

# Preclinical short QT syndrome models: studying the phenotype and drug-screening

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## Abstract

Cardiovascular diseases are the main cause of sudden cardiac death (SCD) in developed and developing countries. Inherited cardiac channelopathies are linked to 5–10% of SCDs, mainly in the young. Short QT syndrome (SQTS) is a rare inherited channelopathy, which leads to both atrial and ventricular tachyarrhythmias, syncope, and even SCD. International European Society of Cardiology guidelines include as diagnostic criteria: (i) QTc ≤ 340 ms on electrocardiogram, (ii) QTc ≤ 360 ms plus one of the following, an affected short QT syndrome pathogenic gene mutation, or family history of SQTS, or aborted cardiac arrest, or family history of cardiac arrest in the young. However, further evaluation of the QTc ranges seems to be required, which might be possible by assembling large short QT cohorts and considering genetic screening of the newly described pathogenic mutations. Since the mechanisms underlying the arrhythmogenesis of SQTS is unclear, optimal therapy for SQTS is still lacking. The disease is rare, unclear genotype–phenotype correlations exist in a bevy of cases and the absence of an international short QT registry limit studies on the pathophysiological mechanisms of arrhythmogenesis and therapy of SQTS. This leads to the necessity of experimental models or platforms for studying SQTS. Here, we focus on reviewing pre-clinical SQTS models and platforms such as animal models, heterologous expression systems, human-induced pluripotent stem cell-derived cardiomyocyte models and computer models as well as three-dimensional engineered heart tissues. We discuss their usefulness for SQTS studies to examine genotype–phenotype associations, uncover disease mechanisms and test drugs. These models might be helpful for providing novel insights into the exact pathophysiological mechanisms of this channelopathy and may offer opportunities to improve the diagnosis and treatment of patients with SQT syndrome.

## Keywords

Sudden cardiac death • Genetics • Short QT syndrome • Human-induced pluripotent stem cell-derived cardiomyocytes • Short QT models • Ion channel • Drug screening

## Introduction

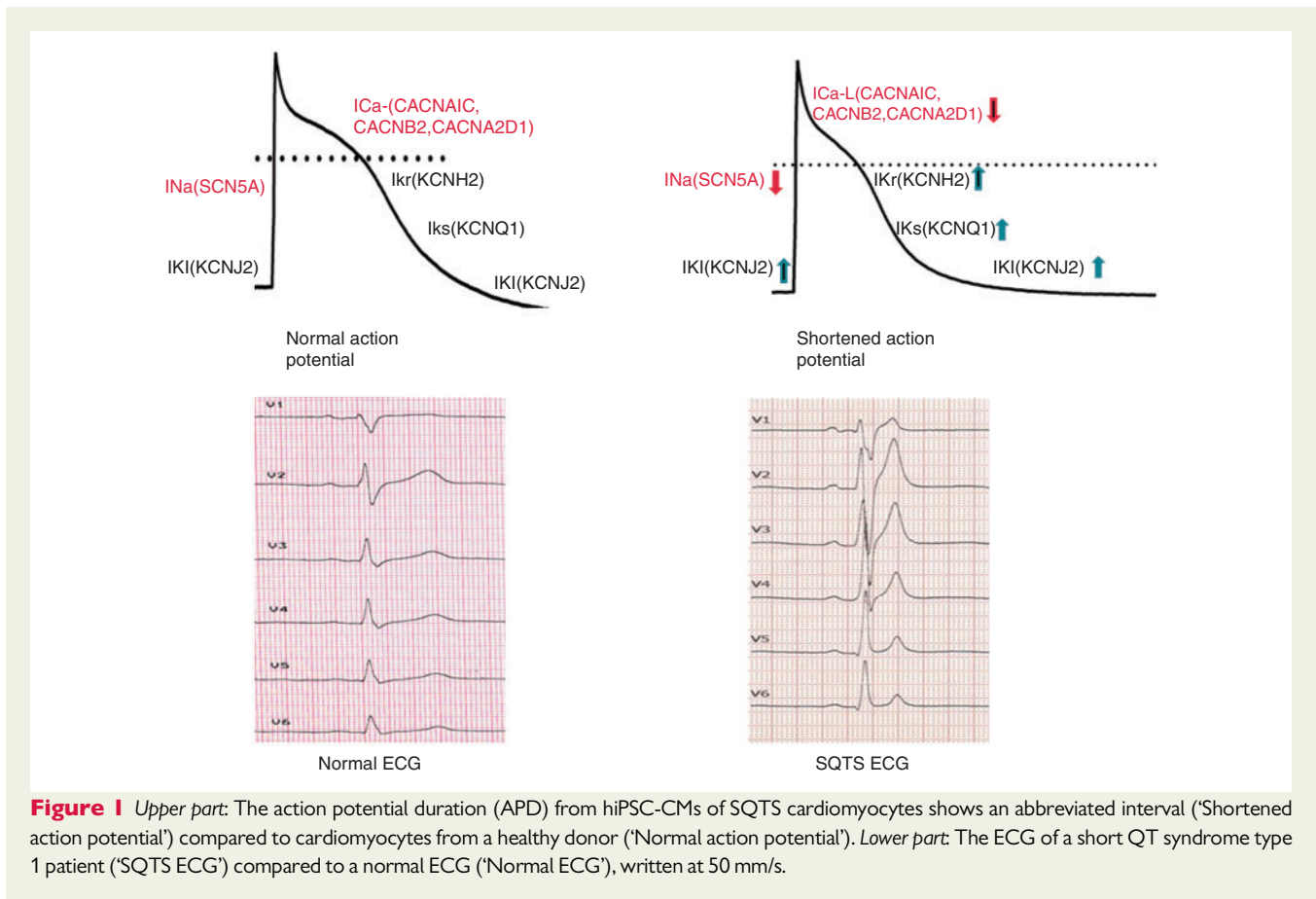
Sudden cardiac death (SCD) in the young can be related to channelopathies. These inherited disorders mainly include Brugada syndrome (BrS), long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and short QT syndrome (SQTS).<sup>1</sup>

The first clinical cases of the SQTS were described by Gussak *et al.*<sup>2</sup> in 2000 showing the main feature of an abbreviated QTc interval on the electrocardiogram (ECG) (Figure 1). In the described report, four SQTS patients suffered from atrial fibrillation (AF) and/or SCD caused by ventricular tachycardia (VT) or ventricular fibrillation (VF).<sup>2</sup> SQTS is diagnosed usually in symptomatic adults with a family

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**Figure 1** Upper part: The action potential duration (APD) from hiPSC-CMs of SQTs cardiomyocytes shows an abbreviated interval ('Shortened action potential') compared to cardiomyocytes from a healthy donor ('Normal action potential'). Lower part: The ECG of a short QT syndrome type 1 patient ('SQTs ECG') compared to a normal ECG ('Normal ECG'), written at 50 mm/s.

