Long QT syndrome — a genetic cardiac channelopathy

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INTRODUCTION

Long QT-syndrome (LQTS) is a genetic cardiac channelopathy characterised by a prolonged QT interval on a surface electrocardiogram (ECG), syncope, T-wave abnormalities, ventricular tachycardia of the torsades de pointes (TdPVT) type (Fig. 1) and an increased risk of sudden death [1]. LQTS has variable clinical presentation and is genetically characterised by incomplete penetrance, as seen in many other cardiac genetic conditions [2].

Historically, LQTS is divided into a congenital and an acquired form. Four clinical types of congenital LQTS (cLQTS) have been defined. The commonest is the Romano-Ward syndrome (RWS), with autosomal dominant inheritance and a prevalence of approximately 1 in 2,500 [1]. The other three variants known are much rarer. These are: Jervell-Lange Nielsen syndrome (JLNS), wherein LQTS is associated with congenital deafness and the pattern of inheritance is autosomal recessive; Andersen syndrome (AS), where LQTS is variably present together with other arrhythmias, periodic paralysis and malformations; and the very rare Timothy syndrome (TS), characterised by a more malignant form of LQTS, cardiac and other somatic malformations, and autism [3, 4].

The acquired form of LQTS (aLQTS) presents itself with a normal QT-interval on the ECG under normal conditions, but a prolonged interval under the influence of drug-intake or structural heart disease [5]. The aLQTS occurs much more frequently than cLQTS, and (interestingly) may have a genetic element that makes the individual more susceptible to certain drugs.

The clinical diagnosis of LQTS is made using the diagnostic criteria given in Table 1 [6]. As seen, it comprises ECG findings, a clinical history of syncope and a family history of LQTS or sudden cardiac death. Importantly, the QT interval increases with decreasing heart rate, making it necessary to use a rate-corrected QT interval termed QTc (QTc = QT/√RR) when assessing whether the interval is prolonged or normal [7]. Presently, the diagnostic criteria do not involve the results from genetic testing, but such testing is necessary to identify asymptomatic carriers and relatives of affected individuals who may otherwise present clinically with sudden death as the first symptom [8]. As beta-adrenergic blockade or the application of an ICD unit may dramatically reduce the risk of cardiac events, there exists a real treatment option in LQTS [9].

The aim of this review is to give an update on the expanding number of genes known to be associated with LQTS and their pathophysiological mechanisms.

GENES INVOLVED IN LQTS

Changes in the QT interval duration are caused by an altered time course of the cardiac action potential (AP). An AP consists of depolarisation, plateau and repolarisation phases which reflect the electrical activity across the cardiomyocyte plasma membrane during one contraction, i.e. from systole until the next diastole. This activity is generated by a number of ion channels and can be influenced by various effector systems such as the autonomous nervous system. The most significant ion channels involved in forming the AP are given in Figure 2, along with their individual time-voltage relationship. The LQTS can be described as a cardiac channelopathy resulting from elevated inward depolarising currents or diminished outward repolarising currents of the AP that lead to a prolongation of the QT interval [10].
To date, 12 different genes have been associated with LQTS. These include sodium, potassium and calcium channels as well as interactors of the various channels and channel subunits. Mutations in these genes may result in the loss [11–22] or gain of function [23, 24] and are summarised in Table 2.

**METHODS OF MUTATION SCREENING**

A variety of techniques are employed to detect the genetic variants in LQTS. For the application of these methods in a clinical setting, it is important that they are inexpensive, rapid and that their sensitivity as well as their specificity exceeds 97%. Direct sequencing by capillary array electrophoresis (CAE) is still considered the ‘gold standard’ although it is too expensive to be a first line mutation detection method. Single strand conformation polymorphism (SSCP) analysis and denaturing high performance liquid chromatography (DHPLC) provide cheaper options [25–27]. These techniques can be optimised and modified for greater sensitivity and specificity and to cut down the labour required, although in certain cases it still takes a lot of time for analyses. Resources required for screening can be reduced by sensibly selecting genes for analysis [28]. However, compound heterozygotes, digenic inheritance, and modifying genes highlight the importance of a comprehensive screening strategy including all genes [29, 30].
GENOTYPE-PHENOTYPE CORRELATION
Correlations between the genotype and phenotype in the LQTS are complicated by the fact that in many LQTS genes, very few mutations have been identified. Intra-allelic heterogeneity also plays a role, as mutations in the same gene may confer different risks of cardiac events due to the location of the mutation, and the phenotype of the LQTS-gene associated with the disease may vary between affected family members. Complex phenotypes such as BrS, CCD as well as structural heart disease, (such as DCM), involving LQTS have been reported [31, 32].

