Chapter 2

Update on the Genetic Basis of Long QT Syndrome

Oscar Campuzano¹,²,³*, Georgia Sarquella-Brugada⁴, Sergi Cesar⁴, Esther Carro⁴, Anna Fernandez-Falgueras¹, Josep Brugada⁴,⁵ and Ramon Brugada¹,²,³,⁶

¹Cardiovascular Genetics Center, University of Girona-IDIBGI, Spain
²Medical Science Department, School of Medicine, University of Girona, Spain
³Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Spain
⁴Arrhythmia Unit, Hospital Sant Joan de Déu, Spain
⁵Cardiovascular Institute, Hospital Clinic, University of Barcelona, Spain
⁶Cardiology Service, Hospital Josep Trueta, Spain

*Corresponding Author: Oscar Campuzano, Cardiovascular Genetics Center, Institut d’Investigació Biomèdica Girona (IDIBGI), C/ Dr Castany s/n, Parc Hospitalari Martí i Julià (M-2), 17190, Salt-Girona, Spain, Email: oscar@brugada.org

First Published May 29, 2017
Acknowledgment: This work was supported by Obra Social “La Caixa” and by grant 201505-10 from La Fundació la Marato TV3. The CIBERCV is an initiative of the ISCIII, Spanish Ministry of Economy and Competitiveness (Fonfos FEDER).

Copyright: © 2017 Oscar Campuzano, et al.

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source.

Abstract

Long QT syndrome is a rare cardiac inherited channelopathy characterized by a prolongation of the QT interval in the electrocardiogram. The prolongation of the QT interval may induce torsades de pointes and ventricular fibrillation, leading to syncope and even sudden cardiac death, usually in young population. Treatment of patients may include β-blockers administration, left cardiac sympathetic denervation, and cardioverter defibrillator implantation in combination with life-style modification such as prohibition of competitive exercise and avoidance of QT-prolonging drugs. Currently, hundreds of rare variants have been associated with the disease in 20 different genes. These genes encode for ion channels and associated
proteins, being responsible for 80%-85% of all diagnosed cases. Recently, other genetic alterations have been also associated with the disease, being responsible for 2%-5% of all cases. A comprehensive genetic analysis could be performed nowadays using new massive sequencing technologies in a cost-effective way but most part of variants remain classified as ambiguous in clinical practice. Current international guidelines include genetic analysis in risk stratification but only definite pathogenic alterations should be considered. One of the current challenges is clarify the role of genetic variants identified, and translation into clinics should be done with caution.

Keywords
Sudden Cardiac Death; Arrhythmias; Long QT Syndrome; Genetics

Introduction
Sudden death (SD) is definite as a natural and unexpected decease that occurs in apparently healthy people, or whose disease was not severe enough to expect a fatal outcome. The event can be due to several pathologies, usually of cardiac cause and called sudden cardiac death (SCD). Myocardial infarction the most common causes of SCD and usually occurs in people more than 40 years old. In infants and young people, cardiomyopathies (such as Hypertrophic Cardiomyopathy, Dilated Cardiomyopathy, and Arrhythmogenic Cardiomyopathy), and channelopa-
thies (such as Brugada Syndrome –BrS-, Long QT syndrome –LQT-, Catecholaminergic Polymorphic Ventricular Tachycardia -CPVT-, and Short QT syndrome -SQT-) are the more common causes [1]. The first group of diseases is due to by pathogenic variants identified in genes encoding cardiac structural proteins such as sarcomere, desmosome, and cytoskeleton. The second group of diseases is originated due to pathogenic variants identified in genes encoding ion channels (sodium, potassium and calcium) or associated proteins [2]. Both groups of disease lead to malignant arrhythmias, ventricular fibrillation, syncope and even SCD. Achieving a proper and effective cardiac rhythm require coordination between ion channels and structural proteins. The complexity of electrical transmission through myocytes remains the major limitation for understanding cellular mechanisms responsible of arrhythmogenesis. Unfortunately, SCD could be the first manifestation of the diseases, being early identification and prevention a crucial point in current medical practice of arrhythmias [3]. This chapter focuses on genetics basis of LQT syndrome.

