Emerging therapeutic targets in the Short QT Syndrome

by

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Highlights

- The short QT syndrome (SQTS) is a rare condition associated with atrial and ventricular arrhythmias and a risk of sudden death. Implantable devices and antiarrhythmic pharmacology are used to treat the syndrome.
- There are 8 successfully genotyped variants, involving gain-of-function mutations to $K^+$ ion channel genes (SQT1-3) or loss-of-function mutations to $Ca^{2+}$ channel subunit genes (SQT4-6), to a $Na^+$ channel gene (SQT7) and to an anion exchanger (SQT8).
- Information from patients and in vitro and in silico experiments indicates important roles for abbreviated refractoriness, dispersion of repolarisation and a shortened wavelength for re-entry in arrhythmia substrates in the SQTS.
- A combination $K^+$ current inhibition during the action potential plateau, with sodium channel inhibition, collectively resulting in delaying repolarisation and refractoriness is likely to be valuable in prolonging effective refractory period and wavelength for re-entry in the SQTS. In vitro and in silico data point to the feasibility of genotype-specific pharmacology, though selective agents against each known target are not yet in clinical use.
- Approximately three-quarters of genotyped cases have not yielded mutations in ion channel target genes. Exome or genome sequencing may thus be warranted to identify the underlying culprits in SQTS cases where targeted ion genotyping is unsuccessful. This is likely to reveal new modulators of repolarisation and potentially new intervention points to target in the SQTS.
Abstract

**INTRODUCTION:** Short QT Syndrome (SQTS) is a rare but dangerous condition characterised by abbreviated repolarisation, atrial and ventricular arrhythmias and risk of sudden death. Implantable cardioverter defibrillators (ICDs) are a first line protection against sudden death, but adjunct pharmacology is beneficial and desirable.

**AREAS COVERED:** The genetic basis for genotyped SQTS variants (SQT1-SQT8) and evidence for arrhythmia substrates from experimental and simulation studies are discussed. The main ion channel/transporter targets for antiarrhythmic pharmacology are considered in respect of potential genotype-specific and non-specific treatments for the syndrome.

**EXPERT OPINION:** Potassium channel blockade is valuable for restoring repolarisation and QT interval, though genotype-specific limitations exist in the use of some K⁺ channel inhibitors. A combination of K⁺ current inhibition during the action potential plateau, with sodium channel inhibition that collectively result in delaying repolarisation and post-repolarisation refractoriness is likely to be valuable in prolonging effective refractory period and wavelength for re-entry. Genotype-specific K⁺ channel inhibition is limited by a lack of targeted inhibitors in clinical use, though experimentally available selective inhibitors now exist. The relatively low proportion of successfully genotyped cases justifies an exome or genome sequencing approach, to reveal new mediators and targets, as demonstrated recently for SLC4A3 in SQT8.

Abstract: 199 words; Limit 200 words
1. Introduction

The QT interval of the electrocardiogram (ECG) corresponds to the period from the initiation of ventricular depolarisation to completion of ventricular repolarisation. It is well recognised that prolongation of the rate-corrected QT (QT<sub>c</sub>) interval beyond 440-460 ms (in males and females respectively) due either to genetic mutations in ion channels or to pharmacological blockade of potassium (K<sup>+</sup>) channels is associated with an increased risk of ventricular arrhythmia [1-3]. QT<sub>c</sub> prolongation beyond 500 ms appears to be particularly associated with increased arrhythmia risk [3]. Since 2000, a distinct genetic syndrome involving abnormally abbreviated QT intervals (and hence accelerated ventricular repolarisation) has been identified [4-6]. Patients with the genetic short QT syndrome (SQTS) typically exhibit: abbreviated QT intervals (circa or <320 ms [6]); poor rate-adaptation of the QT interval; tall, upright T waves; shortened atrial and ventricular effective refractory periods, and an increased risk of ventricular and atrial arrhythmias and of sudden death - in the absence of structural heart disease [4;7-10]. Congenital forms of SQTS are distinct from acquired forms of QT interval shortening, such as those produced by catecholamines, acetylcholine, hypercalcaemia, hyperthermia, cardiac glycosides, carnitine deficiency, or anabolic steroid use [11-17]. The present review considers what is known about the underlying basis of SQTS and arrhythmogenesis in the syndrome as a platform then to discuss existing and potential targets for therapeutic, particularly pharmacological, intervention.

2. What denotes a “short” QT interval?

Very short QT intervals are rare in the general population. For example, in 10,822 middle-aged Finnish subjects, only 43 people had a rate-corrected QT interval of <340 ms, and only 11 people <320 ms [18]. In a large hospital-based population of 114,334, the lowest 0.15 percentile had QT<sub>c</sub> intervals of ≤362 and 369 ms for males and females, respectively [19], with a biphasic distribution with age (i.e. the shortest QT<sub>c</sub> intervals in young and old age). In a separate study of 18,825 healthy people between 14 and 35, the prevalence of a QT<sub>c</sub> ≤320 ms was found to be 0.1% [20]. It is, however, worth noting that the prevalence of short QT<sub>c</sub> intervals may be affected by the rate correction formula adopted for ECG analysis [21]. Cohort analysis has highlighted differences in a number of individuals meeting SQTS
diagnosis criteria between Bazett, Fridericia, Hodges and Framingham rate correction methods [21]. Whilst congenital forms of SQTS are rare, the risk of sudden death [9;10;22;23] makes it important that congenital SQTS cases are identified and treated. Diagnostic criteria for the SQTS are considered in detail elsewhere [9;20;21;24;25]. Current guidelines from the European Society of Cardiology suggest diagnosis of SQTS with a QT_c interval of \( \leq 340 \) ms [25]. A longer QT_c interval of \( \leq 360 \) ms can be used if there is additional evidence of one or more of: a familial history of SQTS; a confirmed pathogenic mutation; a family history of sudden death below 40 years of age; survival from ventricular tachycardia (VT) or fibrillation (VF) in the absence of structural heart disease [25]. Interestingly, PQ segment depression has been reported in 52 of a cohort of 64 (>80 %) SQTS patients and may potentially constitute an additional marker for the syndrome [26], though further work is required to investigate this. Recently, a refractory period cut-off of 200 ms in the right ventricular outflow tract during invasive testing and pacing at basic cycle length of 500 – 600 ms has been proposed as a means of identifying true SQTS from individuals without the syndrome who possess borderline QT intervals [27].

### 3. SQTS genotypes

In order to consider therapeutic targets for intervention in the SQTS, it is first necessary to consider what is known about the underlying genetic basis for the condition. Inherited SQTS mutations are transmitted in an autosomal dominant fashion, with genotyped patients being heterozygous for identified mutations [28].

