitation.

Moreover, TWA quantification permits comparison of risk levels among different populations and disease conditions, whose range of TWA levels correlates with established degrees of risk (Fig. 19.5) [32]. For example, TWA is markedly elevated (≥60 μV) in patients who receive dialysis, even in the absence of a prior cardiovascular event, a finding consistent with their well-known heightened risk for SCD [79]. Also, patients with drug-resistant epilepsy exhibit markedly elevated TWA levels during the interictal period, in the range of 70 microvolts [56, 57, 80], consistent with the >threefold increased risk for SCD over the general population [81, 82] and with emerging evidence of cardiovascular comorbidities [83].

## TWA Assessment to Guide Device-Based Therapy

The primary contemporary application foreseen for microvolt TWA was to identify patients with reduced LVEF who would not benefit from ICD placement. This application was spearheaded by investigators using the spectral method and was based on the presence or absence of criterion levels of TWA. Unfortunately, the overall experience did not prove satisfactory as the MASTER trial (NCT 00305240, NCT00305214) [84] and the TWA substudy of the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT, NCT00000609) [85] did not demonstrate prediction. One potential explanation for this failure is washout of beta-adrenergic blocking agents prior to the test to allow patients to achieve the target heart rate requirement of 105 to 110 beats/min and resumption of these agents following the test. In studies using the spectral method in which beta-blockers were washed out, the hazard ratios were four-fold lower than those in which beta-blockade was maintained (Fig. 19.6) [86]. The present recommendation is that TWA testing should be performed while patients maintain chronic medications including beta-blockers [1]. Employing ICD discharge as a surrogate endpoint for SCD has also been suggested as the explanation for the absence of prediction [87] in the MASTER trial [84] and SCD-HeFT TWA substudy [85]. It is worth noting that the Alternans Before Cardiac Defibrillator (ABCD) trial (NCT00187291) indicated similar 1-year success as electrophysiologic study in identifying patients who would not benefit from ICD placement [88].

The Risk Estimation Following Infarction Noninvasive Evaluation-ICD (REFINE-ICD) randomized controlled trial (NCT00673842), which is testing whether ICD placement guided by both MMA-based TWA and the autonomic indicator heart rate turbulence (HRT) can alter SCD risk, is ongoing in Canada. The available data indicate that the presence of both abnormal TWA and impaired HRT in patients with relatively preserved left ventricular function identifies patients with a nearly tenfold increased risk

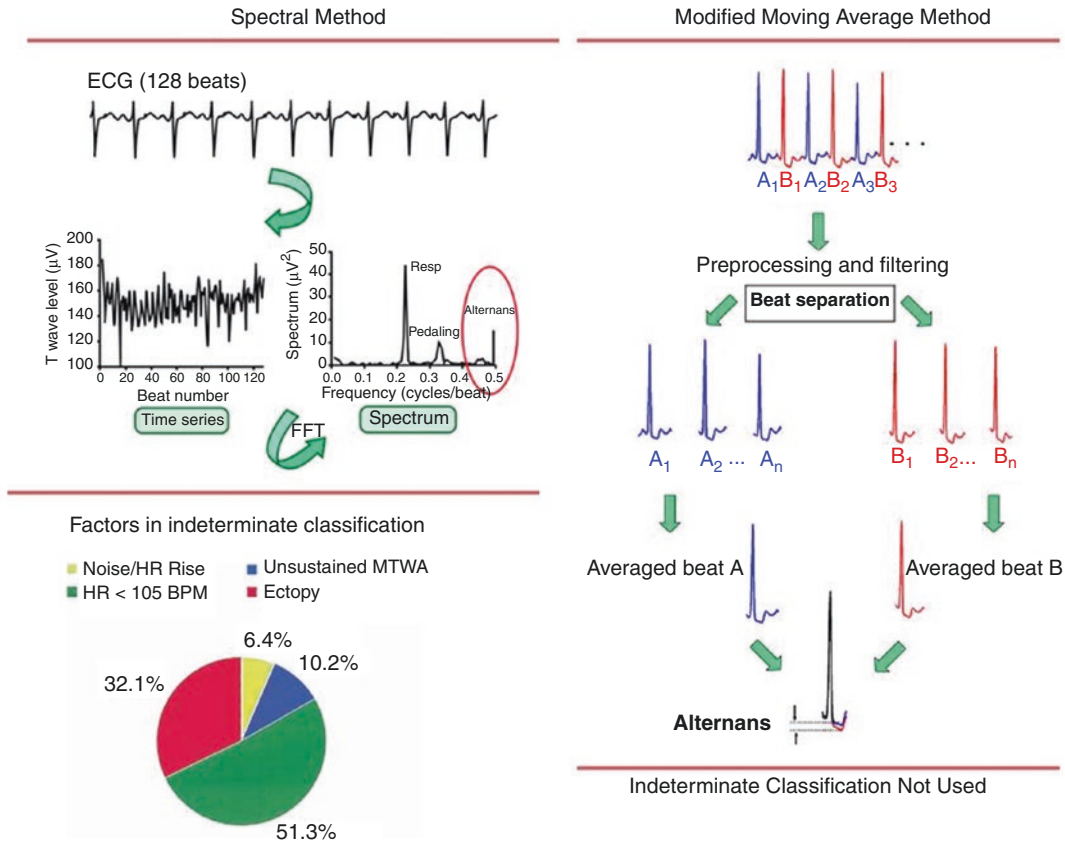


**Fig. 19.4** Left panel, upper figure: Schematic representation of T-wave alternans (TWA) assessment of the electrocardiogram (ECG) with the Spectral Method. FFT, fast Fourier transform. (Reproduced with permission from Elsevier from Verrier et al. [1])
Left panel, lower figure: Causes of indeterminate microvolt TWA (MTWA) tests. Only 6.4% of the indeterminate MTWA results were due to technical factors; the remainder was due to patient factors. BPM, beats/min; HR, heart rate. (Reproduced with permission from Elsevier from Kaufman et al. [72]). Right panel: Flow chart of the major components of the Modified Moving Average Method of TWA analysis. (Reproduced with permission from Elsevier from Verrier et al. [1])

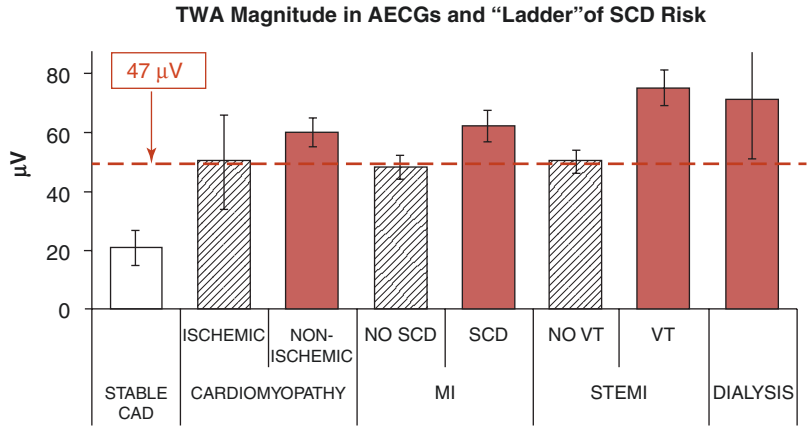


**Fig. 19.5** TWA magnitude determined by the MMA method in ambulatory ECGs showing "ladder" of risk. Mean values of peak TWA are from patients with stable coronary artery disease (CAD), cardiomyopathy, acute post-myocardial (MI) patients with and without sudden cardiac death (SCD), ST-elevation MI (STEMI) patients with and without ventricular tachycardia (VT), and dialysis patients. (Reproduced with permission from Elsevier from Verrier and Ikeda [32])

of cardiac death compared to those who do not meet both of these criteria [89]. The sizeable Comparative Effectiveness Research to Assess the Use of Primary ProphylacTic Implantable Cardioverter Defibrillators in Europe (EU-CERT-ICD, NCT02064192) study, which employs MMA-based TWA together with HRT, has completed enrollment and the results are pending.

