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SYMPOSIUM REVIEW

Long QT syndrome: beyond the causal mutation

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Abstract Congenital long QT syndrome (LQTS) is caused by single autosomal-dominant mutations in a gene encoding for a cardiac ion channel or an accessory ion channel subunit. These single mutations can cause life-threatening arrhythmias and sudden death in heterozygous mutation carriers. This recognition has been the basis for world-wide staggering numbers of subjects and families counselled for LQTS and treated based on finding (putative) disease-causing mutations. However, prophylactic treatment of patients is greatly hampered by the growing awareness that simple carriership of a mutation often fails to predict clinical outcome: many carriers never develop clinically relevant disease while others are severely affected at a young age. It is still largely elusive what determines this large variability in disease severity, where even within one pedigree, an identical mutation can cause life-threatening arrhythmias in some carriers while in other carriers no disease becomes clinically manifested. This suggests that additional factors modify the clinical manifestations of a particular disease-causing mutation. In this article, potential demographic, environmental and genetic factors are reviewed, which, in conjunction with a mutation, may modify the phenotype in LQTS, and thereby determine, at least partially, the large variability in disease severity.

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Abbreviations 3′UTR, 3′ untranslated region; 5′UTR, 5′ untranslated region; EAD, early afterdepolarization; ECG, electrocardiogram; GWAS, genome-wide association studies; LQTS, long QT syndrome; LQT1, long QT syndrome type 1; LQT2, long QT syndrome type 2; LQT3, long QT syndrome type 3; MAF, minor allele frequency; miRNA, microRNA; QTc, heart rate-corrected QT interval; SNP, single nucleotide polymorphism; TdP, torsades de pointes ventricular tachycardia; TDR, transmural dispersion of repolarization; VF, ventricular fibrillation.

Congenital long QT syndrome (LQTS) is a cardiac repolarization disease that is characterized by prolonged heart rate-corrected QT interval (QTc) on the electrocardiogram (ECG) and cardiac events (syncope, out-of-hospital cardiac arrest, and sudden cardiac death (SCD)) due to torsades de pointes ventricular tachycardia (TdP) and ventricular fibrillation (VF). The discovery of rare variants in genes encoding for cardiac ion channels as

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Table 1. Genetic basis of the long QT syndrome (LQTS)

Type	Occurrence (or % of genotyped)	Gene	Protein	Protein function	Affected current	Reference
1	42–54%	*KCNQ1*	$K_v7.1$	α-subunit I_{Ks} channel	I_{Ks} decrease	Wang *et al.* 1996
2	35–45%	*KCNH2*	$K_v11.1$	α-subunit I_{Kr} channel	I_{Kr} decrease	Curran *et al.* 1995; Sanguinetti *et al.* 1995
3	1.7–8%	*SCN5A*	$Na_v1.5$	α-subunit I_{Na} channel	I_{NaL} increase	Wang *et al.* 1995
4	<1%	*ANK2*	Ankyrin-B	Adaptor protein	None	Mohler *et al.* 2003
5	<1%	*KCNE1*	minK	β-subunit I_{Ks} channel	I_{Ks} decrease	Barhanin *et al.* 1996; Sanguinetti *et al.* 1996
6	<1%	*KCNE2*	MiRP1	β-subunit I_{Kr} channel	I_{Kr} decrease	Abbott *et al.* 1999
7	Rare	*KCNJ2*	Kir2.1	α-subunit I_{K1} channel	I_{K1} decrease	Plaster *et al.* 2001
8	Rare	*CACNA1C*	$Ca_v1.2$	α-subunit $I_{Ca,L}$ channel	$I_{Ca,L}$ increase	Splawski *et al.* 2004
9	Rare	*CAV3*	Caveolin-3	Component of caveolae (co-localizes with $Na_v1.5$)	I_{NaL} increase	Vatta *et al.* 2006
10	<0.1%	*SCN4B*	β4	β-subunit I_{Na} channel	I_{NaL} increase	Medeiros-Domingo *et al.* 2007
11	Rare	*AKAP9*	Yotiao	Mediates $K_v7.1$ phosphorylation	Inadequate I_{Ks} increase during β-adrenergic stimulation	Chen *et al.* 2007
12	Rare	*SNTA1*	α1-syntrophin	Regulates I_{Na} channel function	I_{NaL} increase	Ueda *et al.* 2008
13	Rare	*KCNJ5*	Kir3.4	Subunit K_{ACh} channel	I_{KACh} decrease	Yang *et al.* 2010
14	Rare	*CALM*	Calmodulin	Calmodulin	Defective Ca^{2+} signalling	Crotti *et al.* 2013

disease-causing mutations in families with LQTS in 1995 (Curran *et al.* 1995; Wang *et al.* 1995), more than 30 years after the first description of the disease (Romano *et al.* 1963; Ward 1964), created great interest for genetic studies in a few research laboratories across the world. This has resulted in the identification of hundreds of mutations in 14 LQTS-susceptibility genes in single individuals or families with LQTS (Table 1). In the last few years, genetic testing has become available for clinical use (Kapplinger *et al.* 2009) and this has been the basis for a rapidly increasing number of individuals and families worldwide being counselled for LQTS. However, the use of results from genetic testing to identify individuals at the highest risk for prophylactic treatment is greatly hampered by the growing awareness that carriership of a mutation often fails to predict clinical outcome, since many carriers remain asymptomatic (incomplete penetrance), some carriers only show QTc prolongation without cardiac events, while others experience serious cardiac events at a young age (variable expressivity). It remains largely elusive what determines this large variability in disease severity, where even family members that carry an identical mutation display large differences in phenotype and disease severity (Fig. 1). This suggests that additional factors modify the phenotypic/clinical manifestations of a particular disease-causing mutation. This article aims to review potential genetic and non-genetic factors (demographic and environmental) that, in conjunction with a mutation, may modify the phenotype in LQTS, and thereby determine, at least partially, the large variability in disease severity (Fig. 2).

The ventricular repolarization

Ventricular repolarization, reflected by the QT interval on the ECG (Fig. 3*A*), is a complex process which results from a concerted interplay of ion channels or ion channel accessory subunits that are present (expressed) in the sarcolemma or in the cytoplasm. In individual myocytes, repolarization is the net result of inward depolarizing and outward repolarizing currents during phases 1–3 of the action potential (Fig. 3*B*). The cardiac Na^+ channel ($Na_v1.5$), encoded by *SCN5A*, is responsible for the depolarization during phase 0 of the action potential by permitting an inward current (I_{Na}). Phase 1 is accomplished by the repolarizing transient outward K^+ current (I_{TO}). Phase 2 reflects a balance between the depolarizing L-type inward Ca^{2+} current ($I_{Ca,L}$) and the repolarizing rapidly activating delayed outward rectifying currents (I_{Kr}) and the slowly activating delayed outward rectifying current (I_{Ks}). Phase 3 results from the predominance of the delayed outward rectifying currents after inactivation of the L-type Ca^{2+} channels. Repolarization ends by K^+ efflux through the I_{K1} channels (Fig. 3*C*; Amin *et al.* 2010b).