#### 3.1 SQT1

The first study to identify a genetic cause for the SQTS was published in 2004 and implicated KCNH2 encoded “hERG” (human Ether-à-go-go-Related Gene) channels in the condition [29]. hERG is responsible for the pore-forming subunit of ion channels that carry the “rapid delayed rectifier” potassium current, \( I_{Kr} \), that plays a key role in regulating cardiac action potential duration (APD) [30;31] (Figure 1). Candidate gene screening was performed in
three SQTS families and in two of them nucleotide substitutions were identified in KCNH2 that led to a common (asparagine to lysine) substitution (N588K) in the external S5-Pore linker region of the hERG channel [29]. A subsequent study identified the same mutation in another family [32]. Affected individuals exhibited short QT intervals, paroxysmal atrial fibrillation (AF), abbreviated atrial and ventricular refractory periods and susceptibility to arrhythmia induced by programmed electrical stimulation [29;32]. hERG channels have uniquely fast voltage-dependent inactivation that contributes normally to shaping the contribution of $I_{Kr}$ to ventricular repolarisation [33-36]. The S5-Pore linker region of hERG plays a role in hERG current ($I_{hERG}$) inactivation [37;38]. In initial biophysical characterization at ambient temperature, the N588K mutation appeared to eliminate the ability of $I_{hERG}$ to inactivate, which would increase greatly the contribution of the current early during the ventricular action potential (AP) [29]. Subsequent biophysical analyses, at both ambient and physiological temperature, demonstrated that the N588K mutation does not eliminate the inactivation process, but profoundly shifts inactivation to more positive voltages, also with a modest increase in relative Na/K ion permeability [39;40]. AP voltage clamp experiments have shown a profound increase in $I_{hERG}$ during ventricular APs and a shift in timing of peak current to earlier in the AP plateau. Current during atrial and Purkinje fibre APs has also been found to be increased for N588K $I_{hERG}$ [39-42]. Simulation studies have demonstrated a causal relationship between the mutation and AP and effective refractory period (ERP) shortening [43-45]. In 2009, a distinct C terminal hERG mutation (R1135H) was reported in a 34 year old male whose ECG showed a mixed Brugada/SQTS phenotype (QTc interval of 329 ms) [46]. His brother had a QTc of 377 ms and a non-documented arrhythmia, whilst his mother had a QTc of 379 ms and exhibited bradycardia. They also had the R1135H mutation [46]. In vitro recordings from R1135H-hERG showed slowed deactivation for this mutant. Simulations showed how this could contribute to both abbreviated repolarisation and a Brugada phenotype [47]. A third, N terminal, hERG mutation (glutamate → aspartate; E50D) was reported in 2009 in a 22 year old man who had suffered syncope whilst driving [48]. The lowest value of QTc interval duration during Holter monitoring in hospital was 366 ms and on treadmill testing poor rate adaptation of his ECG was observed [48]. Whilst detailed biophysical data on this mutation have not yet been published, a recent article refers to unpublished data suggesting that E50D $I_{hERG}$ density is increased compared to that
of the WT channel and that deactivation is slowed and inactivation modestly positive-shifted [49].

The T618I-hERG, mutation, which occurs at a highly conserved site in the pore-loop of the hERG channel, was originally found in a male whose father and 2 sisters had been victims of sudden death [50]. He had a QTc interval of 298 ms, reduced rate-adaptation of the QT interval and a markedly shortened ERP, all in the absence of structural abnormalities of the heart. Programmed electrical stimulation induced VT/VF [50]. Affected offspring also had QTc intervals of <320 ms. This mutation has now been identified in 7 unrelated, geographically dispersed (Europe, USA, Canada, China, Japan) families [49], with a mean QTc interval for probands and other carriers of 313 ms, poor rate adaptation of the QTc interval, tall peaked T-waves, no gender preference in terms of carriers and 100% penetrance [49]. Affected families have a high incidence of sudden death and aborted sudden death [49]. A distinct U wave has been reported to be present in precordial leads of ~70% of carriers. Interestingly, similar to N588K hERG, T618I carriers are vulnerable to VT and VF; however, in contrast to N588K hERG, no reported T618I probands or carriers have experienced AF (cf. 60% probands for N588K hERG) [49]. This mutation has been established to be the most common clinically occurring mutation of any SQTS variant (accounting for 25.9% of genotyped probands, with N588K the second most commonly occurring at 18.5% [49]). Biophysical analysis of T618I-hERG at room temperature showed a marked increase in current during depolarising voltage commands, accompanied by reduced I-V relation rectification, a negative shift in voltage-dependent activation, accelerated deactivation, a positive shift in voltage-dependent hERG inactivation, slowed development of inactivation but accelerated recovery from inactivation [50]. At 37°C, however, a +15 mV positive shift in voltage dependent activation, no change in rate of activation, but significantly accelerated deactivation, a moderate positive shift in voltage dependent inactivation and slowed rate of onset of inactivation were seen [51]. Ventricular “AP clamp” at 37 °C showed a marked positive shift in peak current during the AP and a near-doubling of peak current during repolarisation [51]. A very recent study with room temperature recordings [49] has reported a negative voltage shift in activation for T618I hERG, accelerated time-course of activation (changes not seen at 37°C [51]) and did not report data for inactivation [49]. In short, whilst
T618I is clearly a gain-of-function mutation, the precise mechanisms underlying this effect appear to differ between studies and recording conditions.

In 2015 a 64 year old man with paroxysmal AF and atrial flutter was diagnosed with SQTS (QT<sub>c</sub> interval of 319 ms and peaked T waves on the precordial leads). His father and brother had died suddenly. Genetic testing uncovered an isoleucine to threonine mutation (I560T) in the transmembrane segment of the hERG channel [52]. In vitro analysis of I560T in h<sub>ERG</sub> revealed an increase in h<sub>ERG</sub> magnitude, without changes to voltage-dependent activation, but with a modest positive shift in voltage-dependent inactivation of the current [52]. Computer simulations confirmed that these changes could result in abbreviated repolarisation [52]. In 2017, a link was established between a pore serine to alanine mutation (S631A) and SQTS [53]. The S631A mutation had been studied in vitro previously as an experimental mutation to impair hERG inactivation [54], profoundly positively-shifting the inactivation process [55] and producing an alteration to h<sub>ERG</sub> during the ventricular AP [36] akin to that seen for N588K [39;40]. This mutation has now been identified clinically for the first time, in a family with abbreviated QT intervals and history of sudden death [53]. The index patient exhibited a QT<sub>c</sub> interval of <320 ms at age 6 and 323 ms at age 18. Her younger sister had a QT<sub>c</sub> interval of 340 ms and slightly reduced ejection fraction (42%, MR tomography). Her father had a QT<sub>c</sub> of 324 ms, whilst an asymptomatic dizygotic twin brother had no QT interval shortening [53]. Both the index patient and her asymptomatic mother possessed and additional SCN10A mutation, but as this was absent from other affected individuals it was unlikely to be causative of the SQTS phenotype.