## TWA Assessment to Guide Antiarrhythmic Drug Therapy

Sakabe and colleagues [90] demonstrated that administration of antiarrhythmic agents does not interfere with the capacity of TWA to predict outcomes. Using the Spectral Method, the investigators prospectively evaluated patients with ischemic or nonischemic dilated cardiomyopathy and a history of sustained VT or VF during empirically guided therapy with amiodarone, beta-blockade, and/or angiotensin-converting enzyme inhibitors. TWA but not LVEF predicted the recurrence of VT during the ensuing 13 ± 11 months. Effects of different classes of antiarrhythmic drugs on TWA have been reviewed in detail [91] and are summarized below, providing evidence of TWA's suitability to serve as a therapeutic target for antiarrhythmic medications.

### Sodium Channel Blockade

Traditional sodium channel blocking agents have not been shown to be effective in reducing malignant ventricular arrhythmias [47], and accordingly, there is little or no evidence in the literature of an effect of this class of agents on TWA. By contrast, inhibition of the late $I_{Na}$ current has yielded promising results. Increases in late $I_{Na}$, which are known to be arrhythmogenic because they result in excess intracellular calcium, have been shown to occur under diverse pathologic conditions, specifically myocardial ischemia, heart failure, reperfusion, and the long QT3 syndrome [92]. The prototypical late $I_{Na}$ inhibitor, ranolazine, was shown using the MMA method to reduce ischemia-induced TWA in parallel with a protective effect on VF threshold in a large animal model of coronary artery stenosis [48]. Conversely, in the same model, flecainide increased ischemia-induced TWA concurrently with an increase in the incidence of spontaneous VF [50], a finding consistent with the drug's known proarrhythmic effects and its contraindication in patients with CAD [51]. Clinically, ranolazine suppressed ventricular tachyarrhythmias in the Metabolic Efficiency with Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndrome-Thrombolysis in Myocardial Infarction 36 (MERLIN TIMI 36, NCT00099788) trial [64]. A reduction in 1-year mortality in patients with TWA ≥47 μV in the MERLIN TIMI 36 TWA substudy was found using the MMA method [27].

### Beta-Adrenergic Blockade

Using the Spectral Method, a decrease in TWA by beta-adrenergic blockade was demonstrated during electrophysiologic study with atrial pacing, indicating a heart rate-independent antiarrhythmic effect as one mechanism underlying its well-known capacity to reduce susceptibility to SCD [58, 59]. Specifically, metoprolol, a relatively cardioselective beta-blocker, and d,l-sotalol, a beta-blocker with Class III antiarrhythmic actions, similarly reduced TWA in patients with malignant VT and diminished the incidence of positive TWA tests by 67% (from 12 to 4) in 54 patients [59]. Esmolol reduced positive TWA tests by 50% (from 42 to 21) in 60 patients with ischemic cardiomyopathy and inducible sustained VT [60]. Propranolol decreased the number of positive TWA tests by 36% (from 11 to 7) in 15 patients with ventricular tachyarrhythmia [61]. In response to graded exercise, metoprolol, carvedilol, or other beta-blockers reduced the incidence of positive TWA tests by 31% (from 26 to 18) in 26 patients with nonischemic heart disease [62].

### Calcium Channel Blockade

Although calcium channel blocking agents decrease TWA in experimental models in parallel with reducing ischemia-induced ventricular tachyarrhythmia [63], their antiarrhythmic actions may be offset by their negative inotropic

effects in clinical studies, particularly in individuals with myocardial infarction complicated by pulmonary congestion [93]. Clinical investigations relevant to effects of this class of compounds on TWA have been limited to patients with vasospastic angina. Shimada and colleagues [21] using the MMA method reported high levels of TWA during asymptomatic phases, especially in patients with tachyarrhythmias during vasospasm, but not in matched control patients; calcium channel blockade decreased arrhythmias and TWA level. The hazard ratio for TWA in predicting ventricular tachyarrhythmias was 15.5 (95% confidence interval, 1.7 to 142.0, $p < 0.01$). The authors proposed that repolarization abnormalities monitored even during the asymptomatic period could indicate increased risk for life-threatening ventricular arrhythmias during ischemic attacks.

## T-Wave Alternans in Detecting Proarrhythmia

Case reports of TWA in association with proarrhythmia have recurred [49, 91, 94]. Notably, Hohnloser [94] reported visible TWA in association with torsade de pointes (TdP) in patients who received high doses of amiodarone to manage atrial fibrillation. Subsequent administration of magnesium sulfate suppressed both ventricular arrhythmia and TWA. It is well-known that d-sotalol, which inhibits $I_{Kr}$, proved to be proarrhythmic in the Survival With Oral d-Sotalol (SWORD) trial [48]; inhibition of $I_{Kr}$ has also been shown experimentally to increase MMA-based TWA during myocardial ischemia [47]. Penicillin, an antibiotic that can inhibit $I_{Kr}$, was reported to induce macroscopic TWA followed by TdP during hospitalization for pneumonia (Fig. 19.7) [49]. Such observations may represent the tip of the iceberg. The potential use of TWA in identifying proarrhythmia deserves systematic study, particularly using quantitative measures of microvolt levels of TWA rather than relying solely on the appearance of visible, macroscopic TWA or a positive TWA test by a binary analysis approach.

## Perspective and Future Directions

Based on a 30-year experience in studying the phenomenon of TWA, both in the experimental laboratory and in analyses of clinical data, the following perspective is provided. The most effective use of TWA should involve quantitative analysis and be performed in the context of provocative testing with either exercise or AECG monitoring to incorporate stimuli of daily activities including mental and physical stressors.

When elevated TWA is detected, the primary objective should be to determine the root cause. A number of prevalent clinical conditions associated with elevated TWA can be readily addressed prior to the decision to place an ICD. Primary, manageable causes of elevated TWA are the persistence of myocardial ischemia [95–97], which can be addressed by revascularization. Compliance with anti-SCD medications such as beta-blockers [98], which is standard of care following myocardial infarction and in LQTS patients, is reported to decrease by 43% during the high-risk period at 30–90 days post-MI [99], by >50% at 4 years following MI [100], and by 51% in LQTS patients [101]. As beta-blockade significantly reduces TWA, this parameter could fill an unmet need to determine whether SCD risk is present due to noncompliance to prescription medications. Another practical area of future application of TWA is to serve as a therapeutic target for antiarrhythmic drug efficacy and drug-induced proarrhythmia, which has been implicated in the context of antibiotics, particularly agents with $I_{Kr}$ inhibition (Fig. 19.7) [49] and amiodarone [94]. An important implication of TWA's sensitivity to antiarrhythmic therapy is that retesting must be performed after a new drug regimen is instituted (Fig. 19.8) [71].

