Cardiac and Vascular Biology 7
Editor-in-chief: Markus Hecker

Jeanette Erdmann
Alessandra Moretti *Editors*

Genetic Causes of Cardiac Disease

Springer

Cardiac and Vascular Biology

Volume 7

The book series gives an overview on all aspects of state-of-the-art research on the cardiovascular system in health and disease. Basic research aspects of medically relevant topics are covered and the latest advances and methods covering diverse disciplines as epigenetics, genetics, mechanobiology, platelet research or stem cell biology are featured. The book series is intended for researchers, experts and graduates, both basic and clinically oriented, that look for a carefully selected collection of high quality review articles on their respective field of expertise.

More information about this series at http://www.springer.com/series/13128

Jeanette Erdmann • Alessandra Moretti
Editors

Genetic Causes of Cardiac Disease

Springer

Editors
Jeanette Erdmann
IIEG
University of Lubeck
Lübeck, Germany

Alessandra Moretti
I Medical Dept (Cardiology)
Klinikum Rechts der Isar of the Technical
University Munich
München, Bayern, Germany

ISSN 2509-7830 ISSN 2509-7849 (electronic)
Cardiac and Vascular Biology
ISBN 978-3-030-27370-5 ISBN 978-3-030-27371-2 (eBook)
https://doi.org/10.1007/978-3-030-27371-2

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Genetics of Adult and Fetal Forms of Long QT Syndrome

1

Lia Crotti, Alice Ghidoni, and Federica Dagradi

Contents

Abstract

Long QT syndrome (LQTS) is an inherited cardiac disease characterized by prolongation of QT interval at surface ECG, T-wave abnormalities, and high risk of life-threatening arrhythmias in otherwise healthy young individuals. Currently the LQTS diagnosis is genetically confirmed in nearly 75–85% of LQTS patients, revealing a good knowledge of the genetic bases of the disease.

L. Crotti (✉)
Istituto Auxologico Italiano, IRCCS, Laboratory of Cardiovascular Genetics, Center for Cardiac Arrhythmias of Genetic Origin, Milan, Italy

Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy

Istituto Auxologico Italiano, IRCCS, Department of Cardiovascular, Neural and Metabolic Sciences, San Luca Hospital, Milan, Italy

A. Ghidoni · F. Dagradi
Istituto Auxologico Italiano, IRCCS, Laboratory of Cardiovascular Genetics, Center for Cardiac Arrhythmias of Genetic Origin, Milan, Italy

J. Erdmann, A. Moretti (eds.), *Genetic Causes of Cardiac Disease*, Cardiac and Vascular Biology 7, https://doi.org/10.1007/978-3-030-27371-2_1

The main LQTS genes are *KCNQ1*, *KCNH2*, and *SCN5A* encoding potassium and sodium cardiac ion channels responsible of the cardiac action potential duration. Minor contributors of LQTS genetic background include genes encoding other cardiac ion channels, ancillary subunits, and protein components forming channels' macromolecular complexes.

Fetal and neonatal forms of LQTS are the most aggressive form of the disease, frequently associated with typical ECG features as very prolonged QTc, 2:1 functional atrioventricular block, T-wave alternans, and life-threatening arrhythmias. The genetic basis of these early-onset cases is peculiar. Indeed, while potassium channel mutations are the most commonly observed causes of adult LQTS, fetal and neonatal forms of the disease are mainly due to aggressive sodium channel mutations or to mutations affecting calcium channel activity, as in Timothy syndrome, triadin knockout syndrome, and calmodulin-LQTS. Aggressive forms of LQTS can also cause sudden infant death syndrome (SIDS) or intrauterine fetal death.

