

Induced pluripotent stem cell technology and inherited arrhythmia syndromes



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Inherited arrhythmia syndromes, including familial long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, and Brugada syndrome, can cause life-threatening arrhythmias and are responsible for a significant proportion of sudden deaths in the young. Identification of genetic mutations and pathophysiological changes that underlie disease development can inform clinical practice and guide novel drug development. However, disease mechanisms in a large number of patients remain elusive and pharmacologic treatment is suboptimal, so many patients rely on implantable cardioverter–defibrillator therapy. Induced pluripotent stem cell models of disease facilitate analysis of disease mechanisms in patient-specific cardiomyocytes, overcoming limitations of animal models and human tissue restrictions. This review outlines how studies using induced pluripotent stem cell–derived cardiomyocytes are contributing to our understanding of the mechanisms that underpin disease pathogenesis and their potential to facilitate

new pharmacologic therapies and personalized medicine.

KEYWORDS Gene mutation; Induced pluripotent stem cell model; Inherited arrhythmia; Personalized medicine

ABBREVIATIONS AP = action potential; ARVC = arrhythmogenic right ventricular cardiomyopathy; BrS = Brugada syndrome; CPVT = catecholaminergic polymorphic ventricular tachycardia; CRISPR = clustered regularly interspaced short palindromic repeats; iPSC = induced pluripotent stem cell; iPSC-CM = induced pluripotent stem cell–derived cardiomyocyte; LQTS = long QT syndrome

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Introduction

Inherited arrhythmia syndromes, including long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS), present with heterogeneous clinical profiles that range from palpitations to syncope, arrhythmias, and sudden cardiac death. Cumulatively they are responsible for a significant proportion of sudden cardiac deaths in the young.¹ Despite progress in our understanding of the mechanisms that underlie inherited arrhythmia syndromes, therapies focus on addressing symptoms and complications. Many patients require implantable cardioverter–defibrillator therapy to prevent sudden cardiac death.

Animal studies have made substantial contributions to our understanding of disease but are limited by species differences.² For example, the antiaddiction drug ibogaine can

significantly prolong the QT in humans despite its ability to reduce the action potential (AP) duration in guinea pig cardiomyocytes.³ Assessment of human cardiac tissue is the gold standard; however, adequate samples of heart tissue are difficult to obtain. Induced pluripotent stem cells (iPSCs) provide an alternative source of species- and patient-specific cardiomyocytes.

Many studies have shown that induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) can effectively recapitulate inherited arrhythmia syndromes at the cellular level. Multiple techniques, including calcium imaging and AP analysis, are used to identify electrophysiological abnormalities. The introduction of clustered regularly interspaced short palindromic repeats (CRISPR), a highly efficient and relatively simple to use gene-editing platform, has facilitated the development of more advanced applications of iPSC-CMs.⁴ CRISPR can be used to alter gene expression and introduce and correct genetic variation. Despite considerable expense, the use of iPSC-CMs in the pharmacologic industry is rapidly expanding, and drug toxicity risk can be determined in patient-derived iPSC-CMs.^{5–7} iPSC-CMs have the potential to facilitate personalized medicine with the aim of targeting the most effective therapy for the patient.⁸ However, iPSC technology has limitations that

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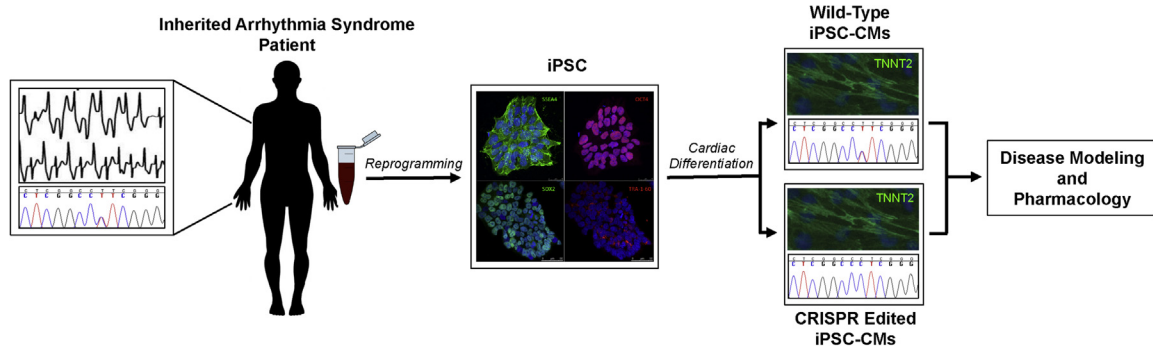