The potential value of TWA in tracking effectiveness of cardiac rehabilitation therapy should be duly considered, as this intervention has been shown to reduce TWA significantly [102]. Other new applications include TWA's capacity to sense risk for rehospitalization during discharge in patients with heart failure [31, 103], as an aid to diagnose ARVD/C [104], and

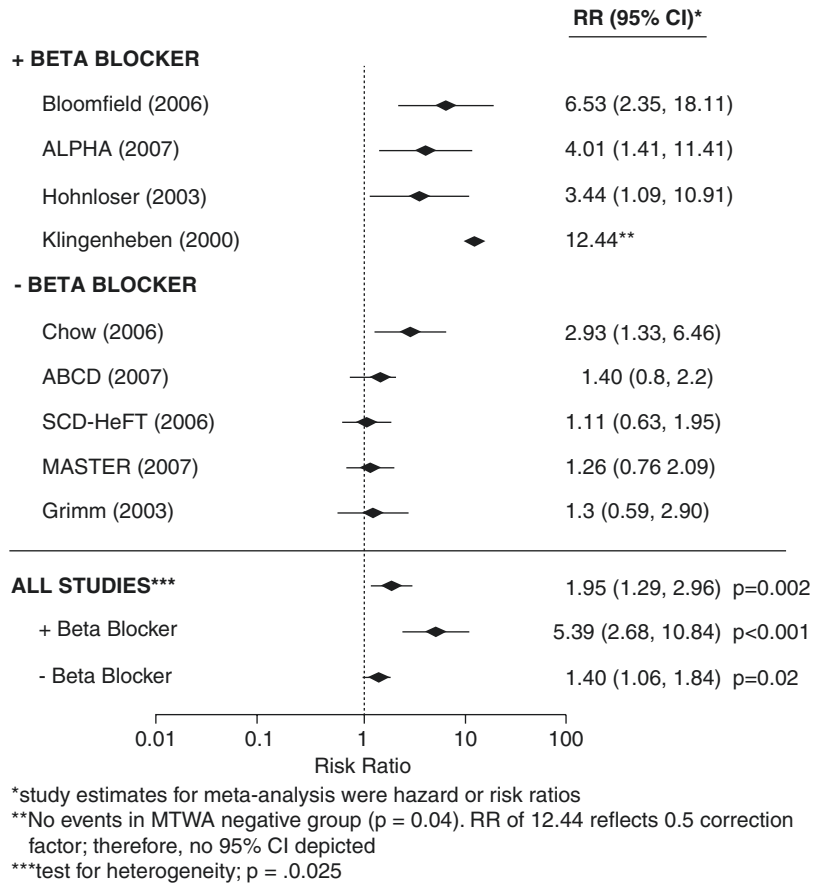


**Fig. 19.6** Relative risks of TWA for ventricular arrhythmic events, stratified by continuation or discontinuation of beta-blocker therapy. Significant heterogeneity was observed among studies ($p = 0.025$). Subgroup analyses found a strong, consistent, fivefold increased risk for ventricular arrhythmic events in studies where beta-blocker therapy was not discontinued prior to TWA assessment. In contrast, a weak association between an abnormal TWA test and risk for arrhythmic events was observed in studies that withheld beta-blocker therapy prior to TWA assessment and then resumed therapy. (Reproduced with permission from Wiley from Chan et al. [86])

to detect coronary artery stenosis [95–97] as well as scar tissue [57, 69].

Neural triggers acting on a vulnerable myocardial substrate are a critical factor in SCD. Combined monitoring of TWA and autonomic parameters, particularly HRT, could prove particularly valuable. Recent evidence that combined assessment of HRT and TWA can prove useful in assessing risk for hospital readmission and cardiovascular mortality in patients with heart failure carries important implications and deserves further exploration [31, 103].

Finally, there is considerable merit in moving from a "snapshot" approach for TWA monitoring to more frequent and sustained assessment of the phenomenon by taking advantage of ECG patch monitors [105]. These devices, with or without capacity for mobile cardiac telemetry, can extend monitoring to 2 weeks to include initiation of therapy and recovery of cardiac electrical stability in patients after myocardial infarction as well as in epilepsy patients with sporadic VT [106].

Collectively, the body of knowledge that has been accrued in the recent three decades using state-of-the-art signal processing techniques points to promising expanding horizons for TWA testing, including the broad spectrum of pathologic conditions predisposing to SCD, supported by evidence of TWA's predictive capacity in Table 19.1.

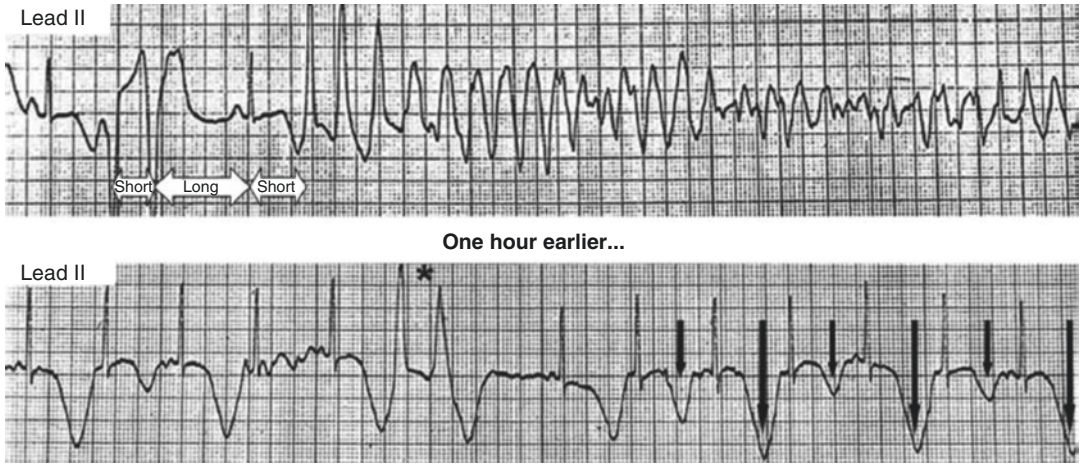


**Fig. 19.7** Top rhythm strip: TdP culminating in ventricular fibrillation in an 83-year-old female in the intensive care unit with pneumonia. Intravenous erythromycin was commenced several hours prior to cardiac arrest. Lower rhythm strip, ECG at 1 hour before the development of TdP reveals pronounced prolongation of the QT interval (QTc in cycles with larger T waves = 730 ms), a ventricular couplet (∗), and macroscopic T-wave alternans (TWA, vertical arrows). If these indicators of risk for TdP had been recognized, termination of the culprit agent and administration of magnesium might have prevented the subsequent cardiac arrest. (Reprinted with permission from Wolters Kluwer from Drew et al. [49])

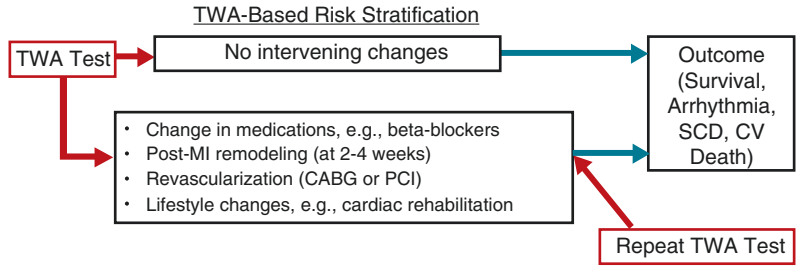


**Fig. 19.8** A number of established clinical factors that affect TWA and thereby impact on prediction. These include changes in medications [83], post-MI remodeling [77], revascularization [25], and exercise rehabilitation [98]. Thus, TWA testing should be repeated following any of these significant changes. The availability of routine exercise testing and ambulatory ECG monitoring using standard electrodes facilitates retesting TWA. Key: CABG coronary artery bypass graft surgery, CV cardiovascular, MI myocardial infarction, PCI percutaneous coronary intervention, SCD sudden cardiac death, TWA T-wave alternans. (Reprinted with permission from Elsevier from Verrier and Sroubek [71])

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# Action Potential Dynamics in Human Atrial Fibrillation

20

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## Introduction

While there is intense debate over the mechanisms for atrial fibrillation (AF), a clear consensus exists on an apparent interplay between triggers and substrate [1]. While triggers (focal beats) for AF often lay at or near the pulmonary veins [2], the mechanisms responsible for maintenance (substrate) of AF appear to be remote to these areas based on the limited success of pulmonary vein isolation in such patients and the results of newer specific mapping techniques (Fig. 20.1) [3]. Electrical remodeling of the atria and structural changes are recognized as key substrates for persistence of atrial fibrillation. While studies increasingly demonstrate the structural substrate for AF using imaging techniques [4], its electrical characterization has for years focused on clinical electrograms. Intracardiac electrograms, however, are not direct markers of atrial action potentials, can be challenging to interpret for their precise activation time (especially during AF), and do not provide insight into atrial *repolarization* and thus the determinants of block, reentry or after depolarizations.