1.1 Introduction

Long QT syndrome (LQTS) is an inherited cardiac disease characterized by prolongation of QT interval at surface ECG, T-wave abnormalities (biphasic or notched T waves), and high risk of life-threatening arrhythmias. The typical ventricular tachyarrhythmia that underlies cardiac events in LQTS is the torsades de pointes (TdP). This type of ventricular tachycardia can produce transient syncope, when it is self-limited, or can degenerate into ventricular fibrillation and cardiac arrest, mainly precipitated by emotional or physical stress. A sign of major electrical instability in LQTS patients is represented by T-wave alternans, a beat-to-beat alteration in polarity, and amplitude of the T wave [1]. Long QT syndrome is considered one of the leading causes of sudden death in young (<35 years) [2]. Unfortunately, the disease can remain clinically silent for a long time, and sudden cardiac death (SCD) may be the first manifestation in some cases.

The congenital form of the disease has been largely studied over the years and includes two main hereditary variants. The Romano-Ward (RW) variant, described for the first time in 1964 [3], represents the autosomal dominant form of the disease, and it is relatively common, with a prevalence of 1:2000 live births [4]. The Jervell and Lange-Nielsen (JLN) syndrome is an extremely severe form of the disease, associated with congenital deafness and higher mortality [5, 6]. The JLN has an autosomal recessive mode of inheritance, more frequently associated with homozygous and rarely compound heterozygous mutations. This syndrome is very rare and affects around 2–3 out of 1000 individuals with congenital deafness [6].

Since the main feature of LQTS is the prolongation of QT interval, it is not surprising that cardiac ion channels responsible for action potential (AP) duration are the main molecular players of the syndrome.

In particular, three genes (*KCNQ1*, *KCNH2*, *SCN5A*), encoding cardiac sodium and potassium channels, are the major genetic contributors underlying LQTS.

Table 1.1 Long QT syndrome variant types so far described and relative LQTS-associated genes

LQTS variant type	Syndrome	Gene	OMIM ID	Locus	Protein	Functional effect
LQT1	RWS, JLNS	*KCNQ1*	*607542	11p15.5-p15.4	$K_V7.1$	$\downarrow I_{Ks}$
LQT2	RWS	*KCNH2*	*152427	7q36.1	$K_V11.1$	$\downarrow I_{Kr}$
LQT3	RWS	*SCN5A*	*600163	3p22.2	$Na_V1.5$	$\uparrow I_{Na}$
LQT4	RWS, ANKB syndrome	*ANKB*	*106410	4q25-q26	Ankyrin B	$\uparrow [Ca^{2+}]_i$
LQT5	RWS, JLNS	*KCNE1*	*176261	21q22.12	MinK	$\downarrow I_{Ks}$
LQT6	RWS	*KCNE2*	*603796	21q22.11	MiRP1	$\downarrow I_{Kr}$
LQT7	ATS	*KCNJ2*	*600681	17q24.3	Kir2.1	$\downarrow I_{K1}$
LQT8	TS	*CACNA1C*	*114205	12p13.33	$Ca_V1.2$	$\uparrow I_{CaL}$
LQT9	RWS	*CAV3*	*601253	3p25.3	Caveolin-3	$\uparrow I_{Na}$
LQT10	RWS	*SCN4B*	*608256	11q23.3	Sodium channel β4-subunit	$\uparrow I_{Na}$
LQT11	RWS	*AKAP9*	*604001	7q21.2	Yotiao	$\downarrow I_{Ks}$
LQT12	RWS	*SNTA1*	*601017	20q11.21	α1-Syntrophin	$\uparrow I_{Na}$
LQT13	RWS	*KCNJ5*	*600734	11q24.3	Kir3.4	$\downarrow I_{KACh}$
LQT14	Calmodulinopathy	*CALM1*	*114180	14q32.11	CaM	$\uparrow I_{CaL}$
LQT15	Calmodulinopathy	*CALM2*	*114182	2p21	CaM	$\uparrow I_{CaL}$
LQT16	Calmodulinopathy	*CALM3*	*114183	19q13.32	CaM	$\uparrow I_{CaL}$
LQT17	TRDN knockout syndrome	*TRDN*	*603283	6q22.31	Trisk32	$\uparrow I_{CaL}$

RWS Romano-Ward syndrome, *JLNS* Jervell and Lange-Nielsen syndrome, *ATS* Andersen-Tawil syndrome, *TS* Timothy syndrome. For each gene, the Online Mendelian Inheritance in Man (OMIM) gene ID, the locus, and the encoded protein are reported. Functional effect and impact of gene mutations on cardiac ionic current derived from in vitro cellular studies are reported as ↓ loss of function or ↑ gain of function

However, many other genes, detailed in Table 1.1, have been so far associated with the disease and will be described in Sects. 1.2 and 1.3.