Mutations in genes encoding for ion channels or their accessory subunits are linked to different types of LQTS (Table 1). Approximately 90% of LQTS mutation carriers (i.e. genotype-positive patients) carry a mutation in *KCNQ1* (type 1 LQTS; LQT1), *KCNH2* (type 2 LQTS; LQT2), and *SCN5A* (type 3 LQTS; LQT3). Loss-of-function mutations in *KCNQ1*-encoded $K_v7.1$ channels and *KCNH2*-encoded $K_v11.1$ channels lead to a decrease in I_{Ks} and I_{Kr}, respectively. Normally, due to its fast inactivation (voltage-dependent closing), $Na_v1.5$ does not conduct current (or only minimally) during the repolarization phases of the action potential. However, LQT3-linked mutations in *SCN5A* impair the fast inactivation of $Na_v1.5$, and this results in a late (sustained or persistent) depolarizing Na^+ current ($I_{Na,L}$). Thus, LQTS-linked mutations cause a decrease in the repolarizing currents (LQT1 and LQT2) or an increase in the depolarizing currents (LQT3), thereby delaying the repolarization of the ventricular action potential. This is reflected by QTc durations that are longer than designated as normal by international guidelines (i.e. in men $\geq$450 ms and in women $\geq$460 ms; Rautaharju *et al.* 2009). When the repolarization process is delayed long enough, $Ca_v1.2$ channels may be able to recover from inactivation and reactivate during phases 2 or 3 of the action potential, thereby triggering early afterdepolarizations (EADs), the cellular substrate for the initiation of TdP (Amin *et al.* 2010a).

Non-genetic factors

Age and sex. Age and sex are well-known modifiers of QTc duration and symptomatology in LQTS. Cardiac events in young patients (<20 years) may usually be triggered by adrenergically or vagally mediated stimuli (exercise, arousal, and/or sleep), while in older patients ($\geq$40 years) environmental triggers (e.g. drugs or hypokalaemia) are more often required to express or aggravate the phenotype (Schwartz *et al.* 2001; Sakaguchi *et al.* 2008). Moreover, cohort studies have found an age–sex interaction, with male LQTS patients experiencing 90% of the first cardiac events before adolescence, while female patients experience their first events in the post-adolescence period (Locati *et al.* 1998). The mechanisms underlying the sex-related differences are still poorly understood. It is partially attributed to the effects of sex hormones on the expression of genes encoding cardiac ion channels and their accessory subunits. In female rabbit cardiomyocytes I_{K1} and I_{Kr} are smaller compared to male myocytes (Liu *et al.* 1998). Similarly, I_{K1} and I_{Ks} are less expressed in female guinea pig cardiomyocytes compared to male myocytes (Zicha *et al.* 2003;

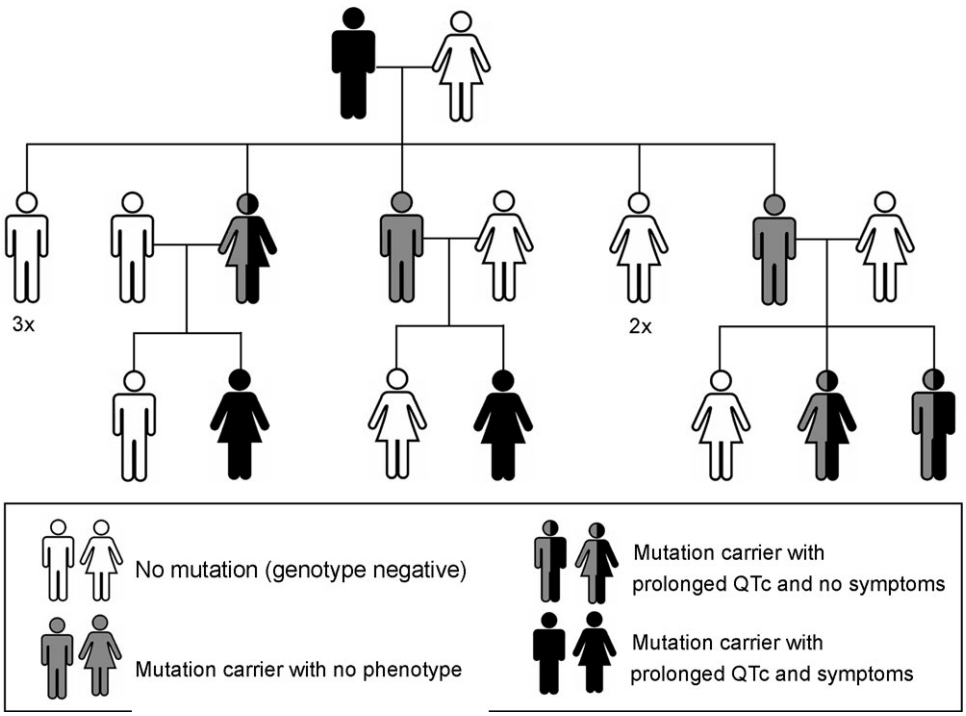

Figure 1. Incomplete penetrance and variable expressivity
The structure of a representative multigenerational pedigree with type 1 long QT syndrome (LQT1) from the Academic Medical Center (AMC) in Amsterdam. LQT1 is caused by the IVS7+5G>A (c.842+5G>A) mutation in intron 7 of *KCNQ1*. The mutation displays 75% disease penetrance. Note the differences in disease severity (variable disease expressivity) between the genotype-positive (mutation-carrying) family members.