3.2 SQT2

SQT2 results from mutations to KCNQ1, which encodes the pore-forming subunit of I<sub>Ks</sub> (slow delayed rectifier) potassium channels (the KCNQ1 protein combining with KCNE1 to form functional proteins [31]) (Figure 1). The first identified form of SQT2 resulted from a mutation (V307L) in the pore-helix (P-loop) of KCNQ1, which is associated with left-ward voltage-shifted and faster time-dependent activation and slower deactivation of I<sub>Ks</sub> (KCNQ1+KCNE1 channels) [56;57]. The 70 year old male patient in whom the mutation was identified experienced aborted sudden death (VF) and abbreviated ventricular
repolarisation with a QTc interval of 302 ms [56]. Simulation data have verified a causal link between this mutation and QT interval shortening and susceptibility to ventricular arrhythmia [58;59]. A second variant was observed in utero, with bradycardia and irregular rhythm and ECG analysis showing abbreviated QT interval and episodes of AF [60]. Genetic analysis revealed a de novo mutation in the S1 transmembrane domain of the KCNQ1 protein (V141M). Biophysical analysis of this mutation revealed that the mutation induced an instantaneous component of KCNQ1+KCNE1 current that was absent in wild-type (WT) channels [60]. Simulation data confirmed that this mutation can shorten QT interval and slow pacemaker rate [60;61]. The R295H mutation was found in a 20 year old proband with a QTc interval of 310 ms who had undergone aborted cardiac arrest [62]. His mother possessed the same mutation and a QTc interval of 300 ms and had experienced paroxysmal VT. Biophysical analysis showed increased current density, faster activation kinetics and slowed deactivation kinetics for $I_{Ks}$ (KCNQ1+KCNE1) channels incorporating the R295H mutation [62]. The F279I KCNQ1 mutation was identified in the 23 year old son of a 37 year old man who had died suddenly [63]. The son’s ECG showed sinus bradycardia, prominent T waves in V2–V4 and a QTc interval of 356 ms, shortening to 350 ms under an exercise load. The F279I mutation resides in the S5 transmembrane segment of the KCNQ1 protein and resulted in KCNQ1+KCNE1 current that exhibited a negative shift in voltage dependent activation and accelerated activation kinetics and the interaction between KCNQ1 and KCNE1 was impaired for mutant channels [63]. Computer simulations demonstrated a causal relationship between the F279I mutation and accelerated repolarisation [63].

3.3 SQT3

SQT3 results from mutations to the $KCNJ2$ gene that encodes the Kir2.1 protein. Kir2.1 contributes to inwardly rectifying ($I_{K1}$) K$^+$ channels that set the resting potential in non-pacemaker cells and that contribute to action potential terminal repolarisation [31;64] (Figure 1). The first SQT3 mutation was found in an asymptomatic 5 year old girl with a markedly abbreviated QT interval and tall, asymmetric T-waves; her father had a history of lamenting tachycardia and palpitations [65]. This form of the SQT3 was linked to a mutation (D172N) in $KCNJ2$-encoded Kir2.1 [65]. The affected residue resides in the transmembrane pore of Kir2.1 and is involved in the Mg$^{2+}$ and polyamine block that underpins voltage-
dependent rectification of $I_{K1}$ [65;66]. In D172N-Kir2.1 channels, this process appears impaired, consequently leading to augmentation of outward but not inward current [65;67]. Simulations showed that the changes to $I_{K1}$ consequent to this mutation would accelerate the final stages of ventricular repolarisation and abbreviate AP duration, though to a lesser extent than the V307L KCNQ1 or N588K hERG mutations [65]. The abrupt abbreviation of the final stage of repolarisation accounted for the asymmetric T waves seen clinically [65]. The authors of the study that identified D172N-Kir2.1-linked SQT commented that “the D172N mutation creates a vulnerable substrate that may facilitate development of atrial and ventricular tachyarrhythmias even in a heterozygote substrate.” [65]. Subsequent action potential voltage-clamp experiments showed that the mutation augments Kir2.1 current during both ventricular and atrial APs [67] and simulations have shown how the mutation can produce a substrate for ventricular arrhythmia [68]. A second SQT3 KCNJ2 (M301K-Kir2.1) mutation was identified in an 8 year old girl with a markedly shortened QTc interval (194 ms), who suffered from paroxysmal AF [69]. When expressed alone, M301K channels did not pass current, but when co-expressed with WT channels (mimicking the heterozygous state of the proband), the inward rectification properties of Kir2.1 were markedly impaired, leading to significantly greater current over physiological repolarisation voltages [69]. A third SQT3 mutation to KCNJ2 (E299V-Kir2.1 reported in 2013) results in a more profound lack of inward rectification of Kir2.1 current compared to the M301K-Kir2.1 mutation [70] and simulations showed it to produce a much more marked AP abbreviation compared to the D172N mutation [70].

3.4 SQT4 and SQT5

SQT4 and SQT5 involve mutations to subunits that comprise channels mediating L-type Ca$^{2+}$ current ($I_{Ca,L}$) (Figure 1). 82 probands with Brugada syndrome were screened for ion channel mutations and 7 were identified with mutations to the $\alpha$ and $\beta_{2b}$ subunits of L-type channels; of these 7, 3 exhibited QTc intervals of 360 ms or less [71]. The first proband was a 25 year old male who presented with aborted sudden cardiac death. He had a QTc interval of 330 ms and coved ST-segment elevation in V$_1$ and V$_2$ ECG leads. A total of 6 of 10 family members showed ST-elevation and somewhat abbreviated QTc intervals [71]. He (and other
phenotype-positive family members) showed a \textit{CACNB2b} mutation absent in 400 ethnically matched control alleles, that led to a S481L substitution in the CACNB2b protein [71]. The second proband was a 41 year old male who presented with AF and a QT$_c$ interval of 346 ms. His brother had died of sudden cardiac arrest at 45 yrs. of age [71]. The QT interval showed poor rate dependence and ST segment elevation in ECG leads V$_1$ and V$_2$ was enhanced with ajmaline. There was no structural heart disease but monomorphic VT could be elicited by programmed electrical stimulation. He had a mutation of \textit{CACNA1C}, which led to a G490R substitution in the Ca$_{v1.2}$ protein, but absent in 640 ethnically matched control alleles. His 2 daughters also possessed the mutation and shortened QT$_c$ intervals. He possessed additional polymorphisms (P1280L and V1821M) that were found in healthy controls and so unlikely to be causally linked with his pathology [71]. The third proband was a 44 year old male with prominent ST elevation in V$_1$ and saddleback ST elevation in V$_2$ and a QT$_c$ interval of 360 ms. His mother had died suddenly at 48. He had a \textit{CACNA1C} mutation that led to an A39V mutation in Ca$_{v1.2}$. In biophysical experiments, all three mutations led to marked reductions in $I_{Ca,L}$. The loss of current with the A39V mutation (but not the other two) was associated with a reduction of surface expression, consistent with a trafficking defect [71]. All three mutations were thus loss of function mutations that led to a mixed Brugada/SQTS phenotype. In a more recent study a distinct \textit{CACNA1C} mutation (leading to a R1937P mutation in Ca$_{v1.2}$) has been identified in a 52 year old male with an early-repolarisation pattern ECG and QT$_c$ of 356 ms [72;73]. He suffered from atrioventricular block and severe left ventricular hypertrophy and dysfunction and possessed 2 additional, distinct mutations (E234K in desmin, \textit{DES}; R989H in myopallidin, \textit{MYPN}) that also likely contributed to his overall pathology. His daughter also possessed all mutations, though with a milder phenotype. In functional analysis, R1937P channels showed a marked loss-of-function [72].