This chapter will discuss key experimental data from monophasic action potentials (MAPs) during rapid pacing, the mechanistic sequelae of this with respect to initiation of atrial fibrillation in humans, and implications for the atrial substrate.

## Action Potential Duration

Atrial action potentials can be measured in vivo in patients using the monophasic action potential (MAP) catheter. Early work by Franz et al. validated this recording to correspond to transmural action potential [6].

Precise measurement of total atrial action potential duration is difficult because of its asymptotic terminus. Clinically, human atrial AP duration is usually determined at a repolarization level of 90% (or another fraction) with respect to the AP amplitude, i.e., recovery from plateau voltage to 90% of fully repolarized "resting membrane potential." The monophasic AP (MAP) amplitude is defined as the distance from the baseline to the crest of the MAP plateau voltage, not its upstroke peak (Fig. 20.2) [6]. The intersection between the diastolic baseline and a

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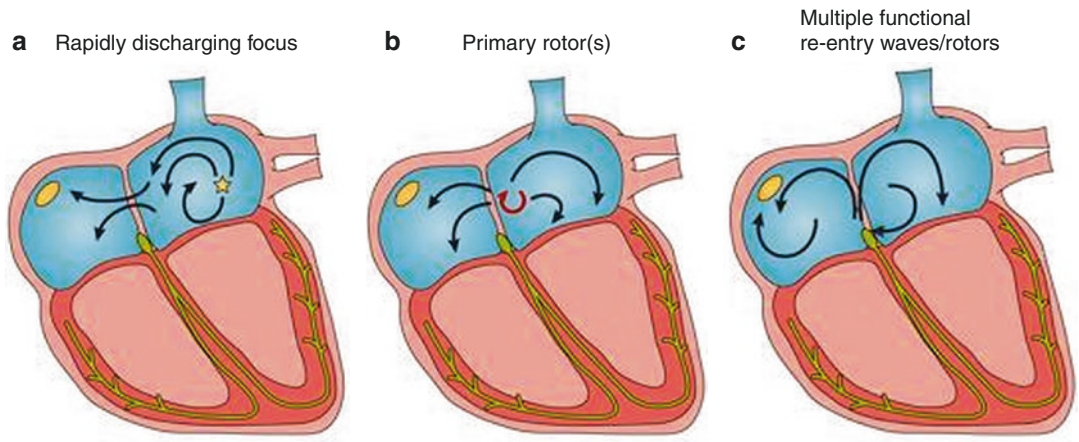


**Fig. 20.1** Summary of AF mechanisms. (**a**) Rapidly discharging focal beats, often near pulmonary veins, act as triggers. (**b**) Primary reentrant driver (rotor) driving downstream fibrillatory conduction, often remote to pulmonary veins, still represents a localized substrate. (**c**) Multiple functional reentries, a global distributed substrate. (Reprinted with permission from Springer Nature: Lip et al. [5])

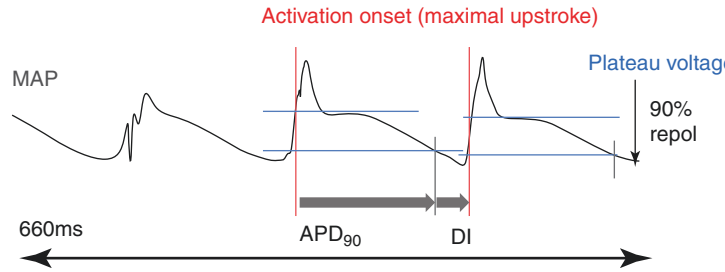


**Fig. 20.2** Graphical representation of monophasic action potential (MAP) recorded from a patient with atrial fibrillation. Note notation of action potential duration 90% (APD90). DI = diastolic interval

tangent placed on phase three of the action potential may also determine MAP duration, but typically yields more arbitrary results. In human atrium, MAPD90 (the duration of time for the MAP to return to 90% of its repolarized voltage) has been correlated to effective refractory period during pacing [7].

## Action Potential Restitution

An electrical restitution curve (ERC) is obtained by pacing at a constant S1–S1 interval, followed by an S2, a single premature stimulus. S1–S2 coupling interval is progressively shortened until block of the premature pulse or the effective refractory period. The graphical relationship between APD 90% (or another fraction) to its preceding DI or CL is the ERC (Figs. 20.3, 20.4 and 20.5) [8, 9]. A modified technique for describing the APD-CL relationship, referred to as the "dynamic" restitution curve (DRC), exhibits the relationship between APD and different steady cycle lengths determined during the early phases of rate adaptation.

## Initiation of Human AF May Be Preceded by Atrial APD Alternans

CL-dependent changes in APD are proposed mechanisms for arrhythmogenesis and, in particular, fibrillation [12, 13]. As ERCs are described, progressive shortening of CL and DI accentuate changes in APD. Increasing APD "alternans" leads to larger wavelength oscillations and points of wavebreak resulting in temporal heterogeneity

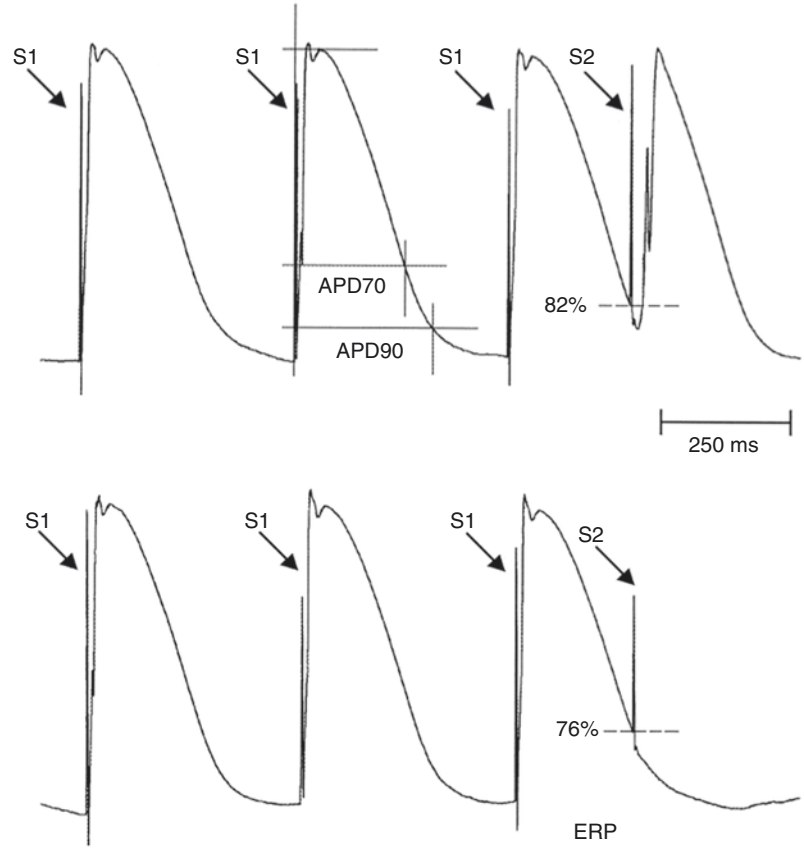


**Fig. 20.3** Simultaneous measurement of atrial ERP and $APD_{70}$ and $APD_{90}$ with the combination catheter. S1 and S2 denote the basic and extrastimulus artifacts. Repolarization levels (n%) at which extrastimuli are superimposed onto the preceding repolarization phase are visualized. (From Bode et al. [10]. Used with permission from Elsevier)

and reentry. The mechanisms by which APD alternans may contribute to electrical instability have been described utilizing computer and numeric simulations [14, 15]. The demonstration of APD alternans at high stimulus rates provides mechanistic support for this concept [16–18]. APD alternans has also been linked to ventricular fibrillation vulnerability [19, 20]. Evidence now exists that APD alternans may contribute to the onset and potentially maintenance of atrial fibrillation and its transition from atrial flutter in animal models [21, 22], in numerical simulations [23], and directly in humans [24], discussed later in this chapter.