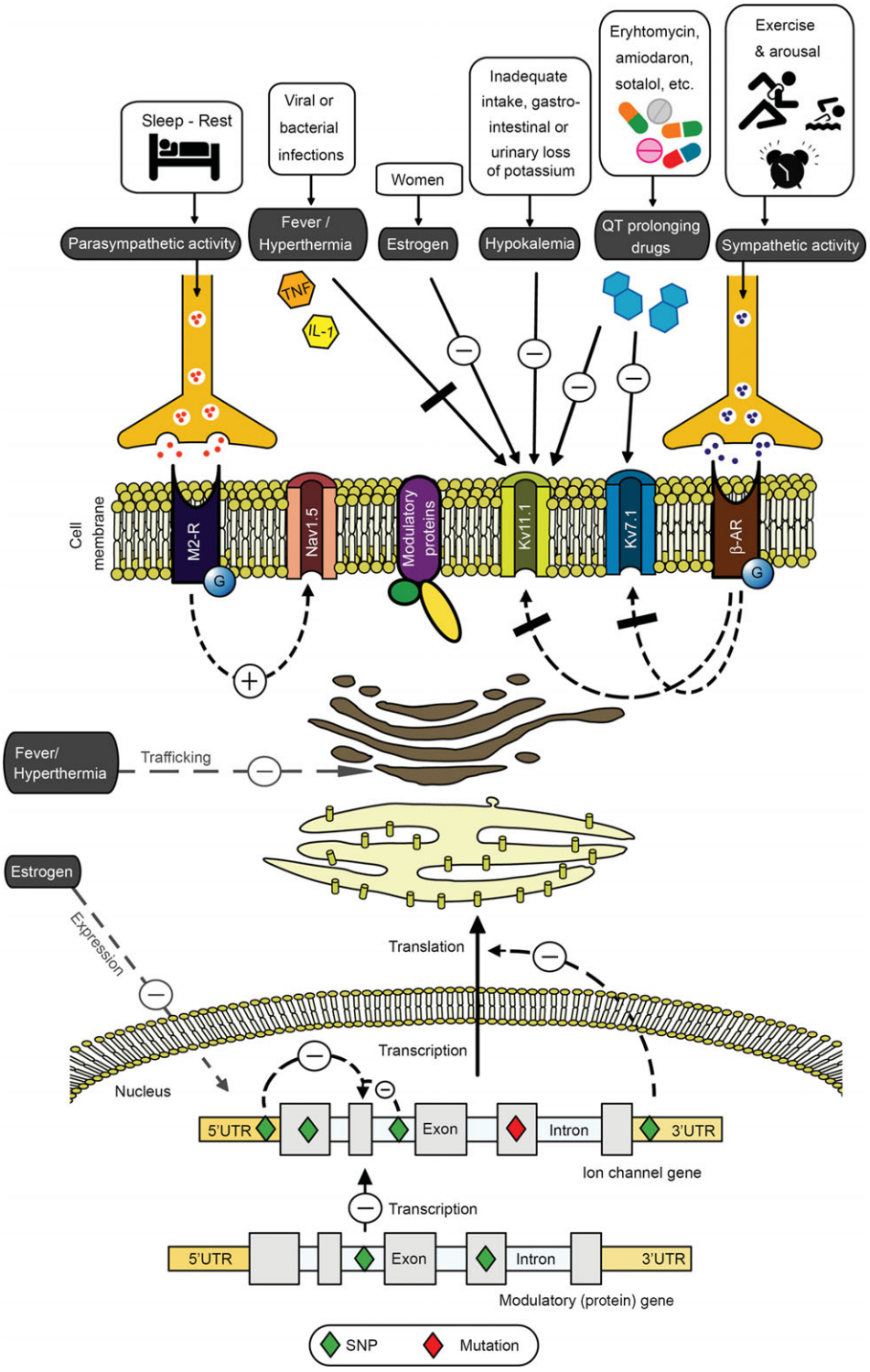

Figure 2. Modifiers of phenotype in long QT syndrome
Schematic representation of genetic and non-genetic factors that are known to modify the phenotype in the long QT syndrome, thereby contributing to incomplete penetrance and variable expressivity. Straight line reflects a direct effect (e.g. direct channel block). Dashed line reflects an indirect effect (e.g. altering gene transcription, messenger RNA translation, or channel phosphorylation). ⊕, stimulatory effect; ⊖, inhibitory effect; 5′UTR, 5′ untranslated region; 3′UTR, 3′ untranslated region; M2-R, M2 muscarinic acetylcholine receptor; β-AR, β-adrenergic receptor; $Na_v1.5$, I_{Na} channel protein; $K_v11.1$, I_{Kr} channel protein; $K_v7.1$, I_{Ks} channel protein; TNF, tumour necrosis factor; IL-1, interleukin-1.

James *et al.* 2004). In line with animal data, messenger RNA (mRNA) and protein expression levels of channel subunits of I_{Kr} ($K_v11.1$), I_{TO} ($K_v1.4$), I_{K1} (Kir2.3) and I_{Ks} (minK, the β-subunit) were lower in non-diseased transplant donor hearts of females *versus* males (Gaborit *et al.* 2010). In addition, direct (non-genomic) effects of sex hormones on cardiac ion currents are also reported. Testosterone rapidly shortens the action potential duration in guinea pig ventricular myocytes through enhancement of I_{Ks} and I_{K1}, and inhibition of $I_{Ca,L}$ (Bai *et al.* 2005). Progesterone shortens the action potential duration in guinea pig ventricular myocytes through enhancement of I_{Ks} under basal conditions and inhibition of $I_{Ca,L}$ under cAMP-stimulated conditions (mimicking β-adrenergic activity; Nakamura *et al.* 2007). Conversely, oestrogen prolongs the action potential duration in guinea pig ventricular myocytes by suppression of $I_{Ca,L}$ and inhibition of I_{Kr} and I_{Ks} (Tanabe *et al.* 1999; Kurokawa *et al.* 2008). Based on these data, we suggest that the net effect of sex hormones on the expression and function of cardiac ion channels is a lower repolarization reserve in women than in men, rendering women more prone to QTc prolongation and TdP occurrence in the presence of a LQTS-causing mutation.

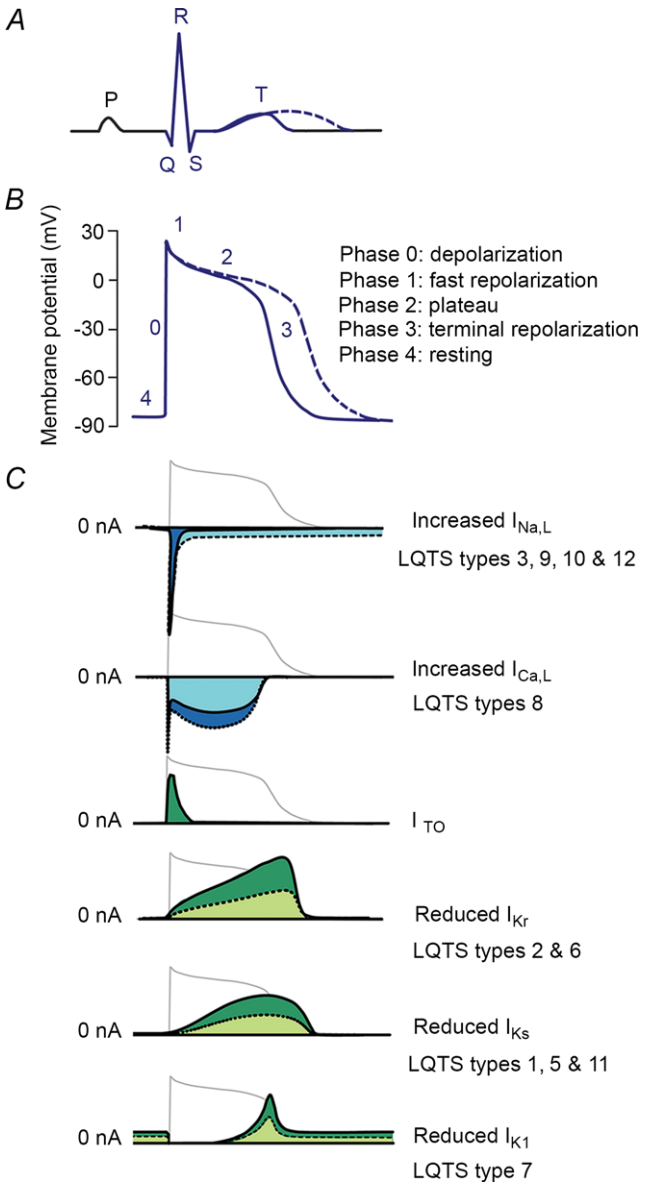

Figure 3. The cardiac electrical activity and the long QT syndrome
A, prolongation of the QT interval on the surface electrocardiogram. *B*, prolongation of the action potential duration. *C*, schematic representation of major inward and outward currents that contribute to action potential formation in ventricular myocytes in health (straight lines), and ion current dysfunctions linked to different types of long QT syndrome (dashed lines).