Figure 1 Generation of iPSC-CMs from patients with inherited arrhythmia syndromes. CRISPR = clustered regularly interspaced short palindromic repeats; iPSC = induced pluripotent stem cell; iPSC-CM = induced pluripotent stem cell-derived cardiomyocyte.

need to be overcome before its true potential can be realized. This review highlights what we have learned from iPSC technology and its clinical role in the inherited arrhythmia syndromes.

Basics of iPSC technology

Development of iPSC technology

iPSCs are embryonic stemlike cells that self-renew and possess the ability to differentiate into cells of all 3 embryonic germ layers.⁹ iPSCs have been generated from many somatic cells including peripheral blood mononuclear cells and fibroblasts through introduction of the Yamanaka pluripotency factors (OCT-4, SOX-2, KLF-4, and C-MYC).¹⁰ iPSC and CRISPR gene-editing technologies can be combined to create patient-specific isogenic controls,¹¹ in which an individual's genome can be studied with and without the presence of specific genetic variation (Figure 1). In the future, iPSC and CRISPR technologies may be used in the clinical cardiac setting with the ultimate goal of curing disease. In some fields of medicine, gene-editing technology is rapidly moving into the clinic.¹²

Cardiac differentiation of iPSCs

iPSCs can be differentiated into iPSC-CMs through chemical manipulation of cardiac developmental pathways (Figure 1).¹³ iPSC-CMs are identified according to the presence of cardiac markers, and the ability to develop cardiac APs and display appropriate drug responses.¹⁴ To create models that most accurately reflect disease pathology, iPSC-CMs can be purified and enriched for subtypes, including atrial-, nodal-, and ventricular-like iPSC-CMs.¹³ Although the majority of iPSC-CMs are ventricular-like and can be identified by MLC2v expression and specific AP characteristics, different protocols produce heterogeneous populations of iPSC-CMs, which can introduce variability in cell analysis.¹⁵ Cell characteristics can be subtype specific.¹⁶ A number of assays can be performed in iPSC-CMs, including analysis of ion channels, calcium handling, APs, and gene expression (Figure 2).

iPSC-CM models of the inherited arrhythmia syndromes

iPSC-CM models of LQTS

Congenital LQTS, characterized by prolongation of ventricular repolarization, has a prevalence of up to 1 in 2,000 individuals and is primarily considered to be a monogenic disease with autosomal dominant inheritance.¹⁷ Importantly, genetic testing can inform clinical practice and in LQTS has a yield of 60%–75%. The majority of patients present with LQTS types 1–3 (LQT1–3). LQT1 patients are likely to experience exercise-related symptoms and carry mutations in potassium channel subfamily Q member 1 (*KCNQ1*), which together with *KCNE1* encodes the slowly activating delayed rectifier potassium channel. Patients with LQT2 most commonly have events triggered by sudden adrenergic stimuli, such as emotional stress, and carry mutations in potassium channel subfamily H member 2 (*KCNH2*), which encodes the

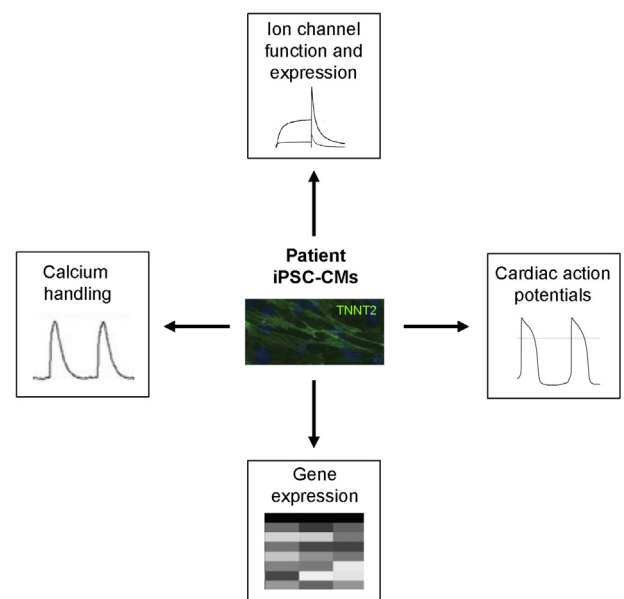


Figure 2 Assessment of cellular phenotype in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from patients with inherited arrhythmia syndromes.