3.5 SQT6

SQT6 was identified from a 17 year old female who suddenly lost consciousness in church. Ventricular fibrillation was terminated by defibrillation. In hospital, her ECG was found to exhibit a short QT$_c$ interval (329 ms) and tall, narrow T waves [74]. Programmed electrical stimulation could elicit AF and VT. Genetic screening revealed a S755T substitution in the
CACNA2D1 encoded Ca\textsubscript{v}\textsubscript{1.2}\textdelta\textsubscript{1} subunit of the L-type Ca\textsuperscript{2+} channel. Coexpression of the mutant Ca\textsubscript{v}\textsubscript{1.2}\textdelta\textsubscript{1} subunit with Ca\textsubscript{v}1.2\textalpha1 and Ca\textsubscript{v}\textbeta2b led to reduced \textit{I}_{Ca,L} (using Ba\textsuperscript{2+} ions as charge carrier) compared to the WT control, without an obvious effect on surface expression, suggestive of a modification of single channel properties by the S755T Ca\textsubscript{v}\textsubscript{1.2}\textdelta\textsubscript{1} [74].

### 3.6 SQT7

In 2012, a novel mixed SQT/Brugada phenotype was identified that is caused by a missense mutation to the \textit{SCN5A} gene, which encodes the \textalpha\ subunit of channels carrying cardiac sodium current, \textit{I}_{Na} [75] (Figure 1). A 40 year old man was admitted to hospital for a non-cardiac injury and was found to have a Brugada-like ECG, accompanied by a short QT interval (QT of 320 ms at 71 beats min\textsuperscript{-1}). His father had died suddenly at age 39. An R689H mutation was identified in \textit{SCN5A}; biophysical analysis showed that \textit{SCN5A} protein incorporating the R689H mutation was unable to mediate \textit{I}_{Na}, indicating loss of function [75]. As \textit{I}_{Na} mediates the ventricular action potential upstroke and can influence APD via a late current component, the loss of function associated with R689H could affect both conduction and repolarisation. The authors of this study discussed the fact that the R689H mutation had previously been associated with a \textit{long} QT phenotype and noted the potential for some genetic defects to have different phenotypic manifestations [75]. A subsequent experimental study reported the R689H mutation to increase late \textit{I}_{Na} [76], which raises a question as to whether or not the mutation is able on its own to account for a SQT phenotype.

### 3.7 SQT8

Candidate ion channel screening has resulted in positive genotyping in <30% of SQTS cases [9]. Exome or genome sequencing can thus be predicted to uncover novel, unexpected genetic associations with SQTS. This is well-illustrated by the discovery in 2017 of a new SQTS variant, found in 2 unrelated families that possess mutations in the anion exchanger (AE3) gene \textit{SLC4A3} [77]. The index patient from the first family presented at 31 years of age
with cardiac arrest during sleep followed by an episode of VF in hospital. He had a QTc interval duration of 320 ms in the absence of structural heart disease. Four relatives had experienced episodes of syncope in their second decade; two had died suddenly at 41 and 42 years of age [77]. In the second family, the proband died at the age of 22 at rest. His brother had a structurally normal heart, but a QTc interval of 320 ms. Two further relatives had short QTc intervals [77]. Normal gene panel screening revealed no mutations in candidate ion channels. However, exome screening of six SQTS individuals and 5 healthy controls revealed a missense variant in SLC4A3, leading to the R370H mutation in a conserved motif of the SLC4 family [77]. Cascade screening identified other carriers of the mutation who had a mean QTc lower than non-carriers [77]. Expression studies of recombinant wild-type and mutant AE3 showed the mutant form to have reduced surface localization, suggestive of impaired trafficking. HCO₃⁻ transport was impaired in cells expressing the mutant form of AE3. Knockdown of slc4a3 in zebrafish embryos replicated QTc shortening and also resulted in raised intracellular pH in zebrafish embryo hearts [77]. Finally, intracellular alkalization and reduced intracellular chloride concentration ([Cl⁻]ᵢ) abbreviated repolarisation in rabbit hearts [77]. Collectively, these observations suggest that intracellular alkalization and reduced [Cl⁻] as a result of the R370H AE3 mutation contribute to accelerated repolarisation in this form of SQTS [77]. The processes affected by these changes to effect faster repolarisation remain to be elucidated.

Table 1 summarises known mutations in SQT1-SQT8.

4. Arrhythmia mechanisms

There are no genotypically accurate mammalian models of the SQTS. Information on underlying arrhythmia mechanisms in the syndrome has been gleaned from studies using in vitro preparations and potassium channel activators and also from computer modelling based on changes to ion channel properties seen in recombinant channel experiments. The K_ATP channel activator pinacidil produces a short QT phenotype when applied to canine left ventricular wedge preparations or to intact rabbit hearts [78-80]. Pinacidil was seen to produce heterogeneous APD abbreviation across the canine left ventricular wall and thus augment transmural dispersion of repolarisation (TDR) and increase susceptibility to
provoked polymorphic VT [78]. In intact rabbit hearts, pinacidil abbreviated QT interval and ERP, which was associated with increased susceptibility to VF [79;80]. Application of the hERG/\(I_{Kr}\) activator PD118057 to canine left ventricular wedge preparations to mimic SQT1 abbreviated QT interval and ERP and augmented TDR and arrhythmia susceptibility [81]. Enhanced TDR has been seen in SQTS patients [82]. Multilevel modelling of N588K-linked SQT1 has shown reductions in APD and ERP, with localised increases in maximal ventricular transmural voltage heterogeneity (\(\delta V\)) in the SQT1 setting, increasing vulnerability to unidirectional conduction block [45]. A reduced ventricular substrate size needed to sustain re-entry facilitated spiral and scroll wave lifespan/stability in 2D and 3D simulations [45]. Parallel observations have been made in simulations of SQT2 [58;59]. Biophysical modelling has also confirmed the ventricular proarrhythmic nature of \(KCNJ2\) SQTS mutations [65;68;70]. For example, D172N Kir2.1 leads to abbreviated APD and ERP and to steeper restitution curves for these parameters [68]. The D172N mutation reduces tissue excitability at slow rates but increases it at higher ones, also increasing temporal vulnerability to initiation of re-entry whilst reducing the substrate size required to maintain re-entry [45].

Heterogeneous effects of SQT mutations on the magnitude of repolarising current during ventricular and Purkinje fibre APs have been observed and the possibility raised that these may contribute to the pronounced U waves reported in some SQTS patients [39;41]. However, it is very probable that altered electromechanical coupling is likely to be of principal importance in this regard: echocardiographic analysis has revealed a dissociation between the end of mechanical systole and ventricular repolarisation in SQTS patients [83]. In SQTS the U wave was found to coincide with the end of mechanical systole and beginning of isovolumetric relaxation [83]. The corresponding electrical changes that contribute to the U wave were not established, but may involve delayed after-depolarisations or early phase 3 afterdepolarisations [84]. A combination of Doppler imaging and speckle-tracking echocardiography has revealed a modest decrease in left ventricular contraction and increased mechanical dispersion in SQTS patients [85;86]. \textit{In silico} simulations predict a reduced systolic \(Ca^{2+}\) transient and contraction in models incorporating SQT mutant \(K^+\) channels [87;88] and, consistent with this, a modest reduction in ventricular myocyte...
shortening accompanies AP abbreviation with the hERG/I\(_{Kr}\) activator PD118057 to mimic SQTS (Figure 2A).