The risk for cardiac events is reduced in women with LQT2 during pregnancy but significantly increased during the 9 month postpartum period (Khositseth *et al.* 2004). Risk is also higher in women with LQT2 during the peri-menopausal period (Buber *et al.* 2011). It is not yet known why the postpartum period and the perimenopausal period are associated with increased arrhythmogenicity only in LQT2 (and not for example in LQT1). However, β-blockers reduce the risk for cardiac events during these high-risk periods (Seth *et al.* 2007; Buber *et al.* 2011).

The autonomic nervous system. It is well established that the autonomic nervous system influences the phenotype in LQTS. The duration of the ventricular repolarization in healthy hearts is shorter during increased sympathetic activity. This is mainly accomplished by an increase of I_{Ks} during β-adrenergic stimulation through $K_v7.1$ channel phosphorylation (Amin *et al.* 2010b). Genotype–phenotype correlations have shown differences in the effects of the autonomic nervous system on the phenotype in different types of LQTS. In LQT1, patients are at increased risk for cardiac events in conditions when sympathetic activity is elevated, i.e. during exercise (in particular during swimming; Schwartz *et al.* 2001). In LQT2, arousal by abrupt auditory triggers, such as the sound of a telephone or an alarm clock, trigger most events. In LQT3, cardiac events occur often during rest or sleep. Furthermore, onset of TdP differs among LQTS genotypes on the ECG level. TdP in LQT1 occurs usually at fast heart rates, while TdP in LQT2 is often preceded by a pause (68% in LQT2 *versus* 0% in LQT1; Tan *et al.* 2006), with the R–R interval immediately before TdP (pause interval) being significantly longer in LQT2 than in LQT1 patients. The mechanisms underlying the variable responses to the sympathetic nervous activity in different types of LQTS are not precisely known. Clinical studies indicate that β-adrenergic stimulation with adrenaline (epinephrine) infusion causes a persistent increase of QTc in LQT1, an initial transient (but dramatic) increase

of QTc in LQT2 at peak adrenaline levels (with return of QTc to baseline levels at steady state), and a small and transient increase of QTc in LQT3 followed by shortening of QTc to baseline levels (or even below baseline levels) at steady state (Noda *et al*. 2002; Shimizu *et al*. 2003). Studies in arterially perfused wedge preparations of canine left ventricle have aimed to define these mechanisms by using β-adrenergic agonists and antagonists for sympathetic modulation and selective blockers of I_{Ks}, I_{Kr} or enhancers of $I_{Na,L}$ for mimicking different types of LQTS (Shimizu & Antzelevitch, 1998, 2000). In the LQT1 model, β-adrenergic stimulation with isoproterenol (isoprenaline) resulted in a persistent prolongation of the QT interval and increase in the transmural dispersion of repolarization (TDR), due to regional (transmural) differences in the action potential duration, which facilitated occurrence of TdP. Of note, in the LQT1 model TDR was not increased under basal conditions, i.e. in the absence of isoproterenol. In contrast, in the LQT2 and LQT3 models, TDR was increased and TdP occurred under basal conditions. In the LQT2 model, isoproterenol caused an initial further prolongation of action potential duration and QT interval and also a further increase in TDP and TdP incidence. However, this was followed by a shortening of the action potential duration and QT interval and a decrease in TDR and TdP, most likely due to an adequate augmentation of I_{Ks} in the LQT2 model during continuous β-adrenergic stimulation. Finally, in the LQT3 model isoproterenol caused a decrease in TDP and TdP incidence. Persistent increase of the action potential duration and TDR during β-adrenergic stimulation is in line with the higher risk of cardiac events in LQT1 patients during exercise. However, based on the pause dependence of TdP in LQT2, and the transient dramatic increase of the action potential duration and TDR upon β-adrenergic stimulation, we assume that the occurrence of a ventricular ectopic beat upon sudden arousal initiates TdP in the LQT2 patients. In LQT3, decreased action potential duration and TDR during β-adrenergic stimulation supports the higher incidence of cardiac events in these patients during rest or sleep when the sympathetic activity is low. Finally, the above-mentioned data provide a rationale for the effectiveness of β-blockers to prevent events in LQT1 patients, and to a lesser extent in LQT2 patients (Schwartz *et al.* 2001).

Furthermore, recent studies suggest that differences in autonomic responses modify disease severity in LQT1. A study in 166 carriers of the A341V mutation in *KCNQ1* showed that subjects with lower heart rates are at lower risk of developing symptoms (Brink *et al.* 2005). In addition, another study in 56 carriers of this mutation showed that the baroreflex sensitivity (measured as the response of blood pressure and heart rate to bolus phenylephrine injections) is lower in asymptomatic than symptomatic mutation carriers, suggesting that a weakened autonomic response is protective in LQT1 (Schwartz *et al.* 2008). Interestingly, higher baroreflex sensitivity in this cohort was associated with a polymorphism in the α2-adrenergic receptor gene resulting in loss of synaptic autoinhibitory feedback and thereby increased presynaptic release of noradrenaline (norepinephrine), and a polymorphism in the β1-adrenergic receptor gene leading to enhanced β1 activity.

Fever/hyperthermia. In 2008, we first described the repeated occurrence of fever-induced TdP and VF in two related LQT2 patients (father and son) with the A558P mutation in *KCNH2* (Amin *et al.* 2008). ECG analysis showed increased QTc with fever in both patients. Molecular analysis revealed that the A558P mutation disrupts the intracellular trafficking of mutant K_v11.1 proteins, and (when co-expressed with normal K_v11.1 proteins) that the A558P mutant proteins exert dominant-negative effects on the intracellular trafficking of normal K_v11.1 proteins and reduce the temperature-dependent increase of the normal K_v11.1 current. Thus, the mutant K_v11.1 current did not increase to the same extent as the normal K_v11.1 current did at higher temperatures. These findings suggest that the blunted increase of mutant I_{Kr} in patients with LQT2 during fever, while the depolarizing $I_{Ca,L}$ is significantly enhanced, upsets the balance between the depolarizing and the repolarizing forces in favour of depolarization, and thereby contributes to the development of QTc prolongation and TdP/VF at febrile temperatures (Amin *et al.* 2008). In line with our data, Burashnikov and colleagues showed that, when I_{Kr} is blocked by a K_v11.1 channel blocking agent, hyperthermia can induce EADs and TdP in canine left ventricle wedge preparations (Burashnikov *et al.* 2008). Together, these data imply that fever can trigger TdP/VF in conditions when the repolarization reserve of myocardium is compromised by defective K^+ channels, as in patients with LQT1 and LQT2. However, this notion awaits further clinical confirmation.