Besides congenital LQTS, an acquired form of the disease (aLQTS) has been described as well [7] and refers to patients in which QT prolongation is secondary to hypokalemia or QT-prolonging drugs (www.azcert.org). A genetic basis of aLQTS is recognized as well. Indeed, a third of these patients carries rare variants in the three main congenital LQTS-associated genes, with *KCNH2* being the gene most frequently involved [8]. Furthermore, a sum of common polymorphisms, known to modulate QT interval in the general population [9], has been shown to predict the degree of drug-induced QT prolongation in acquired LQTS patients [10].

The therapy of choice in congenital LQTS is represented by beta-blockers (BBs), which are effective in preventing life-threatening arrhythmias in the vast majority of patients, with the highest efficacy obtained with propranolol and nadolol [11]. Whenever a failure of BB therapy is observed, left cardiac sympathetic denervation (LCSD) offers additional protection with a 91% reduction in cardiac events [12]. ICD therapy is rarely indicated in LQTS, as available therapies are highly effective [13]. A subgroup of LQTS patients that represent an exception at what previously stated are those patients with cardiac events in the first year of life. These patients represent a small subgroup of LQTS cohorts, 2% in the LQTS International Registry [14], but they are at very high risk to have a subsequent cardiac arrest/sudden cardiac death in the following 10 years of life and are poor responders to beta-blocker therapy [14]. The genetic basis of these most severe forms of the disease will be treated in details in Sect. 1.3.

1.2 Part I: Genetics of Adult Forms of Long QT Syndrome

1.2.1 Major LQTS Genes

The three main genes responsible for LQTS (*KCNQ1*, *KCNH2*, *SCN5A*) were identified between 1995 and 1996 [15–17]. They encode the $Na_v1.5$ sodium channel (SCN5A) and the two alpha subunits of the delayed-rectifier potassium channels (KCNQ1, KCNH2), respectively, involved in the depolarization ($Na_v1.5$) and repolarization (K^+ channels) phases of AP. They represent the major genes responsible for LQTS as they account for approximately 90% of all genotype-positive cases [18].

The ***KCNQ1*** gene, located on chromosome 11, encodes the α-subunit of the slow delayed-rectifier potassium channel ($K_v7.1$) responsible for the depolarizing I_{Ks} current, which is essential for QT adaptation when heart rate increases [15]. Four alpha subunits encoded by *KCNQ1* co-assemble with two beta subunits to form the functional K^+ channel. The typical effect of *KCNQ1* mutations is a decrease of the outward potassium current (loss of function), leading to ventricular repolarization delay and QT prolongation. Since I_{Ks} current is the major determinant of QT adaptation during heart rate increase, when I_{Ks} is diminished or dysfunctional, the QTc fails to adequately shorten during sympathetic activation, and this creates a potential arrhythmogenic substrate. Heterozygous *KCNQ1* mutations cause the

dominant Romano-Ward LQT1 syndrome, while *KCNQ1* homozygous or compound heterozygous mutations cause the recessive JLN variant, characterized also by deafness due to the reduced I_{Ks} in the inner ear.

The gene responsible for LQTS type 2 (LQT2) is ***KCNH2*** [16], encoding the α-subunit of the rapid delayed-rectifier potassium channel (K_v11.1, hERG), which conduces I_{Kr} current. Similar to the slow rectifier potassium channel, four alpha subunits, each encoded by *KCNH2* gene, co-assemble to form a functional channel. Mutations in *KCNH2* gene mainly cause a rapid closure of potassium channels and I_{Kr} decrease (loss of function), resulting in delayed ventricular repolarization and QT prolongation.