A proportion of SQTS patients exhibit AF [22;23;52]: approximately 63% of SQT2 patients and 21% of non-SQT2 patients [52]. Experiments on a perfused canine atrial preparation, using PD118057 to mimic a SQT phenotype have shown AP and ERP abbreviation and an increase in spatial dispersion of repolarisation, which collectively increased susceptibility to AF induced by premature stimulation [89]. \textit{In silico} investigation of the SQT1 N588K mutation found this to decrease ERP and re-entry wavelength (WL) [90]. Our own simulations of the D172N and E299V SQT3 Kir2.1 mutations found both to decrease re-entry WL through reducing ERP and conduction velocity [91]. However, the two mutations, which produce qualitatively different effects on \(I_{K1}\) differentially affected spatial dispersion of APD (with D172N producing increased spatial heterogeneities in some regions and E299V reducing global dispersion of repolarisation), with consequences for stability of re-entry in the two situations (D172N resulting in greater re-entry stability) [91].

Harrell and colleagues investigated genotype-specific characteristics amongst 65 genotyped individuals [52]. The mean age of manifestation appears to be significantly later for SQT1 patients (35 yrs) than for SQT2 (17 yrs) and SQT3-6 (19 yrs), whilst QT\(_{c}\) intervals are comparable. In this study, non-genotyped patients had a mean age of manifestation of 28 yrs. Sick sinus syndrome/bradyarrhythmia appears particularly prominent in SQT2 (75% of SQT2 versus 9% non-SQT2 patients [52]) and this may involve \(I_{Ks}\) with KCNQ1 mutations (particularly V141M) stabilising membrane potential/resisting diastolic depolarisation in sinus node cells ([60], Whittaker \textit{et al}, unpublished). Aside from these features and the greater proportion of SQT2 patients with AF, other clinical characteristics were not seen to differ between genotypes [52]. Thirteen families with SQT1-3 showed a penetrance of 90% compared to 58% for SQT4-6 [52]. The N588K and T618I hERG SQT1 mutations have been reported to exhibit 100% penetrance [49].

General arrhythmia mechanisms in SQTS are summarised in Figure 2B.
5. Therapeutic strategies and options

The SQTS carries a high risk of sudden death, with a >40% probability of a cardiac arrest by the age of 41 years, of 4% during the first year of life alone and of 1.3% per year between 20 and 40 [92]. Documented cardiac arrest events appear to be particularly common at rest, during sleep or undemanding routine activities [92]. Unsurprisingly, therefore, the primary treatment for SQTS patients is the use of implantable defibrillator devices (ICDs; [8;23;93;94]). The changes in T wave morphology in the SQTS can pose a challenge to ICD use, however, in that there is a recognised risk of inappropriate shocks due to T wave oversensing in SQTS patients [8;23;93;95]. Moreover, the incidence of inappropriate ICD shocks has been observed to be significantly higher in paediatric SQTS patients than in an adult cohort [24], indicating a particular challenge in the use of ICD devices in young patients. Whilst such issues can be addressed through ICD reprogramming [93], ICDs do not normalize repolarisation or the arrhythmogenic substrate(s) per se and so there is a strong case for the use of adjunct pharmacological approaches.

5.1 $I_{Kr}$ blockade

$I_{Kr}$ block is predominantly responsible for repolarisation delaying actions of many class Ia and III antiarrhythmic drugs and underpins entirely the actions of methanesulphonanilide class III drugs, including dofetilide and sotalol [96;97]. In the initial study that identified N588K-hERG linked SQT1, sotalol was administered, but did not restore the QT_c interval towards normal [29]. A number of antiarrhythmic drugs were tested in a subsequent study (sotalol, ibutilide, flecainide, hydroquinidine) but only hydroquinidine was found to be successful at prolonging QT_c interval, ventricular ERP and protecting against VF [32]. The reason for the difference in effectiveness between sotalol and quinidine could in principle involve effects of quinidine on additional targets than $I_{Kr}$ and/or differences in effects of the N588K mutation on HERG/$I_{Kr}$ sensitivity to the drug: evidence in support of the latter comes from the observation that the IC$_{50}$ for $h_{HERG}$ block by sotalol was increased by ~20-fold compared to ~5.8 fold for quinidine [98]. The basis for this difference is likely to reside in the inactivation-dependency of block by the two compounds: hERG block by methanesulphonanilide class III agents is known to depend strongly, directly or indirectly, on
hERG channel inactivation gating [99-102]. Quinidine is much less dependent on channel inactivation for binding to hERG to occur [99;103]. Consistent with this, the class Ia drug disopyramide is also comparatively less dependent on inactivation to bind to hERG [104] and when tested against N588K-hERG showed an IC$_{50}$ only 1.5-fold that of the WT channel [105]. In direct comparison, the methanesulphonanilide E-4031 showed an IC$_{50}$ against N588K-hERG 11.5-fold that of the WT channel [105]. Like quinidine, disopyramide has been found to exert beneficial effects in SQT1 patients on QT$_c$ interval, its rate dependence and on ventricular ERP [106]. It has also been found to be effective in a patient with SQTS of unknown genotype [107]. In the ventricular wedge model of SQT1 induced by $I_{Kr}$ activation with PD118057, quinidine was found to prolong QT interval and ERP without altering TDR [81]. Originally, it was proposed that the additional $I_{Ks}$ blocking action of quinidine might contribute to its efficacy in SQT1 [29;108]. Recent computational analysis of actions of both quinidine and disopyramide on human ventricular electrophysiology in the setting of N588K-linked SQT1, incorporating their known actions and kinetics on $I_{Kr}$ and $I_{Na}$, as well as effects on other known targets, has demonstrated that beneficial effects on the QT interval are mostly due to their $I_{Kr}$ blocking effect, whilst prolongation of the ERP derives from a combination of effects on $I_{Kr}$ and $I_{Na}$ [109]. Thus, for the most severe SQT1 phenotypes (which involve the greatest impairment to hERG/$I_{Kr}$ channel inactivation) $I_{Kr}$ inhibitors that do not depend strongly on the process of channel inactivation for binding should, in principle, be effective, particularly when combined with a class Ia type antiarrhythmic action. Consistent with this, in a canine right atrial experimental model of SQT1, combined $I_{Kr}$ and $I_{Na}$ block but neither action alone was effective at preventing AF [89].

The likely effectiveness of $I_{Kr}$ block in other types of SQTS can be inferred from the important role $I_{Kr}$ normally plays in repolarisation and the fact that the kinetics of $I_{Kr}$, and hence drug binding, should be intact in non-SQT1 forms of the syndrome. Consistent with this, drugs with $I_{Kr}$-blocking properties have been reported to be effective in pinacidil-induced SQTS models [78-80] and in patients with non-SQT1 forms of the syndrome (e.g. [22;71]). Indeed, comparison of SQT1 and non-SQT1 forms of the syndrome suggests efficacy of quinidine in both settings, but with greater effects in SQT1 patients [22]. Our simulation work on SQT3 suggests that $I_{Kr}$ block is likely to be beneficial against both D172N and E299V
Kir2.1 mutations when combined with inhibition of the ultrarapid $I_k$ current, $I_{kur}$, and also on its own against E299V [91].