Long QT Syndrome

In 1957, Jervell and Lange–Nielsen described, for the first time, a family showing prolongation of the QT interval, congenital deafness, and an increased risk of SD [4]. In 1964, Romano and Ward reported a family showing prolongation of the QT interval and SCD but absence of
deafness [5]. Currently, more than 8400 articles have been published concerning LQT syndrome (PubMed [LONG and QT and SYNDROME]), and it is classified as a rare (1:2500) cardiac disease characterized by elongated repolarization which induces a prolongation of the QT interval on the electrocardiogram (ECG). The ECG measures electrical activity which induces the movement of the heart. The QT interval shows the time elapsed from the initiation of ventricular depolarization to the end of ventricular repolarization. In addition, the QT interval shortens with increasing heart rate therefore requiring a normalization, or “correction,” (QTc) [6]. The current guidelines state that diagnosis of LQT syndrome should be done in a QTc >480 ms in children and >500 ms in adults, but a male showing >470 ms and >480 ms for females are considered QTc borderline and additional test should be performed in order to discard or diagnose the syndrome [7]. The ventricular action potential results from the summation of several ion channel and associated proteins that control the cellular membrane potential. Ion channels and associated proteins are the entities underlying most ionic currents (mainly sodium, potassium and calcium), allowing trafficking, stabilization, signaling, and function. Coordination among all these elements allows the ionic balance and subsequent electrical spread through the myocytes.
In recent years, acquired or hereditary (congenital) LQT syndrome has been reported, arising from a complex interaction between genetic and environmental factors. Both categories produce a potentially dangerous substrate.
for polymorphic ventricular tachycardia (Torsades-de-Pointes, -TdP-), syncope and even SCD. One of hallmarks of the disease is variable expressivity because phenotypes may range from asymptomatic to ventricular fibrillation and SCD, sometimes as the first manifestation of the disease [8]. It is one of the main diseases associated with deceases in infant (sudden infant death syndrome –SIDS-), juvenile and young population, and usually exercise is the trigger for the lethal arrhythmia. Acquired LQT syndrome is associated with an adverse response to medication (drug-induced LQT syndrome, diLQT syndrome), metabolic abnormalities or bradyarrhythmias, but also depends substantially on genetic predisposition to drug interaction in pathophysiological cellular pathways. The inherited LQT syndrome is associated with pathogenic alterations in genes encoding ion channels or associated proteins of myocyte. Two patterns of inheritance have been described for congenital LQT syndrome: autosomal recessive, a minority form described by Jervell and Lange-Nielsen, and autosomal dominant form, the most common and described by Romano and Ward. In 2017, more than 1,000 genetic alterations have been associated with LQT syndrome. These alterations may cause an increase or decrease in ion channel function, disrupting normal ionic balance leading to pathological electrical activity in the heart [9]. All these alterations have been identified in 20 genes encoding ion channel proteins or functional subunits associated with ion channels (AKAP9, ANK2,
CACNA1C, CALM1, CALM2, CALM3, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, RYR2, SCN1B, SCN4B, SCN5A, SNTA1, TRDN, and TRPM4). Concretely, 80%-85% of families diagnosed with LQT syndrome cases carry a pathogenic alteration in KCNQ1 (LQT syndrome type 1, LQTS1), KCNH2 (LQTS2), or SCN5A (LQTS3). All other reported genes together only explain an additional 2%-5%. Recently, Copy Number Variants (CNVs) have been associated with the disease but explaining less than 5% of cases. Therefore, nearly 15% of patients diagnosed with LQT syndrome remain without genetic diagnosis.