Table 1 Cellular disease phenotypes present in iPSC-CMs derived from patients with inherited arrhythmia syndromes

Disease	Phenotype characteristics	Disease genes	Reference
LQT1	Prolongation of APD, increased susceptibility to EADs and arrhythmias, reduced I_{Ks} activation	<i>KCNQ1</i>	11,18,19,28
LQT2	Prolongation of APD, increased susceptibility to EADs and triggered beats, reduced I_{Kr} density	<i>KCNH2</i>	6,11,14,20,27,28,61
LQT3	Prolongation of APD, increased susceptibility to EADs and recovery from sodium channel inactivation	<i>SCN5A</i>	26,62
LQT15	Prolongation of APD, impaired inactivation of L-type Ca^{2+} channels, reduced beating rate	<i>CALM1, CALM2</i>	23–25
CPVT1	Increased susceptibility to DADs and spontaneous sarcoplasmic reticulum Ca^{2+} release, abnormal Ca^{2+} transients, reduction in RYR2 complexes	<i>RYR2</i>	32–34,36–38
CPVT2	Increased intracellular Ca^{2+} concentration, susceptibility to DADs, oscillatory prepotentials and diastolic Ca^{2+} leak	<i>CASQ2</i>	34,39,63
BrS	Increased beating interval variation and abnormal Ca^{2+} transients, reduced sodium current and maximal upstroke velocity of action potential	<i>SCN5A</i>	43,44,47

APD = action potential duration; BrS = Brugada syndrome; CPVT1, 2 = catecholaminergic polymorphic ventricular tachycardia type 1 or 2; DAD = delayed afterdepolarization; EAD = early afterdepolarization; iPSC-CM = induced pluripotent stem cell–derived cardiomyocyte; LQT1, 2, 3, 15 = long QT syndrome type 1, 2, 3, or 15.

alpha-subunit of the rapidly activating delayed rectifier potassium channel. Mutations in delayed rectifier potassium channels can prolong the QT interval by reducing the repolarizing current. Patients with LQT3 often experience symptoms at low heart rates (eg, during sleep) and carry mutations in *SCN5A*, which encodes the pore-forming subunit cardiac sodium channels. Gain-of-function *SCN5A* mutations are thought to cause LQT3 by increasing the late sodium depolarizing current. Despite differences in clinical presentation and underlying genetic etiology, the majority of LQTS subtypes are primarily treated with beta-blockers, most commonly nadolol and propranolol.

Since the first iPSC-CM model of LQTS was reported in 2010,¹⁸ numerous studies have shown that iPSC-CMs demonstrate key features of LQTS, including AP prolongation and early afterdepolarizations (Table 1). Consistent with the genetic etiologies that underlie LQTS, iPSC-CMs display abnormal expression of specific disease-related ion channels.^{14,18–20}

Genetics and molecular mechanisms that underlie LQTS

Functional studies using iPSC-CMs can provide evidence regarding the pathogenicity of gene mutations. Identification of a clinically meaningful mutation relies on meeting classification criteria with determinants of pathogenicity, including rarity, previous association with disease, and segregation and experimental data.²¹ iPSC-CM models have been used to better understand the pathogenic nature of mutations identified in families with LQTS.^{20,22} With the introduction of CRISPR, the number of studies that use iPSC-CMs and

gene editing to delineate disease-causing mutations from background noise is likely to increase substantially.

Patient-derived iPSC-CMs can also be used to identify the pathophysiological changes that link gene mutations to disease phenotypes. Analysis of patient-derived iPSC-CMs revealed that heterozygous mutations in calmodulin (*CALM1-3*) cause LQT15 through dominant negative mutation mechanisms.^{23–25} This discovery explains why mutations in just 1 of 6 identical calmodulin alleles cause such a severe LQTS phenotype and may facilitate development of new gene therapies.^{24,25} Future exploration of mutation mechanisms with iPSC-CMs may help us understand why specific mutations, or mutations in the same genes, can cause distinct disease phenotypes, best illustrated by gain-of-function *SCN5A* mutations, which cause LQT3, whereas loss-of-function *SCN5A* mutations result in BrS.

