
Article:
Hancox, JC, Whittaker, DG orcid.org/0000-0002-2757-5491, Du, C et al. (2 more authors) (2018) Emerging therapeutic targets in the short QT syndrome. Expert opinion on therapeutic targets, 22 (5). pp. 439-451. ISSN 1472-8222

https://doi.org/10.1080/14728222.2018.1470621

Emerging therapeutic targets in the Short QT Syndrome

Main text (without highlights, abstract, references or legends): 6838 words. (7353 revised version)

Abstract (limit 200 words): 199 words.

Key words: atrial fibrillation; atrial-selective; *CACNA1C*, *CACNA2D1 CACNB2b* hERG; KCNH2; KCNJ2; KCNQ1; Kir2.1; short QT syndrome; *SLC4A3;* sudden death; ventricular fibrillation

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_c) 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^+) channels is associated with an increased risk of ventricular arrhythmia [1-3]. QT_c 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_c intervals of ≤ 362 and 369 ms for males and females, respectively [19], with a biphasic distribution with age (i.e. the shortest QT_c intervals in young and old age). In a separate study of 18,825 healthy people between 14 and 35, the prevalence of a QT_c ≤ 320 ms was found to be 0.1% [20]. It is, however, worth noting that the prevalence of short QT_c intervals may be affected by the rate correction formula adopted for ECG analysis [21]. Cohort analysis has highlighted differences in 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 ≤ 340 ms [25]. A longer QT_c interval of ≤ 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 (QT_c interval of 329 ms) [46]. His brother had a QT_c of 377 ms and a non-documented arrhythmia, whilst his mother had a QT_c 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 QT_c 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 QT_c 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 QT_c interval for probands and other carriers of 313 ms, poor rate adaptation of the QT_c 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 I_{hERG} inactivation, slowed development of inactivation but accelerated recovery from inactivation [50]. At 37^{o}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 ^{o}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^{o}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_c 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 I_{hERG} revealed an increase in I_{hERG} 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 I_{hERG} 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_c interval of <320 ms at age 6 and 323 ms at age 18. Her younger sister had a QT_c interval of 340 ms and slightly reduced ejection fraction (42%, MR tomography). Her father had a QT_c 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_{Ks} (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_{Ks} (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 QT_c 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 prolong QT interval and slow pacemaker rate [60;61]. The R295H mutation was found in a 20 year old proband with a QT_c interval of 310 ms who had undergone aborted cardiac arrest [62]. His mother possessed the same mutation and a QT_c 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 V_2-V_4 and a QT_c 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 SQTS 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 α and β_{2b} subunits of L-type channels; of these 7, 3 exhibited QT_c 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 QT_c 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 QT_c intervals [71]. He (and other

phenotype-positive family members) showed a *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 *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 *CACNA1C* mutation that led to an A39V mutation in Ca_v 1.2. 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 *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, *DES*; R989H in myopallidin, *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 $Ca_v\alpha_2\delta$-1 subunit of the L-type Ca^{2+} channel. Coexpression of the mutant $Ca_v\alpha_2\delta$-1 subunit with $Ca_v1.2\alpha1$ and $Ca_v\beta_{2b}$ led to reduced $I_{Ca,L}$ (using Ba^{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 $Ca_v\alpha_2\delta$-1 [74].

3.6 SQT7

In 2012, a novel mixed SQT/Brugada phenotype was identified that is caused by a missense mutation to the *SCN5A* gene, which encodes the *α* subunit of channels carrying cardiac sodium current, 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^{-1}). His father had died suddenly at age 39. An R689H mutation was identified in SCN5A; biophysical analysis showed that SCN5A protein incorporating the R689H mutation was unable to mediate I_{Na}, indicating loss of function [75]. As 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 *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 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 *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 QT_c 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 QT_c 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 QT_c 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. HCO_3^- transport was impaired in cells expressing the mutant form of AE3. Knockdown of *slc4a3* in zebrafish embryos replicated QT_c 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 $[Cl^-]_i$ 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 (δV) 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]. *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]. *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 depolarization in sinus node cells ([60], Whittaker *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 I_{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 QT_c 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 pentamadine 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 repolarization, 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 QT_c 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 proarrhythmia. 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 proarrhythmic 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}/hERG block, whilst its slow Na channel disassociation kinetics (as a class Ic agent; [96]) would nevertheless have prolonged post-repolarisation refractoriness, 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 QT interval in a patient with a hERG mutation [22] but was effective combined with β-blockade in protecting against malignant arrhythmia in a patient with SQTS of unknown genotype [134] and *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].

Acknowledgements.

The authors thank the British Heart Foundation (FS/14/5/30533, PG/15/106/31915) and SADS (Sudden Arrhythmic Death Syndrome) UK for funding. JCH also acknowledges a University of Bristol Research Fellowship.

References

[1] Modell SM, Lehmann MH: The long QT syndrome family of cardiac ion channelopathies: a HuGE review. Genet Med 2006;8:143-155.

[2] Sanguinetti MC, Mitcheson JS: Predicting drug-hERG channel interactions that cause acquired long QT syndrome. TIPS 2005;26:119-124.

[3] Yap YG, Camm AJ: Drug induced QT prolongation and torsades de pointes. HEART 2003;1363-1372.

[4] Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SI, Chaitman BR, Bjeerregaard P: Idiopathic short QT interval: a new clinical syndrome? Cardiology 2000;94:99-102.