Extracellular K^+ concentration. Alterations in extracellular K^+ concentrations ($[K^+]_o$), influence the QTc duration in healthy subjects and in patients with LQTS. The Nernst equation predicts smaller outward K^+ currents during hyperkalaemia, i.e. when $[K^+]_o$ is elevated. However, I_{K1} (at potentials higher than the resting membrane potential) and I_{Kr} display a 'paradoxical' increase when $[K^+]_o$ is elevated. I_{Kr} increases as a result of a depolarizing shift in the voltage dependence of inactivation (i.e. towards more positive membrane potentials), whereas the effect of K^+ on I_{K1} is complex and beyond the scope of this review (McAllister & Noble, 1966; Sanguinetti & Jurkiewicz, 1992; Choy *et al*. 1997).

These findings suggest shortening of the action potential duration upon elevation of $[K^+]_o$. Indeed, intravenous K^+ administration has been shown to decrease the QTc duration in seven LQT2 patients (Compton *et al.* 1996). However, it must be noted that the patients in the study of Dr Compton and colleagues had markedly prolonged QTc (mean ± SD 627 ± 92 ms), which is much longer than the mean QTc reported in large LQT2 cohorts (∼470 ms to ∼510 ms; Tester *et al.* 2005; Shimizu *et al.* 2009). The extreme QTc values in this study may have positively biased the effect of intravenous K^+ administration because of the higher chance of spontaneous regression of QTc values towards the mean after the therapy (Morton & Torgerson, 2003). Furthermore, hypokalaemia has been associated with increased arrhythmogenesis *in vivo* (Sabir *et al.* 2007), and with further QTc prolongation and cardiac events in a relatively small cohort of LQTS patients (Sakaguchi *et al.* 2008).

QTc-prolonging drugs. Drugs possess the ability to unmask or aggravate the phenotype in patients with LQTS or to cause acquired LQTS (Sakaguchi *et al.* 2008; Kannankeril *et al.* 2010). The underlying mechanism is almost always block of the $K_v11.1$ (I_{Kr}) channels. Because of their molecular structure, $K_v11.1$ channels are susceptible to direct block by drugs with a wide variety of chemical structures and sizes (Kannankeril *et al.* 2010). Moreover, drugs can bind to $K_v11.1$ proteins in the cytoplasm and disrupt their intracellular trafficking to the sarcolemma (Rajamani *et al.* 2006). In addition, some drugs may prolong repolarization by increasing the inward depolarizing currents $I_{Ca,L}$ and $I_{Na,L}$ (Kühlkamp *et al.* 2003; Kuryshev *et al.* 2006).

Genetic factors

Although the above-mentioned non-genetic (demographic and environmental) factors are important determinants of phenotype in LQTS and can relatively easily be detected by routine medical examination, they explain only a small part of the incomplete disease penetrance and variable expressivity in LQTS. Literature provides several examples of pedigrees with LQTS in which members of the same sex and generation display large differences in disease severity, ranging from a lifelong asymptomatic state to SCD at a young age, with a disease penetrance ranging from 25% to 100% (Vincent *et al.* 1992; Priori *et al.* 1999; Viadero *et al.* 2011). In addition, significant overlap in QTc duration may exist between genotype-positive members and genotype-negative members of a particular LQTS pedigree (Vincent *et al.* 1992). Moreover, variability in the QTc duration is not limited to the LQTS, since apparently healthy subjects from the general population may display prolonged QTc duration (>460 ms), while patients with a mutation in a LQTS susceptibility gene can have normal QTc duration (Taggart *et al.* 2007).

In the early nineties, the discovery that the risk for SCD is increased in the first-degree relatives of SCD victims, along with the association of QTc prolongation with an increased risk for SCD in the general population (Algra *et al.* 1991), led to the hypothesis that QTc variability may have a heritable component. This created the basis for considerable interest and extensive efforts to identify genetic factors that may modify the QTc duration. Ever since, large scale genome-wide association studies (GWAS) and candidate gene studies have searched for genetic variants that impact the QTc duration in the general population (Arking *et al.* 2006; Newton-Cheh *et al.* 2009; Pfeufer *et al.* 2009; Marroni *et al.* 2009), while relatively small genetic studies have searched for the co-inheritance of (putatively) functional genetic variants that modulate the QTc duration in LQTS cohorts (Crotti *et al.* 2005, 2009; Nishio *et al.* 2009). These studies have greatly increased our knowledge about the heritable component of the QTc variability.

The genetic architecture of the QTc duration. Although the genetic architecture of the QTc duration turns out to be pretty complex, it is thought to consist of a spectrum of genetic variants ranging from very rare variants, with <1% minor allele frequency (MAF) but large effect on the QTc duration, to common variants with >5% MAF but small effect on the QTc duration. In between are the 'common rare' variants with 1–5% MAF and an intermediate effect on the QTc duration (Fig. 4; Sauer & Newton-Cheh, 2012). Rare variants are expected to be disease-causing mutations in genes encoding ion channels and their accessory proteins that cause abnormally prolonged (or shortened) QTc durations and increase the risk of TdP/VF and SCD. Mutations that are traditionally linked to LQTS in small genetic studies in single pedigrees are examples of such rare variants. These rare variants are not commonly found in the general population. Instead, common variants, identified through GWAS in the general population, are expected to minimally influence (prolong or shorten) the ventricular repolarization (and thus the QTc duration), and confer only small impact on the risk of SCD individually. However, the presence of multiple common variants with the same small effect on the repolarization in one person may theoretically affect the repolarization time to an extent that might lead to manifestation of disease phenotype. For example, co-inheritance of common variants with minor prolonging effect on the repolarization may explain a prolonged QTc duration in a subject with no mutation in one of the LQTS susceptibility genes. Inversely, co-inheritance of common variants with small shortening effect on the repolarization

may theoretically weaken the effect of a LQTS-causing mutation, leading to a normal QTc duration in a genotype-positive patient. However, such a concerted effect of common variants on disease severity has not been described in humans yet. Finally, 'common rare' variants with 1–5% MAF are expected to exert an intermediate effect on QTc duration. Examples include disease-causing mutations found in LQTS families with low penetrance and variable expressivity (Priori *et al.* 1999), variants associated with strong modifying effects on the QTc duration in the general population (Arking *et al.* 2006; Newton-Cheh *et al.* 2009; Pfeufer *et al.* 2009; Marroni *et al.* 2009), and variants associated with disease only in the co-presence of a non-genetic trigger (a 'second hit'), such as those found in subjects with drug-induced LQTS (Kannankeril *et al.* 2010).