Current international guidelines recommend genetic analysis of only main genes (KCNQ1, KCNH2 and SCN5A) associated with LQT syndrome to identify the cause of the disease in families clinically diagnosed [7]. It is mainly due to cost-effective genetic analysis of all other genes LQT-associated. However, current genetic technology (next generation Sequencing -NGS-) allows perform a comprehensive genetic analysis in a low cost and in a reduced time. Current guidelines also recommend genetic analysis in all relatives, including asymptomatic family members that could be at risk of SCD even showing a normal ECG. In addition, genetics has been also included in risk stratification but only if a definite pathogenic role of genetic variant is reported. However, few reports focus on how to differentiate genetic causality from genetic back-
ground noise. This is one of the main current challenges in genetic analysis because most of the genetic data identified remain of ambiguous clinical significance, and translation into clinical practice could be dangerous if a clear role is not stated. In order to clarify this point, familial screening and genotype-phenotype correlation is a crucial way.

**Genetics of LQTS**

Nowadays, 20 genes encoding ion channel subunits or regulatory proteins have been associated with LQT syndrome. Concerning genes encoding potassium channels subunits: \textit{KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, and KCNQ1}; for sodium channel subunits: \textit{SCN1B, SCN4B, and SCN5A}; for calcium channel subunits: \textit{CACNA1C and RYR2}; and for cardiac ion channel-interacting proteins: \textit{ANK2, AKAP9, CALM1, CALM2, CALM3, CAV3, SNTA1, TRDN and TRPM4}.

**LQT Syndrome Type 1**

The \textit{KCNQ1} gene (RefSeq Gene ID: 3784) is the second most prevalent gene associated with LQT syndrome. It encodes the Kv7.1 voltage-gated potassium channel α subunit, responsible for the slowly delayed outward rectifying current (IKs). A reduction in IKs could slow the repolarization phase causing LQT syndrome. To date, more than 550 variations in this gene have been associated with LQT syndrome, inducing a loss-of-function. Of all vari-
ants identified, most part follows an autosomal dominant pattern of inheritance. Despite this fact, homozygous variations and compound heterozygous variations have been also reported, following an autosomal recessive pattern [10,11]. Concerning CNV alterations, only few reports have been reported families suffering of LQT syndrome due to CNV in the $KCNQ1$ gene [12-14]. However, further analysis should be performed to establish the real role of CNV in LQT syndrome. It well accepted that cardiac events and SCD in patients suffering of LQT syndrome due to pathogenic variants in $KCNQ1$, usually happens during exercise (75%), followed by emotional triggers (15%). In addition, variations in this gene have been also associated with SIDS, familial atrial fibrillation and SQT syndrome.

### LQT Syndrome Type 2

The most common gene associated with LQT syndrome is $KCNH2$ (RefSeq Gene ID: 3757). The $KCNH2$ gene encodes for KV11.1 (also called hERG1), the voltage-gated potassium α subunit, which forms a tetramer responsible for the rapid delayed outward rectifying current (IKr). Loss-of-function variations in Kv11.1 reduce the IKr in the phase 2 of the AP leading to a QT prolongation. To date, more than 750 variants have been identified in $KCNH2$ associated with LQT syndrome [15, 16]. Concerning CNV alterations, only few reports have been reported families suffering of LQT syndrome due to CNV
in the \textit{KCNH2} gene [12-14]. However, further analysis should be performed to establish the real role of CNV in LQT syndrome. Cardiac events and SCD in families diagnosed with LQT syndrome type 2 usually occurs after a sudden noise such an alarm, followed by emotional triggers (37%). In addition, variants in this gene have been also associated with SIDS, BrS, atrial fibrillation, and SQT syndrome.