One cautionary note in respect of $I_{hERG}/I_{kr}$ block is that whilst *in vitro* data on T618I hERG have pointed towards effectiveness of a range of drugs with $I_{kr}$ inhibitory effects including both sotalol and quindine [50;51], these *in vitro* predictions have not translated universally to patients. Thus, sotalol has been tried but found to be ineffective at prolonging QTc interval in T618I carriers [49;110]. Quinidine has been found to prolong QTc interval but not to prevent arrhythmias in all T618I patients receiving it [49]. Bepridil, which has not been tested *in vitro* against T618I hERG, has been found to be effective in a T618I patient with VF refractory to other treatment [49]. Further work is required to understand the deviation between experimental and *in vivo* pharmacology in respect of T618I hERG SQT1 carriers.

### 5.2 $I_{ks}$ blockade

In principle, $I_{ks}$ inhibition could have value in the treatment of both SQT2 and non-SQT2 SQTS variants. The V307 residue lies in a region of the KCNQ1 protein that has been identified to be the interaction site for canonical (chromanol-based) $I_{ks}$ inhibitors, and the V307L SQT2 mutation significantly reduces potency of inhibition by chromanol 293B [111]. By contrast, mefloquine, which may not actually require channel gating for binding to occur [112], was found to be effective at inhibiting recombinant $I_{ks}$ channels incorporating the V307L mutation [57]. By contrast with V307L, the V141M mutation has been reported to show increased sensitivity to the $I_{ks}$ inhibitor HMR-1556, as has a proximate heritable AF gain-of-function mutation S140G [113]. In recent *in silico* simulations, modelling ~60% inhibition of $I_{ks}$ was required to normalize repolarisation in the setting of heterozygous V307L mutation, whilst in 3D ventricle simulations 58% $I_{ks}$ inhibition was able to normalize the lifespan of re-entry in the heterozygous V307L condition to that in the WT condition [59]. Taking together the available information, $I_{ks}$ inhibition is predicted to be an effective strategy in SQT2, with the efficacy in practice depending on the location of SQT2 mutations relative to drug binding site(s). The overall importance of $I_{ks}$ to repolarisation reserve [114] might suggest a role for $I_{ks}$ block in other SQTS variants, though the degree to which $I_{ks}$ is recruited during abbreviated APs might be reduced and hence provide reduced potential for
AP prolongation. At present, selective $I_{KS}$ inhibition remains a theoretical treatment possibility, as there are no selective $I_{KS}$ inhibitors in clinical use.

### 5.3 $I_{to}$ Blockade

For SQTS variants with a mixed SQTS/Brugada phenotype, selective $I_{to}$ (transient outward potassium current) block could be useful in correcting early repolarisation [28]. Selective $I_{to}$ inhibitors are not clinically available, though quinidine inhibits $I_{to}$ as well as $I_{Kr}$ and $I_{Na}$ [31] and was successful in treating an SQT4 patient [71]. Vernakalant, which combines $I_{to}$ and $I_{Na}$ block with actions on $I_{Kr}$ and $I_{Kur}$ has been found to suppress VF in a pinacidil model of SQTS [79].

### 5.4 $I_{K1}$ Blockade

Unlike $I_{Kr}$ and $I_{KS}$ channels, those mediating $I_{K1}$ normally carry little current over the ventricular AP plateau phase, with the principal repolarisation contribution of $I_{K1}$ being during terminal repolarisation [31;115]. It is possible, therefore, that $I_{K1}$ block might not be an optimal target for APD/QT interval prolongation in non-SQT3 forms of the SQTS. For SQT3, on the other hand, $I_{K1}$ could represent a genotype-specific target. There are no selective $I_{K1}$ inhibitors in clinical use, but the antimalarial drug chloroquine has been found to be an effective inhibitor of D172N Kir2.1 and to normalise ventricular repolarisation and to prolong ventricular ERP in silico in the D172N-linked SQT3 variant [67;116;117]. Additional inhibitory effects on $I_{Kr}$ may supplement the drug’s actions on $I_{K1}$ [116;117]. Chloroquine binds in the cytoplasmic pore of Kir2.1 [118] and the M301A mutation was not found to impair chloroquine block and so it is likely that M301K mutant channels would retain drug sensitivity [118]. On the other hand, the E299A mutation significantly impaired chloroquine block [118], so E299V channels might not retain WT channel sensitivity to chloroquine. Recently, a pentamidine analogue, PA-6, has been identified that inhibits Kir2.1 selectively at submicromolar concentrations [119]. It has been shown also to be able to inhibit D172N Kir2.1, albeit with a modest reduction in potency [120]. PA-6 exerts an agonist effect on Kir2.1 protein expression, but this action is likely to be outweighed by its
acute channel blocking effect [120]. The encouraging in vitro results with PA-6 point towards the potential for selective $I_{K1}$ inhibitors in SQT3. Our atrial electrophysiology in silico modelling results with SQT3 mutants found a 50% reduction in $I_{K1}$ to be insufficient to terminate re-entry for D172N conditions, but this was sufficient to prevent sustained re-entry for E299V conditions [91]. For both mutants, the simulated combination of $I_{K1} + I_{Kr}$ was effective at terminating re-entry, indicating synergistic effects of combining the two actions [91].

5.5 L-type Ca channel activation

In simulations of atrial effects of SQT3 mutations, increasing $I_{Ca,L}$ by 100% decreased the dominant frequency of atrial re-entrant excitation but failed to terminate re-entry. An increase to 250% was sufficient to terminate atrial re-entry [91]. However, neither atrial electromechanical coupling nor ventricular consequences of such a large increase in $I_{Ca,L}$ were considered. In principle, abbreviated repolarisation in SQT4-6, involving reductions to $I_{Ca,L}$ could be rectified by pharmacological augmentation of $I_{Ca,L}$, but (i) selective agonists of $I_{Ca,L}$ are not available clinically, (ii) augmentation of $I_{Ca,L}$ beyond required levels may be proarrhythmic, and (iii) $I_{Ca,L}$ agonism may also produce extracardiac side effects.

5.6 AE3 activation

The recent discovery of impaired AE3 function in SQT8 highlights a potential new target for treatment, at least in this variant of the SQTS [77]. However, there is no current pharmacological approach that could restore normal function of mutant AE3. AE3$^{-/-}$ knockout mice have been reported to have similar cardiac function to WT controls at baseline [121;122] and, whilst steady-state pH$_i$ was reported to be similar between WT and AE3$^{-/-}$ cardiomyocytes, the knockout myocytes were slower to recover from induced alkalosis [122]. Repolarisation mechanisms differ markedly between mouse and human, however, making mice unsuitable to explore AE3 in the context of SQTS. Given the promising initial results using zebrafish embryos [77] and the comparatively human-like ventricular action potentials of adult zebrafish [123], this species would likely be of
significant value to explore the consequences of mutant AE3 in SQT8. There is potential utility in further research to establish the downstream targets that mediate delayed repolarisation in this setting, as this may identify alternative intervention points.

