Chapter 12. Genome Editing and Inherited Cardiac Arrhythmias

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Abstract

Inherited arrhythmic disorders are a group of heterogeneous diseases predisposing to life-threatening arrhythmias and sudden cardiac death. Their diagnosis is not always simple due to incomplete penetrance and genetic heterogeneity. Furthermore, the available treatments are usually invasive and merely preventive. Genome editing and especially CRISPR/Cas9 technologies, have the potential to correct the genetic arrhythmogenic substrate, thereby offering a cure for these fatal diseases. To date, genome editing has allowed reproducing cardiac arrhythmias *in vitro*, providing a robust platform for variant pathogenicity, mechanistic and drug-testing studies. However, *in vivo* approaches still need profound research regarding safety, specificity and efficiency of the methods.

Key words: Sudden cardiac death, primary arrhythmia, channelopathy, gene editing, CRISPR/Cas9, LQTS, Brugada syndrome, CPVT, SQTS

Introduction

Sudden cardiac death (SCD) in young individuals with anatomically normal hearts has been reported for decades[1]. Due to inability to identify a causal relationship, this SCDs were initially termed as “idiopathic ventricular fibrillation”[2]. However, the discovery of the first long QT syndrome susceptibility genes in the late 90s had transformative effects in their management, introducing what we now call primary arrhythmia syndromes[3–5]. These inherited arrhythmic disorders have a low prevalence and patients suffer from increased predisposition to life-threatening arrhythmias, which arise spontaneously or upon a trigger in the absence of structural cardiac abnormalities. As most of the genes affected encode cardiac ion channels, they are also referred to as cardiac

channelopathies. Their diagnosis is not always simple due to incomplete penetrance and genetic heterogeneity. The available treatments are merely preventive and involve life-long or invasive approaches like the implantable cardiac defibrillator (ICD) or left cardiac sympathetic denervation (LCSD) [6]. This is a big challenge for clinicians, whose decision may have life-altering consequences for the patients, especially for those with an inconclusive diagnosis. For these reasons, the lack of highly effective pharmacological treatment makes inherited cardiac arrhythmias the perfect candidate for genome editing based approaches, potentially reversing the genetic substrate and offering a 'cure' for the disease.

As the availability and use of genetic testing increases, so does the probability that rare variants of uncertain significance (VUS) are found. Regarding channelopathies, to date genome editing has mainly been used to either directly introduce the desired VUS mutation in *in vitro* models, particularly human induced pluripotent stem cells (hiPSCs), or to generate the proper isogenic controls from patient-derived lines. Both approaches result in isogenic sets of cells, allowing the elimination of epigenetic differences and unknown genetic modifiers that may introduce phenotype variability (Figure 12.1). Consequently, genome editing and especially CRISPR/Cas9, provides a robust platform to study genotype-phenotype correlations, being able to identify causality or association of the variant to the disease. Furthermore, this system allows molecular and mechanistic studies, identification of regulatory elements and comparative studies of different mutations.

In summary, this chapter focuses on the knowledge that CRISPR/Cas9 technologies have helped acquire regarding the four major channelopathies: long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and short QT syndrome (SQTS).

Long QT Syndrome

Long QT syndrome (LQTS) is the most frequent primary arrhythmia with a prevalence of up to 1:2000. The inheritance is autosomal dominant, although some very rare and extremely severe recessive variants have also been described[7, 8]. LQTS comprises a heterogenous family of diseases characterized by a prolonged QT interval and T-wave abnormalities in the electrocardiogram (ECG) (Figure 12.2b). Especially upon adrenergic stimulation, these patients are at a high risk of ventricular tachycardia, which can end in sudden cardiac death due to Torsade de Pointes.

To date, congenital LQTS has been classified based on mutations associated with up to 17 genes[9]. The QT prolongation arises mainly from loss of function mutations of the K^+ channels, which cause a decrease in repolarizing potassium current in phase 3 of the action potential; or gain of function mutations of Na^+ and Ca^{2+} channels, which cause a late inward entry of positive ions in the cardiomyocyte. LQT1-3 subtypes comprise about 75% of all the patients with LQTS and affect KCNQ1, KCNH2 and SCN5A genes, respectively[10]. KCNQ1 and KCNH2 both encode for the alpha-subunits of K^+ channels conducting the slow and rapid delayed rectifying current, I_{ks} and I_{kr}. SCN5A, on the other hand, encodes for the alpha-subunit of the cardiac sodium channel, conducting the depolarizing sodium inward current (I_{Na})[11]. These major genes were first identified in 1995[3–5] and, as 20% of LQTS remained genetically elusive, the past 25 years have experienced an exponential growth in publications reporting LQTS-associated genes and mutations, describing more than 600 genetic variants[10, 12].