Genetic variants modifying QTc duration in the general population. In general, two types of genetic studies have associated genetic variants with the QTc duration in the general population. Initially, candidate gene studies have searched for associations between the QTc variability in the community and relatively small numbers of variants in ion channel-encoding genes, often non-synonymous (i.e. leading to an amino acid change) single nucleotide polymorphisms (SNPs). More recently, by using maps of hundreds of thousands of common SNPs spread throughout the genome, GWAS have systematically examined whether genomic variations are associated with the QTc variability in the general population. This unbiased approach of GWAS has the advantage of identifying genomic regions that are not previously linked to the QTc duration, but GWAS do not provide information on the underlying causative mechanism.

The most important SNPs that are associated with the QTc duration and/or SCD in the general population by candidate gene approach include K897T in *KCNH2*-encoded K_v11.1 (Bezzina *et al.* 2003; Pfeufer *et al.* 2005; Newton-Cheh *et al.* 2007; Marjamaa *et al.* 2009), D85N in *KCNE1*-encoded minK (Gouas *et al.* 2005; Marjamaa *et al.* 2009), and SNPs in the non-coding regions of *KCNQ1* and *KCNH2* (Newton-Cheh *et al.* 2009; Pfeufer *et al.* 2009). GWAS have not only provided further support for the association of K897T, D85N and some of the non-coding SNPs that were found in these small studies, but have also uncovered novel associations between SNPs and the QTc duration in the general population (Table 2). As expected, some of these SNPs are located at genomic regions harbouring ion channel-encoding genes that are implicated in LQTS (*KCNQ1*, *KCNH2*, *SCN5A* and *KCNJ2*). Moreover, the first GWAS in a community-based cohort from Germany introduced the power of GWAS in discovering unexpected genetic associations by showing a strong association between QTc variability and SNPs in *NOS1AP*, the gene that encodes the nitric oxide synthase 1 adaptor protein (Arking *et al.* 2006). *NOS1AP* had not been linked to cardiac electrical activity before. Importantly, two subsequent meta-analyses of GWAS data of QTc duration from several population-based cohorts of European descent replicated the association between QTc variability and SNPs in *NOS1AP* (Newton-Cheh *et al.* 2009; Pfeufer *et al.* 2009).

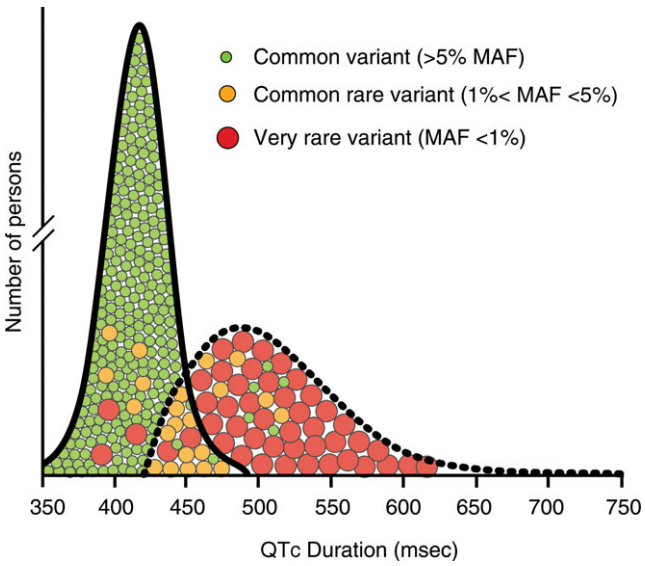

Figure 4. Distribution of QTc duration in health and in long QT syndrome
Continuous line represents QTc distribution in health. Dashed line represents QTc distribution in long QT syndrome. The circles display the genetic architecture of the QTc duration consisting of very rare variants with <1% minor allele frequency (MAF) but large effect on the QTc duration, common rare variants with 1–5% MAF with an intermediate effect on QTc duration, and common variants with >5% MAF but small effect on the QTc duration. Rare variants are mutations that are traditionally linked to the long QT syndrome; however, they may be found in persons with normal QTc duration. Common variants are expected to minimally influence (prolong or shorten) the QTc duration. Common rare include variants associated with strong modifying effects on the QTc duration in the general population and variants associated with LQTS only in the co-presence of a non-genetic trigger (a 'second hit').

Genetic variants modifying QTc duration in LQTS. Genetic studies aiming to unravel QTc- and phenotype-modifying genetic variants in LQTS are mostly based on the theory that co-inheritance of a second genetic variant that influences the ventricular repolarization may affect the final outcome of a disease-causing mutation in an individual carrier, a so-called 'second hit' or 'double hit' theory.

Double hits may involve mutations in the same gene (compound heterozygosity), mutations in different LQT genes (digenic heterozygosity), or a mutation and a common rare variant in the same gene or in different

Table 2. Common variants associated with the QTc duration

Locus	SNP	MAF	Location	Nearest gene	Function	QTc effect
1q	rs12143842	0.16	Intergenic	*NOS1AP*	Nitric oxide synthase 1 adaptor protein	↑
	rs2880058	0.26	Intergenic			↑
	rs10494366	0.33	Intron			↓
	rs12029454	0.11	Intron			↑
	rs16857031	0.15	Intron			↑
	rs4657178	0.18	Intron			↑
1q	rs10919071	0.11	Intron	*ATP1B1*	β-subunit Na^+/K^+ ATPase	↑
1p	rs846111	0.26	3′UTR	*RNF207*	Ring finger protein	↑
3p	rs11129795	0.34	Intergenic	*SCN5A*	α-subunit I_{Na} channel ($Na_v1.5$)	↓
	rs12053903	0.29	Intron			↓
6q	rs12210810	0.08	Intergenic	*C6orf204*	Phosphorylation	↓
	rs11970286	0.47	Intergenic			↑
7q	rs2968863	0.26	Intergenic	*KCNH2*	α-subunit I_{Kr} channel ($K_v11.1$)	↓
	rs4725982	0.18	Intergenic			↑
	rs1805123	0.24	Exon (K897T)			↑↓
11p	rs12296050	0.23	Intron	*KCNQ1*	α-subunit I_{Ks} channel ($K_v7.1$)	↑
	rs12576239	0.16	Intron			↑
	rs2074238	0.08	Intron			↓
12q	rs3825214	0.22	Intron	*TBX5*	Transcription	↑
13q	rs2478333	0.35	Intergenic	*SUCLA2*	Mitochondrial enzyme	↑
16p	rs8049607	0.49	Intergenic	*LITAF*	Tumour necrosis factor	↑
16q	rs37062	0.27	Intron	*CNOT1*	RNA transcription	↓
17q	rs17779747	0.32	Intergenic	*KCNJ2*	α-subunit I_{K1} channel (Kir2)	↓
17q	rs2074518	0.49	Intron	*LIG3*	DNA ligase III	↓
21q	rs1805128	0.03	Exon (D85N)	*KCNE1*	β-subunit I_{Ks} channel (minK)	↑

SNP, single nucleotide polymorphism; MAF, minor allele frequency; ↑, QTc prolongation; ↓, QTc shortening.

genes. The prevalence of compound heterozygosity and digenic heterozygosity in LQTS is between 5% and 10%, and is associated with a more severe phenotype (i.e. extensive QTc prolongation and early onset of cardiac events; Schwartz *et al.* 2003; Westenskow *et al.* 2004). So far, compound heterozygosity and digenic heterozygosity have only been described for mutations that affect the QTc duration in the same direction by an additive on the ion channel function (i.e. two mutations that exert a prolonging effect on the QTc duration).