\textbf{LQT Syndrome Type 3}

The third most common gene associated with LQT syndrome is \textit{SCN5A} (RefSeq Gene ID: 6331). The \textit{SCN5A} gene encodes for the cardiac voltage-gated sodium channel $\alpha$ subunit (Nav1.5), responsible for the sodium current in the depolarization phase (INa) and also for the small amount of late sodium current (INaL) in the cardiac AP phases 2 and 3. The QT elongation is due to gain-of-function variations. So far, more than 250 pathogenic variations in this gene have been associated with LQT syndrome, usually inducing arrhythmias and SCD during sleep (80%), followed by emotional stress triggers (15%) [17,18]. Variants in this gene have been also associated with BrS, cardiac conduction disease (CCD), SIDS, and atrial fibrillation.

\textbf{LQT Syndrome Type 4}

This type of LQT syndrome is a multisystem form of the disease, also known as Ankyrin-B Syndrome because variants have been identified in the \textit{ANK2} gene.
(RefSeq Gene ID: 287). This gene encodes for Ankyrin-B protein. Ankyrins are adaptor proteins that link membrane proteins, transporters, and cell adhesion molecules to cytoskeleton. Variations in the ANK2 gene result in a dysfunctional ankyrin-B that cause an increment of intracellular Na+ and Ca+2 ions, producing cellular early and delayed afterdepolarizations in response to catecholamine. To date, 13 variations have been identified in the ANK2 gene associated with inherited LQT syndrome, and one related to diLQT syndrome [19,20]. Patients with variations in this gene suffer different grades of cardiac dysfunction with sinus node dysfunction (sinus bradycardia or junctional escape beat called sick sinus syndrome), idiopathic ventricular fibrillation (IVF), and CPVT.

**LQT Syndrome Type 5**

This type of LQT syndrome occurs in families carrying pathogenic variants in the KCNE1 gene (RefSeq Gene ID: 3753). It encodes for Mink protein (minimal potassium channel, also called Isk) which together with Kv7.1 forms the voltage gated potassium channel that conducts IKs. To date, a total of 36 pathogenic variants have been reported in the KCNE1 gene associated with LQT syndrome, being one of them related to diLQT syndrome (p.D85N). All these variants lead to reduced IKs [21,22]. Finally, variations in the KCNE1 gene gave been also associated with others cardiac arrhythmogenic diseases as atrial fibrillation.
LQT Syndrome Type 6

This type of LQT syndrome is associated with pathogenic variants in the *KCNE2* gene (RefSeq ID: 9992). It encodes the protein MiRP1 (MinK-related peptide 1). MiRP1 is a small transmembrane β subunit protein that colocalize and regulates Kv11.1 in the conduction of IKr, and also modulates Kv7.1, Kv4.3, and Cav1.2. Pathogenic variants in *KCNE2* reduce IKr by slow activation, and/or accelerate inactivation kinetics. To date, nearly 20 pathogenic variations in the *KCNE2* gene have been associated with LQT syndrome, being one of them associated with diLQT syndrome (p.T8A) [23,24]. Finally, variations in the *KCNE2* gene have also been associated with atrial fibrillation and SIDS cases.

LQT Syndrome Type 7

This type of LQT syndrome is also named Anderson-Tawil Syndrome (ATS). Genetic alterations have been identified in the *KCNJ2* gene (RefSeq Gene ID: 3759) which encodes for Kir2.1 potassium channel protein. LQT syndrome type 7 is associated with mild forms of prolonged QT, with prominent U waves in the ECG while ATS is characterized by periodic paralysis, cardiac arrhythmias (including asymptomatic LQT syndrome, bi-directional ventricular tachycardia, syncope, and recurrent TdP), and dysmorphic features. To date, nearly 60 variations have been associated with ATS, inducing a functional failing of
the channel [25-27]. Finally, Kir2.1 variations were also related to SQT syndrome, atrial fibrillation and CPVT.