For LQT1, LQT2 and LQT3 correlations of the genotype with prognosis and risk of cardiac events have been performed [33]. These three types of LQTS also have characteristic T-wave differences on the ECG [34]. Repeating these investigations with other LQT types is difficult, as there is limited data available. However, LQT7 patients exhibit a distinct ECG with characteristic U-waves [35]. JLNS and TS (LQT8) carry a particularly high risk of sudden cardiac death from early childhood onwards [23, 36].

CLINICAL SIGNIFICANCE OF MUTATION IDENTIFICATION IN LQTS-ASSOCIATED GENES
The simple findings of mutations in LQTS-associated genes are not sufficient to claim that they are the cause of the disease. Apart from single mutations, there are numerous polymorphisms which contribute to the number of sequence variants observed. Many of these variants have been associated with repolarisation as well as cardiac conduction defects and have been proposed as disease-modifying factors. The pathological significance of a mutation is only very occasionally supported by genetic linkage. Evidence for pathogenicity can be obtained if functional analysis is performed to determine the effect of a particular LQTS-associated mutation. This can be achieved by doing electrophysiological studies of mutated ion channel subunits expressed in vitro in order to compare it to known pathophysiological mechanisms.
LQTS-ASSOCIATED GENES AND THE PATHOPHYSIOLOGICAL MECHANISM OF MUTATIONS

KCNQ1
The first reported LQTS-associated mutations were found in the potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) gene (MIM# 607542). The KCNQ1 gene is located on the short arm of chromosome 11 and contains 16 exons which range in size from 47 to 1,122 bp [37]. This gene belongs to a large family of genes that provide instructions for making potassium channels. It encodes a 75 kDa protein consisting of 676 amino acids [37]. This is an alpha-subunit of the slow producing voltage-gated potassium channel (Kv7.1) (Fig. 3A) which conducts the slow delayed rectifier K+ current (IKs) (Fig. 2). It contributes to the repolarisation of the cell, terminating the plateau phase of cardiac action potential (AP) and thereby also the heart’s contractions [38]. Kv7.1 co-assembles with a beta subunit called minK which plays an important role in modulating the current of this channel [38].

To date, more than 250 mutations in KCNQ1 have been implicated with LQTS type 1 (LQT1 – MIM# 192500) [9]. It has been shown that mutations of Kv7.1 alter the function of I\textsubscript{\text{Kr}} due to defective trafficking and dominant-negative loss-of-function effects [39, 40]. Moreover, several mutations have been reported that affect the binding of interacting proteins [41].

KCNH2
Another frequently mutated gene in LQTS is KCNH2, a potassium voltage-gated channel, subfamily H, member 2 gene (MIM# 15427). This gene was mapped to chromosome 7 and contains 15 exons [13, 42]. It encodes a protein consisting of 1,159 amino acids and is highly expressed in the he-
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Figure 4. Schematic drawing of the sodium voltage-gated channel Nav1.5 containing four homologous domains, DI-DIV, each of which has six putative membrane-spanning regions. DI-IV — domains 1-4; PDZ — PDZ structural domain; PH1 — pleckstrin homology domain 1; PH2 — pleckstrin homology domain 2; SU — syntrophin unique domain.

Sodium channel dysfunctions in congenital LQTS are largely due to mutations in the SCN5A gene. This sodium voltage-gated channel, type V, alpha subunit gene (MIM# 600163), is involved in a number of functions, such as the transport of ions across the cell membrane, including the regulation of the cardiac action potential [11]. When this channel’s ability to conduct electrical current across the cell membrane is inhibited or compromised by the application of drugs or by occurring mutations, it can result in LQTS [43].

To date, more than 300 mutations have been reported in KCNH2 associated with LQTS type 2 (LQT2 – MIM# 152427) [9]. Mutations in KCNH2 result in many different dysfunctions of the Kv11.1 channel, including trafficking deficiencies and the formation of non-functional channels or channels with altered gating properties [44].

SCN5A

Sodium channel dysfunctions in congenital LQTS are largely due to mutations in the SCN5A gene. This sodium voltage-gated channel, type V, alpha subunit gene (MIM# 600163) was mapped to chromosome 3 by fluorescence in situ hybridisation [45]. It consists of 28 exons that span approximately 80 kb, and 65% of LQT3-causing mutations are found in exons 20–28 [46]. SCN5A encodes a protein of 2,016 amino acids which is structurally very similar to that of other depolarising sodium channels. This protein forms the alpha-subunit of the cardiac sodium channel Nav1.5 (Fig. 4). Nav1.5 conducts the sodium inward current (I Na) (Fig. 2) which is responsible for the initial depolarisation of cardiomyocytes [9].