## Quantifying Atrial APD Alternans

APD alternans was historically assessed by visual inspection [25], but this does not allow for rapid assessment or quantification. More recently, APD alternans has been detected and quantified spectrally. APs for a contiguous number of beats are each denoted by beat number and time sample. APs are time-aligned such that APs for successive beats (as voltage-time series) can be visualized in a third dimension for the number N of beats (Fig. 20.6).

A fast Fourier transform is used to compute power spectra *across beats* at each time sample to produce a spectrum for that time sample, which are then summated across all time samples to produce a spectral estimate of oscillations for the entire AP [26]. The magnitude of APD alternans is represented by the dimensionless k-score (Fig. 20.6).

It has been suggested that the slope of the ERC (see Fig. 20.5) may produce APD alternans and hence wavebreak and serve as an electrical substrate for reentry. A steeper ERC slope means

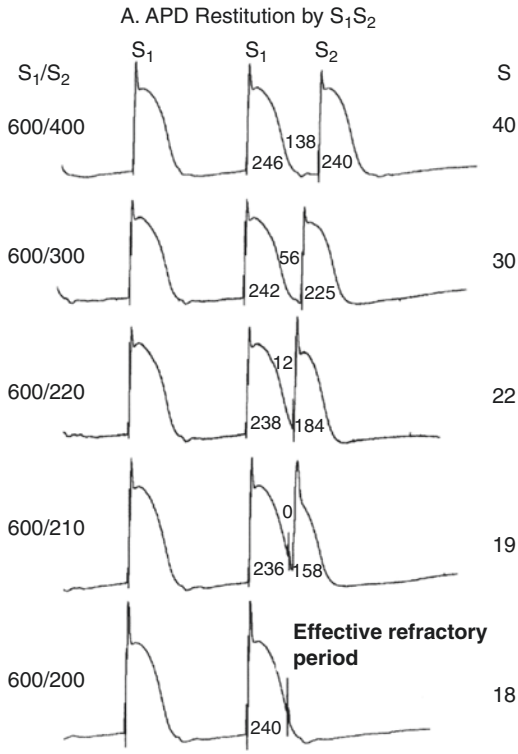


**Fig. 20.4** Examples of MAP recorded at the high lateral wall of the right atrium by $S_1S_2$ in patients with persistent AF. A single extrastimulus ($S_2$) was given at progressively shorter $S_1S_2$ intervals. As diastolic interval decreased from 138 to 0 ms, the $APD_{90}$ decreased from 240 to 158 ms and then reached effective refractory period (ERP) with a coupling interval of 200 ms. (From Kim et al. [11]. Used with permission from Elsevier)

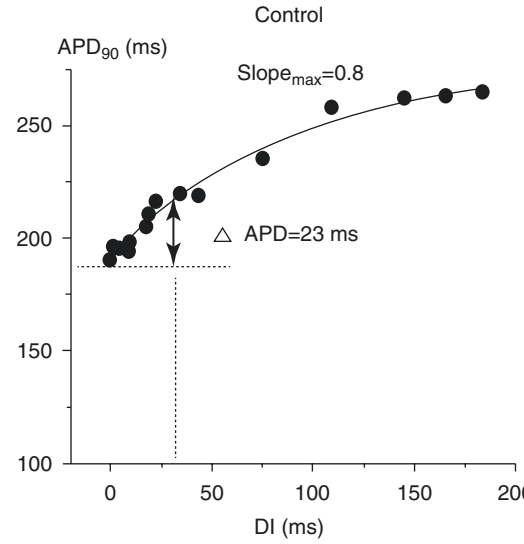


**Fig. 20.5** An ERC by S1S2. The decrease in APD90 with increasing prematurity resulted in an ERC slope of 0.8. (From Kim et al. [11]. Used with permission from Elsevier)

that a relatively small change in DI may render large changes in APD and refractoriness which in turn cause reciprocal changes in DI for the next cycle, causing an opposite APD change and thus producing oscillations. The "restitution hypothesis" suggests that when the ERC slope >1, augmented APD "alternans" results in larger wavelength oscillations and points of wavebreak. Alternatively, when ERC slope <1, oscillations progressively decrease and ultimately achieve a new steady state [12, 28–30].

However, several findings question this otherwise attractive hypothesis for ventricular [26] and atrial [29, 31] arrhythmias. A major issue is that patients with advanced persistent AF appear to have less steep atrial APD restitution curves than patients with less advanced paroxysmal AF [29]. Accordingly, work from our laboratory and others have moved to examine the shape of the human atrial action potential as a potentially more revealing pathophysiological marker than the single point of MAPD alone. In Fig. 20.6 we applied spectral analysis to quantify alternans across the entire atrial MAP duration, which we have related more recently to atrial electrical remodeling and potential cellular mechanisms [32].

## Atrial Action Potentials to Mark Electrophysiologic Remodeling in Atrial Fibrillation

Atrial electrophysiologic remodeling as a consequence of AF was significantly advanced by work conducted in animal models [33, 34]. Further studies also suggested that AF induced atrial APD shortening and action potential plateau phase depression in animals [35, 36] as well as

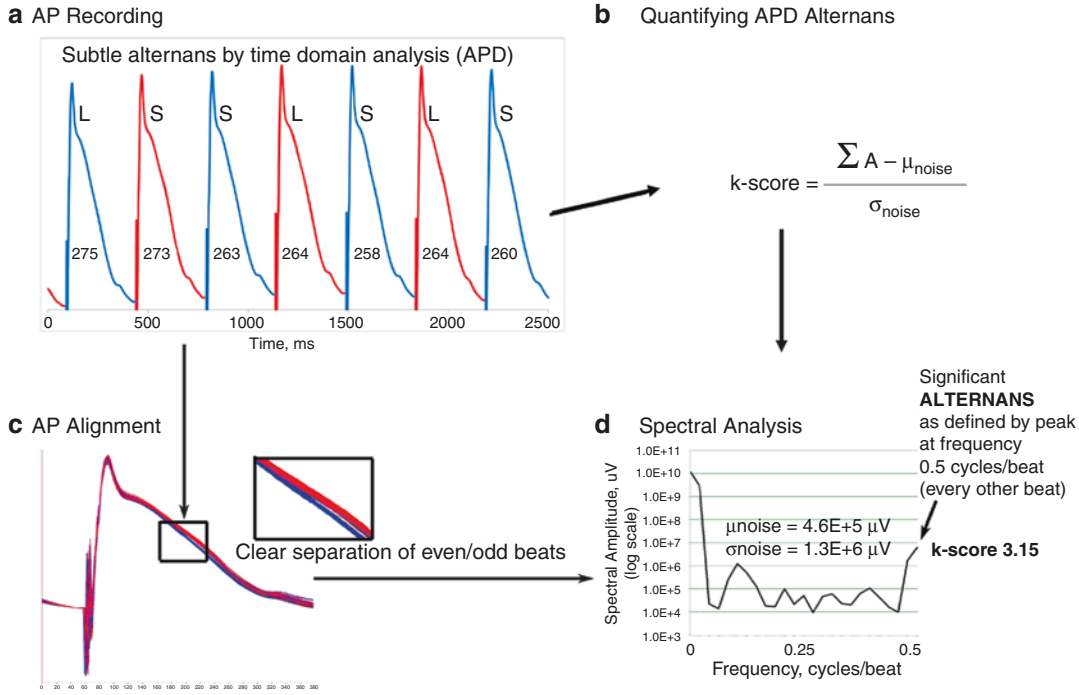


**Fig. 20.6** Measurement of atrial alternans. (**a**) Left atrial monophasic action potentials during atrial fibrillation, with APD values listed beneath. (**b**) APD alternans quantification. ΣA is spectral magnitude at 0.5 cycles/beat, and μ noise and σ noise are the mean and standard deviation of noise. A k > 0 indicates that alternans exceeds noise. (**c**) AP amplitude shows marked alternans, shown by the separation of even (blue line) and odd (red line) beats. (**d**) Significant AP alternans with k-score 3.15. (From: Lalani et al. [27]. Used with permission from Wolters Kluwer)