Common rare variants that are proposed as genetic modifiers in LQTS include SNPs in *KCNH2* (K897T), *KCNE1* (D85N) and *NOS1AP*. SNP rs1805123 (A→C) in *KCNH2* has a MAF of ∼24% in populations of European descent in *KCNH2* and leads to the substitution of lysine at residue 897 to threonine (K897T) in $K_v11.1$. Dr Crotti and colleagues showed in a multigenerational pedigree with LQT2 due to the low-penetrant *KCNH2* mutation (A1116V) that disease was only manifested in members who co-inherited the minor C allele of SNP rs1805123 on the non-mutant *KCNH2* allele (i.e. K897T in the healthy $K_v11.1$ proteins; Crotti *et al.* 2005). They also showed that the minor C allele caused a reduction of the $K_v11.1$ current in heterologous expression systems. However, controversy exists about the effect of K897T on ventricular repolarization, since the minor C allele was associated with a shorter QTc duration in GWAS (Pfeufer *et al.* 2005), and caused an increase of $K_v11.1$ current when expressed in different experimental settings (Bezzina *et al.* 2003).

SNP rs1805128 (G→A) in *KCNE1* is present in ∼1% of the general (white) population and leads to the substitution of aspartic acid at residue 85 to asparagine (D85N) in minK, the β-subunit of the I_{Ks} channel. In addition, although controversy exists on the role of minK on the regulation of the I_{Kr} channel *in vivo*, minK has been shown to bind to the *KCNH2*-encoded protein and affect the I_{Kr} current density *in vitro* (McDonald *et al.* 1997; Ohno *et al.* 2007). D85N has not only been shown to cause LQTS, but also to contribute to disease penetrance and variable expressivity in patients with LQT1 (Nishio *et al.* 2009; Lahtinen *et al.* 2011). Moreover, experimental studies suggest that D85N causes a significant reduction of I_{Ks} and I_{Kr} (Nishio *et al.* 2009; Nof *et al.* 2011), providing a molecular mechanism for the more severe phenotype in LQT1 patients who also carry the minor A allele of SNP rs1805128.

After the discovery of genetic variants in *NOS1AP* as modifiers of QTc duration in the general population, two studies have established the role of *NOS1AP* as a genetic modifier of disease severity in LQTS. A study in a South African LQT1 cohort, segregating the A341V

mutation in *KCNQ1* (including 205 mutation carriers and 295 non-carriers), showed that the minor alleles of two SNPs in the non-coding regions of *NOS1AP* are associated with longer QTc durations and more symptoms (cardiac arrest and SCD) in this population (Crotti *et al.* 2009). This study suggested that mutation carriers who also carried the minor allele of one of these two SNPs had a greater risk for experiencing life-threatening cardiac events than the mutation carriers without the minor allele. A second study in 901 LQTS patients, mainly LQT1, LQT2 and LQT3, showed that genetic variation in *NOS1AP* also modifies disease severity in other types of LQTS (Tomás *et al.* 2010). Finally, the recent association of SNPs in *NOS1AP* with drug-induced LQTS further supports the 'second hit' theory; in this case disease manifestation through the conjoint effect of a common rare genetic variant with an environmental trigger (i.e. a QT-prolonging drug; Jamshidi *et al.* 2012). Since SNPs in *NOS1AP* associated with disease severity in LQTS are located in the non-coding regions of the gene, they are presumed to influence the transcription of the *NOS1AP* allele in which they are located (*cis*-acting regulation). Cellular studies suggest