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

[5] McPate MJ, Witchel HJ, Hancox JC: Short QT Syndrome. Future Cardiology 2006;2:293-301.

[6] Bjerregaard P, Jahangir A, Gussak I: Targeted therapy for short QT syndrome. Expert Opin Ther Targets 2006;10:393-400.

[7] Gussak I, Brugada P, Brugada J, Antzelevitch C, Osbakken M, Bjerregaard P. ECG phenomenon of idiopathic and paradoxical short QT intervals. Cardiac Electrophysiology Review 2003;6:49-53.

[8] Schimpf R, Wolpert C, Gaita F, Giustetto C, Borggrefe M: Short QT syndrome. Cardiovasc Res 15-8-2005;67:357-366.

[9] Gollob MH, Redpath CJ, Roberts JD: The short QT syndrome: proposed diagnostic criteria. J Am Coll Cardiol 15-2-2011;57:802-812.

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

[10] Giustetto C, Di MF, Wolpert C, Borggrefe M, Schimpf R, Sbragia P, Leone G, Maury P, Anttonen O, Haissaguerre M, Gaita F: Short QT syndrome: clinical findings and diagnostic-therapeutic implications. Eur Heart J 2006;27:2440-2447.

[11] Garberoglio L, Giustetto C, Wolpert C, Gaita F: Is acquired short QT due to digitalis intoxication responsible for malignant ventricular arrhythmias? J Electrocardiol 2007;40:43-46.

[12] Bidoggia H, Maciel JP, Capalozza N, Mosca S, Blaksley EJ, Valverde E, Bertran G, Arini P, Biagetti MO, Quinteiro RA: Sex differences on the electrocardiographic pattern of cardiac repolarization: possible role of testosterone. Am Heart J 2000;140:678-683.

[13] Charbit B, Christin-Maitre S, Demolis JL, Soustre E, Young J, Funck-Brentano C: Effects of testosterone on ventricular repolarization in hypogonadic men. Am J Cardiol 15-3-2009;103:887-890.

[14] Bigi MA, Aslani A, Aslani A: Short QT interval: A novel predictor of androgen abuse in strength trained athletes. Ann Noninvasive Electrocardiol 2009;14:35-39.

[15] Hancox JC, Choisy SC, James AF: Short QT interval linked to androgen misuse: wider significance and possible basis. Ann Noninvasive Electrocardiol 2009;14:311-312.

[16] Cheng TO: Digitalis administration: an underappreciated but common cause of short QT interval. Circulation 9-3-2004;109:e152.

[17] Roussel J, Labarthe F, Thireau J, Ferro F, Farah C, Roy J, Horiuchi M, Tardieu M, Lefort B, Francois BJ, Lacampagne A, Richard S, Fauconnier J, Babuty D, Le Guennec JY: Carnitine deficiency induces a short QT syndrome. Heart Rhythm 2016;13:165-174.

[18] Anttonen O, Junttila MJ, Rissanen H, Reunanen A, Viitasalo M, Huikuri HV: Prevalence and prognostic significance of short QT interval in a middle-aged Finnish population. Circulation 14-8-2007;116:714-720.

[19] Miyamoto A, Hayashi H, Yoshino T, Kawaguchi T, Taniguchi A, Itoh H, Sugimoto Y, Itoh M, Makiyama T, Xue JQ, Murakami Y, Horie M: Clinical and electrocardiographic characteristics of patients with short QT interval in a large hospital-based population. Heart Rhythm 2012;9:66-74.

[20] Dhutia H, Malhotra A, Parpia S, Gabus V, Finocchiaro G, Mellor G, Merghani A, Millar L, Narain R, Sheikh N, Behr ER, Papadakis M, Sharma S: The prevalence and significance of a short QT interval in 18,825 low-risk individuals including athletes. Br J Sports Med 2016;50:124-129.

[21] Providencia R, Karim N, Srinivasan N, Honarbakhsh S, Vidigal Ferreira MJ, Goncalves L, Marijon E, Lambiase PD: Impact of QTc formulae in the prevalence of short corrected QT interval and impact on probability and diagnosis of short QT syndrome. HEART 2018; 104:502-508.

[22] Giustetto C, Schimpf R, Mazzanti A, Scrocco C, Maury P, Anttonen O, Probst V, Blanc JJ, Sbragia P, Dalmasso P, Borggrefe M, Gaita F: Long-term follow-up of patients with short QT syndrome. J Am Coll Cardiol 2-8-2011;58:587-595.

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

[23] Maury P, Extramiana F, Sbragia P, Giustetto C, Schimpf R, Duparc A, Wolpert C, Denjoy I, Delay M, Borggrefe M, Gaita F: Short QT syndrome. Update on a recent entity. Arch Cardiovasc Dis 2008;101:779-786.

[24] Villafane J, Atallah J, Gollob MH, Maury P, Wolpert C, Gebauer R, Watanabe H, Horie M, Anttonen O, Kannankeril P, Faulknier B, Bleiz J, Makiyama T, Shimizu W, Hamilton

R, Young ML: Long-Term Follow-Up of a Pediatric Cohort With Short QT Syndrome. J Am Coll Cardiol 2013;61:1183-1191.

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

[25] Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, Elliott PM, Fitzsimons D, Hatala R, Hindricks G, Kirchhof P, Kjeldsen K, Kuck KH, Hernandez-Madrid A, Nikolaou N, Norekval TM, Spaulding C, van Veldhuisen DJ: 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J 2015;36:2793-2867.