Mutations in SCN5A that are associated with LQTS (LQT3 – MIM# 603830) characteristically produce an increased late I Na and consequently prolonged repolarisation [47]. The SCN5A LQTS-associated mutations mainly act through a gain-of-function mechanism which means that although the mutant channel functions normally, certain properties are altered, the most frequent being fast inactivation [47].

ANK2

The first protein implicated in a congenital long QT syndrome that is not an ion channel or channel subunit is called Ankyrin B. It is coded for by ANK2 (MIM# 600919). The ANK2 gene is located on the long arm of chromosome 4 and consists of 46 exons, of which exon 38 is brain-specific [48]. Ankyrin B is a member of a larger family of versatile membrane adapters (ankyrin-R, ankyrin-B, ankyrin-G and tissue-specific splice forms) required for organising, transporting and anchoring membrane protein complexes to the actin/spectrin cytoskeleton. Among other molecules, ankyrins bind a number of ion motive proteins essential for cardiac electrophysiology in general including the Na+/Ca2+ exchanger; inositol 1,4,5-triphosphate receptor and Na+/K+ ATPase [49].

The loss-of-function mutations identified in ANK2 (LQT4 – MIM# 600919) are associated with dominantly inherited type LQT4 in humans [50]. All these mutations result in abnormal co-ordination of multiple functionally-related ion channels and transporters such as the Na+/Ca2+ exchanger which is involved in Ca2+ release during cardiac excitability and can lead to a complex of phenotypes including LQTS, bradycardia, cathcolaminergic polymorphic ventricular tachycardia, idiopathic ventricular fibrillation and sudden death [15, 51].

KCNE1 and KCNE2

Two other genes involved in the congenital long QT syndrome encode ion channel beta-subunits of the KCNE family. Their protein products form single trans-membrane domain ancillary subunits that co-assemble with voltage-gated potassium (Kv) channel alpha-subunits modifying their function. The first one is called KCNE1, which is the potassium voltage-gated channel alpha-subunits modifying their function. The first one is called KCNE1, which is the potassium voltage-gated channel, IsK related subfamily member 1 gene (MIM# 176261). KCNE1 is located on the long arm of chromosome 21. It consists of three exons, with the third exon encoding the 129 amino acid protein [37, 52]. This protein, called the minimal potassium channel (minK), co-assembles with alpha-subunits of Kv7.1 (Fig. 2) forming channels that conduct the slowly activating delayed rectifier K+ (I Ks) current (Fig. 2) [38].

Mutations in the KCNE1 gene that are associated with LQTS (LQT5 – MIM# 176261) are characterised by reducing the potassium flux. There is evidence to suggest that KCNE1 plays a role in channel recycling which alters the I Ks current [53]. The importance of minK in regulating the function of the Kv7.1 channel is emphasised by the fact that a number of inherited mutations in KCNE1 result not only in long QT syndrome but also in deafness due to the reduced I Ks in the inner ear [17, 54]. The combination of deafness with LQTS is called JLNS and has a very poor prognosis [36].
The second member of the KCNE family is KCNE2. KCNE2 is the potassium voltage-gated channel, IsK-related subfamily, member 2 gene (MIM# 603796). This gene is located on the long arm of chromosome 21 and consists of two exons. The second exon encodes the 123 amino acid protein [11]. This protein, known as the minimum potassium ion channel related peptide 1 (MiRP1), functions as a small integral membrane β-subunit associated with the α-subunits of the Kv11.1 ion channels (Fig. 3B). Together, these subunits conduct the rapidly activating delayed rectifier (Ikr) current (Fig. 2) [11, 55].

Mutations in KCNE2 associated with LQTS (LQT6 – MIM# 603796) are characterised by a reduction of the potassium flux generated by the Ikr current resulting in delayed repolarisation [11, 17]. Furthermore, KCNE2 mutations may also cause the acquired form of the LQTS [56].

**KCNJ2**

A further potassium channel involved in LQTS and influencing the cardiac action potential is encoded by the KCNJ2 gene. KCNJ2 is the potassium inward rectifying channel, subfamily J, member 2 gene (MIM# 600681). This gene is located on the long arm of chromosome 17 and contains two exons which span approximately 10 kb [57]. The KCNJ2 gene encodes a 427 amino acid protein (Kir2.1) (Fig. 5) which forms a voltage-dependent inwardly rectifying potassium ion channel responsible for conducting a significant part of the inwardly rectifying Iin current (Fig. 2). Iin is important for stabilising the resting membrane potential, defining the excitation threshold and modulating repolarisation [58]. The activity of the Kir 2.1 channel is highly dependent on the integrity of channel interactions with phosphatidylinositol 4,5-bisphosphate (PIP2) [59].