history of SCD. The age ranges from a few months to older age. There are almost about 250 cases and nearly 200 families reported so far worldwide and the incidence of SCD at the age of 40 years in this disease is nearly 40%.<sup>3</sup> Due to the limited number of reported cases and the possibility of overlooking SQTs cases, the real prevalence of SQTs is difficult to be determined in the world population. Iribarren et al.<sup>4</sup> stated that the prevalence of a short QT interval (<300 ms) was highest in Blacks (5.8 per 100 000), followed by Caucasians (3.2 per 100 000), Latinos (1.8 per 100 000), and Asian/Pacific Islanders (1.6 per 100 000 persons). Pooled analysis suggested that male patients presented more often with syncope compared with females by analysing 145 patients diagnosed with SQTs between 2000 and 2017.<sup>5</sup> However, over follow-up females seem to suffer more frequently from heart rhythm disorders and cardiac arrest. This suggests that higher testosterone levels in males and genes located on the X chromosome could be involved in QTc interval regulation.<sup>6,7</sup>

The SQTs diagnosis criteria have been debated in the last decade. Gollob et al.<sup>8</sup> developed in 2011 formal SQTs diagnostic criteria based on family history, clinical history, 12-lead ECG and genotype. Currently, a significant clinical diagnosis of SQTs should be considered in the presence of a shortened QT interval ( $QTc < 340$  ms), or in the presence of a  $QTc \leq 360$  ms accompanied by one or more of the following: (i) a confirmed pathogenic mutation, (ii) a family history of SQTs, (iii) a family history of sudden death until the age of 40 years, and (iv) survival from a VT/VF episode in the absence of heart disease

according to the European Society of Cardiology (ESC) guidelines in 2015.<sup>9</sup> Importantly, SQTs could be overlooked in ECG, therefore caution is required especially in the cases of unclear syncope, symptoms of arrhythmia, or the presence of paroxysmal or persistent AF in young patients.<sup>10</sup> For the treatment of SQTs, implantable cardioverter-defibrillator (ICD) is the first choice for prevention and treatment of SCD in SQTs patients. It is recommended for those, who survive cardiac arrest or are at high risk of SCD. However, several reports indicate that ICDs have a number of specific problems, such as an increased risk of inappropriate shock due to sinus tachycardia, T-wave oversensing or lead failure and AF.<sup>11–13</sup> El-Battrawy et al.<sup>11</sup> reported that inappropriate shocks (33% of SQTs patients with complications) were particularly due to T-wave oversensing (8.7%), supraventricular tachycardia (19%), lead failure and fracture (21%) over a long-term follow-up of 57 SQTs patients treated with an ICD. Therefore, young patients, especially children for whom ICD had been rejected or had been contraindicated, may take antiarrhythmic medication as an alternative therapy to prevent symptomatic AF. To date, the exact pathophysiological mechanism and treatment strategy of SQT are still unclear. Several models such as animal models, heterologous expression systems, computational model simulations and human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used to model the disease. Some antiarrhythmic drugs have been used to treat SQTs. Hydroquinidine (HQ) can prolong the QTc interval and should be considered in SQTs patients, who refuse to receive ICD or have a highly symptomatic

disease course. HQ seems to be effective in preventing ventricular tachyarrhythmia induction during electrophysiological study and tachyarrhythmic events during long-term follow-up.<sup>14–16</sup> However, other antiarrhythmics such as carvediol, metoprolol, and sotalol are not useful for the treatment of SQTS.<sup>16</sup> Of note, these data are based on small case series and/or preclinical studies. Here, we will discuss how preclinical SQTS models and platforms can be helpful to better understand the genotype–phenotype associations and the underlying mechanisms. But we will also discuss which drugs could be used for patient-specific therapies, including the repurposing of drugs already approved for other uses.

## Genetic basis and state of research

Identification of genetic mutations and pathophysiological changes that underlie the SQTS often remain elusive and pharmacologic treatment is suboptimal. The ability to associate individual cases of SQTS with specific gene mutations for preclinical studies is of great value in determining causality and potential treatments. It is recognized that the identification of genetic variants of unknown significance may be problematic.<sup>17</sup> Short QT syndrome is inherited with an autosomal dominant trait with genetic heterogeneity. Until recently, a total of six SQTS subtypes have been described. Six genes encoding potassium channels (KCNH2, KCNQ1, KCNJ2) and calcium channels (CACNA1C, CACNB2, CACNA2D1) were found to be associated with SQTS.<sup>18</sup> In addition, an SCN5A genetic variant from a Chinese patient with the family history of SCD caused the loss of function of cardiac Na channels, resulting in a subclinical phenotype of BrS and short QT interval on ECG.<sup>19</sup> This was described as SQTS type 7.<sup>3,16,20</sup> In 2017, Thorsen *et al.*<sup>21</sup> described a mutation (R370H) in the anion exchanger Solute Carrier Family 4 Member 3 (SLC4A3) gene, encoding a Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>-exchanger (AE3) as a novel genetic aetiology for SQTS. This mutation could change the function of AE3, increase intracellular pH (pHi) and reduce intracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]<sub>i</sub>). High pHi and low [Cl<sup>-</sup>]<sub>i</sub> shorten cardiac action potential (AP) in zebrafish embryos, which confirmed the functional association of AE3 with SQTS. To date, only this rare variant R370H has been identified in the SLC4A3 gene associated with SQTS, consistent with SQTS type 8. Currently, although a large number of mutations have been reported to be related or possibly related to SQTS, only a small part of them have been investigated experimentally to explore their functional significance. More than 30 variants have been identified in these eight genes and are assumed to cause SQTS because their functional consequences were uncovered in experimental studies (Table 1). Campuzano *et al.*<sup>22</sup> analysed all published cases with clinical diagnosis of SQTS in order to translate genetic findings to the clinical setting. They revealed that only 28.12% of reported variants have a conclusive role in SQTS. Gene variants encoding potassium channels (KCNQ1, KCNH2, and KCNJ2) seem to have a definite deleterious role for the clinical SQTS phenotype. However, clinical data did not show a relevant difference of the outcome related to the genotype. Short QT syndrome patients with positive genotype have no higher long-term risk of arrhythmic events and/or SCD than genotype negative ones except that AF is more prevalent in genotype-

positive patients.<sup>23</sup> But caution is required since not in all cases genetic screening was standardized.