Multiple studies have used iPSC-CMs to explore the physiological changes that occur in LQTS. Moretti et al¹⁸ used iPSC-CMs derived from an LQT1 patient with the *KCNQ1* mutation p.R190Q to show that the primary change underlying the LQT1 phenotype was a reduction in the slow outward potassium current. Studies have also identified increased recovery from inactivation of depolarizing sodium channels in iPSC-CMs derived from LQT3 patients with mutations in *SCN5A*.^{22,26} In these cases, effective treatment with mexiletine further supports the role of inappropriate sodium channel activity in pathological AP prolongation.

The rise in genetic testing has resulted in increased identification of asymptomatic mutation carriers, which highlights the

Table 2 Cellular responses to drug treatment in iPSC-CMs derived from patients with inherited arrhythmia syndromes

Disease	Compounds	Drug class	Outcome	Reference
LQT1	Isoproterenol	Beta-adrenergic agonist	Increased APD and arrhythmia susceptibility	18
	Propranolol	Beta-blocker	Decreased proarrhythmic effects after isoproterenol treatment	18
	ML277	I _{Ks} activator	Increased activation of I _{Ks} , reduction in APD	19
LQT2	E-4031, cisapride	K ⁺ channel antagonists	Prolonged APD, increased number and complexity of EADs	6,14
	Nifedipine	Ca ²⁺ channel antagonist	Reduced APD, eliminated EADs and triggered beats	14
	Pinacidil, Nicorandil	K ⁺ channel agonists	Reduced APD, eliminated EADs and triggered beats	6,14
	Ranolazine	Late Na ⁺ channel agonist	No effect on APD, reduced number of EADs and triggered beats	14
	Isoprenaline	Beta-adrenergic agonist	Reduced APD, increased susceptibility to EADs	6
	LUF7346	Allosteric modulator of hERG	Reduced APD and EAD development	28
	Mexiletine, ranolazine, phenytoin	Na ⁺ channel antagonists	Reduced APD, eliminated EADs	26,62
LQT3	Verapamil, amlodipine	Ca ²⁺ channel antagonists	Reduced QTc	23
LQT15	Isoproterenol	Beta-adrenergic agonist	Negative chronotropic response, increased arrhythmias, DADs, Ca ²⁺ sparks, and diastolic intracellular Ca ²⁺ concentration	32–34
CPVT1	Dantrolene	RYR2 antagonist	Reduced Ca ²⁺ sparks and beat irregularity with beta-adrenergic stimulation	38
	Nadolol	Beta-blocker	Negligible reduction in Ca ²⁺ waves and sparks	36
	Flecainide	Na ⁺ channel antagonist	Reduced frequency and duration of Ca ²⁺ waves and sparks	36

Abbreviations as in Table 1.

issue of incomplete penetrance. Analysis of iPSC-CMs from asymptomatic mutation carriers may help us to understand the variable disease penetrance seen in LQTS. Lahti et al²⁷ generated iPSC-CMs from an asymptomatic carrier of LQT2 Finnish founder mutation *KCNH2* p.R176W with a significant family history of sudden cardiac death. Despite the asymptomatic nature of the patient, iPSC-CMs displayed significant AP prolongation and potassium current reduction in comparison to controls. These findings suggest that asymptomatic mutation carriers have increased susceptibility to developing LQTS and that multiple genetic or environmental factors may be required for full disease onset.

Pharmacologic treatment of LQTS

iPSC-CMs derived from LQTS patients show promise for enhanced pharmacologic development and safety testing (Table 2). Importantly, patient and patient-derived iPSC-CM drug responses appear to be consistent.^{6,22} Patient-derived iPSC-CMs demonstrate responses to adrenergic stress, such as QT prolongation and early afterdepolarizations, which are effectively treated with beta-blocker therapy.¹⁸

Patient-derived iPSC-CMs have been used to assess the potential of different drug classes, including calcium and late sodium channel antagonists and potassium channel agonists, to reduce AP duration and ameliorate the LQTS phenotype (Table 2). Manipulation of hERG to increase the delayed

rectifying potassium current has demonstrated efficacy in congenital and drug-induced forms of LQTS and is being extensively investigated.²⁸ Significant work is still required to move these therapeutic approaches into the clinic, specifically to determine how to identify optimal drug concentrations and prevent the development of a short QT interval. However, these early studies with patient-derived iPSC-CMs are facilitating the exploration of new, much needed drugs for LQTS.