- **Contains useful working definition/criteria for SQTS diagnosis**

[26] Tulumen E, Giustetto C, Wolpert C, Maury P, Anttonen O, Probst V, Blanc JJ, Sbragia P, Scrocco C, Rudic B, Veltmann C, Sun Y, Gaita F, Antzelevitch C, Borggrefe M, Schimpf R: PQ segment depression in patients with short QT syndrome: a novel marker for diagnosing short QT syndrome? Heart Rhythm 2014;11:1024-1030.

[27] Rollin A, Gandjbakhch E, Giustetto C, Scrocco C, Fourcade C, Monteil B, Mondoly P, Cardin C, Maupain C, Gaita F, Maury P: Shortening of the Short Refractory Periods in Short QT Syndrome. J Am Heart Assoc 31-5-2017;6. pii: e005684. doi: 10.1161/JAHA.117.005684

[28] Patel C, Yan GX, Antzelevitch C: Short QT syndrome: from bench to bedside. Circ Arrhythm Electrophysiol 2010;3:401-408.

[29] Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu YS, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C: Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation 2004;109:30-35.

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

[30] Sanguinetti MC, Tristani-Firouzi M: hERG potassium channels and cardiac arrhythmia. Nature 23-3-2006;440:463-469.

[31] Tamargo J, Caballero R, Gomez R, Valenzuela C, Delpon E: Pharmacology of cardiac potassium channels. Cardiovasc Res 2004;62:9-33.

[32] Hong K, Bjeerregaard P, Gussak I, Brugada R: Short QT syndrome and atrial fibrillation caused by mutation in KCNH2. J Cardivas Electophysiol 2005;16:394-396.

- **Useful insight into N588K-hERG linked SQT1 .**

[33] Sanguinetti MC, Jiang C, Curran ME, Keating MT: A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. Cell 1995;81:299-307.

[34] Smith PL, Baukrowitz.T., Yellen G: The inward rectification mechanism of the HERG cardiac potassium channel. Nature 1996;379:833-836.

[35] Hancox JC, Levi AJ, Witchel HJ: Time course and voltage dependence of expressed HERG current compared with native 'rapid' delayed rectifier K current during the cardiac ventricular action potential. Pflugers Archiv - European Journal of Physiology 1998;436:843-853.

[36] Hancox JC, Witchel HJ, Varghese A: Alteration of HERG current profile during the cardiac ventricular action potential, following a pore mutation. Biochem Biophys Res Comm 1998;253:719-724.

[37] Jiang M, Zhang M, Maslennikov IV, Liu J, Wu DM, Korolkova YV, Arseniev AS, Grishin EV, Tseng GN: Dynamic conformational changes of extracellular S5-P linkers in the hERG channel. J Physiol 15-11-2005;569:75-89.

[38] Clarke CE, Hill AP, Zhao J, Kondo M, Subbiah RN, Campbell TJ, Vandenberg JI: Effect of S5P alpha-helix charge mutants on inactivation of hERG K+ channels. J Physiol 2006;573:291-304.

[39] Cordeiro JM, Brugada R, Wu YS, Hong K, Dumaine R: Modulation of I_{Kr} inactivation by mutation N588K in KCNH2: a link to arrhythmogenesis in short QT syndrome. Cardiovas Res 2005;67:498-509.

- **Detailed biophysical analysis of N588K-hERG SQT1 mutation.**

[40] McPate MJ, Duncan RS, Milnes JT, Witchel HJ, Hancox JC: The N588K-HERG K^+ channel mutation in the 'short QT syndrome': mechanism of gain-in-function determined at 37 ^oC. Biochem Biophys Res Comm 2005;334:441-449.

- **Detailed biophysical analysis of N588K-hERG SQT1 mutation.**

[41] McPate MJ, Zhang H, Ideniran I, Cordeiro JM, Witchel HJ, Hancox JC: Comparative effects of the short QT N588K mutation at 37^oC on hERG K^+ channel current during ventricular, Purkinje fibre and atrial action potentials: an action potential clamp study. J Physiol Pharmacol 2009;60:23-41.

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

[42] McPate MJ, Zhang H, Cordeiro JM, Dempsey CE, Witchel HJ, Hancox JC: hERG1a/1b heteromeric currents exhibit amplified attenuation of inactivation in variant 1 short QT syndrome. Biochem Biophys Res Commun 14-8-2009;386:111-117.

[43] Zhang H, Hancox JC: In silico study of action potential and QT interval shortening due to loss of inactivation of the cardiac rapid delayed rectifier potassium current. Biochem Biophys Res Commun 2004;322:693-699.

[44] Itoh H, Horie M, Ito M, Imoto K: Arrhythmogenesis in the short-QT syndrome associated with combined HERG channel gating defects: a simulation study. Circ J 2006;70:502-508.

[45] Adeniran I, McPate MJ, Witchel HJ, Hancox JC, Zhang H: Increased vulnerability of human ventricle to re-entrant excitation in hERG-linked variant 1 short QT syndrome. PLoS Comput Biol 2011;7:e1002313.

- • **Multilevel modelling study providing insight into arrhythmia substrate in SQT1.**

[46] Itoh H, Sakaguchi T, Ashihara T, Ding WG, Nagaoka I, Oka Y, Nakazawa Y, Yao T, Jo H, Ito M, Nakamura K, Ohe T, Matsuura H, Horie M. A novel KCNH2 mutation as a modifier for a short QT interval. Int J Cardiol 2009;137:83-85.