Short QT syndrome type 1 (SQTS1) is the most frequently described form. Short QT syndrome type 1 was firstly reported by studying three families with hereditary SQTS in 2004.<sup>24</sup> Brugada *et al.* described 2 different missense mutations that lead to the same amino acid change (N588K) at the S5-P loop region of the cardiac HERG (KCNH2) channel. The genetic mutations in HERG attenuate the inactivation and increase the HERG channel current ( $I_{Kr}$ ) ('gain of function'), leading to alternating the repolarization pattern and shortening of QT intervals.<sup>22,25</sup> The incidence of AF in KCNH2-N588K was higher than in all KCNH2-T618I carriers.<sup>25,26</sup> Mutations of N588K and T618I in the pore region are associated with shorter QTc intervals compared to mutations of E50D in N-terminal and R1135H in C-terminal, suggesting that the KCNH2 P-loop may be the critical region for a clear SQTS phenotype.<sup>25</sup> The variant in KCNH2, R1135H, was linked to a potential impairment of repolarization pattern from studies in HEK cells and CHO cells.<sup>25,27</sup> The finding of a novel missense mutation in KCNH2 (Glu50Asp) provided supportive clinical evidence by diagnosing patients suffering from SQTS.<sup>28</sup> The I560T mutant in KCNH2 caused an increase in peak current density and AP shortening and created an arrhythmogenic substrate for VF.<sup>25,29</sup>

The second reported gene associated with SQTS is KCNQ1. Bellocq *et al.*<sup>30</sup> identified the SQTS type 2 (SQTS2) caused by a gain of function substitution G>C at nucleotide 919 (GTG>CTG) in the KvLQT1 ( $I_{Ks}$ ) channel of the KCNQ1 gene. This results in the substitution of valine at position 307 by leucine (V307L). The mutation KCNQ1-V141M shortens ventricular AP and enhances transmural action potential duration (APD) heterogeneity under beta-adrenergic stimulation, showing earlier onset and frequent complications of bradyarrhythmia.<sup>29,31</sup> However, quinidine was effective at terminating arrhythmic excitation waves associated with the V307L mutation in the KCNQ1 gene but not V141M mutation.<sup>31–33</sup> An additional rare variant R259H has been identified which is potentially associated with SQTS 2.<sup>34</sup>

Inward rectifier potassium channels ( $I_{K1}$  current) are linked to SQTS type 3 (SQTS3). Priori *et al.*<sup>35</sup> reported about this new form of SQTS3, which was associated with a D172N substitution in the Kir2.1 channel and characterized by asymmetrical T waves. The genetic defect in the KCNJ2 gene significantly increases the outward component of  $I_{K1}$ , which was displayed by transfecting 1.6 µg plasmid DNA of Kir2.1 WT or D172N mutant into Chinese Hamster Ovarian (CHO) cells. Furthermore, a novel KCNJ2 gain-of-function mutation, M301K, associated with SQTS, increases the channel current only in co-expression with wild type but not in the M301K homozygous channels.<sup>36</sup> In addition, it was described that the E299V and the V93I mutations are associated with AF but not with VT/VF. The reason for these findings needs to be analysed.<sup>37–39</sup>

In 2007, Antzelevitch *et al.*<sup>3,40</sup> considered the alpha and beta subunits of the voltage-dependent L-type calcium channel expressed by the CACNA1C- and CACNB2b-genes as linked to SQTS type 4 and SQTS type 5 overlapping with BrS, reporting missense mutations in CACNA1C (A39V and G490R) and CACNB2 (S481L) responsible for loss of function of the L-type calcium channel. The R1937P mutant in CACNA1C dramatically decreased peak current density by ~68%, without significant influence on channel kinetics.<sup>41</sup>

**Table 1** SQTS genes related to experimental researches

SQTS type	Gene	Protein	Ion channel	Ionic current	Mutation site	Effect of mutation on channel function	Gain/loss of function
SQTS1	KCNH2	Potassium voltage-gated channel subfamily H member 2	hERG	$I_{kr}$	N588K <sup>23,24</sup> T6181 <sup>24,25</sup> R1135H <sup>24,26</sup> E50D <sup>24</sup> Glu50Asp <sup>27</sup> I560T <sup>28</sup>	$I_{kr}$ ↑	Gain of function
SQTS2	KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	KvLQT1	$I_{ks}$	V307L <sup>29-31</sup> V141M <sup>28,30</sup> R259H <sup>32</sup>	$I_{ks}$ ↑	Gain of function
SQTS3	KCNJ2	Inward rectifier potassium channel 2	Kir2.1	$I_{k1}$	D172N <sup>34,38</sup> M301K <sup>35</sup> E299V <sup>36,38</sup> V93I <sup>37</sup>	$I_{k1}$ ↑	Gain of function
SQTS4	CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	L-type calcium	$I_{ca-L}$	A39V <sup>39</sup> G490R <sup>39</sup> R1973P <sup>40</sup>	$I_{ca-L}$ ↓	Loss of function
SQTS5	CACNB2	Voltage-dependent L-type calcium channel subunit beta-2	L-type calcium	$I_{ca-L}$	S481L <sup>39</sup>	$I_{ca-L}$ ↓	Loss of function
SQTS6	CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1	L-Type calcium	$I_{ca-L}$	S755T <sup>41</sup>	$I_{ca-L}$ ↓	Loss of function
SQTS7	SCN5A	Sodium channel protein type 5 subunit alpha	Nav1.5	$I_{Na}$	R689H <sup>19</sup>	$I_{Na}$	Loss of function
SQTS8	SLC4A3	Anion exchange protein 3		Anion exchanger AE3	R370H <sup>21</sup>		Loss of function

CACNA1C, calcium voltage-gated channel subunit alpha1 C; CACNA2D1, calcium voltage-gated channel auxiliary subunit alpha2delta 1; CACNB2, calcium voltage-gated channel auxiliary subunit beta 2; KCNH2, potassium voltage-gated channel subfamily H member 2; KCNJ2, potassium inwardly rectifying channel subfamily J member 2; KCNQ1, potassium voltage-gated channel subfamily Q member 1; SCN5A, sodium voltage-gated channel alpha subunit 5; SLC4A3, Homo sapiens solute carrier family 4 member 3.

In 2011, Templin et al.<sup>42</sup> identified SQTS type 6 (SQTS 6) related to a variant in the  $\alpha$ -subunit of the voltage-dependent L-type calcium channel, associated with the CACNA2D1 gene. The heterozygous transition c.2264G>C predicting replacement of serine by threonine at position 755 (p.Ser755Thr) is the only reported CACNA2D1 gene mutation associated with SQTS. Therefore, additional studies need to be performed for proving genetic associations of this subtype of SQTS.

A mixed SQT and BrS phenotype caused by the heterozygous missense mutation R689H in the SCN5A gene was first reported in 2012.<sup>19</sup> The patient with a short QT interval (QT of 320 ms at 71 beats min<sup>-1</sup>) was found to have a Brugada-like ECG and biophysical analysis showed that SCN5A protein incorporating the R689H mutation was unable to mediate  $I_{Na}$ , indicating loss of function of Na

channels. However, there are no conclusive data available regarding the association of this variant with SQTS.

Although the pathogenic effects of some rare variants have been reported, it still needs to be cautious when interpreting their clinical relevance. Thus, a personalized genetic interpretation should be done, for example, the SQTS patient receives genetic testing and undergoes gene-specific therapy. Variant-specific therapeutic strategies may need to be implemented in the future.

Even though it is acceptable for patients to receive the results of their genetic testing within weeks, at the present stage of technology, it is clearly unfeasible to introduce long-term strategies based on patient-specific cellular models for the majority of our patients. High-throughput platforms often encounter the bottleneck of a low-throughput functional biology.