There are different mechanisms through which mutations in *KCNQ1* and *KCNH2* can cause reduction or complete loss of the I_K current, the major determinant of the phase 3 of the cardiac AP. The first two mechanisms described, haploinsufficiency and dominant negative effect, are relevant to both *KCNQ1* and *KCNH2*. Haploinsufficiency is a mechanism causing a ~50% reduction of current density due to an overall decreased production of functional channels into the cell membrane, whereas dominant negative effect is elicited by the negative interaction of mutated subunits with the wild-type ones and can cause more than 50% reduction of current density [19]. More recently, mutations in *KCNH2* have been classified into four types on the basis of the channel biophysical property that was impaired. Specifically, class 1 mutations disrupt the synthesis or the translation of K_v11.1 α-subunits, class 2 mutations reduce the intracellular transport or trafficking of K_v11.1 proteins to the cell membrane, and class 3 and 4 mutations affect K_v11.1 channel gating and permeation [20].

The third major LQTS gene is ***SCN5A*** [17], encoding the α-subunit of the cardiac sodium channel (Na_v1.5) involved in the genesis of depolarizing sodium inward current (I_{Na}) and responsible for the phase 0 of AP. In vitro expression studies showed that *SCN5A* mutations lead to LQTS phenotype (LQT3 variant type) through a gain-of-function mechanism, by increasing the delayed Na^+ inward current, resulting in the prolongation of AP duration and QT interval.

Alterations in the sodium channel are also associated with other genetic disorders like Brugada syndrome, atrial fibrillation, sick sinus node syndrome, and the Lev-Lenègre disease. As a further complexity, some *SCN5A* mutations can show a pleiotropic behavior, i.e., the same mutation may associate with more than one phenotype, leading to the so-called overlap syndromes [21, 22].

Overall, the yield of genetic testing for the three main genes in clinically definite LQTS patients is approximately 75% [23], while the prevalence of LQTS variant types among genotype-positive patients is estimated to be 43% for LQT1 (*KCNQ1*), 32% for LQT2 (*KCNH2*), and 13% for LQT3 (*SCN5A*) [18].

These three major LQTS variant types have been associated with specific arrhythmic triggers [24]. LQT1 patients are at higher risk during physical or emotional stress, with swimming being particularly dangerous and specific [24]. Indeed, the majority of patients (99%) that experienced cardiac events while swimming were LQT1. By contrast, LQT2 and LQT3 patients, who have a normal level of I_{Ks}, are at low risk during physical exercise and sport activity. LQT2 patients are more

sensitive to sudden noises, such as alarm clocks or telephone ringing, especially during sleep, whereas LQT3 patients tend to have their events at rest or while asleep, when the heart rate decreases [24].

The clinical manifestations of LQTS may also vary according to the different genetic background. The first large study suggesting interactions between genotype, QTc, and gender reported that the risk of cardiac events was higher for LQT2 females and LQT3 males and further increases in the presence of marked QT prolongation (QTc $>$ 500 ms) [25]. LQT1 patients experienced less frequently cardiac events, probably because a very high percentage of them has a QTc $<$ 440 ms [25]. These findings were confirmed some years later, in another study that showed that female gender, QTc interval $>$ 500 ms, and syncopal events were associated with significantly increased risk of life-threatening cardiac events in adulthood [26]. However, the severity of the disease and the relative risk of cardiac events are also influenced by the type of mutation, the location of the mutation in the protein, and the effect produced on cellular function [19, 27, 28].

1.2.2 Minor LQTS Genes

After the identification of the three main LQTS genes, several others have been associated with the disease. They collectively account for a small portion of LQTS (nearly 5%); thereby they are considered as minor genes [23].