Patient-derived iPSC-CMs provide an unprecedented opportunity to explore individual responses to drug therapies. Consistent with clinical observations, iPSC-CMs derived from LQT3 patients are more effectively treated with different sodium channel inhibitors than with beta-blockers.²⁶ Additionally, using iPSC-CMs from an LQT3 patient with suboptimal drug therapy, Terrenoire et al²² found that a combination of cardiac pacing and mexiletine treatment was more beneficial than addition of a second sodium channel inhibitor. Importantly, these *in vitro* findings are consistent with best clinical treatment of the patient to date. Overall, these studies support preclinical assessment of drug efficacy with patient-derived iPSC-CMs for the development of personalized treatment strategies.

iPSC-CM models of CPVT

CPVT is a severe arrhythmia syndrome with an estimated prevalence of 1 in 10,000 individuals.²⁹ Beta-blockers are the main form of treatment of CPVT but are ineffective in

a large proportion of patients. CPVT is primarily caused by mutations in the cardiac ryanodine receptor (*RYR2*) and cardiac calsequestrin 2 (*CASQ2*).³⁰ Mutations in *RYR2* cause type 1 CPVT (CPVT1), which accounts for 50%–60% of CPVT cases. Mutations in *CASQ2* cause type 2 CPVT (CPVT2), a more rare autosomal recessive form of CPVT. A small number of *CASQ2* mutations that cause an autosomal dominant form of CPVT2 have been identified.³⁰ *RYR2* and *CASQ2* proteins are major components of a complex that controls coordinated release of calcium from the sarcoplasmic reticulum during systole. Consistently, genetic etiologies and animal studies suggest that CPVT primarily results from abnormal storage and release of calcium from the sarcoplasmic reticulum.³¹

Analysis of iPSC-CM lines derived from CPVT patients has revealed a cellular phenotype of CPVT (Table 1). Consistent with the absence of a baseline arrhythmic phenotype in CPVT patients, the majority of CPVT iPSC-CMs require beta-adrenergic stimulation with drugs such as isoproterenol to induce arrhythmic features.³² However, underlying abnormalities in calcium handling have been identified in CPVT1 iPSC-CMs at baseline.³³ Isoproterenol-treated iPSC-CMs from a CPVT1 patient displayed a negative chronotropic response with development of arrhythmias.³³ This abnormal response to beta-adrenergic stimulation is consistent with arrhythmia development in CPVT patients and iPSC-CMs derived from both CPVT1 and CPVT2 patients.³⁴

Molecular mechanisms that underlie CPVT

The main mechanism of CPVT development is considered to be aberrant diastolic calcium release from the sarcoplasmic reticulum.^{35,36} Analysis of iPSC-CMs derived from CPVT1 patients suggests that disease-causing *RYR2* mutations are gain-of-function mutations that result in calcium leak due to increased open probability of *RYR2*.^{32,33} Calcium handling abnormalities identified in CPVT1 iPSC-CMs include reduced calcium transient amplitudes and sarcoplasmic reticulum calcium stores and increased *RYR2*-mediated SR calcium leak and fractional calcium release.³² Evidence for a pathogenic increase in the open probability of *RYR2* supports the use of compounds such as S107, which can increase binding of calstabin-2 to *RYR2* promoting the closed *RYR2* state.³⁷ Dantrolene, which stabilizes the closed state of *RYR2*, has been shown to prevent arrhythmia development in iPSC-CMs derived from CPVT1 patients.³⁸ The efficacy of dantrolene, which increases interdomain interactions in *RYR2*, supports the domain unzipping mechanism, whereby the interactions between domains in *RYR2* are weakened due to *RYR2* mutations and result in an increased open probability.