**Table 2** Preclinical models for SQTS

Models	Advantages	Disadvantages
Animals (rabbits, guinea pigs, canine, zebrafish)	Electrical and mechanical cardiac function similar to the human heart; study on the whole-heart level; mimic clinical features of human SQTS	High cost and time consuming; energetics, myofibrillar composition, beating rates, expression of key ion channels and electrophysiology, as well as $\text{Ca}^{2+}$ homeostasis different from human
Heterologous expression (HEK293 cells, <i>Xenopus</i> oocytes, tsA201 cells, CHO cells)	More suitable for studying the impact of mutations on ion channel currents and channel gating kinetics; easier to feed	Lack of macromolecules ion channel complex; lack of the ability to generate action potential
Human native cardiomyocytes	Same as or similar to the biological setting in patients	Ethical limitations
Computational model	Stimulate the electrophysiological properties of a single cell, tissues, and the whole heart; investigate pharmacotherapeutic effects of anti-arrhythmic drugs on SQTS variants	Not capture sufficiently the complex cardiac microstructure of the human cardiomyocytes; not apply to all forms of SQTS; different drug concentrations from experiments and computer simulations to meaningful clinical concentrations
hiPSC-CMs	Available patient-specific cardiomyocytes; easy genetic and genome editing; filling the gap between animal models and human clinical experiments	Immature phenotype of cells; possible differences between hiPSC-CMs and native CMs

CM, cardiomyocyte; hiPSC-CM, human-induced pluripotent stem cell-derived cardiomyocyte; SQTS, short QT syndrome

## Experimental models of short QT syndrome

Due to the sparse number of identified patients suffering from SQTS, the experience with antiarrhythmic drug therapy is very limited. Currently reported genetic animal models cannot accurately reproduce a clinically identified SQTS genotype and may not enable detailed assessments of underlying pathophysiological mechanisms. For a clear and deep understanding of the pathogenesis of SQT syndrome, researchers initially relied on animal models either transgenic or drug-induced, heterologous cell models, and computer models. Notably, each model has its own unique advantages and limitations which will be discussed in the present review (Table 2).

### Animal models

Animal models of SQTS are a commonly used approach for disease modelling and drug testing. They have unique advantages, e.g. for *in vivo* studies and generation of transgenic animals.  $I_{K-ATP}$  channel opener (pinacidil), which can cause a significant abbreviation of ventricular repolarization such as reducing QT interval, APD and ventricular refractory periods, was employed to establish SQTS models in intact rabbit hearts,<sup>43,44</sup> guinea pig hearts,<sup>45</sup> and canine left-ventricular wedge preparations.<sup>46</sup> Increasing concentrations (50–100  $\mu\text{M}$ ) of pinacidil reduced APD and QT interval in 48 Langendorff-perfused rabbit hearts, mimicking SQTS. In this drug-induced model, antiarrhythmic effects of the  $I_{K_r}$  blocker sotalol, quinidine and the sodium channel blocker flecainide<sup>43</sup> were studied. Although there are obvious differences between this experimental model and the known heterogeneous genetic model, the rabbit heart model provides mechanistic insights into the electrophysiological mechanisms of arrhythmogenesis on the whole-heart level. This rabbit model can be

used to analyse intercellular coupling effects on the pathomechanism of arrhythmias, which cannot be studied in single-cell experiments. Therefore, the pinacidil-induced SQTS model is more widely used in experimental studies such as at the cellular level and in isolated hearts. Another drug, trapidil, was used to simulate the electrophysiological characteristics of SQTS2 with an increase of  $I_{K_s}$  current, reducing APD and ventricular refractory periods in guinea pigs.<sup>47</sup> The zebrafish model is similar to a human model in aspects of cardiac electrophysiology. In addition, the zebrafish is suitable for rapidly analysing the bioactivity of small molecules and their therapeutic potential *in vivo* because of its size and the high number of progenies. Therefore, zebrafish has emerged as a novel vertebrate model for SQTS. Hassel and colleagues established the zebrafish mutant *reggae* (*reg*) line displaying clinical features of human SQTS as the first valuable animal model for human SQTS, confirming the gain-of-function effect of the *reg* mutation carrying a missense mutation (L499P) on HERG channel function *in vivo*.<sup>48</sup> In addition, SLC4A3 knockdown in zebrafish increased cardiac  $\text{pH}_i$ , showed short QTc and reduced systolic duration, which is rescued by wildtype, suggesting both high  $\text{pH}_i$  and low  $[\text{Cl}^-]_i$  are likely to contribute to the SQTS.<sup>21</sup> Up to date, compared with other animal models, rabbits share pronounced similarities with humans in electrical and mechanical cardiac function.<sup>49</sup> Therefore, the rabbit model is a crucial animal model to investigate directly the electrical KCNH2/HERG-N588K phenotype on cellular, tissue, and whole-heart levels. Transgenic SQTS rabbits may be more suitable for studying the pathomechanism of SQTS. In the recently published transgenic SQTS rabbit model, the repolarization pattern was accelerated in the atria, the right and left ventricle of rabbit hearts, like in patients.<sup>50</sup> These transgenic rabbits expressed the mutation N588K of the HERG gene consistent with the clinical phenotype of SQTS1. Quinidine prolonged the QT interval and the APD by reducing  $I_{K_r}$ .<sup>50</sup> Nevertheless, several differences exist between

cardiomyocytes from small animal models and human cardiomyocytes, including energetics, myofilament composition, beating rates, expression of key ion channels and electrophysiology as well as  $\text{Ca}^{2+}$  homeostasis. Therefore, animal model systems cannot completely replace human studies and results from animal models cannot be completely translated into the clinic.

## Heterologous expression systems

More physiological cell models that mimic genetic background are required for advanced mechanistic studies. Transfecting specific mutant genes identified from SQTs patients into cells can help understand the underlying pathophysiological mechanism and provide unique opportunities gaining insights into different forms of SQTs. A striking advantage of the heterologous expression system is that a selected channel gene cloned from other cells can be expressed in a cell that contains no endogenous channel of the same type so that the current conducted by the channel can be easily separated from other channel currents. Therefore, heterologous expression systems are more suitable for studying the impact of mutations on ion channel currents and channel gating kinetics. To date, there are some studies on the expression of specific mutant genes in primary cultured cardiomyocytes such as neonatal rat ventricular myocytes to assess the functional modulation of mutant channels. Over-expressed KV1.5 in cardiomyocytes of Sprague-Dawley rats on foetal 18–19 days extremely shortened APD and triggered rapid electrical activities. This suggests this model being able to be used to study the arrhythmogenic substrate of SQTs,<sup>51</sup> but the precise mechanism of rapid beatings of the myocytes with over-expression of Kv1.5 was not fully studied. Heterozygous overexpression of M301K, a KCNJ2 mutation, in neonatal rat ventricular myocytes exhibited markedly shorter APDs than the WT alone.