- **Identifies R1135H -hERG as potential mediator of SQT1.**

[47] Wilders R, Verkerk AO: Role of the R1135H KCNH2 mutation in Brugada syndrome. Int J Cardiol 2009; 144(1):149-51

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

[48] Redpath CJ, Green MS, Birnie DH, Gollob MH: Rapid genetic testing facilitating the diagnosis of short QT syndrome. Can J Cardiol 2009;25:e133-e135.

- **Reports that E50D-hERG can produce SQTS phenotype.**

[49] Hu D, Li Y, Zhang J, Pfeiffer R, Gollob MH, Healey J, Harrell DT, Makita N, Abe H, Sun Y, Guo J, Zhang L, Yan G, Mah D, Walsh EP, Leopold HB, Giustetto C, Gaita F, Zienciuk-Kraja A, Mazzanti A, Priori SG, Antzelevitch C, Barajas-Martinez H: The Phenotypic Spectrum of a Mutation Hotspot Responsible for the Short QT Syndrome. JACC: Clinical Electrophysiology 2017;in press:h t t p : / / d x . d o i . o r g / 1 0 . 1 0 1 6 / j . j a c e p . 20 1 6 . 1 1 . 0 1 3.

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

[50] Sun Y, Quan XQ, Fromme S, Cox RH, Zhang P, Zhang L, Guo D, Guo J, Patel C, Kowey PR, Yan GX: A novel mutation in the KCNH2 gene associated with short QT syndrome. J Mol Cell Cardiol 2011;50:433-441.

- **First report implicating T618I-hERG in the SQTS.**

[51] El Harchi A, Melgari D, Zhang YH, Zhang H, Hancox JC: Action Potential Clamp and Pharmacology of the Variant 1 Short QT Syndrome T618I hERG K^+ Channel. PLoS One 2012;7:e52451.

• **Suggests potentially effective pharmacology against T618I hERG mutation.**

[52] Harrell DT, Ashihara T, Ishikawa T, Tominaga I, Mazzanti A, Takahashi K, Oginosawa Y, Abe H, Maemura K, Sumitomo N, Uno K, Takano M, Priori SG, Makita N: Genotype-dependent differences in age of manifestation and arrhythmia complications in short QT syndrome. Int J Cardiol 2015;190:393-402.

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

[53] Akdis D, Saguner AM, Medeiros-Domingo A, Schaller A, Balmer C, Steffel J, Brunckhorst C, Duru F: Multiple clinical profiles of families with the short QT syndrome. Europace 19-7-2017. doi: 10.1093/europace/eux186

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

[54] Schoenherr R, Heinemann SH: Molecular determinants for activation and inactivation of HERG, a human inward rectifier potassium channel. J Physiol 1996;493:635-642.

[55] Zou A, Xu QP, Sanguinetti MC: A mutation in the pore region of HERG K channels expressed in *Xenopus* oocytes reduces rectification by shifting the voltage dependence of inactivation. J Physiol 1998;509:129-137.

[56] Bellocq C, van Ginneken AC, Bezzina CR, Alders M, Escande D, Mannens MM, Baro I, Wilde AA: Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. Circulation 2004;109:2394-2397.

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

[57] El Harchi A, McPate MJ, Zhang YH, Zhang H, Hancox JC: Action potential clamp and mefloquine sensitivity of recombinant 'I_{KS}' channels incorporating the V307L KCNQ1 mutation. J Physiol Pharmacol 2010;61:123-131.

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

[58] Zhang H, Kharche S, Holden AV, Hancox JC: Repolarisation and vulnerability to re-entry in the human heart with short QT syndrome arising from KCNQ1 mutation--a simulation study. Prog Biophys Mol Biol 2008;96:112-131.

[59] Adeniran I, Whittaker DG, El HA, Hancox JC, Zhang H: In silico investigation of a KCNQ1 mutation associated with short QT syndrome. Sci Rep 16-8-2017;7:8469.

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

[60] Hong K, Piper DR, az-Valdecantos A, Brugada J, Oliva A, Burashnikov E, Santos-de-Soto J, Grueso-Montero J, az-Enfante E, Brugada P, Sachse F, Sanguinetti MC, Brugada R: De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. Cardiovasc Res 2005;68:433-440.

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

[61] Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, Jin HW, Sun H, Su XY, Zhuang QN, Yang YQ, Li YB, Liu Y, Xu HJ, Li XF, Ma N, Mou CP, Chen Z, Barhanin J, Huang W: KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 10-1-2003;299:251-254.

[62] Wu ZJ, Huang Y, Fu YC, Zhao XJ, Zhu C, Zhang Y, Xu B, Zhu QL, Li Y: Characterization of a Chinese KCNQ1 mutation (R259H) that shortens repolarization and causes short QT syndrome 2. J Geriatr Cardiol 2015;12:394-401.

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

[63] Moreno C, Oliveras A, de la CA, Bartolucci C, Munoz C, Salar E, Gimeno JR, Severi S, Comes N, Felipe A, Gonzalez T, Lambiase P, Valenzuela C: A new KCNQ1 mutation at the S5 segment that impairs its association with KCNE1 is responsible for short QT syndrome. Cardiovasc Res 2015;107:613-623.

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

[64] Dhamoon AS, Jalife J: The inward rectifier current (I_{K1}) controls cardiac excitability and is involved in arrhythmogenesis. Heart Rhythm 2005;2:316-324.