In this race for genotype-phenotype association, the advances in hiPSCs culture and differentiation together with CRISPR-Cas9 genome editing tools have undoubtedly provided an easy and rapid method to study the causality of genetic variants in a dish. For example, the missense mutation T983I in KCNH2 was initially classified as VUS due to very low population frequency and lack of prior clear phenotypic data. Isogenic sets of cells consisting of patient-derived and CRISPR/Cas9 corrected hiPSC-Cardiomyocytes (hiPSC-CMs), allowed the reclassification of this variant to likely pathogenic. The mutant cells showed prolonged action potential (AP), reduced I_{Kr} and a greater propensity to proarrhythmia upon high risk torsadogenic drugs. On the other hand, correction of the mutation through genome editing restored the aberrant cellular phenotype. In a complementary set of experiments, the mutation was introduced in homozygosis in healthy hiPSCs, getting rid of the patient's genetic background, and hallmark features of the LQTS disorder were again recapitulated[13]. Missense mutations in KCNH2, also called hERG, usually have a dominant negative effect and result in inappropriate maturation of the potassium channel and reduced I_{Kr}. The dominant mechanism associated with the protein loss of function has been reported to be the generation of trafficking deficient channels[14]. To gain further insight, a missense mutation known to cause LQT2 was introduced in homozygosis in control hiPSC-CMs using CRISPR/Cas9, and compared to unedited cells and heterozygous patient-derived hiPSC-CMs[15]. hERG immunostaining showed similar intracellular presence of the channel for all the cells but reduced fluorescence intensity in the plasma membrane in both LQT2 models, suggesting

trafficking defects. This same mutation had been previously studied in heterologous systems, and its transient expression showed insufficient processing in the Golgi apparatus[14]. Therefore, the reduced I_{Kr} and prolongation of AP duration (APD) phenotype observed in the mutant hiPSC-CMs may be the result of a non-glycosylated hERG that fails to be transported to the plasma membrane[16]. The fact that the mutation in homozygosis aggravated the phenotype, supports not just its pathogenic role, but also the use of patient-independent models to confidently study disease pathogenesis.

VUSs in less common LQTS-associated genes have also been studied with this same approach. The R518C mutation in the CACNA1C gene was reported to be the genetic substrate of cardiac-only timothy syndrome (TS)[17, 18]. TS is a very rare and severe variant of LQT8 in which there is a coexistence of LQTS, cardiomyopathy and extracardiac phenotypes. The affected gene encodes for the heart's voltage-gated L-type calcium channel, LTCC. The ion current studies of patient-derived hiPSC-CMs (CACNA1C-R518C) and its CRISPR/Cas9 corrected isogenic control showed that this gain-of-function mutation is sufficient to cause the patient's QT prolongation due to an increase in LTCC late current and delayed calcium transient resolution. This prolongs the plateau phase of the myocyte action potential, leading to delayed repolarization, and is the monogenetic substrate for the LQTS phenotype in the patient[19]. Furthermore, a patient-independent model in which another CACNA1C VUS was introduced into hiPSCs using CRISPR/Cas9 also showed prolonged AP due to reduced LTCC voltage-dependent inactivation[20]. Not only do these results support the pathogenicity of this specific variant but also, together with the previous report, support that CACNA1C is a susceptibility gene for LQTS.

Taking a step further, Yoshinaga et al. proposed the use of these patient derived hiPSC-CMs together with their corresponding CRISPR/Cas9 corrected controls to develop a novel method for LQTS phenotype-based classification. It consists of specific currents blockade and their electrophysiological assessment using multielectrode array systems (MEA), which allow multiple simultaneous recordings at once. Studying cells from patients suffering from the 3 main subtypes of LQTS and their CRISPR/Cas9-corrected controls, they observed that LQT1-3 could be distinguished by I_{Ks}, I_{Kr} and I_{Na} blockade, respectively [21]. This strategy reduces variability compared to traditional single cell patch clamp recordings, allowing the detection of subtle electrophysiological differences. Therefore, it could potentially allow high throughput screening, efficient recognition of pathogenic variants and phenotype-based diagnosis of LQT subtypes.