6. Expert Opinion

The existence, at the time of writing, of eight distinct SQTS genotypes firmly establishes the SQTS as a primary genetic syndrome. The relatively low proportion (approximately 1 in 4 tested [124]) of successfully genotyped cases makes it important to differentiate clearly congenital from acquired causes of the syndrome and that any potential acquired causes (including hypercalcaemia, cardiac glycoside, anabolic steroid use [11-17]) are eliminated from the picture (and treated, where necessary) during diagnosis. SQTS as a consequence of carnitine deficiency has been associated with mutations to the SLC22A5 gene that encodes the OCTN2 carnitine transporter [17] and so, arguably, SQTS could be considered to be secondary to SLC22A5 mutations in such cases. Mice with induced carnitine deficiency showed both structural remodelling and abbreviated ventricular repolarisation, substantiating a causal link [17]. Where carnitine deficiency associated SQTS is observed dietary carnitine supplementation may be beneficial [17]. The poor rate adaptation of the QT interval in congenital SQTS patients makes cases likely to be more apparent on ECGs at low/resting heart rates [124] and the presence of mixed Brugada-SQTS phenotypes in some SQT variants, together with evidence for a high prevalence (~65%) of early-repolarisation in SQTS patients [125], means that in some instances SQTS may exist as an ‘overlap’ syndrome. Given the potential for differences in calculated QTc interval to arise dependent on the rate correction method used [21], and as highlighted by the authors of [21], to mitigate such issues it is important that ECG measurements are repeated in individuals with suspected SQTS at rates as close to 60 beats min$^{-1}$ as possible.

On the basis of the in vitro and in silico evidence considered above, at least in respect of K$^+$ channel-linked variants, genotype-specific pharmacology to restore normal repolarisation is possible. In practice, however, this is limited by the fact that entirely selective inhibitors of $I_{Ks}$ and $I_{K1}$ are not yet clinically available and that for SQT1 variants with severe inactivation-lesions, purely $I_{Kr}$-selective compounds (methanesulphonanilides) may not be effective.
Pharmacological strategies selectively to increase $I_{Ca,L}$ are not clinically available and are potentially fraught with issues of extra-cardiac side effects and, potentially, of proarrrhythmia. Further work is probably warranted to determine whether atrial-selective strategies, such as $I_{Kur}$ inhibition [126], might be viable against paroxysmal AF in different SQTS variants. Whilst, as considered in section 4, there is some evidence for modest contractile deficiency in some SQTS patients, as this is likely to be secondary to the abbreviated repolarisation (except in SQT4-6, in which $I_{Ca,L}$ is directly affected), correcting abnormal repolarisation might be expected to reverse such deficits.

Although the orientation of this review is target- rather than drug- based, it is inescapable that the single agent that has showed most effectiveness in the treatment of the SQTS is (hydro)quinidine. Recent cohort data have demonstrated a high level of effectiveness of hydroquinidine in preventing life-threatening proarrrhythmic events during long term follow up of SQTS patients [127]. As noted in section 5.1, our simulation work on the class 1a agents quinidine and disopyramide, incorporating detailed binding kinetics of both drugs and supplementary effects against multiple targets, highlights dominant roles for both $I_{Kr}$ and $I_{Na}$ inhibition in helping restore repolarisation and refractoriness [109]. Although it is less potent against $I_{Kr}$/hERG than is quinidine [103;105], disopyramide may offer an alternative where quinidine’s gastrointestinal side effects provide problematic, and/or in locations where quinidine has been withdrawn [127;128]. Simplified simulations that do not incorporate detailed binding kinetics may not accurately reproduce clinical efficacy of such drugs [129]. We suggest that systematic in silico investigation of other SQTS variants would be beneficial incorporating both drug potency and the kind of binding kinetics that we have in SQT1 simulations [109], in order to establish clearly the target qualities that maximise effectiveness (and indeed limitations) of combined $I_{Kr}$ and $I_{Na}$ block in different SQTS variants. $I_{Ks}$ and/or $I_{K1}$ inhibition at different potencies could then be systematically incorporated to establish clearly any additional benefit from synergistic $I_{Kr}$ and $I_{Ks}/I_{K1}$ block.

It should be noted that other concomitant pharmacological actions have the potential to mitigate QT prolonging effects. For example, propafenone is at least as potent against $I_{Kr}$/hERG as is quinidine and largely retains efficacy against the N588K SQT1 mutation [103;130]. However, it was found to be effective at treating AF in this SQT1 variant without normalizing the QT interval [32]. One may speculate that the known $I_{Ca,L}$ inhibitory action of
propafenone [131;132] may have offset its \( I_{Kr}/\text{hERG} \) block, whilst its slow Na channel disassociation kinetics (as a class Ic agent; [96]) would nevertheless have prolonged post-repolarisation refactoriness, extended ERP and the wavelength for re-entry. Amiodarone, which affects multiple cellular targets [133], has been used with mixed success in SQTS patients [22]. Amiodarone was reported not to prolong the QT interval in a patient with a hERG mutation [22] but was effective combined with \( \beta \)-blockade in protecting against malignant arrhythmia in a patient with SQTS of unknown genotype [134] and \textit{in silico} studies have suggested potential effectiveness against some SQT2 and 3 mutations [135;136]. Further work is required to understand circumstances in which amiodarone may be of value in treating SQTS.

Perhaps one of the most intriguing questions in respect of the SQTS is what underlies the syndrome in the substantial proportion of patients who have unsuccessfully undergone targeted genotyping? The success of exome sequencing in identifying SQT8 [77] highlights (i) the value of exome (and potentially genome) sequencing in identifying novel causes of the syndrome (and thereby potentially novel therapeutic intervention points) and (ii) that new molecular culprits are likely to be surprising and upstream of electrogenic processes. Ultimately, the best treatments of SQTS, as for other heritable disorders, are likely to involve molecular genetic approaches to correct the underlying mutations. However, such approaches are unlikely to be available in the short- to medium- term, making ICD use together with judiciously chosen pharmacology the approaches of choice for the condition.

Finally, in addition to the peer-reviewed literature discussed in this article, available online resources on the SQTS include [137-139].

\textbf{Acknowledgements.}

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References


**Paper that suggests idiopathic short QT may be new clinical condition.**


**A key publication in the consideration of diagnostic criteria for SQTS.**


- A key overview of long-term clinical characteristics in the SQTS.


25
- An important overview of long-term clinical characteristics children in the SQTS.


- Contains useful working definition/criteria for SQTS diagnosis


- Presents the first genotyped variant (SQT1) of SQTS.


- Useful insight into N588K-hERG linked SQT1.


- Detailed biophysical analysis of N588K-hERG SQT1 mutation.


- Detailed biophysical analysis of N588K-hERG SQT1 mutation.


- Demonstration of heterogeneity of response in N588K-hERG linked SQT1 with different regional action potential configurations.


Multilevel modelling study providing insight into arrhythmia substrate in SQT1.


Identifies R1135H-hERG as potential mediator of SQT1.


Shows that R1135H-hERG can produce Brugada as well as SQTS phenotype.