Patient-derived iPSC-CMs have been used to explore mechanistic differences between CPVT1 and CPVT2.³⁴ Although both CPVT1 and CPVT2 iPSC-CMs displayed immature ultrastructures, reduced ryanodine receptor expression, and increased arrhythmia susceptibility after adrenergic stimulation, underlying differences were identified between the 2 forms of CPVT. CPVT1 and CPVT2 iPSC-CMs demonstrated disparities in the storage and stimulated release of calcium from the

sarcoplasmic reticulum. Importantly, beta-adrenergic stimulation of both CPVT1 and CPVT2 iPSC-CMs with isoproterenol resulted in a marked increase in diastolic intracellular calcium concentration, a key feature of CPVT.

Pharmacologic treatment of CPVT

Current pharmacologic treatment of CPVT is suboptimal, and both patients and clinicians would benefit from preclinical assessment of patient-specific drug efficacy. Supporting the use of iPSC-CM pharmacology testing to guide patient therapy, multiple studies have shown that drug responses in iPSC-CMs correlate strongly with clinical responses in the CPVT patients from whom they were derived.^{7,32,39} Preininger et al³² and Mainzels et al³⁹ evaluated drug responses in iPSC-CMs derived from CPVT1 and CPVT2 patients, respectively, whose arrhythmic events were not clinically treatable with widely used beta-blockers. Consistent with patient clinical responses, iPSC-CMs demonstrated only a minimal antiarrhythmic response to beta-blocker treatment. Alternatively, both patient and iPSC-CMs responded more effectively to flecainide therapy. Further comprehensive studies are required to move this technology into the clinic and to better understand how genetic variation can affect drug efficacy.

Table 2 summarizes some of the drug compounds that have been evaluated with iPSC-CMs derived from CPVT1 patients. Recent iPSC-CM studies and a small clinical trial in CPVT1 patients have provided evidence supporting the use of drugs, such as dantrolene, that increase the closed state of *RYR2*.⁷ However, the efficacy of dantrolene appears to be dependent on mutation location,⁷ which suggests that optimal treatment of CPVT1 may require different pathways to be targeted even among individuals with mutations in the same genes. Patient and iPSC-CMs revealed consistent responses to dantrolene, supporting the ability of iPSC-CMs to accurately model personalized drug responses in the context of CPVT1.⁷

iPSC-CM models of BrS

BrS is characterized by ST-segment elevation and a significant risk of life-threatening ventricular fibrillation and sudden cardiac death.⁴⁰ BrS is increasingly being referred to as an overlap syndrome, following its identification in patients with features of other cardiomyopathies.⁴¹ Management of BrS patients is limited primarily to lifestyle modifications, with symptomatic patients requiring implantable cardioverter-defibrillator therapy. New treatment options, such as quinidine therapy and ablation, are being explored. BrS is considered a monogenic syndrome, although complex genetic mechanisms have been reported. The genetic yield in BrS is only 20%–25% (mainly *SCN5A*), with the majority of BrS cases remaining genetically elusive.

Despite evidence that arrhythmic features of BrS result from tissue-level conduction abnormalities,⁴² cellular features of BrS are identifiable in iPSC-CMs derived from patients who carry *SCN5A* mutations (Table 1). iPSC-CMs derived from BrS patients with *SCN5A* mutations display abnormal AP profiles and a reduction in sodium channel