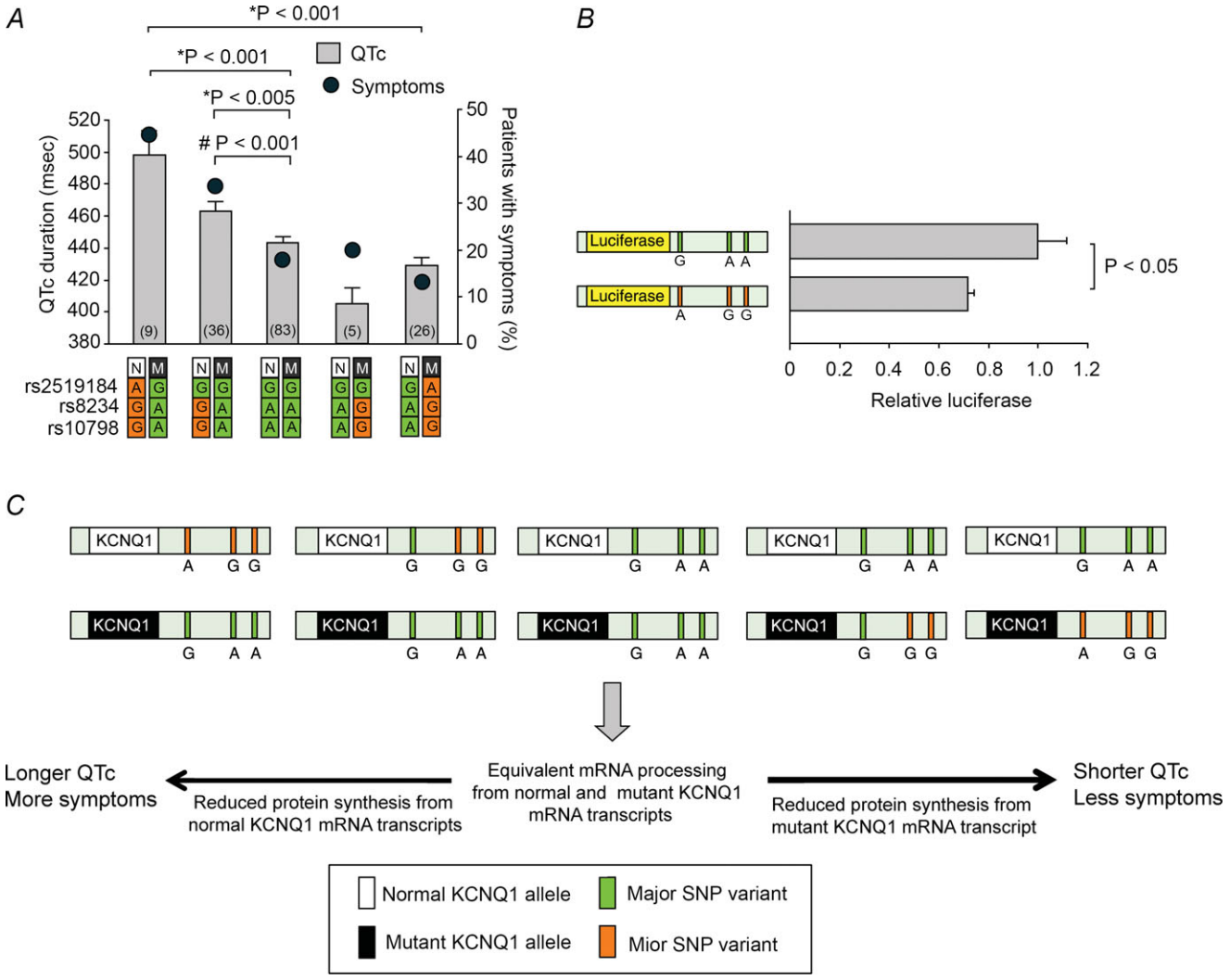

Figure 5. Allele-specific effects of variants in the 3′ untranslated region (3′UTR) of *KCNQ1* on the QTc duration and symptomatology in long QT syndrome type 1 (LQT1)
A, allele-specific effects of SNPs rs2519184, rs8234 and rs10798 in the 3′UTR of *KCNQ1* on the QTc and symptomatology in patients with LQT1. *B*, luciferase assays in primary neonatal rat cardiomyocytes transfected with two independent reporter plasmids containing the major or the minor haplotype of SNPs in 3′UTR of *KCNQ1*. *C*, the possible mechanism where SNPs in the 3′UTR of *KCNQ1* modify phenotype in LQT1. SNPs in the 3′UTR may alter the expression of the normal and the mutant *KCNQ1* alleles in an allele-specific manner, and, as a result, the balance between the normal and the mutant $K_V7.1$ proteins within the tetrameric I_{Ks} channels. Numbers in bars denote group sizes. Data are presented as mean; error bars represent SEM. N, normal *KCNQ1* allele; M, mutant *KCNQ1* allele. Reproduced from Amin *et al.* (2012) © 2012 with permission from Oxford University Press. ∗ indicates comparisons between QTc durations. # indicates comparison between symptomatology.

that *NOS1AP*-encoded protein (CAPON) decreases $I_{Ca,L}$ density and slightly increases I_{Kr} density, leading to a shortening of the action potential (Chang *et al.* 2008). Minor alleles of SNPs in *NOS1AP* may reduce CAPON levels by impairing transcription, thereby attenuating the effect of CAPON on the action potential duration.

Genetic variants modifying QTc duration in LQTS in an allele-specific manner. In 2012, we proposed a novel mechanism for genetic variants to modify disease severity in LQTS by describing an association between SNPs in the 3′ untranslated region (3′UTR) of *KCNQ1* and the QTc duration and symptomatology in patients with LQT1 (Amin *et al.* 2012). The 3′UTR is a region of the mRNA that starts with the nucleotide immediately following the stop codon of the coding region. The 3′UTR contains binding sites for microRNAs (miRNAs). By virtue of the repressive effects of the miRNAs on the translation, the 3′UTR determines in a *cis*-regulatory fashion whether and to what extent mRNA is translated to protein (Chen & Rajewsky, 2006; Huntzinger & Izaurralde, 2011). SNPs in the miRNA binding sites of the 3′UTR have been implicated in the clinical manifestation of various non-cardiac diseases, including asthma, cancer, diabetes mellitus, hypertension and Parkinson's disease (Borel & Antonarakis, 2008). However, most of these SNPs were located outside the gene harbouring the (putative) pathogenic mutation or involved in the pathophysiology of complex diseases. Therefore, clear evidence for allele-specific effects of 3′UTR SNPs, via *cis*-regulatory actions, on the expression of mutation-carrying genes was lacking. We hypothesized that in an autosomal-dominant disease such as LQTS genetic variation in the 3′UTR of either the normal or rather the mutant allele may influence miRNA binding, and thereby alter the relative amount of proteins stemming from either of these alleles. Our group demonstrated in a combined cohort of 168 LQT1 patients with different *KCNQ1* mutations from the United States and the Netherlands that the SNPs rs2519184 (G→A), rs8234 (A→G), and rs10798 (A→G), located in the 3′UTR of *KCNQ1*, were associated with the QTc duration and symptom occurrence (syncope, TdP, VF, SCD) in an allele-specific manner. Patients with the minor 3′UTR haplotype (A-G-G or G-G-G) on their mutant *KCNQ1* allele had shorter QTc and fewer symptoms, while patients with the minor 3′UTR haplotype on their healthy *KCNQ1* allele had significantly longer QTc and more symptoms (Fig. 5*A*). Furthermore, our luciferase reporter assays showed that the expression of *KCNQ1*'s 3′UTR with the minor SNP variants was lower than the expression of the 3′UTR with the major SNP variants (Fig. 5*B*). Taken together, our clinical and experimental data showed that occurrence of 'suppressive' 3′UTR SNPs in *cis* (on the same allele) to the mutation attenuates disease severity by lowering the abundance of mutant $K_v7.1$ proteins, while occurrence of 'suppressive' 3′UTR SNPs in *trans* (on the opposite allele) to the mutation aggravates clinical phenotype by reducing the translation of the normal *KCNQ1* allele (Fig. 5*C*). This discovery not only introduced novel genetic modifiers of LQTS, with an effect outweighing by far that of previously identified modifying SNPs, but it also uncovered a mechanism that greatly influences the severity of disease-causing mutation in a Mendelian inherited disease, since in many cases the unaffected parent may alter disease severity by bringing in naturally occurring variation in the 3′UTR. However, it must be noted that the effects of the above-mentioned 3′UTR SNPs are not yet supported by GWAS or genetic studies in other LQTS cohorts. Furthermore, further research is necessary to unravel the precise mechanism by which 3′UTR SNPs influence gene expression and disease severity, e.g. the role of specific miRNAs.

Conclusion

LQTS is known as a monogenetic disorder, with several well-recognized non-genetic modifiers (among others, age and sex) as important modifiers. In recent years it has also become increasingly clear that other genetic variants play an equally important modulatory role in establishing the severity of the phenotype. Detailed understanding of these modifying (genetic and non-genetic) factors is mandatory for accurate risk stratification and the subsequent therapeutic choices. Active 'cascade screening' in affected families leads to a rapidly increasing number of genotype-positive phenotype-negative individuals, with an ill-defined prognosis. Further appreciation of their actual risk from life-threatening arrhythmias would therefore be more than welcome.

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Additional information

Competing interests

None declared.

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