Reports that E50D-hERG can produce SQTS phenotype.


Contains valuable information on prevalence of different mutations in SQTS and highlights T618I-hERG as a hotspot mutation.


First report implicating T618I-hERG in the SQTS.

• Suggests potentially effective pharmacology against T618I hERG mutation.


• Important in presenting genotype specific information on SQTS variants. Also presents I560T-hERG mutation for the first time.


• Implicates S631A-hERG, a mutation hitherto considered to be human-made for structure-function studies, in SQT1 variant of SQTS.


• Important in presenting first SQT2 (V307L-KCNQ1) variants.


• In vitro demonstration of pharmacological sensitivity to inhibition of SQT2 V307L mutation.


• In silico demonstration of arrhythmia substrates in SQT2 with the V307L mutation and explores I_{Ks} as pharmacological target.

**First report of V141M mutation in SQT2.**


**First report implicating R295H-KCNQ1 in the SQTS.**


**First report implicating F279I-KCNQ1 in the SQTS.**


**First study to report Kir2.1 mutation linked SQT3 variant**


**Demonstrated in vitro efficacy of Kir2.1 block in D172N-Kir2.1 linked SQ**


• Identified M301K mutation in SQT3


• Identified E299V mutation in SQT3


• First study to show loss of function mutations in SQTS (SQT4-SQT5) and that these result in mixed SQTS Brugada phenotype.


• Presents overlapping SQT4 and early repolarisation phenotype


• First report of SQT6


• First report of SQT7 and showed overlapping SQTS/Brugada phenotype


- Important demonstration of role for non-electrogenic process in SQTS and demonstrates power of exome/genome sequencing in identifying new culprits in the SQTS


KATP channel activation as an experimental model for probing arrhythmia substrates in SQTS.


IKr/hERG activation as an experimental model for probing arrhythmia substrates in SQT1.


Evidence for modestly impaired contractile behaviour in SQTS.
Frea S, Pidello S, Giustetto C, Scrocco C, Gaitan F: Author's Reply to "Altered In Vivo Systolic Function In the Short Qt Syndrome Anticipated In Silico". Heart Rhythm 30-6-2015. 12(9):e115-6


**In silico demonstration that SQTS can in principle impair contractile function.**


**In vitro examination of atrial arrhythmia substrate in SQT1**


**Multilevel modelling examination of atrial arrhythmia substrates in SQT3. Also explores I_{K1} inhibition, on its own and with additional targets, as potential therapeutic strategy**


**Highlights the lethality of the SQTS**


• Correlates relative effectiveness of sotalol and quinidine in SQT1 patients with in vitro sensitivity of SQT1 channels to block


• In vitro demonstration of effectiveness of disopyramide in SQT1

**First patient demonstration of effectiveness of disopyramide in SQT1**


**Important patient study of antiarrhythmic pharmacology in SQTs**


**In silico study that provides detailed mechanistic insight into combined I_{Kr} and I_{Na} inhibition effectiveness**


**Patient study that provides counterpoint to in vitro data suggesting effectiveness of sotalol with this SQT mutation.**


**In vitro proof of concept for effectiveness of selective I_{ks} inhibition against gain of function Kir2.1 mutant channels.**


- **In vitro and in silico proof of concept for effectiveness of I\(_K1\) inhibition against SQT3 mutant Kir2.1 channels.**


- **In vitro proof of concept for effectiveness of a selective I\(_K1\) inhibitor against gain of function Kir2.1 mutant channels.**


Sowah D, Brown BF, Quon A, Alvarez BV, Casey JR: Resistance to cardiomyocyte hypertrophy in ae3-/- mice, deficient in the AE3 Cl-/. BMC Cardiovasc Disord 21-7-2014;14:89.


Hancox JC, Mitcheson JS: Inhibition of L-type calcium current by propafenone in single myocytes isolated from the rabbit atrioventricular node. Br J Pharmacol 1997;121:7-14.


http://www.shortqtsyndrome.org (Accessed 13 April 2018)
<table>
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<tr>
<th>SQT Variant</th>
<th>Gene/gene product</th>
<th>Channel (subunit)</th>
<th>Mutation (amino-acid change)</th>
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**Table 1.** List of known SQTS mutations. Effects of amino acid changes on protein function are detailed in the main text of section 3. ?? for SQT7 indicates conflicting evidence for gain- or loss- of function consequence of the mutation.
Figure Legends

Figure 1

Schematic diagram of cardiac ventricular action potential (AP) and the normal profiles of depolarising (inward) and repolarising (outward) currents (not to scale) that are known to be affected by SQT1-SQT7 genotyped variants of the SQTS. The affected gene is shown in italics to the right of the respective ionic current. Table 1 contains a list of known SQTS mutations to these genes.

Figure 2

(A) Upper traces show guinea-pig ventricular myocyte APs recorded at 37°C (at a stimulation frequency of 1Hz) in a standard physiological control solution and in the presence of the hERG/\(I_{Kr}\) activator PD118057, to abbreviate repolarisation and hence mimic SQTS. Lower traces show records of unloaded cell shortening (contraction) for the two APs, illustrating decreased contraction amplitude in the presence of PD118057.

(B) A summary of arrhythmia mechanisms in the SQTS. All variants of the SQTS shorten the action potential duration (APD) and effective refractory period (ERP) - SQT1-3 through increased outward current, SQT4-7 through decreased inward current, and SQT8 through mechanisms that remain to be elucidated. These result in shortening of the QT interval. Heterogeneous APD shortening is a mechanism for increased transmural dispersion of repolarisation (TDR) which increases T wave amplitude. Increased TDR also increases dispersion of refractoriness, which increases susceptibility to unidirectional conduction block - a mechanism for initiation of re-entry. Furthermore, shortened APD reduces the excitation wavelength (WL) in tissue, which facilitates sustenance of re-entry (by reducing the substrate size necessary to sustain re-entry).
Figure 1

Ventricular AP

Current

Gene affected (SQTS variant)

Outward currents

\( I_{Kr} \) \( KCNH2 \) (SQT1)

\( I_{Ks} \) \( KCNQ1 \) (SQT2)

\( I_{K1} \) \( KCNJ2 \) (SQT3)

Inward currents

\( I_{Ca,L} \) \( CACNA1C \) (SQT4)

\( I_{Ca,L} \) \( CACNB2b \) (SQT5)

\( I_{Na} \) \( CACNA2D1 \) (SQT6)

\( I_{Na} \) \( SCN5A \) (SQT7)
Figure 2

A

![Voltage and Cell Shortening Graph](image)

B

<table>
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<tr>
<th>SQT1-3</th>
<th>SQT4-7</th>
<th>SQT8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased outward current</td>
<td>Decreased inward current</td>
<td>???</td>
</tr>
</tbody>
</table>

- **SQT1-3**
  - Increased outward current
  - Reduced WL
  - Shortened QT interval
  - Anatomical or spiral wave re-entry

- **SQT4-7**
  - Decreased inward current
  - Shortened APD/ERP
  - Increased T wave amplitude

- **SQT8**
  - Heterogeneous shortening
  - Increased TDR
  - Dispersion of refractoriness
  - Unidirectional conduction block

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Figure 2