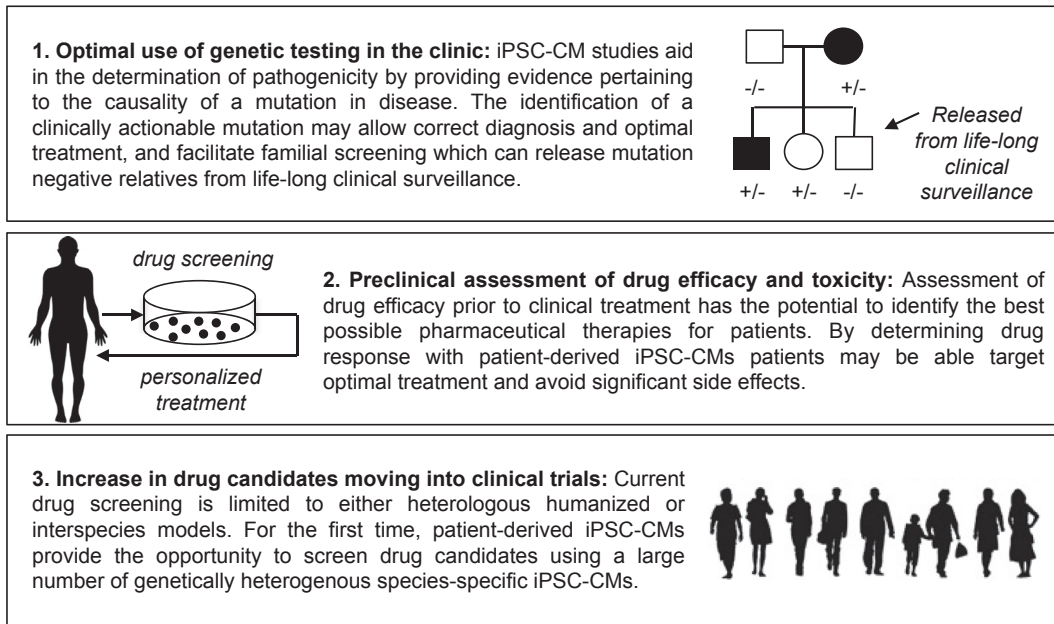


Figure 3 Potential future implications of induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) technology for the clinical cardiac electrophysiologist.

function with significantly slower depolarization, increased triggered activity, and calcium transient abnormalities.⁴³ Encouragingly, gene expression patterns in iPSC-CMs derived from 2 unrelated BrS patients correlated with gene expression patterns identified in cardiac tissue isolated from BrS patients.⁴³

The underlying etiology of BrS remains unclear and is likely a combination of multiple genetic factors interacting with a range of environmental influences. Veerman et al⁴⁴ found that a clear cellular phenotype of BrS was not identifiable in iPSC-CMs derived from genotype-negative BrS patients. This finding may support recent suggestions that BrS can present as an overlap syndrome resulting from a number of different underlying pathophysiological mechanisms.⁴⁵ Furthermore, it suggests that the approximately 80% of patients with inconclusive genetic testing may not have underlying defects in ion channel function, and many oligogenic or nongenetic factors may contribute to BrS development. The BrS phenotype in these patients may be the result of a complex interaction of multiple cell types, genetics, and the environment, and therefore iPSC-CMs may be an unsuitable model. As the technology advances and limitations, primarily with respect to maturation, are overcome, a cellular disease phenotype may become identifiable.

Genetic and molecular mechanisms that underlie BrS

CRISPR gene editing has been used to correct *SCN5A* mutations in patient-derived iPSC-CMs, resulting in resolution of the BrS phenotype with respect to both AP and calcium handling abnormalities.⁴³ Additional analyses comparing the cellular phenotype of corrected iPSC-CMs to control iPSC-CMs would be of value in determining the contribution of genetic background to BrS. Such comparisons will be important because the genetic

etiologies are not well understood, and inheritance patterns do not consistently support a monogenic disease.⁴⁶

iPSC-CMs have been used to assess whether mutations in plakophilin-2 (*PKP2*), a well-described cause of arrhythmogenic right ventricular cardiomyopathy (ARVC), can cause a BrS phenotype.⁴⁷ *PKP2* is a component of the cardiac desmosome, which is important for intercellular communication and myocardium integrity. After screening of 200 otherwise genotype-negative BrS patients, 5 probands were found to carry missense mutations in *PKP2*. Assessment of iPSC-CMs derived from the 5 patients revealed findings consistent with previous BrS studies using animal models and cardiac cell lines. iPSC-CMs with mutant *PKP2* had reduced sodium channel expression and current, thus supporting a role for *PKP2* mutations in BrS. Further work is required to understand the role of *PKP2* mutations in BrS and the underlying disease mechanisms that result in an overlap syndrome of both BrS and ARVC.