Combination of the patient-independent platform together with MEA has also been used to observe intragenotype differences in disease severity attributable to the KCNH2 mutation site. Heterozygous missense mutations known to affect the pore-loop domain (KCNH2-A561T) or the cytoplasmic tail of hERG (KCNH2-N996I) were introduced into control hiPSCs using CRISPR/Cas9. Action potentials (APs) and field potentials (FPs) were recorded using both patch clamp and MEA in single cells and confluent monolayers of hiPSC-CMs, respectively. Furthermore, to mimic the triggering factors that induce arrhythmic events in LQTS, the researchers examined the behavior of the cells upon inhibition with the I_{Kr} blocker E-4031. In summary, the pore-loop mutation had longer APs and FPs and a higher risk of developing an arrhythmic cardiac event upon stimulation with a triggering factor[22]. These results are in line with the fact that pore mutations cause a more severe clinical course due to a dominant negative effect, while usually, C-term mutations cause haploinsufficiency and therefore less severe phenotypes[23]. Although a larger panel of KCNH2 mutations should be assessed to further evaluate this scoring system, it appears to be sufficiently sensitive to detect subtle intragenotype-phenotype mutational differences and could have clinical implications in diagnosis, prognosis and risk stratification of LQTS patients[22].

In combination with next generation exome sequencing, genome editing also enables the identification of plausible genetic causes for families with genotype-phenotype discordances. A large Cleveland family that was studied for 20 years showed a homogenous LQTS population carrying the LQT2 KCNH2-R752W mutation. Nevertheless, out of 26 mutation positive members, only 6 had severely affected phenotypes, making it so variable that clinical analysis did not allow an accurate diagnosis of those individuals carrying these mutations[24]. Whole exome sequencing analysis identified a variant in the GTP-binding protein REM2, common for the severe phenotypes. REM2 encodes for a member of the Ras superfamily, which are well-known modulators of voltage-gated calcium ion channels, suggesting it could be a promising modifier gene in LQTS[25]. Five patients were selected from this family and as their hiPSC-CMs were able to reproduce phenotype discordances, Chai et al. used a CRISPR/Cas9 strategy based on homologous recombination to correct the REM2 variant in the cells from severely affected individuals. The hiPSC-CMs showed enhanced LTCC and prolonged action potentials that were successfully reversed upon genome editing. Therefore, they linked the REM2 gene variants to arrhythmias and concluded that the REM2-driven increased L-type Ca^{2+} current in combination with primary KCNH2

haploinsufficiency is the permutation that produces the full-blown disease phenotype[26]. A similar situation was studied recently in a family in which both father and son were carriers of the same Y111C missense mutation in KCNQ1 gene, but presented opposite clinical phenotypes. The functional and molecular study of their hiPSC-CMs showed impaired trafficking and increased degradation of the mutant KCNQ1 protein in the symptomatic (S) patient. In contrast, for the asymptomatic (AS) patient, the degradation was reduced as a result of a reduced activity of Nedd4L, which is involved in channel protein degradation via the proteasome. Whole exome sequencing found 2 single nucleotide variants (SNVs) on a Nedd4L interactor gene, MTMR4, present in the AS patient and his two siblings, also AS carriers. Correction of the SNVs in AS cells using CRISPR/Cas9 unmasked the LQTS phenotype, showing reduced I_{Kr} density. Furthermore, they confirmed that their presence reduced MTMR4 dephosphorylation activity, thus blunting the proteasomal degradation of KCNQ1 mediated by Nedd4L. In a separate cohort, they found that the same MTMR4 variants were present in 77% of AS Y111C mutation carriers, additionally supporting their protective effect and their role in the incomplete penetrance of Y111C-LQT1[27].

As we mentioned before, dealing with incomplete penetrance is one of the major hindrances to effective clinical diagnosis. At the molecular level, multiple mechanisms may be responsible for the penetrance heterogeneity in LQTS. Introduction of a very low penetrance SCN5A mutation in hiPSCs through CRISPR/Cas9 showed prolonged action potentials and arrhythmogenic delayed afterdepolarizations. The LQT3 phenotype was reversed by using PIP_3, a known sodium late current modulator. This is consistent with the results obtained in heterologous expression systems, in which PIP_3 could also reverse the late current phenotype in this variant. However, a fully penetrant SCN5A mutation did not show sensitivity to PIP_3. Therefore, this penetrance differences from almost 0% to 100% may be the result of distinct molecular mechanisms, which need to be considered when interpreting the severity of a late current derived from sodium channels functional defects[28].