humans [37], due to quantifiable remodeling in ion channels [32, 38]. AF induces abnormal cellular calcium handling that may promote compensatory downregulation of inward L-type $Ca^{2+}$ current ($I_{CaL}$) [39–41]. It is theorized that this reduction in $I_{CaL}$ activity mitigates the plateau phase and largely explains the accentuated shortening of atrial APD [42, 43]. Transient outward $K^+$ current ($I_{TO}$) is also reduced in chronic AF [44], which seems puzzling but may help explain additional effects of AF on APD [32]. Chronic AF also exhibits an increase in inward rectifying $K^+$ current $I_{K1}$ [45] which primarily controls the resting potential of the atrial myocyte. In sum, a complex interplay of currents in atrial AF myocytes renders a shorter APD and mitigation of the plateau phase.

An interesting phenomenon noted by Wijfells et al. [33] was an alteration to physiologic rate adaptation. As expected, normal goats in sinus rhythm exhibited atrial APD shortening at shorter pacing intervals. Goats artificially maintained in AF over 24–48 hours, however, lost physiological adaptation and exhibited either constant or shorter APD at slower heart rates. Further, normal heart rate adaptation was restored within a few days after cardioversion to sinus rhythm. A later study by Van Der Velden et al. added that increasing pacing rates rendered longer APD in a post-cardioversion chronic AF goat model [46]. This constitutes an AF-induced, paradoxical physiological adaptation of the atrial APD to changes in heart rate. This maladaptation of the atrial APD had been observed in prior human studies [24, 47].

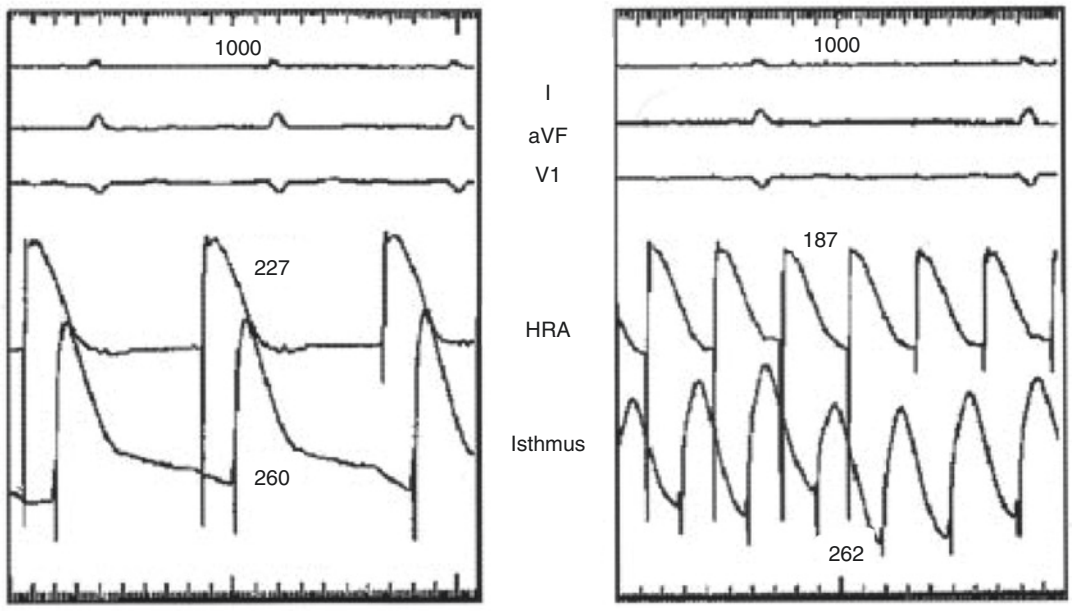


**Fig. 20.7** APD-CL nonuniformity between the isthmus and high right atria (HRA) in a patient who developed AF. APD is longer at the isthmus than the HRA at pacing CL of 800 ms (left) and CL of 300 ms (right). Notably, APD at the isthmus failed to shorten with CL (right), although shortening was appropriate at the HRA. APD rate maladaptation at the isthmus versus HRA eliminated the diastolic interval, caused the MAP to encroach on subsequent beats, and became more pronounced at faster pacing. (From Narayan et al. [24]. Used with permission from Wolters Kluwer)

We documented atrial APD alternans in humans during transitions of atrial flutter (Afl) to AF and noted that rate maladaptation leads to APD alternans preceding all such transitions [24]. Conversely, much less APD alternans was seen in patients in whom AF did not initiate. A critical observation was of spatial heterogeneity within the atria [48], with such APD alternans observed in certain regions (in this case, in the cavotricuspid isthmus, which may explain breakdown of otherwise stable typical AFL; Fig. 20.7) and heterogeneous rate maladaptation in Afl and AF known to contribute to AF [49].

We also demonstrated that continued incremental pacing enhanced APD alternans until beat-to-beat conduction was unable to be maintained physiologically, leading to 2:1 conduction block, which was also noted in prior investigation [11]. We posited that, as a result of a maladaptive APD rate response, spatially heterogeneous APD alternans at


**Fig. 20.8** APD alternans with 2:1 capture block at the isthmus during pacing CL of 230 ms, while 1:1 capture is maintained at the His and distal coronary sinus (DCS) sites; this preceded AF initiation. $APD_{90}$ alternans in the isthmus first appeared in this patient at CL of 250 ms. (From Narayan et al. [34]. Used with permission from Wolters Kluwer)

increasing heart rates leads to wavefront fractionation and wavebreak, conduction block, and the eventual transition to AF [24]. See Figs. 20.8 and 20.9.