Some of the minor LQTS genes concern auxiliary beta subunits that co-assemble with alpha channel subunits encoded by *KCNQ1*, *KCNH2*, and *SCN5A*, to recapitulate sodium and potassium currents. These genes are *KCNE1*, *KCNE2*, and *SCN4B*.

KCNE1 encoding MinK is the single-transmembrane β-subunit of KCNQ1 potassium channel, which contributes as well to generate I_{Ks} current [29]. Mutations in *KCNE1* gene may cause either the dominant RW syndrome (LQT5) when present in heterozygosity or the recessive JLN syndrome if present in homozygosity or compound heterozygosity [30].

KCNE2 gene encodes MiRP1 (MinK-related peptide 1), a small peptide that co-assembles with hERG alpha subunits to form I_{Kr} channel. Mutations in this gene are responsible for the LQT6 variant type and have been associated both with congenital [31] and acquired LQTS [32].

The ***SCN4B*** gene, underlying LQT10 variant type, encodes the beta auxiliary subunit of $Na_v1.5$ channel and contributes to modulate I_{Na} current. The first mutation identified in this gene (*SCN4B*-p.Leu179Phe) segregated in a family whose proband presented with intermittent 2:1 atrioventricular (AV) block and a corrected QT interval of 712 ms, while other two members died for SCD [33]. The mutation showed in vitro to increase the I_{Na} current, resembling LQT3 phenotype [33].

Other minor genes associated with LQTS encode some components of the sodium channel macromolecular complex, such as *CAV3* and *SNTA1*, and represent LQT9 and LQT12 variant types. Mutations in these genes almost mimic the LQT3 phenotype.

The gene ***CAV3*** encodes caveolin-3, a small protein that localizes on caveolae, small microdomains of the plasmalemma involved in vesicular trafficking and in the regulation of signal transduction pathways. Mutations in this gene were first described in adult patients, and it was hypothesized that caveolin proteins associated with sodium channel may influence the I_{Na} depolarizing current [34].

The α1-syntrophin, belonging to dystrophin-associated protein family, is part of the sodium channel macromolecular complex, together with neuronal nitric oxide synthase (nNOS) and the nNOS inhibitor Ca^{2+} ATPase PMCA4b. The gene ***SNTA1*** was firstly implicated in the disease in 2008, with the identification of the p. Ala390Val mutation in a LQTS subject symptomatic for cardiac events, with a QTc of 529 ms [35]. This mutation localizes in the PMCA4b binding domain, resulting in $Na_v1.5$ channel function impairment and I_{Na} current increasing [35].

Additional genes associated with LQTS cases were *AKAP9* (LQT11), *KCNJ5* (LQT13), *KCNJ2* (LQT7), and *ANKB* (LQT4).

The A-kinase anchor protein 9, also known as yotiao, is involved in the phosphorylation of KCNQ1 via PKA and is responsible for the LQT11 variant type [36]. The first ***AKAP9*** mutation (p.Ser1570Leu) identified in a LQTS patient was predicted to weaken the interaction between PKA and KCNQ1, making the channel not responsive to AMPc, lastly causing QT prolongation [36].

More recently another potassium channel, Kir3.4, encoded by ***KCNJ5*** gene, was implicated in LQTS type 13. The Kir3.4 is a G protein-coupled inwardly rectifying potassium channel, with a greater tendency to allow potassium to flow into the cell rather than out of the cell. The gene was identified through a genome-wide linkage analysis performed in a family with autosomal dominant LQTS [37]. Heterologous expression studies of Kir3.4-p.Gly387Arg mutation revealed a loss-of-function phenotype resulting from reduced plasma membrane expression [37].

Long QT syndrome types 4 and 7 refer to *ANKB* and *KCNJ2* genes. Mutations in these genes were associated with complex disorders, in which the QT interval prolongation is a minor feature of the heterogeneous patients' phenotype; therefore they are atypical forms of LQTS. The ***ANKB*** gene encodes a membrane adapter, anchoring different proteins and ion channels to plasmatic membrane. The *ANKB*-p. Glu1425Gly mutation was identified in a large family with modest QT prolongation associated with severe sinus bradycardia and episodes of atrial fibrillation [38].