Although gene correction is the most appealing application of CRISPR/Cas9, knocking out or down genes is also possible. This approach is especially interesting in those diseases affecting redundantly expressed genes. That is the case for calmodulinopathies, since the human genome harbors 3 distinct genes encoding for an identical calmodulin protein (CALM1-3). This protein is a ubiquitous Ca^{2+} sensor that modulates several ion channels, including LTCCs, which inactivation is promoted by the formation of Ca^{2+}-

CaM complexes. As calmodulin is also an LQTS susceptibility gene, in 2017, two groups used this approach to investigate CALM2-LQT15 mutations. On the one hand, Limpitikul et al. used a CRISPRi system in which a dead Cas9 is fused to a suppressor, allowing downregulation of the target gene and avoiding double strand breaks that could permanently alter off-target or downstream elements in the genome. The CRISPRi suppressed patient-specific iPSCs, normalized the prolonged APD and corrected fully the magnitude of LTCC's Ca2+-CaM dependent inactivation. Furthermore, it provided additional evidence that mature cells like cardiomyocytes could potentially be targeted by this approach[29]. On the other hand, Yamamoto et al. leaned towards a mutant allele specific ablation in another LQT15 model of patient-derived iPSCs. This approach used a Cas9 double nickase system to reduce off target effects and premature stop codons. They achieved the rescue of the electrophysiological abnormalities of the LQT15-hiPSC-CMs, indicating that the mutant allele caused dominant negative suppression of LTCC inactivation, resulting in prolonged AP duration[30]. In contrast to the former strategy, this allele-specific approach does not affect the WT allele, and therefore can be used in any other dominant negative disease with no need for genetic redundancy. Both strategies hold great promise in the treatment and diagnosis of LQTS and other inherited diseases, whose management is moving into the realm of precision medicine.

Brugada Syndrome

Like LQTS, Brugada syndrome (BrS) belongs to the group of inherited primary arrhythmia syndromes, predisposing to ventricular fibrillation and sudden cardiac death in the absence of structural heart abnormalities. This channelopathy is characterized by a coved-type ST segment elevation in the right precordial leads of the ECG (Figure 12.2c), occurring spontaneously or upon the intravenous administration of class I antiarrhythmic drugs[31]. The main gene associated to BrS is SCN5A, the alpha subunit of the voltage gated Nav1.5 cardiac sodium channel responsible for phase 0 of the cardiac action potential. More than 350 rare variants have been identified in SCN5A, accounting for 30% of the diagnosed cases [32]. Although BrS remains to be classified as a monogenic disease, incomplete penetrance and variable expressivity suggest a complex mode of inheritance, and most of these genetic variants remain of questionable causality[33]. More information about the pathophysiological mechanism of the disease is needed in order to develop BrS specific treatments, for which the only proven therapeutic option is ICD[6].

BrS genotype-phenotype associations have been studied using genome editing. One of the SCN5A variants examined showed reduced inward sodium current (I_{Na}), abnormal Ca^{2+} transients and increased triggered activity in patient-derived hiPSC-CMs, reproducing the single cell phenotype features of BrS. When this variant was corrected to wild type with CRISPR/Cas9, the maximal upstroke velocity and inter-beat variability were ameliorated, resulting in an improvement of the proarrhythmic phenotype and the disturbances found in AP recordings and Ca^{2+} imaging[34]. In another SCN5A variant, a patient-independent approach was used to study causality of the mutation, irrespective of the patient's genetic background. The loss of function BrS A735V-SCN5A variant was introduced in homozygosis in control hiPSC-CMs using CRISPR/Cas9. Apart from observing strongly reduced upstroke velocities and abnormal APs associated to the mutation, they identified a shifted activation curve of Nav1.5 channels that represents a key mechanism underlying the pathology of the variant[35]. In short, both approaches found evidence to support the association of the mutations to the observed BrS phenotypes.

Furthermore, usage of isogenic pairs of cells has enabled the identification of new BrS susceptibility genes. Whole exome sequencing of a large pedigree with BrS and history of SCD identified a rare non-synonymous variant (R211H) in RRAD, a gene encoding the RAD GTPase, present in all the affected members of the family. Insertion of the variant in an extra-familial control iPSC line with CRISPR/Cas9 technology recapitulated the same phenotype of patient-derived hiPSC-CMs, including persistent Na^{+} current and cytoskeleton disturbances. This confirms the involvement of the RRAD variant in the BrS phenotype, thus identifying a new BrS susceptibility gene[36].