The human embryonic kidney cell line HEK293 is the most widely used cell heterologous expression system to study SQTs. A mutation (T618I) of the KCNH2 ion channel in a Chinese family caused a substantial gain-of-function of hERG channels in HEK 293 cells and it was found that both quinidine (5  $\mu\text{M}$ ) and sotalol (500  $\mu\text{M}$ ) had similar inhibitory effects on steady currents.<sup>26</sup> Transient transfection of E299V-Kir2.1 mutant into HEK293 cells presents an abnormally large outward  $I_{K1}$  at potentials above  $-55$  mV due to a lack of inward rectification and shortens the APD.<sup>37</sup> More mutations related to SQTs genotype have been transfected into HEK293,<sup>21,25,26,36,42,52–55</sup> which showed that this cellular model is an important approach in functional research. This heterologous model provides a good combination for studying molecular biology and electrophysiological functional characteristics.

A mutation (KCNH2-I560T), when expressed in COS-7 cells, showed a 2.5-fold increase in KCNH2 peak current density and a positive shift (+14 mV) of the inactivation curve.<sup>29</sup> Functional characterization of the CACNA1C-R989H mutation was conducted by co-expression of CACNB2B and CACNA2D1 in TSA201 cells, a human embryonic kidney cell line with SV40 transformed and combined with patch-clamp experiments.<sup>56</sup> In addition, the N588K mutation expressed in mammalian TSA201 cells shifted inactivation of HERG towards more positive potentials causing a functional increase in  $I_{Kr}$ .<sup>57</sup> *Xenopus* oocytes were used to study the gating mechanisms of expressed V141M KCNQ1/KCNE1

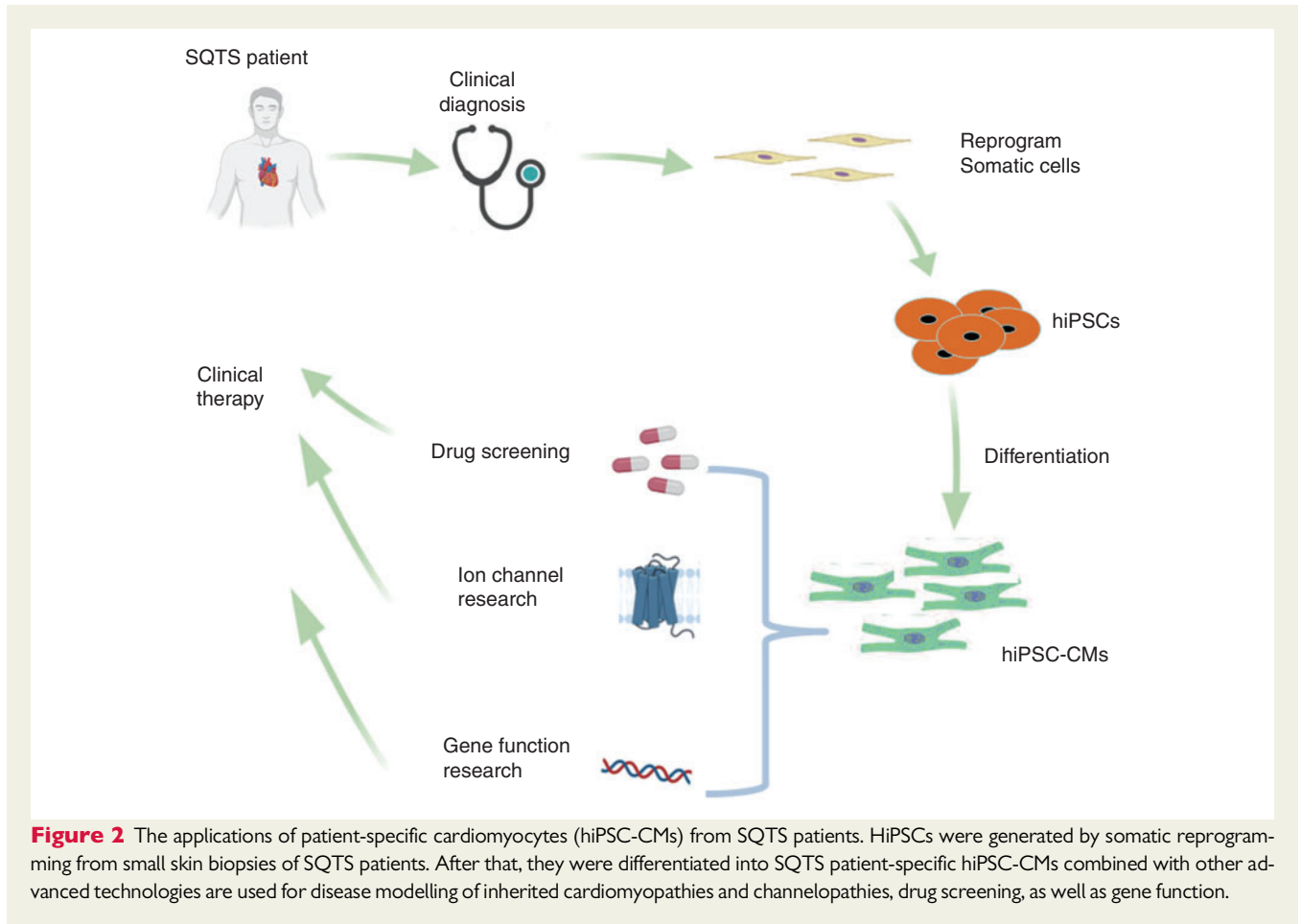
channels.<sup>31</sup> Of note, *Xenopus* oocytes are widely used as heterologous ion channel expression vectors. There are also plenty of meaningful research aspects related to SQTs using *Xenopus* oocytes<sup>58–60</sup> and Chinese hamster ovary (CHO) cells.<sup>33,35,55,61–65</sup> However, an important disadvantage of these cell models is the lack of macromolecular ion channel complexes existing in cardiomyocytes and the lack to generate APs. This leads to failed simulation of exact molecular and electrophysiological cardiomyocyte-specific phenotypes.

## Computational model simulations

Based on changes of ion channel properties seen in recombinant channel experiments, computational model simulations of the heart are beginning to offer a viable way and play a crucial role in the investigations of arrhythmias and anti-arrhythmic drug therapy. Since 2004, this model has provided a good platform for studying the pathophysiological mechanism of SQTs mutations at the cellular level, one-dimensional (1D) transmural ventricular strand model, heterogeneous two-dimensional (2D) tissue level, and heterogeneous three-dimensional (3D) organ scale.