By genetic linkage studies in a large family, Plaster et al. [16] identified mutations in KCNJ2 that were also associated with Andersen syndrome (LQT7 – MIM# 170390). Andersen syndrome (AS) is inherited in an autosomal dominant fashion and is characterised by periodic paralysis, cardiac arrhythmias and dysmorphic features [60]. Furthermore, there is a high variability and incomplete penetrance in AS. LQTS is the primary cardiac manifestation, present in 71% of patients with AS, and dominant-negative loss-of-function mutations in KCNJ2 result in malfunctioning Kir2.1 channels [3]. This is also the reason that LQT7 remains the name of this type of LQT. However, it should be noted that LQT7 is associated with bidirectional ventricular tachycardia (VT), premature ventricular contraction (PVC) and extrasystoles [35]. Sudden death is rare when compared to other LQT-types.

**CACNA1C**

Calcium channel dysfunctions in the congenital LQTS are related to mutations in CACNA1C, the calcium voltage-dependent channel, L type, alpha-1C subunit gene (MIM# 114205). CACNA1C is located on the short arm of chromosome 12 and contains 50 exons [61]. This gene encodes an α1 subunit of a voltage-dependent calcium channel, known as CaV1.2 (Fig. 6) which mediates the depolarising influx of calcium ions (I_{Ca,L} Current) (Fig. 2) into the cell and contributes to the plateau phase of the cardiac action potential. Multiple isoforms of the α1 subunit protein exist and they often have different electrophysiological and pharmacological properties [62].

Mutations in CACNA1C have been described in patients with Timothy syndrome (LQT8 – MIM# 601005) [4, 23]. Timothy syndrome (TS) is a rare autosomal dominant disorder characterised by physical malformations, as well as neurolo-
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AKAP9 and SNTA1

Recently, two additional genes with LQTS-associated mutations involved in controlling ion channels function have been identified. The first encodes a protein which is a member of the A-kinase anchor proteins (AKAPs), while the second is a member of the syntrophin protein family.

AKAP proteins are a group of structurally diverse proteins which target cAMP-dependent protein kinase A (PKA) to facilitate PKA mediated phosphorylation [68]. LQTS-associated mutations were reported in one of the AKAP members known as the A-kinase anchor protein 9 (AKAP9) gene (MIM# 604001). AKAP9 is located on chromosome 7 and comprises 51 exons [69]. This gene encodes two proteins, the human homologue of the rat protein, AKAP120 and a 1,626 amino acid protein also known as yotiao (Fig. 3A) [69]. In the heart, yotiao is involved in the phosphorylation of a number of proteins including the ryanoend receptor, the L-type Ca2+ channel and the potassium channel responsible for the slow repolarising current, \( I_{\text{kr}} \) [70–72].

Chen et al. [12] analysed the AKAP9 gene in 50 LQTS families where no mutations were detected in the other known LQTS-associated genes. They discovered a single mutation located in close proximity to the C-terminal Kv7.1 binding site (LQT11 – MIM# 611820). Further investigation showed that this inherited mutation reduces, but does not eliminate, the interaction between yotiao and Kv7.1. It reduces the cAMP-dependent phosphorylation of Kv7.1 and alters the functional response of \( I_{\text{ks}} \) channels to cAMP, resulting in delayed repolarisation of the ventricular action potential [12].

Syntrophins are cytoplasmic sub-membranos proteins that are components of the dystrophin-associated protein complex containing multiple protein interaction motifs. LQTS-associated mutations were identified in the syntrophin, \( \alpha 1 \) (SNTA1) gene (MIM# 601017) which forms part of this group. The SNTA1 gene is located on chromosome 20 and consists of eight exons [73]. It encodes a 505 amino acid protein called \( \alpha 1 \)-syntrophin (Fig. 4) which acts as a scaffolding protein for neural nitric oxide synthase (nNOS), plasma membrane Ca2+-ATPase (PMCA) and the \( \alpha \)-subunit of Nav1.5 at the C-terminus [74].

The SNTA1 gene was analysed in 50 unrelated LQTS patients for whom no mutations have been reported in the other LQTS-associated genes. One missense mutation was identified in a patient with a seriously prolonged QTc interval on the ECG (LQT12- MIM# 601017) [20]. Functional studies showed that the mutation causes increased nitrosylation of Nav1.5 and increases the late sodium current. This is consistent with previous reports about LQT3-associated mutations in SCN5A. Additionally, the mutation disrupts the link between Ca2+ transporting, plasma membrane 4 (PMCA4b) and the Nav1.5/\( \alpha 1 \)-syntrophin complex [20].
CONCLUSIONS
To date, mutations in 12 genes have been associated with LQTS. The spectrum of genes involved is rapidly increasing, and recent findings point to the significance of proteins interacting or modulating cardiac ion channels. Identification of disease-causing mutations is important as it may help identify asymptomatic gene carriers that could benefit from prophylactic beta-adrenergic blockade or application of an ICD unit.

References