As we have already observed, I_{Na} reductions are characteristic of BrS. Therefore, understanding the molecular mechanisms underlying this reduced current could be of help in the search for potential therapeutic options. Wnt/β-catenin signaling, which is active in heart disease, has been reported to potently inhibit Nav1.5 expression in both neonatal and adult rat cardiomyocytes[37]. Furthermore, chromatin immunoprecipitation showed that TCF4, a downstream effector of the pathway, had binding sites in the SCN5A promoter. Therefore, CRISPR/Cas9 genome editing has been used to induce mutations within these TCF4 binding sites in neonatal rat ventricular myocytes, showing attenuated Wnt-inhibition of SCN5A and demonstrating that those sites were functionally important for Wnt regulation of SCN5A[38]. All in all, strategies to block this intracellular cascade would represent novel methods for cardiac-specific inhibition of the Wnt pathway to

rescue I_{Na} and prevent SCD. Following the regulation of Nav1.5, a conserved regulatory cluster with super enhancer characteristics has been identified downstream of SCN5A. It drives localized cardiac expression and contains conduction velocity associated variants, including BrS variants[39]. Deletion of its component regulatory elements using genome editing in the one cell stage of mouse embryos showed that the cluster and its individual components are selectively required for cardiac SCN5A expression, normal cardiac conduction and normal embryonic development. These studies reveal physiological roles of an enhancer cluster in the SCN5A-SCN10A locus that controls chromatin architecture and SCN5A expression. Thus, alteration of its activity by genetic variants like the ones found in BrS may contribute to the disease phenotype[40].

Catecolaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is the third of the main primary arrhythmia syndromes, characterized by adrenergic-induced bidirectional and polymorphic ventricular tachycardias (Figure 12.2d) in the absence of structural cardiac abnormalities[41]. Treatments for CPVT include beta-blockers, Flecainide and ICD[6]. Two main types of CPVT have been described: an autosomal dominant disease affecting the RyR2 gene (CPVT1)[42], and a less common recessive form involving the CASQ2 gene (CPVT2)[43]. The RyR2 gene encodes the cardiac ryanodine receptor, which is the main intracellular calcium release channel. On the other hand, CASQ2 is involved in the regulation of the RyR2 activity. Mutations in these proteins therefore are associated with defects in Ca^{2+} handling by the sarcoplasmic reticulum (SR) and underlie the pathophysiology of the disease[44]. CPVT1 accounts for approximately 60% of the cases, while CPVT2, usually more severe, accounts for 10-15%[45]. The remaining CPVT cases are due to mutations of known or unknown origin. In this regard, other genes like triadin, calmodulin and TECRL are also being studied as potential susceptibility genes[46–48].
As for the other channelopathies, genome editing tools have helped in generating robust models for studying CPVT *in vitro*. As such, a CPVT1 model generated with CRISPR/Cas9 exhibited aberrant Ca^{2+} signaling properties indistinguishable from those previously recorded in cells derived from patients carrying the same mutation[49, 50]. This supports the pathological effect of the variant as well as the feasibility of the patient-independent model.

With respect to the molecular mechanism underlying CPVT, RyR2 mutations result in an abnormal protein that is prone to spontaneous calcium release from the SR, drives depolarizing Na^{+}-Ca^{2+} exchange, and results in afterdepolarizations that can trigger subsequent action potentials, causing ventricular ectopy and arrhythmias[45]. Gene editing studies in CPVT have been centered into understanding these imbalances in calcium homeostasis, which are triggered upon catecholaminergic stimulation. Studies in mouse models have shown that CaMKII-mediated phosphorylation of RyR2 is able to promote ventricular arrhythmias and its inhibition has proven to be successful in preventing arrhythmogenesis in several CPVT1 mutations *in vitro* and *in vivo*[51–53]. The use of genome editing has further supported this hypothesis, identifying a serine (RYR2-S2814) that induces CPVT1. As clinical arrhythmias emerge from the collective behavior of cardiomyocytes assembled into myocardial tissue, researchers developed a bidimensional model using integrated muscular thin films (MTF) from patient-derived and CRISPR/Cas9-introduced R4651-RyR2 hiPSCs. Together with optogenetics, this enabled simultaneous assessment of myocardial Ca^{2+} transient propagation and contraction. Both patient-derived and engineered MTFs reproduced the CPVT phenotype at the tissue level and implicated CaMKII as a key signaling molecule in the pathogenesis of CPVT. To further study the mechanism of reentry, they used genome editing to replace a critical target serine of CaMKII with alanine (S2814A) in RYR2 alleles, in both WT and R4651I background. By doing so, they blocked the phosphorylation event and observed normalized pacing- and isoproterenol-induced Ca^{2+} propagation speed heterogeneity and relative diastolic Ca^{2+} level, resulting in a substrate that is less vulnerable to reentry[54].