A simple ventricular AP simulation model suggested that a gain of function of KCNH2 is leading to shortening of the APD and that arrhythmogenesis is being linked to both the gain of function and accelerated deactivation of the N588K HERG channel.<sup>66</sup> The simulation studies lay a foundation for understanding the behaviour in the multicellular tissue with complex interactions. However, the heart is both an electrical and mechanical organ and abbreviated repolarization in disease might affect the mechanical function of the heart. Stretch-activated channel current ( $I_{sac}$ ) attenuates the reduced ventricular cell contractility arising from SQTs potassium channel mutations and sustained stretch shortens the APD, providing a possible explanation for dissociation between the end of mechanical systole and ventricular repolarization.<sup>67–69</sup> Short QT syndrome type 2, especially the V141M mutation in KCNQ1 augments the APD dispersion across the whole transmural strand but simulated application of  $I_{K1}$  blocker improved transmural APD heterogeneity and increased QT intervals.<sup>31,70</sup> Computer simulations at various levels of integration using 1D, 2D, and 3D models of cardiac excitation and propagation were conducted, suggesting that the E299V mutation shortens the APD and the QT interval and increases ventricular re-entry vulnerability and the E299V mutation is associated with AF but not with VT/VF.<sup>37</sup> Zhang *et al.*<sup>70</sup> incorporated the channel kinetics of the V307L mutation in the KCNQ1 gene into human ventricular AP models and into 1D and 2D transmural tissue simulations, suggesting that the KCNQ1 V307L mutation is causally associated with QT interval shortening. However, the simulations showed limitations, such as some discrepancies between the simulated transmural APD dispersion and those observed experimentally, not considering complicated anatomical structure of ventricular wall and fibre orientations. In addition, the simulation only used a set of fixed parameters for the stimulus pulse.

Furthermore, computational modelling was used to investigate pharmacotherapeutic effects of anti-arrhythmic drugs on SQTs variants. Disopyramide, quinidine, and propafenone produced effective refractory period (ERP) prolongation in the setting of SQT1. The effect was greatest for quinidine which does not reduce APD



heterogeneity during  $\beta$ -adrenergic stimulation for the V141M mutation but was effective to terminate arrhythmic excitation waves involved in the V307L.<sup>31,32,71</sup> Simulated application of  $I_{K1}$  blocker improved transmural APD heterogeneity and QT interval widening suggesting specific  $I_{K1}$  blockade as a potential antiarrhythmic strategy in SQTs. Based on the Ten Tusscher's model for the human ventricular APs and the 2D tissue models with transmural heterogeneities, Luo *et al.*<sup>72–74</sup> showed that quinidine effectively prolonged APD, exhibiting significantly better therapeutic effects on SQT3 with D172N mutation than disopyramide and E-4031. In addition, they found that amiodarone is causing a QT interval prolongation, decreasing of APD dispersion and membrane potential dispersion ( $\delta V$ ) for D172N mutation on SQTs3. In addition, optogenetics-based treatment can be used to correct pathological abbreviation of atrial APs in SQTs.<sup>75</sup>

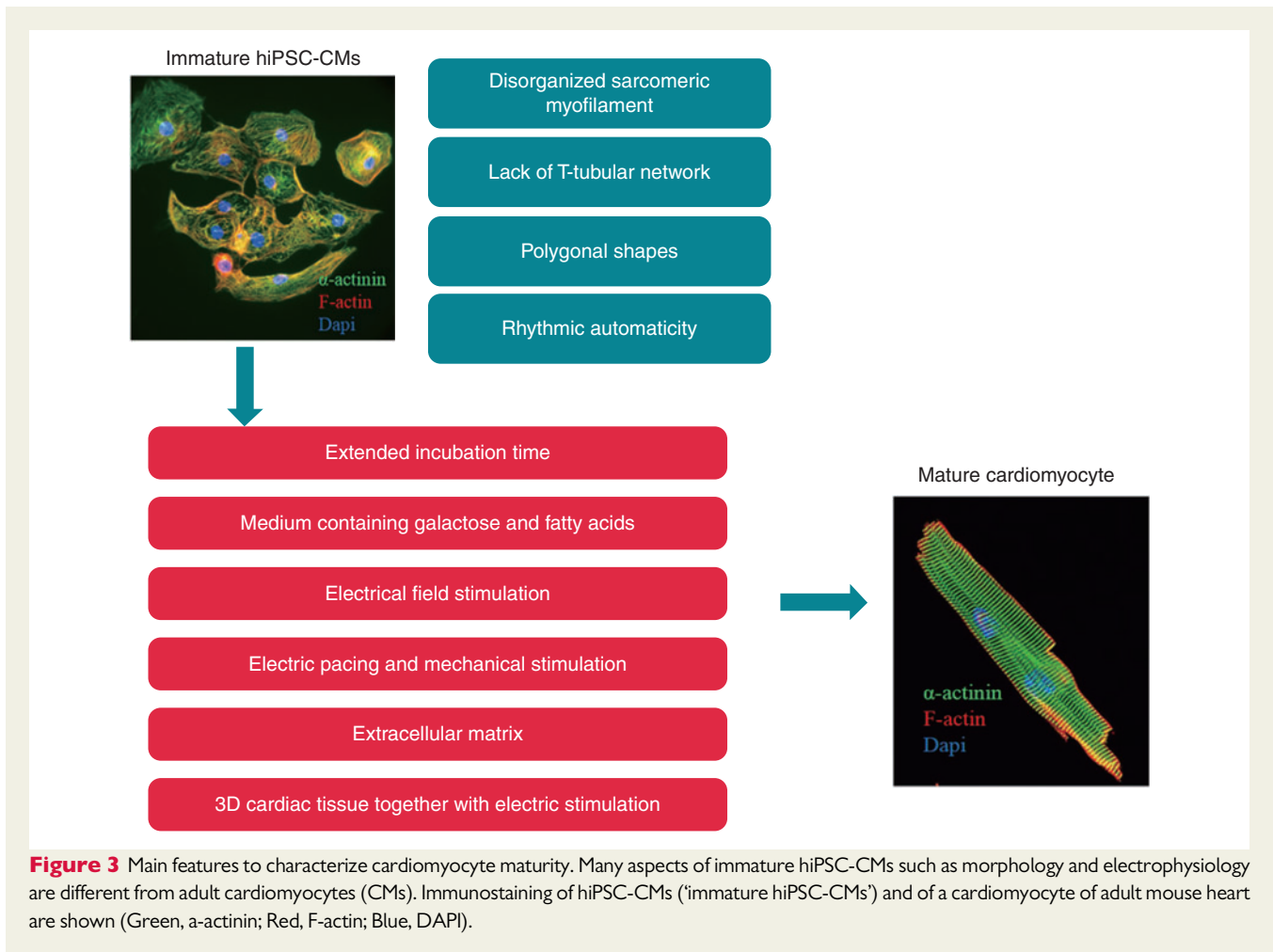
Animal models, the heterologous cell system and computational model simulations are of limited value in fully reflecting the response of human cardiomyocytes to drugs and studying the pathophysiological mechanism of the final comprehensive manifestation from patients around the world. In addition, human primary cardiac myocytes are difficult to obtain and maintain in culture *in vitro*. Thus, an easily accessible human-based cellular model with a higher translational relevance such as human stem

cell-derived cell lines may be more helpful and valuable to solve these issues.

### Human-induced pluripotent stem cell-derived cardiomyocyte

Human-induced pluripotent stem cell-derived cardiomyocytes are widely used in studying basic and profound mechanisms of cardiac channelopathies such as LQTS, familial dilated cardiomyopathy, familial hypertrophic cardiomyopathy, CPVT, arrhythmogenic right ventricular cardiomyopathy, and BrS.<sup>76–87</sup> Implementation of this unique and clinically relevant cell model shows a significant advantage in cardiovascular research. Human-induced pluripotent stem cell-derived cardiomyocytes may overcome limitations of animal models and human tissue restrictions and effectively recapitulate inherited arrhythmia, e.g. SQTs at the cellular level to provide an additional evaluation of human cardiac myocyte function and create a disease-specific model system for searching potential therapeutic targets. Therefore, hiPSC-CMs provide a promising and reliable cell source for disease modelling, tissue engineering, translational, and regenerative medicine (Figure 2).