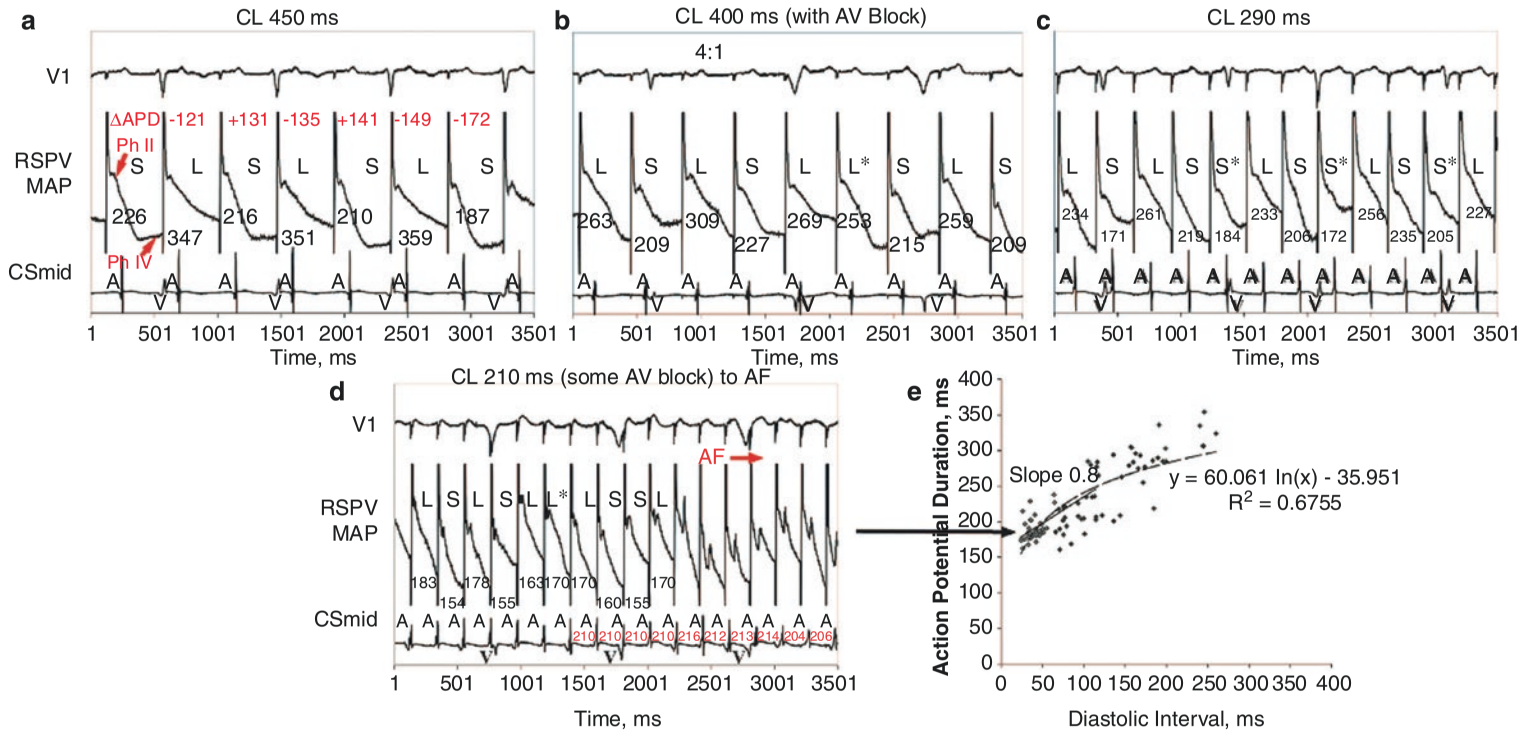


**Fig. 20.9** APD alternans at slow rates in persistent AF, disorganizing to complex oscillations just preceding AF. This patient showed substantial APD alternans at 500 ms (**a**); CL 450 ms (mean APD 120 ms for 10 beats); (**b**) CL 400 ms during intermittent AV block, with a phase reversal (∗); (**C**) CL 290 ms with multiple phase reversals; and (**d**) CL 260 ms, with complex oscillations than AF. (**e**) ERC with maximum ERC slope. (From Narayan et al. [31]. Used with permission from Wolters Kluwer)

## Heterogeneity of APD Maladaptation

We evaluated the concept of heterogeneity of APD maladaptation and AP oscillations during AF initiation in the electrophysiology laboratory in patients with and without clinical AF [31]. We hypothesized that AF-induced atrial remodeling may cause APD alternans at slower heart rates. Using an incremental pacing technique, we studied left and right atrial $APD_{90}$, APD restitution curve, APD alternans, and non-alternating complex APD oscillations on transitions to AF in patients with persistent AF, paroxysmal AF, and control subjects without clinical AF.

The development of APD alternans was elicited at near resting rates in patients with persistent AF, at intermediate rates in those with paroxysmal AF, and only at very rapid rates just prior to AF in controls. Moreover, the amplitude of APD alternans also varied by group and was greatest in those with persistent AF, intermediate in those with paroxysmal AF, and smallest just before AF induction in controls. A representative patient with persistent AF is shown in Fig. 20.9, in whom marked left atrial APD alternans was seen at CL 600 ms (100 beats/min) and CL 500 ms (120 beats/min, illustrated). At faster rates (shorter CLs), APD alternans disorganized to complex APD oscillation immediately preceding AF transition. In stark contrast, Fig. 20.10 shows a representative control subject in whom APD alternans did not arise until very fast rates (CL 200–250 ms), just prior to AF onset. No control subject had APD alternans at CL greater than or equal to 250 ms and, when exhibited, alternans had very small magnitude.

The APD rate-response relationship and APD alternans directly indicated AF "substrates," presumably caused by electrical remodeling. The CL onset of APD alternans and CL range varied among the three groups. As delineated in the figure below, persistent and paroxysmal AF subjects exhibited APD alternans at all rates while controls only exhibited APD alternans around CL 250 ms (Fig. 20.11).

## Refining Electrical APD Restitution in AF

We explored the potential role of APD restitution in patients with different forms of AF and in control subjects without AF. Notably, initiation of APD alternans was observed in some control subjects as well as a few paroxysmal AF subjects in whom ERC slope was >1. This is consistent with, and may explain, spontaneous AF initiation by triggering premature atrial complexes such as those from the PVs (Fig. 20.12).

However, this did not explain AF onset in all patients. The ERC slope in all paroxysmal and persistent AF subjects was <1 at the onset of APD alternans, even if maximum slope was >1 at faster rates. Persistent AF enabled onset of APD alternans at relatively slower heart rates (see Fig. 20.9), suggesting that some other facet of electrical remodeling than ERC slope was operative. As noted earlier, AF is thought to affect several aspects of atrial myocyte character and function, including abnormal intracellular $Ca^{2+}$ handling [42], membrane ion current remodeling changes [50], and atrial conduction slowing [51].

Nevertheless, atrial APD alternans was noted to precede every AF transition, supporting prior studies [29] and suggesting a critical role for APD alternans in AF onset. Accordingly, we suggest that, in addition to spontaneous premature atrial complexes, APD alternans amplification or complex oscillation preceding AF transition at fast rates represent period multiplying in nonlinear systems, another suspected trigger for AF. Proposed mechanisms to elucidate this phenomenon remain under intense scrutiny.

## Future Directions

There is renewed interest in recording action potential-like signals in human cardiac chambers as the ability to view repolarization is a pre-requisite for interpreting complex electrograms and detecting reentry. The interaction of head meets tail at rotational cores requires accu-