Comparative analysis of different disease-causing mutations can also be performed using genome editing. Recently, Zhang et al. explored 3 mutations introduced by CRISPR/Cas9 in different domains of the RyR2 to determine whether the molecular mechanism underlying their pathological effect is dependent on the specific RyR2 mutation site. The mutations where located at the N-terminus, C-terminus and central domains of the protein. All three mutants exhibited CPVT phenotype with prolonged calcium releases. However, in the C-ter and central domain mutations, the SR Ca^{2+} leak was significantly increased and the SR Ca^{2+} content was reduced compared to control cells or the N-ter mutant, which showed moderate leak and Ca^{2+} content. In the C-ter domain this might be explained by the higher fractional Ca^{2+} releases and calcium-induced calcium release (CICR) gains observed. Furthermore, dantrolene, reported to bind to RyR2 N-ter domain,

was more effective in suppressing the SR leak and aberrant Ca^{2+} releases in the C-ter mutation. Although no other drug tested showed mutation-site specificity, these results suggest that the treatment of CPVT1 should move towards personalized medicine, applying mutation specific pharmacotherapy[55].

Short QT syndrome

As opposed to LQTS, short QT syndrome is characterized by a shortened QT interval as a consequence of abbreviated ventricular repolarization (Figure 12.2e). Pathogenic mutations have been identified in both potassium and calcium channel genes and at least 6 subtypes of SQTS have been reported. Like the rest of the primary arrhythmia syndromes it predisposes to life-threatening ventricular arrhythmias and sudden cardiac death. The treatments of choice for SQTS are the class Ia antiarrhythmic drug quinidine or ICD implantation[6].

SQTS is one of the rarest and less studied channelopathies, therefore models for this syndrome developed with genome editing are scarce. Nevertheless, as for the above-mentioned diseases, it has been demonstrated that the phenotype of SQTS can be reproduced *in vitro* in single cells. Compared to its gene corrected isogenic control, SQTS cells (KCNH2-T618I) showed shortened action potential duration and increased beat-beat interval variability. In addition, this particular missense mutation produced gain of function of KCNH2, with increased I_{Kr} and protein expression in the membrane[56].

However, more complex electrophysiological phenomena, such as conduction and re-entrant arrhythmias, need to be studied in the whole tissue, rather than in individual cells. Cardiac cell sheets (CCSs) provide a bidimensional approach that can overcome this restraint[57]. CCSs from SQTS patient-derived and gene corrected hiPSCs allow to study the mechanisms underlying SQT pathophysiology. This approach was used to investigate the most common mutation causing SQTS, KCNH2-N588K. It recapitulated the SQTS disease phenotype in both cells and tissues, including a shortened APD and wavelength, increased susceptibility for induction of re-entrant arrhythmias, and increased arrhythmia complexity as observed by optical mapping in the CCSs. To validate this tissue model further, the effects of several potential SQTS therapies were screened. Interestingly, despite being able to prolong AP in both healthy and isogenic control hiPSC-CMs, sotalol did not show effects on CCSs[58]. This reinforces the importance of using tissue models over single cell ones while studying arrhythmogenic diseases, since sotalol also failed to produce QTc prolongation in SQTS patients[59].

Summary and Future perspectives

For primary arrhythmias, the first report using genome editing dates back to 2014, when zinc finger nucleases were used to correct an LQTS mutation *in vitro*[60]. A couple years later, coinciding with CRISPR/Cas9 bursting applications, more groups interested in arrhythmias slowly started to consider the use of genome editing. Regarding *in vitro* models, we have seen that the generation of isogenic control cells with CRISPR/Cas9 has allowed the identification of new susceptibility genes and variants. Furthermore, the patient-independent approach in which the mutation is introduced by CRISPR/Cas9 in control hiPSCs is much cheaper and rapid than obtaining patient-derived cells for evaluating VUSs. This approach does not require access to human samples, making feasible even *post mortem* studies.