KCNJ2 gene, encoding Kir2.1 channel, is referred like LQT7. However, mutations in this gene result in Andersen-Tawil syndrome, a multisystem disease that includes modest QT interval prolongation secondary to reduction of the potassium repolarization currents (I_{K1}), and polymorphic tachycardia [39]. This current contributes both to the repolarization phase 3 of AP and to the maintenance of resting membrane potential; therefore, channel dysfunctions may lead to a reduction of I_{K1} with consequent QT prolongation.

Finally, the role of different proteins involved in Ca^{2+} transport, signalling, and homeostasis is currently emerging. Most of the Ca^{2+}-related forms are characterized by extremely severe phenotypes, manifesting in perinatal period or during infancy. Therefore, they will be presented in details in Sect. 1.3.2. The following paragraphs

describe in brief the gene function and the first studies that demonstrated an association with LQTS disease.

The main gene regulating Ca^{2+} cellular load is *CACNA1C*, coding the L-type voltage-dependent Ca^{2+} calcium channel $Ca_V1.2$. This gene refers specifically to a malignant form of LQTS known as Timothy syndrome (TS) (LQT8), described for the first time in 1992 as a novel arrhythmia syndrome associated with syndactyly (webbing of fingers and toes) [40, 41]. The molecular basis of the syndrome was described in 2004 by Splawski's group, who identified mutations in *CACNA1C* affecting a single amino acid (p.Gly406Arg), co-segregating with TS phenotype in several families [42]. They also provided exhaustive clinical characterization of the syndrome, including long QT syndrome, life-threatening arrhythmias, congenital cardiac defects, syndactyly, variable penetrance of autism features, craniofacial abnormalities, and hypoglycemia [42]. The spectrum of mutations associated with TS has been enlarged during the following years [43]; however, for some of them, functional evidences supporting their causative role are less clear.

Calmodulin (CaM) is a multifunctional Ca^{2+} binding protein (Fig. 1.1, panel a) essential for intracellular signalling processes in eukaryotic cells [50] that has been recently identified as an additional causative factor for LQTS. It is a ubiquitous protein which transduces Ca^{2+} signals in excitable tissues such heart and brain and therefore influences the activity of ion channels, kinases, and other target proteins [51]. Human calmodulin is highly conserved among vertebrates and is encoded by three separate genes (*CALM1*, *CALM2*, and *CALM3*), producing proteins with identical amino acid sequence [52]. *CALM* genes, when mutated, can cause LQTS (LQT14–16, Table 1.1), cathecolaminergic polymorphic ventricular tachycardia (CPVT) [53], idiopathic ventricular fibrillation, and sudden cardiac death. CALM-LQTS [44] is characterized by very severe forms of the disease with early-onset presentation and recurrent life-threatening arrhythmias [44, 49]. Calmodulinopathy will be discussed in details in Sect. 1.3.2.2.

TRDN is another gene encoding a protein (triadin) implicated in Ca^{2+} channel regulation that was associated to both CPVT [54] and LQTS [55]. Triadin syndrome will be discussed in Sect. 1.3.2.3.

1.2.3 Genetic Modifiers of Long QT Syndrome

Long QT syndrome is a Mendelian disorder, in which the phenotype is primarily explained by a single mutation in one of the main cardiac ion channel genes. However, the disease is also characterized by high clinical heterogeneity within families and among carriers of the same disease-causing mutation. This phenomenon, usually attributed to incomplete penetrance and variable expressivity [56], could be partially due to genetic modifiers. Genetic modifiers are genes or loci, distinct from the primary disease-causing mutation, associated with arrhythmia susceptibility. They act as fine regulators of the arrhythmic risk modulating the effect of the primary disease-causing mutation in a protective or detrimental way. The role of genetic modifiers in LQTS has been largely studied in the last years, and