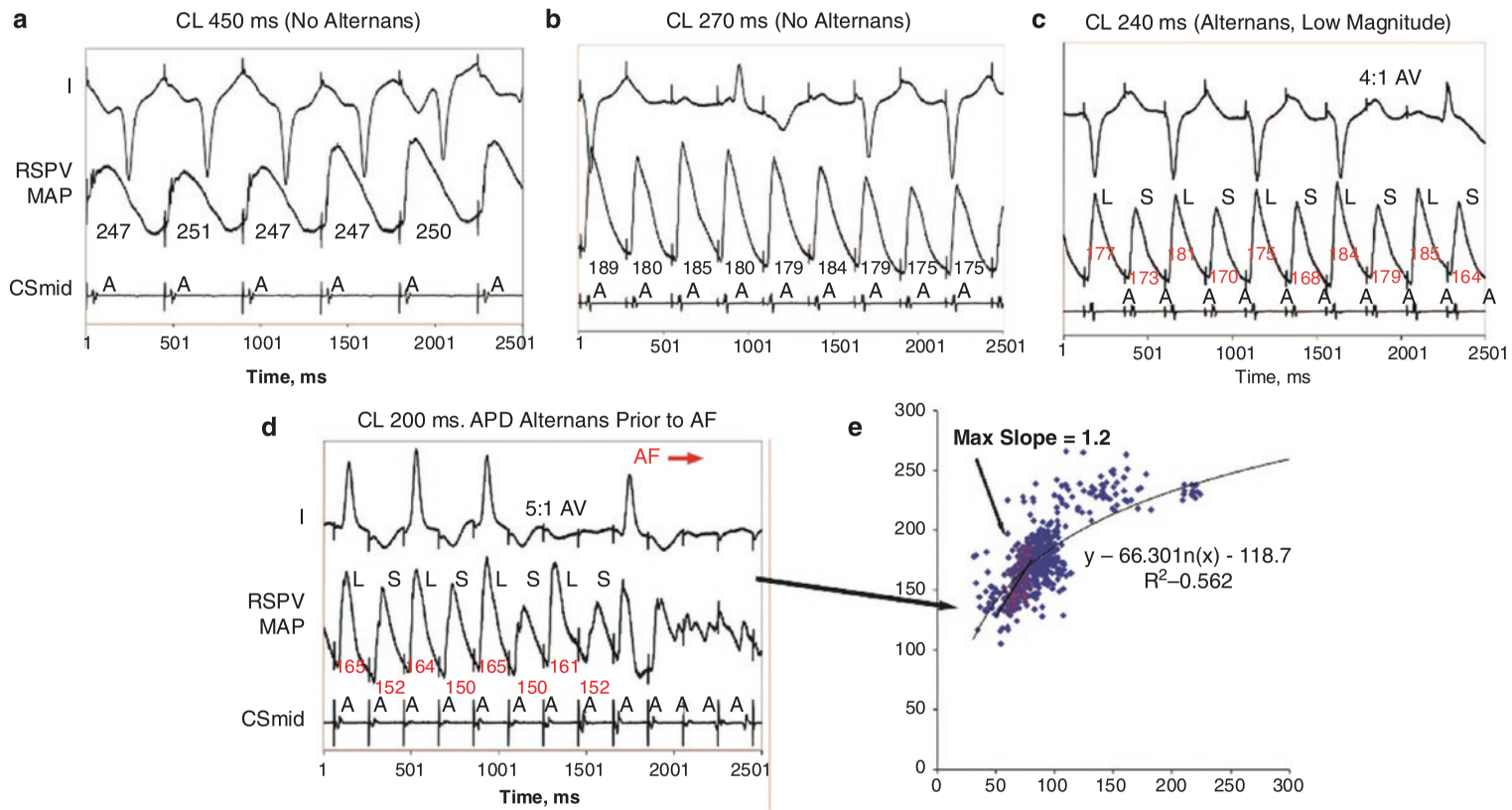


**Fig. 20.10** Control subject with APD alternans only at very fast rates preceding AF. This patient showed no APD alternans at (**a**) CL 500 ms to (**b**) CL 240 ms. (**c**) APD alternans appeared at CL 210 ms during 1:1 and 2:1 AV conduction. (**d**) At CL 180ms, APD alternans amplified to 6.5% of APD immediately preceding AF initiation. ERC slope 1 at the fast rates of APD alternans onset. (From Narayan et al. [31]. Used with permission from Wolters Kluwer)

rate spatial awareness of wavefront curvature and can only be visualized directly by action potential data, such as human heart ex vivo optical mapping [52]. In clinical practice, limited tools exist to quantify repolarization from electrograms, but novel methods are under investigation [53] and may shed further light onto AF mechanisms [54].

Viewing repolarization also is instrumental in assessing conduction velocity (CV) as it relates to the proximity of the preceding depolarization. CV is minimal closest to the refractoriness of the preceding AP and then recovers quickly as the DI increases (CV restitution). CV is the major determinant of the AP wavelength and thus may be important to assess the number of reentry wavelets in play during closely spaced depolarizations (as in AF). There is considerable research interest in measurement of conduction velocity in the human atria [51, 55–57], but measurements during fibrillation itself require knowledge of wavelet direction a priori, which can limit extrapolation from paced data preinitiation.

Because the MAP reflects repolarization at the cellular level, MAP recordings could also be used to define clinical phenotypes anchored on cellular mechanisms, and current modeling and translational studies are exploring this possibility for patients with atrial fibrillation as well as those at risk for sudden death. Finally, assessments of repolarization can also thus be used to suggest individuals who may or may not benefit from anti-arrhythmic drug therapy, for instance, using novel tools such as induced pluripotent

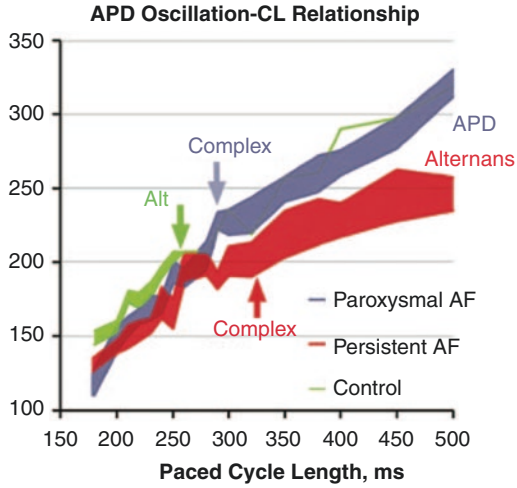


**Fig. 20.11** APD oscillation and CL. AF patients show marked APD oscillations (alternans at slow rates transitioning to complex oscillations at faster rate), whereas controls show APD alternans only at very short CL. (From Narayan et al. [31]. Used with permission from Wolters Kluwer)

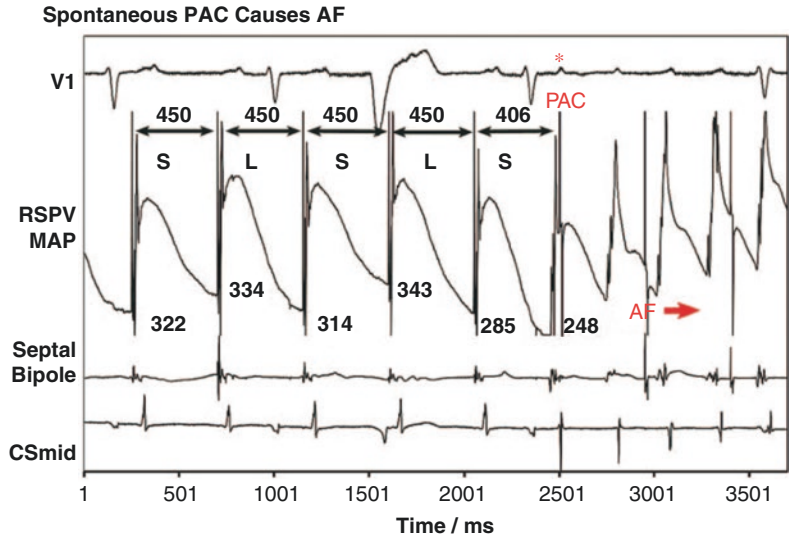


**Fig. 20.12** Spontaneous premature atrial complex (PAC, marked by ∗) initiates AF in the milieu of APD alternans at relatively slow rates. This happened multiple times in this patient at various rates. (From Narayan et al. [31]. Used with permission from Wolters Kluwer)

stem cell-derived cardiomyocytes in individual patients [58].

## Conclusions

Cardiac arrhythmias are dependent upon a complex interplay of impulse formation, spatially heterogeneous conduction slowing, and abnormal repolarization. Monophasic action potential data is uniquely suited to reveal abnormalities in these human tissue properties in vivo that escape traditional unipolar or bipolar electrogram measurements. A number of studies using this technique have demonstrated the role of abnormal electrical restitution, alternans and complex oscillations in action potential shape and duration, and spatial heterogeneities which may directly enable the initiation and maintenance of complex arrhythmias. The MAP can thus be used to delineate novel clinical phenotypes based on cellular remodeling, can be used to assist in mapping for complex arrhythmias, and thus may have an important role in guiding therapy.

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