**LQT Syndrome Type 8**

This type of LQT syndrome is also named Timothy Syndrome (TS). Genetic alterations have been identified in the *CACNA1C* gene (RefSeq Gene ID: 775) which encodes for the α subunit voltage-dependent calcium channel Cav1.2, responsible for the L-type calcium influx current (ICa,L) during the phase 2 of the cardiac action potential. The enlargement of the QT interval could be due to reduced channel inactivation or an increment of Cav1.2 surface expression. Pathogenic variants in this gene could induce only LQT syndrome or TS with QT prolongation [28-30]. In addition, *CACNA1C* pathogenic variations were also associated to BrS and SQTS (Figure 1)[31].

**LQT Syndrome Type 9**

The *CAV3* gene (RefSeq Gen ID: 859) encodes for caveolin-3, which is the main protein that forms the caveolae in cardiac and skeletal muscle. The variants induce an increment of INaL; to date no more than 5 have been reported in LQT syndrome [32-34]. Variations in the *CAV3* gene were also associated to SIDS.
LQT Syndrome Type 10

In this type of LQT syndrome, pathogenic variants have been identified in the *SCN4B* gene (RefSeq Gene ID: 6330). It encodes for the sodium channel β4 subunit (Navβ4), and interacts and modulates the inactivation kinetics of Nav1.5. The 3 pathogenic variants reported so far induce a significant increase of INaL, and then variations in SCN4B could produce LQTS with the similar mechanisms as variations in Nav1.5 itself [35]. In addition, pathogenic variants in this gene have been also associated with atrial fibrillation and SIDS.
LQT Syndrome Type 11

In this type of LQT syndrome, pathogenic variants have been identified in the AKAP9 gene (RefSeq Gene ID: 10142). It encodes for the A-kinase-anchoring proteins (AKAPs) 9, which is a scaffolding protein that determines the localization of protein kinase A (PKA) and other proteins that regulate the PKA (phosphatase or other kinases). The 4 reported variants prolong the AP duration, due to a reduction of the cAMP-dependent phosphorylation of Kv7.1, and the reduction in cAMP stimulation response [36]. Recently, the variation p.Q3531E was related to diLQT syndrome, highlighting that some rare variations in rare genes could predispose to LQT syndrome [37].

LQT Syndrome Type 12

In this case, pathogenic variants have been identified in the SNTA1 gene (RefSeq Gene ID: 20648). It encodes the protein α1-Syntrophin (SNTA1), a member of dystrophin-associated proteins that contains multiple protein interacting motifs. To date, 5 variations have been reported resulting in an increment of INa and INaL, the principal mechanism of sodium-related LQT syndrome [38-40]. Finally, SNTA1 variations were also related to SIDS.

LQT Syndrome Type 13

This type encloses pathogenic variants identified in the KCNJ5 gene (RefSeq Gene ID: 3762). It encodes for
Kir3.4 channel (also called GIRK4), which forms homomeric channels or functional heteromeric channels with other Kir3.x. Pathogenic variants induce a significant reduction of IKACH with an important dominant negative effect in Kir3.4 and Kir3.1 channels, leading to trafficking defects [41,42].

**LQT Syndrome Type 14**

This type encloses pathogenic variants in the RYR2 gene (RefSeq Gene ID: 6262). It encodes for the ryanodine receptor found in cardiac muscle sarcoplasmic reticulum. Pathogenic variants in the RyR2 gene alter the sensitivity of the channel to luminal and/or cytosolic calcium activation, leading to sever calcium spillover from sarcoplasmic reticulum to cytosol, causing cardiac arrhythmias and SCD. Pathogenic variants in this gene have been also associated with CPVT and due to differential diagnosis of CPVT and LQT syndrome could be difficult, it is recommended analysis of RYR2 in LQT syndrome patients, mainly to recognize CPVT patients with masking LQTS diagnosis [43, 44]. Apart from CPVT and LQTS, variations in this gene have been associated with SIDS.