As the number of iPSC-CM studies assessing BrS increases, we will likely see a larger contribution of this technology to our understanding of disease development. In a recent study, Liang et al⁴³ found that iPSC-CMs derived from BrS patients with *SCN5A* mutations have significantly reduced sodium channel function and expression at the cell membrane, which supports previous indications of reduced sodium channel expression in BrS. A reduction in maximum intracellular calcium concentration and rate of depolarization was also identified. Analysis also revealed a decrease in the expression of *KCNJ2*, which encodes Kir2.1 inwardly rectifying potassium channels and may represent a compensatory mechanism.

iPSC models of inherited cardiomyopathies

iPSC-CMs derived from patients with inherited cardiomyopathies such as ARVC have been used to explore disease

mechanisms and drug development.^{48–52} Multiple studies have identified cellular characteristics of ARVC, including reduced PKP2 protein, increased lipid accumulation, and apoptosis in iPSC-CMs.^{53–56} iPSC-CMs have been used to explore the genetics of ARVC, which currently has a genetic yield of 30%–50% and primarily involves genes of the cardiac desmosome.⁵⁷ The pathogenicity of mutant *PKP2*, the most common genetic cause of ARVC, has been demonstrated in iPSC-CMs by the loss of ARVC phenotype after transfection of wild-type *PKP2* protein.^{47,55} Analysis of iPSC-CMs has also provided support for the pathogenicity of single amino acid substitutions in *PKP2* in addition to the known loss-of-function mutation mechanism.⁴⁷ iPSC-CM studies have further demonstrated the importance of abnormal peroxisome proliferator-activated receptor- γ activation in ARVC development⁵⁴ and testosterone in gender differences in ARVC whereby prevalence and severity of disease are increased in males.⁵³

Overall, studies using iPSC-CMs to model ARVC and other inherited cardiomyopathies are significantly contributing to our understanding of the genetic and molecular mechanisms underlying disease development.

Technical challenges and limitations of iPSC-CMs

iPSC-CMs have significant limitations, in particular their immature fetal-like state, isolated culture environment, and challenges with reproducibility. At present, the fetal-like characteristics of iPSC-CMs limit their ability to model inherited arrhythmia syndromes, which generally have an adult onset. Immature iPSC-CMs have important differences, including a lack of T-tubules, abnormal morphology, altered gene expression, and reduced sarcomere organisation.² Although the major cardiac channels, including those implicated in development of inherited arrhythmia syndromes, are present in iPSC-CMs, they have significantly altered expression and current amplitudes.⁵⁸ Importantly, sodium channels contribute less to AP activity as iPSC-CMs have relatively depolarized diastolic membrane potentials.²² Efforts are underway to increase iPSC-CM maturity, and a number of methodological factors, including time in culture, modulation of metabolism, electrical stimulation, and growth as microtissues, have shown improvement.⁵⁵ The *in vitro* nature of iPSC-CM models must also be taken into consideration as cardiac function is largely affected by systemic involvement and the heart is made up of a heterogeneous population of cells in addition to cardiomyocytes.

What is on the iPSC-CM horizon?

Although iPSC-CM models have and will continue to contribute to our understanding of inherited arrhythmia syndromes, the field likely will see an increase in culture of iPSC-CMs as cardiac microtissues.⁵⁹ Cardiac microtissues have significant advantages that increase their physiological relevance, such as enhanced electrical coupling, force generation and maturation, and the ability to co-culture iPSC-CMs with multiple cell types.⁶⁰ In comparison to adult cardiac

tissues, iPSC-derived cardiac microtissues currently remain relatively immature, with reduced cardiac current amplitudes and contraction forces.⁵⁹ At present, the use of cardiac microtissues for disease modeling and drug testing remains limited by significant time and cost restrictions.

Conclusion

Our understanding of the mechanisms that cause inherited arrhythmia syndromes has been limited by accessibility of human tissue and species discordance. Patient-derived iPSC-CMs have the potential to provide an unlimited source of patient-specific cardiomyocytes, and studies to date show that they already are contributing to many aspects of the inherited arrhythmia syndrome field. As cardiac microtissues become more time and cost efficient, we will likely see a shift from 2- to 3-dimensional iPSC-CM-derived models of disease. The insight gained from iPSC-CM studies will likely impact directly on the clinical practice of cardiac electrophysiologists, enhancing aspects of diagnosis and management of inherited arrhythmia patients, with more precise and targeted approaches (Figure 3). Specifically, patient-derived iPSC-CMs will likely be used to aid in the assessment of clinically actionable genetic mutations, to evaluate optimal treatment strategies with personalized preclinical drug testing, and to identify new drug compounds that facilitate better clinical outcomes for patients with inherited arrhythmia syndromes.

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