The iPSC-CM technology has made it possible to make patient-specific cardiomyocytes efficiently available for various research



purposes. This technology provides unlimited specific cell types.<sup>88</sup> Therefore, a lot of research groups have extensively used patient-specific hiPSC-CMs for human cardiovascular disease modelling *in vitro*, drug screening and mechanistic studies. In addition, genome editing combined with patient-specific iPSC-CMs have allowed the identification of putative modified genes.

El-Battrawy et al.<sup>89</sup> described the first hiPSC model of SQTs1 using hiPSC-CMs derived from a patient carrying an N588K mutation in the KCNH2 ion channel, recapitulating the single-cell phenotype of SQTs. Short QT syndrome-hiPSC-CMs showed increased  $I_{Kr}$  density, shortened APD, abnormal calcium transients and increased KCNH2 expression at gene and protein levels. In addition, hiPSC-CMs from SQTs patients carrying the missense mutation T618I in KCNH2 exhibited also abnormal AP phenotype,<sup>89</sup> compared with control and gene-corrected hiPSC-CMs. The gene-corrected hiPSC-CMs had been generated by using the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats-associated 9) genome-editing technology.<sup>90</sup> These studies provide hiPSC-based models with opportunities to further elucidate SQT-related pathophysiological mechanisms and test effective treatment strategies. The introduction of clustered regularly interspaced short palindromic repeats (CRISPR) or transcription activator-like effector nucleases

(TALENs),<sup>90–92</sup> two highly efficient and relatively simple techniques to use gene-editing platforms, has facilitated the development of more advanced applications of hiPSC-CMs by altering gene expression and correct genetic variation.<sup>93,94</sup>

To date, the experimental research using the hiPSC-CMs model of SQTs mainly focuses on SQTs1 with N588K and T618I mutations in the KCNH2 ion channel. However, several other gene mutations can be causative for SQTs. Human-induced pluripotent stem cell-derived cardiomyocytes have become an important platform for preclinical drug tests, elucidating mechanisms of drugs effects, and identifying cardioprotective pathways that could be further explored for the development of new therapy strategies.

### Current limitations

Nevertheless, hiPSC-CMs have several limitations (Figure 3). Firstly, hiPSC-CMs are relatively less mature than isolated adult ventricular cardiomyocytes and mostly resemble cardiomyocytes in the embryonic state.<sup>95,96</sup> They mostly show a disorganized sarcomeric myofilament, lack of t-tubular network, polygonal shapes, and rhythmic automaticity.<sup>97</sup> Until now, there are several methods that have been used to stimulate the maturation of hiPSC-CMs, including extending cell culture time,<sup>98</sup> using a medium containing galactose and fatty



acids,<sup>99</sup> electrical field stimulation,<sup>100</sup> electric pacing and mechanical stimulation,<sup>101</sup> extracellular matrix,<sup>102</sup> and a combination of 3D cardiac tissue together with electric stimulation.<sup>103</sup> Secondly, the human heart consists of smooth muscle cells, endothelial cells, pericytes, leucocytes, and fibroblasts in addition to cardiomyocytes. The 3D tissue engineering platform can promote the application of hiPSC-CM in drug screening, disease modelling, and heart regeneration. The emergence and growth of hiPSC technology has led to countless progress and the generation of hiPSC-CMs<sup>106</sup> from SQTS has already provided many advances in the understanding of the genetic and molecular pathophysiology of cardiac disorders.

## Drug screening

The use of iPSC models has recently been successfully tested for the identification of mutation-specific drugs, especially for long QT syndrome.<sup>104,105</sup> A recent example of drug-repurposing has been the discovery that Lumacaftor, a drug used to treat cystic fibrosis, is able to re-establish the HERG trafficking defect in iPSC-CMs of patients with LQTS2 type 2 syndrome and shorten the AP *in vitro*.<sup>107</sup> Importantly, administration of lumacaftor plus ivacaftor in two LQT2 patients with the same trafficking defect resulted in shortening of QTc duration, even though the quantitative changes were much smaller than what observed *in vitro*.<sup>108</sup> These results seem promising, even if preliminary, and reinforce the notion that the iPSC model can be of help also for other channelopathies such as SQTS. Drug therapy may be the main modality to restore the normal QT interval and protect patients against arrhythmia. At present, little is known on its pharmacological treatment. Some new or already marketed drugs are being used to target-specific mechanisms underlying different SQTS types. The hiPSCs have provided a good platform for drug discovery and screening purposes.<sup>109</sup> Quinidine exerts a critical role in leading to prolongation of repolarization pattern, ERP, post-repolarization refractoriness (PRR) and reducing dispersion of repolarization, compared with flecainide and sotalol.<sup>43</sup> Hydroquinidine may not be suitable for all SQTS types, since the QT prolongation effect may depend on genotype.<sup>16,25,62,110</sup> But both quinidine and sotalol may be therapeutic options for patients with the T618I HERG mutation.<sup>26</sup> Quinidine exhibited significantly better therapeutic effects on SQT3 associated with the Kir2.1 D172N mutation than disopyramide and E-4031. This indicates that quinidine is not only helpful to terminate re-entry but also decreases the susceptibility of human ventricular tissue to the genesis of re-entry in computer simulation models.<sup>72</sup> Additionally, quinidine also exhibited significantly better therapeutic effects on SQTs1 related to N588K mutation in the HERG gene than E-4031 and disopyramide.<sup>111</sup> Furthermore, quinidine normalized the APD in hiPSC-CMs of an SQTs patient with T618I or N558K mutation in the HERG gene. Importantly, shortened APD phenotype observed in SQTs hiPSC-CMs was effectively rescued by the short-peptide scorpion toxin BmKkx2 targeting KCNH2.<sup>90</sup> Quinidine but not sotalol or metoprolol could normalize the abnormal rhythmic activities of SQTs-hiPSC-CMs derived from a patient carrying the mutations N588K and T618I in KCNH2.<sup>89,90</sup> In addition, ivabradine, mexiletine, and ajmaline but not flecainide, ranolazine, or amiodarone prolonged APD, inhibited KCNH2 channel currents significantly and reduced the epinephrine-induced