[65] Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dhamoon A, Napolitano C, Anumonwo J, di Barletta MR, Gudapakkam S, Bosi G, Stramba-Badiale M, Jalife J: A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. Circ Res 2005;96:800-807.

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

[66] Abrams CJ, Davies NW, Shelton PA, Stanfield PR: The role of a single aspartate residue in ionic selectivity and block of a murine inward rectifier K^+ channel Kir2.1. J Physiol 15-6-1996;493 (Pt 3):643-649.

[67] El Harchi A, McPate MJ, Zhang YH, Zhang H, Hancox JC: Action potential clamp and chloroquine sensitivity of mutant Kir2.1 channels responsible for variant 3 short QT syndrome . J Mol Cell Cardiol 2009;137:83-85.

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

[68] Adeniran I, El HA, Hancox JC, Zhang H: Proarrhythmia in KCNJ2-linked short QT syndrome: insights from modelling. Cardiovasc Res 1-4-2012;94:66-76.

[69] Hattori T, Makiyama T, Akao M, Ehara E, Ohno S, Iguchi M, Nishio Y, Sasaki K, Itoh H, Yokode M, Kita T, Horie M, Kimura T: A novel gain-of-function KCNJ2 mutation

associated with short-QT syndrome impairs inward rectification of Kir2.1 currents. Cardiovasc Res 2012;93:666-673.

- **Identified M301K mutation in SQT3**

[70] Deo M, Ruan Y, Pandit SV, Shah K, Berenfeld O, Blaufox A, Cerrone M, Noujaim SF, Denegri M, Jalife J, Priori SG: KCNJ2 mutation in short QT syndrome 3 results in atrial fibrillation and ventricular proarrhythmia. Proc Natl Acad Sci U S A 2013;110:4291-4296.

- **Identified E299V mutation in SQT3**

[71] Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros EP, Jr., Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu LF, Haissaguerre M, Schimpf R, Borggrefe M, Wolpert C: Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation 2007;115:442-449.

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

[72] Chen Y, Barajas-Martinez H, Zhu D, Wang X, Chen C, Zhuang R, Shi J, Wu X, Tao Y, Jin W, Wang X, Hu D: Erratum to: Novel trigenic CACNA1C/DES/MYPN mutations in a family of hypertrophic cardiomyopathy with early repolarization and short QT syndrome. J Transl Med 11-5-2017;15:101.

[73] Chen Y, Barajas-Martinez H, Zhu D, Wang X, Chen C, Zhuang R, Shi J, Wu X, Tao Y, Jin W, Wang X, Hu D: Novel trigenic CACNA1C/DES/MYPN mutations in a family of hypertrophic cardiomyopathy with early repolarization and short QT syndrome. J Transl Med 2017;15:78.

- **Presents overlapping SQT4 and early repolarisation phenotype**

[74] Templin C, Ghadri JR, Rougier JS, Baumer A, Kaplan V, Albesa M, Sticht H, Rauch A, Puleo C, Hu D, Barajas-Martinez H, Antzelevitch C, Luscher TF, Abriel H, Duru F: Identification of a novel loss-of-function calcium channel gene mutation in short QT syndrome (SQTS6). Eur Heart J 2011;32:1077-1088.

- **First report of SQT6**

[75] Hong K, Hu J, Yu J, Brugada R: Concomitant Brugada-like and short QT electrocardiogram linked to SCN5A mutation. Eur J Hum Genet 2012;20:1189-1192.

- **First report of SQT7 and showed overlapping SQTS/Brugada phenotype**

[76] Sottas V, Rougier JS, Jousset F, Kucera JP, Shestak A, Makarov LM, Zaklyazminskaya EV, Abriel H: Characterization of 2 genetic variants of Na(v) 1.5-arginine 689 found in patients with cardiac arrhythmias. J Cardiovasc Electrophysiol 2013;24:1037-1046.

[77] Thorsen K, Dam VS, Kjaer-Sorensen K, Pedersen LN, Skeberdis VA, Jurevicius J, Treinys R, Petersen IMBS, Nielsen MS, Oxvig C, Morth JP, Matchkov VV, Aalkjaer C, Bundgaard H, Jensen HK: Loss-of-activity-mutation in the cardiac chloride-bicarbonate exchanger AE3 causes short QT syndrome. Nat Commun 2017;8:1696.

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

[78] Extramiana F, Antzelevitch C: Amplified transmural dispersion of repolarization as the basis for arrhythmogenesis in a canine ventricular-wedge model of short-QT syndrome. Circulation 2004;110:3661-3666.

- **K_{ATP} channel activation as an experimental model for probing arrhythmia substrates in SQTS.**

[79] Frommeyer G, Ellermann C, Dechering DG, Kochhauser S, Bogeholz N, Guner F, Leitz P, Pott C, Eckardt L: Ranolazine and Vernakalant Prevent Ventricular Arrhythmias in an Experimental Whole-Heart Model of Short QT Syndrome. J Cardiovasc Electrophysiol 2016;27(10):1214-1219.

[80] Frommeyer G, Weller J, Ellermann C, Kaese S, Kochhauser S, Lange PS, Dechering DG, Eckardt L: Antiarrhythmic properties of ivabradine in an experimental model of Short-QT- Syndrome. Clin Exp Pharmacol Physiol 2017;44:941-945.

[81] Patel C, Antzelevitch C: Cellular basis for arrhythmogenesis in an experimental model of the SQT1 form of the short QT syndrome. Heart Rhythm 2008;5:585-590.