**LQT Syndrome Type 15**

The CALM1 gene (RefSeq Gene ID: 801) together with CALM2 and CALM3, encode for calmodulin protein, and their products have identical amino acid sequences, and all three are expressed in human heart left ventricle.
Calmodulin is a multifunctional Ca+2 binding protein essential for transduction of Ca+2 signals to influence the activity of cardiac ion channels, kinases, and other target proteins in heart. To date, only 4 pathogenic variants have been associated with LQT syndrome in this gene, reducing Ca+2 affinities [45, 46]. Finally, CALM1 variations were also reported linked to CPVT.

**LQT Syndrome Type 16**

The CALM2 gene (RefSeq Gene ID: 805) together with CALM1 and CALM3, encode for calmodulin protein, as mentioned before. To date, only 2 pathogenic variants have been associated with LQT syndrome in this gene, reducing Ca+2 affinities [45]. Finally, variations in the CALM2 gene were also reported linked to CPVT.

**LQT Syndrome Type 17**

The SCN1B gene (RefSeq Gene ID: 6324) encodes for two Navβ1 subunit isoforms: Navβ1 isoform alpha and Navβ1 isoform beta. Both isoforms are expressed in human heart and effect on sodium channel function. To date, only 1 pathogenic variant has been identified associated with LQT syndrome, increasing significantly INaL and QT prolongation [47]. Finally, Navβ1b variations were also associated with BrS and atrial fibrillation.

**LQT Syndrome Type 18**

The CALM3 gene (RefSeq Gene ID: 808) together with CALM1 and CALM2, encode for calmodulin pro-
tein, as mentioned before. The family of proteins binds calcium and functions as a enzymatic co-factor. Activity of this protein is important in the regulation of the cell cycle and cytokinesis because mediates the control of a large number of enzymes, ion channels, aquaporins and other proteins by Ca2+. To date, only 1 pathogenic variant has been associated with LQT syndrome in this gene, reducing Ca+2 affinities [45,48]. Finally, CALM3 variations were also reported linked to CPVT.

LQT Syndrome Type 19

The TRDN gene (RefSeq Gene ID: 10345), encodes an integral membrane protein that contains a single transmembrane domain, named triadin. It contributes to the regulation of luminal Ca2+ release via the sarcoplasmic reticulum calcium release channels RYR1 and RYR2, a key step in triggering skeletal and heart muscle contraction. To date, only 1 pathogenic variant has been associated with LQT syndrome in this gene, following a recessive pattern of inheritance [49]. Finally, TRDN variations were also reported associated with CPVT.

LQT Syndrome Type 20

The TRPM4 gene (RefSeq Gene ID: 54795) encodes the Transient Receptor Potential Cation Channel Subfamily M Member 4. The protein encoded by this gene is a calcium-activated nonselective ion channel that mediates transport of monovalent cations across membranes,
thereby depolarizing the membrane. Recently, 2 new rare variants have been associated with LQT syndrome [50]. Other pathologies associated with TRPM4 include Progressive Familial Heart Block and Familial Progressive Cardiac Conduction Defect.

**Conclusion**

Current guidelines in ventricular fibrillation and sudden cardiac death stated that LQT syndrome is one of the most lethal diseases, mainly in young population. In last 15 years the improvements in genetic diagnose of LQT syndrome has allowed the incorporated of genetic analysis into risk stratification of families. Hence, genetic analysis is recommended in families suffering of LQT syndrome in order to identify the cause of the disease but also early identification of relatives who could be at risk of SCD. Nowadays, hundreds of pathogenic variations identified in 20 genes have been associated with LQT syndrome, including CNVs. Despite NGS technology allows the screening of multiple genes in a cost-effective approach, a large part of genetic data identified still remains classified as unknown significance. This fact reinforces the necessity of further studies focused on genotype-phenotype correlation in order to clarify the role of each variant, allowing translation into clinical practice.
References


7. Priori SG, Blomstrom-Lundqvist C. European Society of Cardiology Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death summa-


31. Schwartz PJ, Ackerman MJ, George AL, Wilde