arrhythmic events in hiPSC-CMs from a SQTs1 patient.<sup>112</sup> Application of disopyramide normalized APD and suppressed arrhythmia induction by enhancing  $I_{Ca-L}$ ,  $I_{NCX}$ , late  $I_{Na}$  and reducing  $I_{SK}$  in SQTs1-hiPSC-CMs. These mechanisms may underlie the APD-prolonging and antiarrhythmic effect of disopyramide.<sup>113–115</sup> The researchers suggested that blocking Kir2.1 channels may be potential therapeutic for SQTs3. Lopez-Izquierdo *et al.*<sup>116</sup> showed that 3  $\mu$ M of the anti-malarial drug chloroquine normalized inward rectifier  $K^+$  current magnitude, prolonged APD and increased ERP by *in silico* modelling of the heterozygous WT-D172N Kir2.1 condition, suggesting therapeutic concentrations of chloroquine for treatment of SQT3. Whilst at therapeutic concentration amiodarone significantly increased the APD and the ERP at the single-cell level. It also prolonged the QT interval in pseudo-ECG and prevented the re-entry in tissue, showing anti-arrhythmic effects of amiodarone on SQT3.<sup>74</sup> Importantly, amiodarone is not only effective for SQT3, but also has a therapeutic effect on SQT2 and the effect is more obvious at high doses.<sup>117</sup> El Harchi *et al.*<sup>118</sup> characterized molecular determinants of disopyramide binding within the HERG channel inner cavity using a lanine mutants of HERG S6 and pore helix residues and MthK-based homology modelling and ligand docking. Disopyramide associated with QT prolongation in healthy probands may also be effective to prevent tachyarrhythmias in SQTs1-patients carrying the N588K mutation in the HERG channel by APD-prolongation via enhancing  $I_{Ca-L}$ , late  $I_{Na}$ ,  $I_{NCX}$ , and reducing  $I_{SK}$ .<sup>65,113</sup> Blocking  $I_{K1}$  with chloroquine will not only prolong the expected effect of APD, but could also lead to an increase in resting potential of cells. This side effect may cause arrhythmias not related to APD.

Frommeyer *et al.*<sup>44</sup> described a potential protective effect of both ranolazine and vernakalant therapy in an experimental whole-heart model in rabbits of SQTs, showing that ranolazine led to an increase of QT-interval, APD90 and ERP. Vernakalant showed similar results. Frommeyer *et al.*<sup>119</sup> revealed that ivabradine has anti-arrhythmic effects based on increasing both ERP and PRR. However, the used concentration of ivabradine in this study exceeds the clinical therapeutic concentration in heart failure patients. In addition, only a single dose was administered. Therefore, the application of ivabradine in the clinical setting may require further research. In addition, they analysed the use of mexiletine for eliminating ventricular arrhythmias in the isolated rabbit hearts.<sup>120</sup>

A new  $I_{K1}$  inhibiting compound Pentamidine-Analogue 6 (PA-6) had been developed, which is an efficient and specific  $I_{K1}$  inhibitor that interacts with the cytoplasmic pore region of Kir2.1 ion channel, encoded by the KCNJ2 gene. It inhibits Kir2.1 channels with V93I and D172N mutations and impacts channel expression at the plasma membrane. Thus it can be considered as a candidate drug in treating SQT3.<sup>121,122</sup> Hydrocinnamic acid (HA) as a natural compound from the traditional Chinese medicine has its own advantages, such as a slow affinity and low toxicity, competing with PIP2 to bind at the same sites and thus inhibits the Kir2.1 current.<sup>54</sup> Ellermann *et al.* showed significantly antiarrhythmic effects of antazoline which prevented VF in all hearts that were previously inducible in the setting of SQTs.<sup>123</sup> When using beta-blockers in SQTs patients, we should consider that carvedilol and metoprolol exhibit different effects in cardiomyocytes presenting mutations of SQTs1 or SQTs2. N588K-KCNH2 and V307L-KCNQ1 mutations can reduce the inhibition of carvedilol on  $I_{Ks}$  or  $I_{Kr}$  tail current, but increase the inhibition of

carvedilol on  $I_{Kr}$  at end-pulse and the inhibition of metoprolol on tail current and end-pulse current.<sup>55</sup> Mg(NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>3</sub>)<sub>2</sub>·H<sub>2</sub>O, a taurine-magnesium coordination compound (TMCC), exerted anti-arrhythmic effects with low toxicity. With respect to the potential anti-arrhythmic effects of TMCC on SQTs2, TMCC can extend the repolarization period and inhibit the repolarizing current- $I_{Ks}$  in SQTs2.<sup>124</sup>

With uniform expression of Channelrhodopsin-2 (ChR2) and non-attenuation of light, the optogenetic approach can correct the pathological shortening of AP caused by  $I_{K1}$  gain-of-function mutations. Compared with drug therapy, it can reliably restore AP to its non-disease state. Hence, optogenetic intervention will be an attractive method to replace drug therapy for SQTs.<sup>75</sup> Unfortunately, poor blue-light penetration in cardiac tissue and possible non-uniform distribution of light-sensitive cells are major barriers to the efficacy of optogenetics-based SQTs treatment. Therefore, additional experiments on drug screening for SQTs patients are warranted and more specific and patient-tailored therapies are still needed.

The effects of the above-mentioned drugs on different subtypes and mutations of SQTs will provide strong evidence for clinical use in the future. However, clinical medication should also take into account the differences between different individuals suffering from the same disease.

## Conclusion and future perspectives

Short QT syndrome is a rare and inheritable cardiac channelopathy leading to a shortened QTc and tachyarrhythmias with a high risk of AF, atrial flutter and life-threatening arrhythmias, even SCD. To date, different types of SQTs and more than 200 SQTs-patients with different gene mutations around the world have been reported. To have a better understanding and treatment of SQTs, personalized diagnosis and therapy should be implemented. Currently, the diagnostic and treatment approaches are still challenging due to the low prevalence of this disease. A large number of experimental studies have been conducted to explore its underlying mechanism, but so far, the exact pathophysiological mechanism of SQTs remains controversial. Nowadays, current guidelines recommend an ICD as the first and most effective therapeutic measure in patients who have experienced sustained VT/VF episodes or who are survivors of an aborted cardiac arrest. However, alternative therapies such as drugs are needed, especially in small children and in adults in whom ICD is not feasible or who suffer from repetitive ICD discharges.

The mechanisms of arrhythmogenesis in SQTs are not well understood. Different kinds of platforms and methods are available for studying this disease. The animal models, heterologous expression models and computer models play an important role in the process of studying the pathophysiological mechanisms of SQTs. Undeniably, each model has its own advantages and limitations. Among these models, hiPSCs-CMs from SQTs patients provide a reliable model for studying human genetic diseases *in vitro*, which may fill the gap between animal models and human clinical experiments. Despite several limitations, studies such as CRISPR/Cas9-mediated genome editing using hiPSC-CM-based platforms and tissue engineering technology will help to elucidate the pathogenesis of SQTs. Improving

the development and maturity of hiPSC-CMs, these platforms will also help to develop efficient drugs and to improve our understanding of the underlying disease mechanisms. To date, precision medicine has become very popular, providing many benefits such as a better understanding of pathogenesis and genotype–phenotype relationships in cardiac channelopathies.<sup>125</sup> Accordingly, we expect that, by further refining the accuracy of all different layers used by precision medicine, in the future it will be possible to develop more patient-specific approaches for SQTs therapy and clinical handling.

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