Genetic heterogeneity is very common in channelopathies and as a result, more and more mutations are being discovered and added to the potential list of variants susceptible for genetic testing. Although both patient-dependent and -independent *in vitro* approaches can support the pathogenicity of a variant, further robust scientific and statistical evidence of disease causation must be considered in order to include them in routinely used clinical screening[61, 62]. In addition, even though huge progress has been made into understanding the pathogenesis of inherited cardiac arrhythmias, the recommendations for therapeutic interventions have barely changed in the last four decades[63], including beta-blockers, LCSD or ICD[6]. Being able to introduce distinct disease-causing mutations while keeping the same genetic background has also allowed unbiased comparison of multiple variants. This comparative analysis showed that different mutations in the same gene might be the consequence of distinct molecular mechanisms, reinforcing the concept that the treatment of inherited arrhythmias needs to move into the realm of precision medicine and patient-specific approaches.

Another point worth mentioning is that despite the fact that missense variants are relatively easy to be corrected by CRISPR/Cas9, targeting complex mutations, such as double heterozygosity, may pose additional challenges that still need to be surpassed[64]. Additionally, CRISPR/Cas9 itself has several limitations that have delayed the application of genome editing *in vivo* to the treatment of cardiac arrhythmias. Correcting a mutation requires the activity of the homologous recombination cellular machinery, which is downregulated in terminally differentiated cells like cardiomyocytes, thereby

reducing the chances of success. Furthermore, correction of only a small number of cells might trigger proarrhythmic events and even worsen the patients' clinical scenarios[65]. All in all, what we have learnt from these recent studies of primary arrhythmia syndromes and genome editing is that these diseases can be successfully reproduced in a dish, showing defective ion currents and providing a useful platform for molecular, comparative and drug-testing studies. Although very promising, this technology is still very young and translating it from bench to beside will need additional research to improve safety, efficiency and specificity of the methods. Hopefully, in the upcoming years, more *in vivo* CRISPR/Cas9 research in cardiac channelopathies will help us see its potential to cure these diseases and make precision medicine a reality.