- **I_{Kr}/hERG activation as an experimental model for probing arrhythmia substrates in SQT1.**

[82] Anttonen O, Vaananen H, Junttila J, Huikuri HV, Viitasalo M: Electrocardiographic transmural dispersion of repolarization in patients with inherited short QT syndrome. Ann Noninvasive Electrocardiol 2008;13:295-300.

[83] Schimpf R, Antzelevitch C, Haghi D, Giustetto C, Pizzuti A, Gaita F, Veltmann C, Wolpert C, Borggrefe M: Electromechanical coupling in patients with the short QT syndrome: further insights into the mechanoelectrical hypothesis of the U wave. Heart Rhythm 2008;5:241-245.

[84] Schimpf R, Antzelevitch C, Haghi D, Giustetto C, Pizzuti A, Gaita F, Veltmann C, Wolpert C, Borgreffe M: To the editor response. Heart Rhythm 2008;5:1091-1092.

[85] Frea S, Giustetto C, Capriolo M, Scrocco C, Fornengo C, Benedetto S, Bianchi F, Pidello S, Morello M, Gaita F: New echocardiographic insights in short QT syndrome: More than a channellopathy? Heart Rhythm 2015;12:2096-2105.

- **Evidence for modestly impaired contractile behaviour in SQTS.**

[86] 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

[87] Adeniran I, Hancox JC, Zhang H: In silico investigation of the short QT syndrome, using human ventricle models incorporating electromechanical coupling. Front Physiol 2013;4:166 doi: 10.3389/fphys.2013.00166

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

[88] Hancox JC, Adeniran I, Whittaker DG, Zhang H: To the Editor--Altered in vivo systolic function in the short QT syndrome anticipated in silico. Heart Rhythm 2015;12:e115.

[89] Nof E, Burashnikov A, Antzelevitch C: Cellular basis for atrial fibrillation in an experimental model of short QT1: implications for a pharmacological approach to therapy. Heart Rhythm 2010;7:251-257.

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

[90] Loewe A, Wilhelms M, Fischer F, Scholz EP, Dossel O, Seemann G: Arrhythmic potency of human ether-a-go-go-related gene mutations L532P and N588K in a computational model of human atrial myocytes. Europace 2014;16:435-443.

[91] Whittaker DG, Ni H, Harchi AE, Hancox JC, Zhang H: Atrial arrhythmogenicity of KCNJ2 mutations in short QT syndrome: Insights from virtual human atria. PLoS Comput Biol 2017;13:e1005593.

- **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**

[92] Mazzanti A, Kanthan A, Monteforte N, Memmi M, Bloise R, Novelli V, Miceli C, O'Rourke S, Borio G, Zienciuk-Krajka A, Curcio A, Surducan AE, Colombo M, Napolitano C, Priori SG: Novel insight into the natural history of short QT syndrome. J Am Coll Cardiol 8-4-2014;63:1300-1308.

- **Highlights the lethality of the SQTS**

[93] Schimpf R, Wolpert C, Bianchi F, Giustetto C, Gaita F, Bauersfeld U, Borggrefe M: Congenital short QT syndrome and implantable cardioverter defibrillator treatment: inherent risk for inappropriate shock delivery. J Cardiovasc Electrophysiol 2003;14:1273-1277.

[94] Schimpf R, Bauersfeld U, Gaita F, Wolpert C: Short QT syndrome: successful prevention of sudden cardiac death in an adolescent by implantable cardioverter-defibrillator treatment for primary prophylaxis. Heart Rhythm 2005;2:416-417.

[95] Anttonen O, Junttila J, Giustetto C, Gaita F, Linna E, Karsikas M, Seppanen T, Perkiomaki JS, Makikallio TH, Brugada R, Huikuri HV: T-Wave morphology in short QT syndrome. Ann Noninvasive Electrocardiol 2009;14:262-267.

[96] Hancox JC, Patel KCR, Jones JV: Antiarrhythmics - from cell to clinic: past, present and future. HEART 2000;84:14-24.

[97] Vandenberg JI, Walker BD, Campbell TJ: HERG K+ channels: friend and foe. TIPS 2001;22:240-246.

[98] Wolpert C, Schimpf R, Giustetto C, Antzelevitch C, Cordeiro JM, Dumaine R, Brugada R, Hong K, Bauersfeld U, Gaita F, Borgreffe M: Further insights into the effect of quinidine in short QT syndrome caused by a mutation in HERG. J Cardiovas Electophysiol 2005;16:54-58.

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

[99] Lees-Miller JP, Duan Y, Teng GQ, Duff HJ: Molecular determinant of high affinity dofetilide binding to HERG1 expressed in Xenopus oocytes: involvement of S6 sites. Molecular Pharmacology 2000;57:367-374.

[100] Weerapura M, Hebert TE, Nattel S: Dofetilide block involves interactions with open and inactivated states of HERG channels. Pflugers Arch 2002;443:520-531.

[101] Ficker E, Jarolimek W, Brown AM: Molecular determinants of inactivation and dofetilide block in ether a-go-go (EAG) channels and EAG-related K^+ channels. Mol Pharmacol 2001;60:1343-1348.

[102] Numaguchi H, Mullins FM, Johnson JP, Jr., Johns DC, Po SS, Yang IC, Tomaselli GF, Balser JR: Probing the interaction between inactivation gating and Dd-sotalol block of HERG. Circ Res 2000;87:1012-1018.

[103] McPate MJ, Duncan RS, Hancox JC, Witchel HJ: Pharmacology of the short QT syndrome N588K-hERG K^+ channel mutation: differential impact on selected class I and class III antiarrhythmic drugs. Br J Pharmacol 2008;155:957-966.

[104] Paul AA, Witchel HJ, Hancox JC: Inhibition of HERG potassium channel current by the Class 1a antiarrhythmic agent disopyramide. Biochem Biophys Res Comm 2001;280:1243-1250.

[105] McPate MJ, Duncan RS, Witchel HJ, Hancox JC: Disopyramide is an effective inhibitor of mutant HERG K^+ channels involved in variant 1 short QT syndrome. J Mol Cell Cardiol 2006;41:563-566.

- ***In vitro* demonstration of effectiveness of disopyramide in SQT1**

[106] Schimpf R, Veltmann C, Giustetto C, Gaita F, Borgreffe M, Wolpert C: In vivo effects of mutant HERG K^+ channel inhibition by disopyramide in patients with a short QT-1 syndrome: a pilot study. J Cardiovas Electophysiol 2007;18:1157-1160.

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

[107] Mizobuchi M, Enjoji Y, Yamamoto R, Ono T, Funatsu A, Kambayashi D, Kobayashi T, Nakamura S: Nifekalant and disopyramide in a patient with short QT syndrome: evaluation of pharmacological effects and electrophysiological properties. Pace - Pacing and Clinical Electrophysiology 2008;31:1229-1232.

[108] Gaita F, Giustetto C, Bianchi F, Schimpf R, Haissaguerre M, Calo L, Brugada R, Antzelevitch C, Borgreffe M, Wolpert C: Short QT syndrome: Pharmacological treatment. J Am Coll Cardiol 2004;43:1494-1499.

• **Important patient study of antiarrhythmic pharmacology in SQTS**

[109] Whittaker DG, Ni H, Benson AP, Hancox JC, Zhang H: Computational Analysis of the Mode of Action of Disopyramide and Quinidine on hERG-Linked Short QT Syndrome in Human Ventricles. Front Physiol 2017;8:759. doi: 10.3389/fphys.2017.00759

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

[110] Giustetto C, Scrocco C, Giachino D, Rapezzi C, Mognetti B, Gaita F: The lack of effect of sotalol in short QT syndrome patients carrying the T618I mutation in the KCNH2 gene. HeartRhythm Case Rep 2015;1:373-378.

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

[111] Lerche C, Bruhova I, Lerche H, Steinmeyer K, Wei AD, Strutz-Seebohm N, Lang F, Busch AE, Zhorov BS, Seebohm G: Chromanol 293B binding in KCNQ1 (Kv7.1) channels involves electrostatic interactions with a potassium ion in the selectivity filter. Mol Pharmacol 2007;71:1503-1511.

[112] Kang J, Chen XL, Wang L, Rampe D: Interactions of the antimalarial drug mefloquine with the human cardiac potassium channels KvLQT1/minK and HERG. J Pharmacol Exp Ther 2001;299:290-296.

[113] Campbell CM, Campbell JD, Thompson CH, Galimberti ES, Darbar D, Vanoye CG, George AL, Jr.: Selective targeting of gain-of-function KCNQ1 mutations predisposing to atrial fibrillation. Circ Arrhythm Electrophysiol 2013;6:960-966.

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

[114] Roden DM, Abraham RL: Refining Repolarization Reserve. Heart Rhythm 2011;8(11):1756-1757.

[115] Mitcheson JS, Hancox JC: An investigation of the role played by the E-4031-sensitive (rapid delayed rectifier) potassium current in isolated rabbit atrioventricular nodal and ventricular myocytes. Pflugers Archiv - European Journal of Physiology 1999;438:843-850.

[116] Lopez-Izquierdo A, Ponce-Balbuena D, Ferrer T, Sachse FB, Tristani-Firouzi M, Sanchez-Chapula JA: Chloroquine blocks a mutant Kir2.1 channel responsible for short QT syndrome and normalizes repolarization properties in silico. Cell Physiol Biochem 2009;24:153-160.

- ***In vitro* and *in silico* proof of concept for effectiveness of I_{K1} inhibition against SQT3 mutant Kir2.1 channels.**

[117] Luo C, Wang K, Zhang H: Modelling the effects of chloroquine on KCNJ2-linked short QT syndrome. Oncotarget 5-12-2017;8:106511-106526.

[118] Rodriguez-Menchaca AA, Navarro-Polanco RA, Ferrer-Villada T, Rupp J, Sachse FB, Tristani-Firouzi M, Sanchez-Chapula JA: The molecular basis of chloroquine block of the inward rectifier Kir2.1 channel. Proc Natl Acad Sci U S A 29-1-2008;105:1364-1368.

[119] Takanari H, Nalos L, Stary-Weinzinger A, de Git KC, Varkevisser R, Linder T, Houtman MJ, Peschar M, de Boer TP, Tidwell RR, Rook MB, Vos MA, van der Heyden MA: Efficient and specific cardiac IK(1) inhibition by a new pentamidine analogue. Cardiovasc Res 2013;99:203-214.

[120] Ji Y, Veldhuis MG, Zandvoort J, Romunde FL, Houtman MJC, Duran K, van HG, Zangerl-Plessl EM, Takanari H, Stary-Weinzinger A, van der Heyden MAG: PA-6 inhibits inward rectifier currents carried by V93I and D172N gain-of-function KIR2.1 channels, but increases channel protein expression. J Biomed Sci 2017;24:44.

- ***In vitro* proof of concept for effectiveness of a selective I_{K1} inhibitor against gain of function Kir2.1 mutant channels.**

[121] Prasad V, Bodi I, Meyer JW, Wang Y, Ashraf M, Engle SJ, Doetschman T, Sisco K, Nieman ML, Miller ML, Lorenz JN, Shull GE: Impaired cardiac contractility in mice lacking both the AE3 Cl-/. J Biol Chem 14-11-2008;283:31303-31314.

[122] 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.

[123] Brette F, Luxan G, Cros C, Dixey H, Wilson C, Shiels HA: Characterization of isolated ventricular myocytes from adult zebrafish (Danio rerio). Biochem Biophys Res Commun 12-9-2008;374:143-146.

[124] Bjeerregaard P, Gussak I. Short QT Syndrome. 569-581. 2013. London, Springer. Electrical Diseases of the Heart. Gussak, I. and Antzelevitch, C.

[125] Watanabe H, Makiyama T, Koyama T, Kannankeril PJ, Seto S, Okamura K, Oda H, Itoh H, Okada M, Tanabe N, Yagihara N, Kamakura S, Horie M, Aizawa Y, Shimizu W: High prevalence of early repolarization in short QT syndrome. Heart Rhythm 2010;7:647-652.

[126] Hancox JC, James AF, Marrion NV, Zhang H, Thomas D: Novel ion channel targets in atrial fibrillation. Expert Opin Ther Targets 2016;20:947-958.

[127] Mazzanti A, Maragna R, Vacanti G, Kostopoulou A, Marino M, Monteforte N, Bloise R, Underwood K, Tibollo V, Pagan E, Napolitano C, Bellazzi R, Bagnardi V, Priori SG: Hydroquinidine Prevents Life-Threatening Arrhythmic Events in Patients With Short QT Syndrome. J Am Coll Cardiol 19-12-2017;70:3010-3015.

- **Valuable evidence for effectiveness of quinidine against life threatening events in SQTS**

[128] Brugada P: Short QT Syndrome and Hydroquinidine: Rare Diseases and Unavailable Drugs. J Am Coll Cardiol 19-12-2017;70:3016-3017.

[129] Luo C, Wang K, Zhang H: In silico assessment of the effects of quinidine, disopyramide and E-4031 on short QT syndrome variant 1 in the human ventricles. PLoS One 2017;12:e0179515.

[130] Paul AA, Witchel HJ, Hancox JC: Inhibition of heterolgously expressed HERG potassium channels by flecainide and comparison with quinidine, propafenone and lignocaine. Br J Pharmacol 2002;136:717-729.

[131] Fei L, Gill JS, McKenna WJ, Camm AJ: Effects of propafenone on calcium currents in single ventricular myocytes of guinea-pig. Br J Pharmac 1993;109:178-182.

[132] 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.

[133] Kodama I, Kamiya K, Toyama J: Cellular electropharmacology of amiodarone. Cardiovas Res 1997;35:13-29.

[134] Lu LX, Zhou W, Zhang X, Cao Q, Yu K, Zhu C: Short QT syndrome: a case report and review of literature. Resuscitation 2006;71:115-121.

[135] Cunjin L, Kuanquan W, Henggui Z: Modeling the effects of amiodarone on short QT syndrome variant 2 in the human ventricles. Conf Proc IEEE Eng Med Biol Soc 2017;2017:4273-4276.

[136] Luo C, Wang K, Zhang H: Effects of amiodarone on short QT syndrome variant 3 in human ventricles: a simulation study. Biomed Eng Online 7-6-2017;16:69.

[137] https://www.uptodate.com/contents/short-qt-syndrome (Accessed 13 April 2018)

[138] http://www.shortqtsyndrome.org (Accessed 13 April 2018)

[139] https://lifeinthefastlane.com/ecg-library/basics/short-qt-syndrome/ (Accessed 13 April 2018)

SQT Variant	Gene/gene product	Channel (subunit)	Mutation (amino-acid change)	Gain/Loss of function
SQT1	KCNH2 (hERG)	I_{Kr} (α [pore-forming] sub-unit)	N588K	Gain-of-function
			R1135H	Gain-of-function
			E50D	Gain-of-function
			I560T	Gain-of-function
			T618I	Gain-of-function
			S631A	Gain-of-function
SQT2	KCNQ1 (KCNQ1/KvLQT1)	I_{Ks} (α sub-unit)	V307L	Gain-of-function
			V141M	Gain-of-function
			R259H	Gain-of-function
			F279I	Gain-of-function
SQT3	KCNJ2 (Kir2.1)	I_{K1}	D172N	Gain-of-function
			M301K	Gain-of-function
			E299V	Gain-of-function
			K346T	Gain-of-function
SQT4	CACNA1C ($Ca_V1.2$)	L-type I_{Ca} (α subunit)	A39V	Loss-of-function
			G490R	Loss-of-function
			R1973P	Loss-of-function
SQT5	CACNB2b (β_{2b} subunit)	L-type I_{Ca} (β_{2b} subunit)	S481L	Loss-of-function
SQT6	CACNA2D1	L-type I_{Ca} (α2δ1 subunit)	S755T	Loss-of-function
SQT7	SCN5A	I_{Na} (α subunit)	R689H	Loss-of-function ??
SQT8	SLC4A3	Anion exchanger AE3	R370H	Loss-of-function

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^{o}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).