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Figures

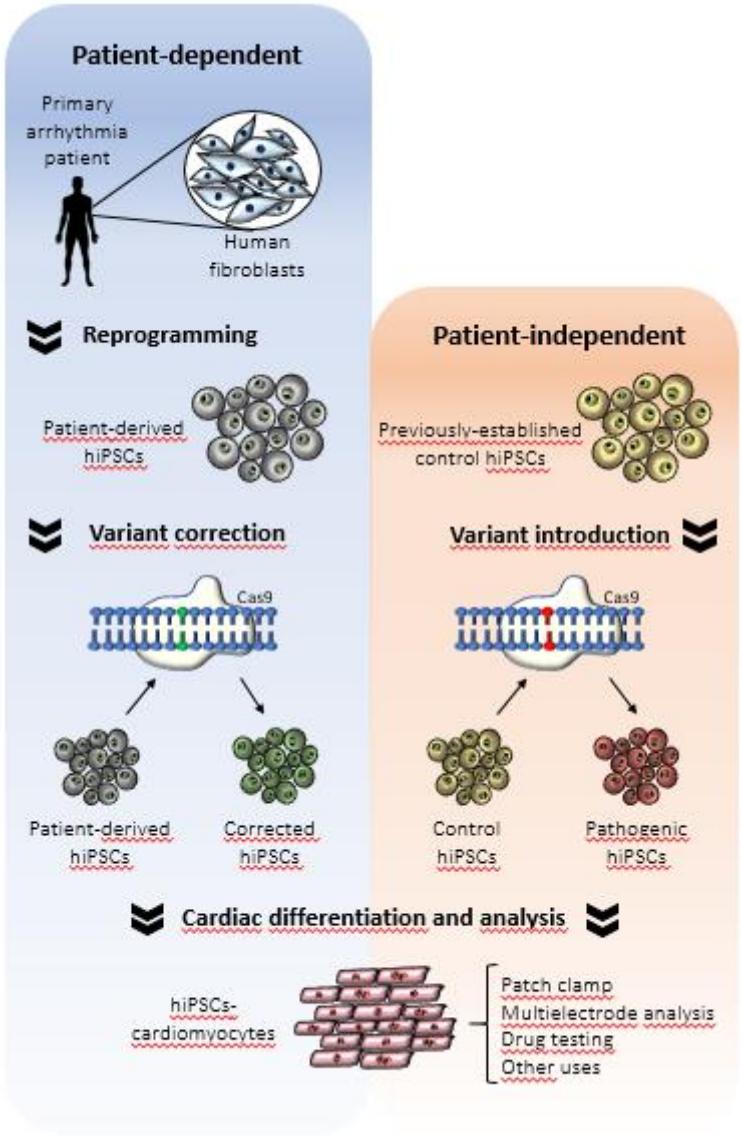

Figure 12.1. Comparison of patient-dependent and independent hiPSCs models in primary arrhythmias. Patient-dependent approach (left) in which the hiPSC line is generated *de novo* from affected fibroblasts and CRISPR/Cas9 genome editing is used to generate the isogenic controls by correcting the variant. A much more rapid patient-independent approach (right) uses previously-established control hiPSCs to introduce the mutation with CRISPR/Cas9. After cardiac differentiation, both models are ready for analysis.

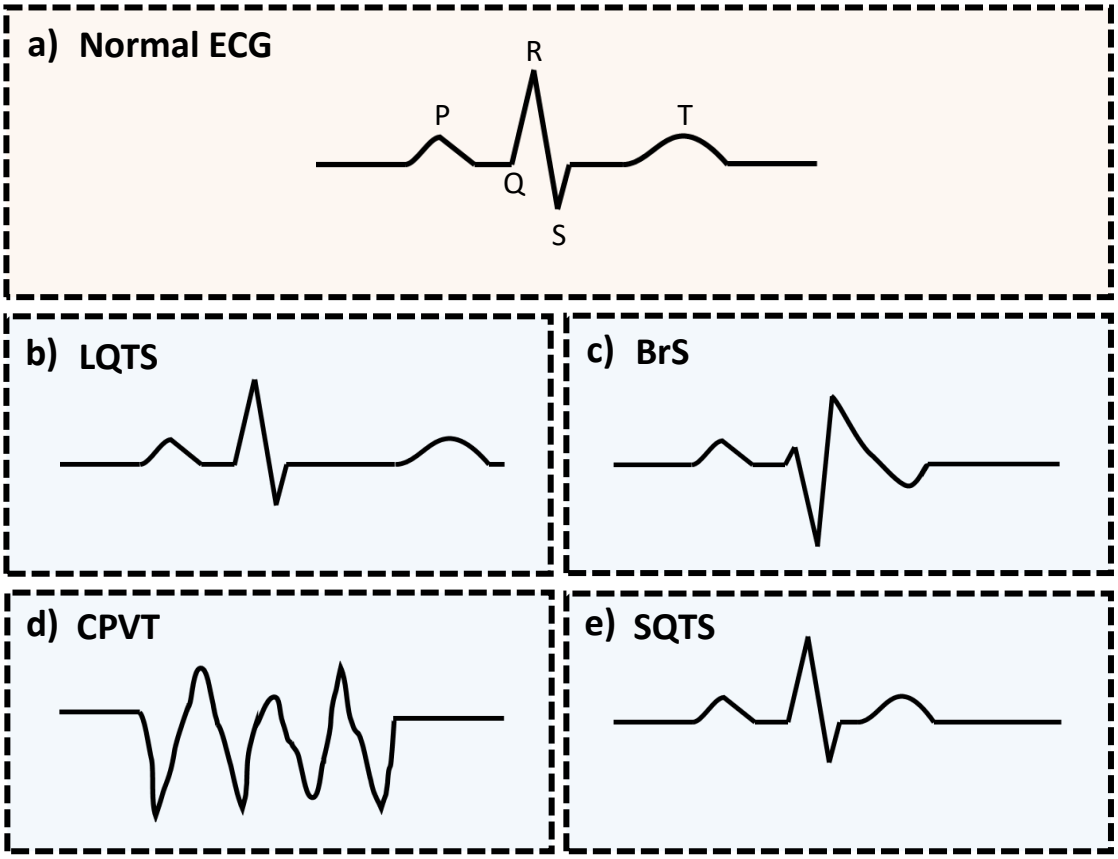

Figure 12.2. Electrophysiological characteristics of the main cardiac channelopathies. a) Normal ECG. b) Prolonged QT interval in long QT Syndrome (LQTS). c) Coved-type ST segment elevation in Brugada Syndrome (BrS). d) Shortened QT interval in Short QT Syndrome (SQTS). e) Polymorphic ventricular tachycardia in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT).