CONTEMPORARY REVIEW

Cardiac potassium inward rectifier Kir2: Review of structure, regulation, pharmacology, and arrhythmogenesis

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Potassium inward rectifier channel Kir2 is an important component of terminal cardiac repolarization and resting membrane stability. This functionality is part of balanced cardiac excitability and is a defining feature of excitable cardiac membranes. “Gain-of-function” or “loss-of-function” mutations in KCNJ2, the gene encoding Kir2.1, cause genetic sudden cardiac death syndromes, and loss of the Kir2 current IK1 is a major contributing factor to arrhythmogenesis in failing human hearts. Here we provide a contemporary review of the functional structure, physiology, and pharmacology of Kir2 channels. Beyond the structure and functional relationships, we will focus on the elements of clinically used drugs that block the channel and the implications for treatment of atrial fibrillation with IK1-blocking agents. We will also review the clinical disease entities associated with KCNJ2 mutations and the growing area of research into associated arrhythmia mechanisms. Lastly, the presence of Kir2 channels has become a tipping point for electrical maturity in induced pluripotent stem cell–derived cardiomyocytes (iPS-CMs) and highlights the significance of understanding why Kir2 is important to consider for Comprehensive In Vitro Proarrhythmia Assay and drug safety testing.

KEYWORDS KCNJ2; KCNJ2 mutation; Kir2; Macromolecular complex; Pharmacology; Potassium inward rectifier

Introduction

Potassium inward rectifier channel Kir2 is an important component of terminal cardiac repolarization and resting membrane stability. This functionality is part of balanced cardiac excitability and is a defining feature of excitable cardiac membranes. “Gain”- or “loss-of-function” mutations in KCNJ2, the gene encoding Kir2.1, cause genetic sudden cardiac death syndromes, and loss of the Kir2 current IK1 is a major contributing factor to arrhythmogenesis in failing human hearts. Here we provide a contemporary review of the functional structure, physiology, and pharmacology of Kir2 channels. Beyond the structure and functional relationships, we will focus on the elements of clinically used drugs that block the channel and the implications for treatment of atrial fibrillation with IK1-blocking agents. We will also review the clinical disease entities associated with KCNJ2 mutations and the growing area of research into associated arrhythmia mechanisms. Lastly, the presence of Kir2 channels has become a tipping point for electrical maturity in induced pluripotent stem cell–derived cardiomyocytes (iPS-CMs) and highlights the significance of understanding why Kir2 is important to consider for Comprehensive In Vitro Proarrhythmia Assay (CiPA) and drug safety testing.

Methods

The research reviewed in this article adheres to the PRISMA guidelines as outlined by Moher et al. Literature cited and research mentioned all have been derived from public domain sources from PubMed. We used the following search terms: Kir2, Potassium inward rectifier 2, KCNJ2, IK1, Andersen-Tawil syndrome (ATS), long QT syndrome 7, short QT syndrome, pharmacologic agents, Kir2 block, and Kir2 pore blockers. Additional search criteria included full-text articles, English language, and some electrophysiological-related concepts in induced pluripotent stem cells (iPSCs). A focus on cardiac electrophysiology was attempted, but when studies involved only neural or other myocytes and described key associations, noncardiac studies were included. Methodical cross-checking of available studies was used, but it is possible that nonpublic domain research has been performed that we could not include.
Structure, function, and cardiac isoforms

The Kir2.x channels are classified within a larger superfamily of potassium inward rectifiers consisting of Kir1-6 (Figure 1). The Kir superfamily contains some parallels in structure and function, but their physiology and distribution are wide-ranging. Kir2, Kir3, and Kir6 subfamilies are known to be important in human cardiac electrophysiology. In concert with SUR subunits, Kir6 channels make up ligand-activated K<sub>ATP</sub>, essential for the cellular response to ischemic preconditioning. Classic strong inward rectification is noted with Kir2 and Kir3 subfamilies. In mammalian hearts, the strong inward rectifiers Kir3.1 and Kir3.4 are the molecular correlates for IKACh, an important receptor-activated current prevalent in the atria and nodal tissue.3

In the mammalian heart, 3 isoforms of Kir2.x are present: Kir2.1, Kir2.2, Kir2.3, and they are encoded by KCNJ2, KCNJ12, and KCNJ4, respectively.4 Kir2.1 (mouse) was the first to be cloned in 1993 and was found to have a structure similar to other known K<sup>+</sup> channels but with 2 transmembrane domains (not 6 like other voltage-gated K<sup>+</sup> channels), with an inner core for ionic passage and a large C-terminal domain.5 Functional channels consist of 4 subunits that can assemble as homotetramers (single isoform) or heterotetramers (combination of isoforms).6 Important tetrameric interaction of N- and C-terminus of separate subunits is necessary for proper membrane trafficking7 and is important for channel regulation (discussed in the section on Regulatory molecules and binding partners).

The Kir2.x channels are the molecular correlates to I<sub>K1</sub> (Figure 1), which in the heart functions to complete phase 3 repolarization and maintain resting membrane potential on a beat-to-beat basis to rapidly achieve membrane polarization and establish potassium and sodium gradients. Inward rectification is the strongly voltage-dependent decrease in K<sup>+</sup> conductance with membrane depolarization. As shown in the current/voltage relationship in Figure 2, at physiologic voltages the channels conduct outward K<sup>+</sup> current with a peak between −60 to −40 mV, which diminishes at more positive voltages.5 The current reverses direction to inward close to E<sub>K</sub> at −80 mV. The original description of inward rectification focused on the large inward current component8 and has been emphasized as the primary characteristic of Kir2 channels historically.6 However, physiologically voltages below −80 mV are not achieved in the heart; therefore, the dominant component of I<sub>K1</sub> for cardiac electrical stability is the outward current. It is the outward current that plays a critical role in phase 3 repolarization to reset the cardiac membrane and allows Na<sup>+</sup> channel recovery from inactivation.9 Kir2 channels depend on binding of phosphatidylinositol

Figure 1 Kir superfamily and role in cardiac action potential. A: Hierarchy plot showing different Kir family members. B: Ventricular cardiac action potential with I<sub>K1</sub> current activity highlighted below.

Figure 2 Kir2 isoform current/voltage profiles shown in comparison with Kir3.1/3.4. (From Anumonwo JMB, Lopatin AN. Cardiac strong inward rectifier potassium channels. J Mol Cell Cardiol 2010;48:45–54.)
4,5-bisphosphate (PIP₂), and reintroduction of PIP₂ to inside-out patches allows for recovery of rundown. Binding of PIP₂ initiates a conformational change in the tetrameric channel to allow ionic conductance. Resolution of Kir2.2 crystal structure has revealed key conserved amino acid residues from interacting separate monomers are necessary for PIP₂ binding and stabilization of the PIP₂ binding site. It is not surprising that mutations of key PIP₂ residues on Kir2.1 have been found in patients with the KCNJ2 mutation–associated disease ATS (discussed in detail in the section on Clinical disease association and arrhythmogenesis related to KCNJ2 mutations).

Homotetrameric Kir2.x channels have distinct biophysical properties, including single channel conductance, rectification, and pH sensitivity. Kir2.1 single channel conductance is approximately 20–31 pS compared to Kir2.2 34–42 pS and Kir2.3 10–14 pS and have differing sensitivity to divalent cation block.

Protein kinase A and protein kinase C regulation
Protein kinase A (PKA) regulates Kir2 channels, with evidence in heterologous systems and native cells. In heterologous expression systems, evidence seems clear that activation of PKA via isoproterenol or “PKA cocktail” (for-skonilin + 3-isobutyl-1-methylxanthine [IBMX]) results in an increase in outward current. Mutation of the putative PKA phosphorylation site at Kir2.1 Ser425 results in lack of response to PKA activation. The 3 members of the Kir2.x family—Kir2.1, Kir2.2, and Kir2.3—are expressed in human heart and underpin cardiac IK₁; therefore, direct comparison with heterologous expression systems expressing only 1 isoform may not recapitulate the effect of PKA activation on IK₁. Older studies of PKA regulation in canine Purkinje myocytes and guinea pig ventricular myocytes suggest that activation of PKA by isoproterenol inhibits Kir2.1. However, it is worth noting that these studies focus on the inward component of IK₁, with little resolution of physiologic voltages. Recent work by our group has characterized mouse, rat (unpublished data), and human (unpublished data) isolated ventricular myocyte response to isoproterenol. In addition, the concentration of isoproterenol used in myocyte studies is high (1 μM), which could result in saturation of the response and a large increase in intracellular calcium, which is known to inhibit IK₁. Therefore, a submaximal dose and more physiologically relevant concentrations revealed that IK₁ is, indeed, increased.

The effect of protein kinase C (PKC) on IK₁ is controversial. Activation of β3-adrenoreceptors with isoproterenol enhanced Kir2.1 and Kir2.2 currents with no effect on Kir2.3 channels. Inhibition of PKA and Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) had no effect on regulation of Kir2.1; however, PKC inhibition suppressed activation of Kir2.1. In contrast, PKA inhibition abolished activation of Kir2.2. This suggests that different isoforms of Kir2.x are differentially regulated by PKC, an effect that was observed when expressed as heteromeric channels and resulted in all channels being activated following isoproterenol. Interestingly, Kir2.3 activity can be inhibited by PKC-mediated phosphorylation at threonine53. When the N-terminus of Kir2.3 was replaced with that of Kir2.1, the chimeric channel lost sensitivity to inhibition via PKC activation. In contrast, treatment of monocytes with phorbol 12-myristate 13-acetate (PMA) activated Kir2.1 and Kir2.2 channels and seemed to be dependent on conversion of PIP₂ to PIP₃. The majority of these studies are performed using Xenopus oocytes, without the full complement of cellular machinery, so it can be argued that in native tissues, the observed effects could be different, resulting in the different effects of both PKA and PKC regulation observed on Kir2.x channels.

Nitric oxide
Nitric oxide plays an important regulatory role within cardiac cells. At physiological levels of nitric oxide, IK₁ increased in atrial myocytes and in Chinese hamster ovary (CHO) cells.

Regulatory molecules and binding partners

PIP₂ regulation
It is well known that lipid molecules are integral for regulation of ion channel activity. PIP₂, a dynamic lipid component of cell membranes, is known to regulate a number of ion channels, including Kir2 channels. Pioneering work from Huang et al. in 1998 revealed that PIP₂ prevented rundown of the channel during patch-clamp experiments. Application of PIP₂ to Kir2.1 expressed in Xenopus oocytes resulted in activation of the channel. Indeed, competition with PIP₂ antibodies resulted in potent inhibition of channel activity. Consistent with direct binding of PIP₂ to the channel, GST-fusion proteins of the C-terminus of Kir2.1 revealed strong binding affinity to PIP₂. The crystal structure of Kir2.2 confirmed that PIP₂ binds to Kir2.2 at the interface between the transmembrane domain and the cytoplasmic domain. In addition to directly modulating Kir2.x activity, PIP₂ has been shown to affect other properties of Kir2.x channels. The pH sensitivity of Kir2.x channels, for example, is dependent on PIP₂ binding affinity. Similarly, PIP₂ is required for Mg²⁺ inhibition of Kir2.x, as in the absence of PIP₂, Mg²⁺ was able to inhibit the channel irreversibly.
Direct nitrosylation at Kir2.1 Cys76 increases channel open probability as well as frequency of channel opening. The effect is membrane hyperpolarization and action potential (AP) shortening in mouse and human atria.

Subcellular localization
Kir2.x channels are regionally distributed in the heart. \( I_{K1} \) is prominent in ventricular myocytes and Purkinje fibers, but is significantly smaller in atrial myocytes (with the exception of mouse atria). Real-time reverse transcriptase polymerase chain reaction of Kir2.x transcripts from human heart revealed the following expression profiles for Kir2.x isoforms: Purkinje fibers: Kir2.1> Kir2.3> Kir2.2; right ventricle: Kir2.1> Kir2.2> Kir2.3; and right atria: Kir2.3> Kir2.2> Kir2.1. Immunostaining techniques show a distinct t-tubular staining pattern for Kir2.1 and Kir2.2 in human left ventricle sections, with Kir2.3 found only at the intercalated disc. Disruption of t-tubules via osmotic shock in ventricular myocytes leads to decrease in \( I_{K1} \), supporting their localization to t-tubular membranes. In addition, co-staining with caveolin-3 (Cav3), critical for the formation of caveolae, revealed colocalization with Kir2.1 at the sarcolemma; with Kir2.1 and Kir2.2 in t-tubules; and with Kir2.1 and Kir2.3 at the intercalated disc. This suggests that Kir2.x isoforms may localize to caveolar domains within cardiac myocytes, which are important for clustering of ion channels and molecular partners required for appropriate excitation–contraction coupling. The interaction between Kir2.x and Cav3 is discussed in the section on Cav3.

Synapse-associated protein-97
Kir2.x channels in macromolecular complexes are known to interact with the membrane-associated guanylate kinase (MAGUK) scaffolding protein family. Synapse-associated protein-97 (SAP-97) belongs to the MAGUK family and is expressed in heart. Kir2.x proteins bind MAGUK proteins through a PDZ binding motif in the C-terminus, except Kir2.4. This binding motif may overlap with the putative PKA phosphorylation site, and phosphorylation of this site results in loss of SAP-97 binding. Immunostaining also demonstrated colocalization of Kir2.x and SAP-97 in cardiac myocytes. Heterologous expression of Kir2.3 and SAP-97 resulted in cellular relocation and increased cell surface expression. Reported single channel conductance for Kir2.3 were variable when coexpressed with SAP-97 without altering the open probability. Interestingly, knockdown of SAP-97 in rat ventricular myocytes decreased \( I_{K1} \) and blunted the response to isoproterenol. Additionally, loss of SAP-97 seemed to be due to a loss of Kir2.x channel abundance, perhaps related to cell surface expression. Coimmunoprecipitation studies show that SAP-97 interacts with PKA and \( \beta_1 \)-adrenergic receptors in cardiac myocytes, suggesting that SAP-97 also regulates the signaling complex involved in regulation of Kir2.x.

Cav3
We have previously shown that Kir2.x channels associate with Cav3, encoded by CAV3 in the heart. Fluorescent resonant energy transfer and coimmunoprecipitation demonstrated an association between Cav3 and Kir2.1. In addition, residues required for the association with Cav3 are conserved in the N-terminal sequence, containing a caveolin-binding motif (CBM) QxQxxxxQ where, Q is an aromatic amino acid (tyrosine, tryptophan, and phenylalanine) and x represents other amino acids. CBM of Kir2.x is required for coimmunoprecipitation, as deletion results in loss of association. Cav3 scaffolding and membrane domains associate with Kir2.x. Disruption of these domains or CBM could influence Kir2.x localization to caveolar domains. Mutations in CAV3 modulate Kir2.x channel activity and localization. The long QT syndrome 9–associated Cav3 mutation F97C, when expressed with homomeric Kir2.1 and Kir2.2, decreased current density by 50%–60% but had no effect on Kir2.3. Heteromeric channels coexpressed with F97C-Cav3 decreased current density of Kir2.1-Kir2.2 and Kir2.2-Kir2.3 heteromers and seems to be related to decreased channel trafficking.

Kir2.1 and Na\(_V\)1.5 macromolecular complex
We and others have demonstrated that Kir2.1 and Na\(_V\)1.5 co-localize in ventricular myocytes in human, rat, and mouse. Both Kir2.1 and Na\(_V\)1.5 interact independently with distinct PDZ domains within SAP97 (discussed in the section on Synapse-associated protein-97) and \( \alpha_1 \)-syntrophin. Functional changes in Kir2.1 were shown to modulate Na\(_V\)1.5 and vice versa, suggesting that they are functionally linked. This interaction is a crucial determinant of cardiac excitability and AP duration, as overexpression of both in neonatal rat ventricular myocyte (NRVM) monolayers resulted in hyperpolarization of the resting membrane potential, shortening of the AP, and an increase in conduction velocity due to Na\(_V\)1.5 availability. Additionally, an increase in the frequency and persistence of reentrant rotor activity was observed in NRVM monolayers when both Na\(_V\)1.5 and Kir2.1 were overexpressed. Interestingly, overexpression of the N-terminal domain of Na\(_V\)1.5 resulted in increased \( I_{Na} \) and \( I_{K1} \) via increasing expression of Na\(_V\)1.5, Kir2.1, and Kir2.2, which seems to mediated by \( \alpha_1 \)-syntrophin. When chimeras of Kir2.1 and Kir2.2 were generated in which PDZ domains were replaced with PDZ domains that bind \( \alpha_1 \)-syntrophin but not SAP-97, cotransfection with Na\(_V\)1.5 increased \( I_{K1} \) inward and outward current. Knockdown of \( \alpha_1 \)-syntrophin in CHO cells and adult rat ventricular myocytes had decreased \( I_{Na} \) and \( I_{K1} \). The reciprocal modulation of Na\(_V\)1.5 and Kir2.1 is due in part to cotrafficking of the channels. Trafficking-deficient Kir2.1\(^{A314–A315} \) mutation reduced expression of Na\(_V\)1.5 at the surface membrane. It has also been shown that endoplasmic reticulum (ER) trafficking-deficient Na\(_V\)1.5 channels associated with Brugada syndrome significantly decreased \( I_{K1} \). Similarly, Golgi trafficking-deficient Na\(_V\)1.5 channels had a
Pharmacology of Kir2.x
A number of pharmacologic agents affect Kir2.x current. As the contribution of IK1 in arrhythmic disease is better understood, the basis for block or enhancement of IK1 is important for both antiarrhythmic and proarrhythmic effects.

Pore blockers
It is well established that Kir2.x channels conduct K\(^+\) ions inwardly through the cell membrane more efficiently than outwardly. Cations have been widely used to investigate the permeability and gating mechanisms of potassium channels.\(^{52}\) Kir2.x channels are particularly sensitive to blockade by divalent cations and have been studied in native tissues and heterologous systems.\(^{53-57}\) These studies identified 2 distinct binding sites: a shallow site that does not sense the membrane electrical field, and a deeper site that is approximately halfway within the membrane electrical field.\(^{54-56,58,59}\) A single ion is all that is required to block the channel at either site. Removal of divalent cations from the external solution decreases the extent of inactivation of Kir2.x channels and of Kir1.1 channels.\(^{53,60}\) Increasing extracellular K\(^+\) can decrease the extent to which external Mg\(^{2+}\) and Ca\(^{2+}\) can block Kir channels.\(^{60-62}\) K\(^+\) antagonizes the effect of Mg\(^{2+}\) in a manner that suggests they compete for an external inactivation site. Mg\(^{2+}\) was identified to reduce inward currents in Kir2.2 in a voltage-dependent manner.\(^{63}\) Molecular dynamic simulations demonstrated that Mg\(^{2+}\) blocks the channel by staying at the selectivity filter and causes a reduction in current as a result. Mutagenesis of key negatively charged residues at the outer mouth of the pore in Kir2.2 decreased the voltage-dependent blockade of inward currents by Mg\(^{2+}\) via electrostatic repulsion.\(^{63}\)

Ca\(^{2+}\) also produces voltage-dependent block of Kir2.x channels, but with less potency than Mg\(^{2+}\).\(^{64}\) Despite reduced potency and low average concentrations of [Ca\(^{2+}\)]\(_i\), there is some evidence that Ca\(^{2+}\) can modulate IK1. Recordings of IK1 during the AP in guinea pig ventricular myocytes, transient increases in Ca\(^{2+}\) lead to inhibition of the current.\(^{65}\) It is proposed that this reduction in current flow is due to a decrease in outward current\(^{66}\) by decreasing the open probability of the main open channel state. The concentrations of Ca\(^{2+}\) that generate changes in rectification are in the range of those observed during Ca\(^{2+}\) transients and may affect Kir2.1 conductance during the cardiac AP.

Polyamines have also been demonstrated to inhibit Kir2.x channels. Mice with altered polyamine biosynthesis, which results in increased spermidine levels, reduced IK1 by approximately 38%, with no effect on rectification.\(^{67}\) Additionally, loss of spermine via disruption of spermine synthase gene resulted in IK1 with weakened rectification and no change in current density. This suggests a role of spermine in the rectification at potentials positive to EK, with spermidine dominating at potentials around and negative to EK. In all Kir2.x isoforms, blockade by spermine is characterized by 3 distinct components: 1 steep and 2 shallow.\(^{68}\) These components are predicted to correspond to spermine blocking at 2 distinct sites in the pore.\(^{69,70}\) The steep component is due to binding to negative residues in the cytoplasmic vestibule of the channel, whereas the slow component is due to binding to the “rectification controller” deep within the pore.\(^{71-73}\) Kir2.x isoforms are differentially regulated by spermine at these 2 sites, with block at shallow site 1 being more potent in Kir2.2 than in Kir2.3 as well as differences in the steep component of rectification.\(^{65}\) It was demonstrated in heterologous experiments with Kir2.1 that polyamines regulate the amplitude of outward IK1 by modifying the proportion of channels with different sensitivities to blockade, and outward current is primarily generated by channels with lower affinity to polyamines.\(^{74,75}\)

Barium is a widely used pore blocker of Kir2.x channels. It blocks Kir2.x channels in a voltage-dependent manner,\(^{75}\) blocking 50% of Kir2.1 current at 3–10 μM when the membrane potential was −80 mV. Barium impairs Kir2.1 rectification via a noncompetitive mechanism. Binding of divalent cations to Kir2.x channels is thought to occur via 2 distinct binding sites. Additionally, 2 mutations in the pore region (E125N and T141A) impaired Ba\(^{2+}\) entry and binding to the channel.\(^{75}\) Ba\(^{2+}\) was found to bind to the deeper site within the membrane electrical field.\(^{54-56,58,59,76}\) Expression of Kir2.1 and Kir2.3 homomeric channels in Xenopus oocytes resulted in similar Ba\(^{2+}\) sensitivities, but they were less sensitive than native cardiac IK1.\(^{77}\) Kir2.2 showed similar sensitivity to cardiac IK1, but blocking kinetics were faster than native currents. Coexpression of Kir2.x subunits had similar Ba\(^{2+}\) sensitivities and blocking kinetics to native IK1. However, Ba\(^{2+}\) is known to inhibit other types of K\(^+\) channels, including the delayed inward rectifier, native inward K rectifier of starfish eggs and frog skeletal muscle, and Ca\(^{2+}\)-activated K\(^+\) channels.\(^{69}\) Additionally, Ba\(^{2+}\) is a potent blocker of the BK channel pore.\(^{78}\) Ba\(^{2+}\) also acts as a charge carrier for L-type Ca\(^{2+}\) channels.\(^{79}\) Therefore, although Ba\(^{2+}\) is a useful pharmacologic tool for studying IK1, in certain cell systems, its effect on other ion channels may confound results.

Chloroquine is an important therapeutic treatment of malaria as well as adjunct therapy for systemic inflammatory disorders. However, despite its use clinically, it has a narrow safety margin. It is known to cause prolongation of QT and QRS on surface electrocardiograms (ECGs).\(^{80,81}\) At higher concentrations, chloroquine can lead to ventricular ectopy and ventricular arrhythmias.\(^{82}\) These clinical outcomes are the result of prolongation of the cardiac AP duration, enhancement of automaticity, and a decrease in maximum diastolic potential at the cellular level.\(^{83,84}\) These cellular changes are due, in part, to blockade of IK1 and IKr.\(^{85,86}\) Like many of the pore blockers discussed here, chloroquine blocks Kir2.x channels from the cytoplasmic surface in a voltage- and K\(^{+}\)-dependent manner.\(^{81}\) Interestingly, even when Kir2.1 channels were pre-blocked with polyamines, chloroquine was still able to reach
its binding site, suggesting that it binds at a site distinct from polyamines. Despite the potentially fatal effects of chloroquine, there may be some therapeutic potential for certain cardiac conditions. In patients with persistent atrial fibrillation, a 14-day regimen of chloroquine decreased burden of atrial fibrillation.\textsuperscript{86} which may be related to blocking $I_{K_{ACh}}$ and $I_{K_1}$ to prolong atrial APs.

**PIP$_2$ interference**

PIP$_2$ is required for Kir2.x channel function (discussed previously in the section on PIP$_2$ regulation). Several pharmacologic agents exert their effects via interference with PIP$_2$—Kir2.x channel interaction. Quinacrine, which originally was developed as an antimalarial drug, has been demonstrated to inhibit Kir2.x channels in guinea pig neurons.\textsuperscript{88} Quinacrine has high lipophilicity and interacts with membrane phospholipids directly. It differentially inhibits Kir2.3 to a greater degree than Kir2.1 channels. It is proposed that this occurs via direct pore blockade and disruption of PIP$_2$—Kir channel interaction. Evidence for disruption of this interaction was due to the slow onset of blockade by quinacrine, in addition to increasing or decreasing the affinity of the channel for PIP$_2$, respectively, which resulted in either decreased or increased sensitivity to blockade. In addition, application of PIP$_2$ with quinacrine resulted in decreased inhibition of Kir2.x channels.

Carvedilol is a commonly used $\beta$- and $\alpha$-adrenoceptor antagonist used in the treatment of congestive heart failure, hypertension, and myocardial infarction.\textsuperscript{89,90} In addition to its effects on adrenoceptors, it acts as a multichannel blocker that can inhibit $I_{K_1}$, $I_{Ks}$, $I_{KATP}$, $I_{Ca-L}$, $I_{Ca-T}$, and $I_{Na}$ with variable potency.\textsuperscript{91–94} Studies in HEK293 cells demonstrated that carvedilol inhibits $K_{ATP}$ and $K_{ACh}$ channels, with no direct effect on $I_{K_1}$.\textsuperscript{95} The high lipophilicity and alpha-hydroxyl secondary amine functional group of carvedilol may insert into the membrane and interfere with PIP$_2$-channel interaction and thereby may inhibit Kir2 channels.\textsuperscript{95} Kir2.3 has lower affinity for PIP$_2$ compared to Kir2.1. It is inhibited by carvedilol with $IC_{50} = 0.49$ $\mu$M, which is 100-fold higher than the $IC_{50}$ of Kir2.1 ($>50$ $\mu$M). Inhibition was concentration- and voltage-dependent. Increasing the affinity of Kir2.3 for PIP$_2$ resulted in decreased inhibition by carvedilol.\textsuperscript{96} Additionally, addition of exogenous PIP$_2$ decreased the inhibitory effect.

Interestingly, gambogic acid, which is an anticancer agent, showed both PIP$_2$ interference and pore-block effects on homomeric and heteromeric channels.\textsuperscript{97} Gambogic acid showed slow inhibition of monomeric and heteromeric channels at low micromolar concentrations; however, it did not reach saturation during the course of experiments in *Xenopus* oocytes and was irreversible. Site-directed mutation of amino acids involved in polyamine block resulted in reduced inhibition in both homomeric and heteromeric channels, suggesting a direct pore-block mechanism. In addition, mutation of residues to alter the affinity for PIP$_2$ resulted in increased inhibition when affinity was increased (I214L Kir2.3).\textsuperscript{97} This effect was more pronounced in Kir2.3 and Kir2.2 channels than in Kir2.1 channels, which may be explained by their differences in PIP$_2$ affinity.

**Cross-reactivity**

Dronedarone is a Class III antiarrhythmic drug used to treat atrial fibrillation.\textsuperscript{98} It was shown in guinea pig ventricular myocytes that dronedarone is a multichannel blocker that inhibits $I_{Na}$, $I_{Ca-L}$, $I_{Kr}$, $I_{Ks}$, and $I_{K_1}$.\textsuperscript{99} Dronedarone inhibited $I_{K_1}$ in a dose-dependent manner, with incomplete block at 10 and 30 $\mu$M in guinea pig ventricular myocytes. Expression of heterogeneous channels in *Xenopus* oocytes revealed inhibition of Kir2.1 but not Kir2.2 or Kir2.3.\textsuperscript{100} The onset of block was slow and reversible upon washout. Blockade had no voltage- or frequency-dependence. Kir2.1 mutation of E224 within the cytoplasmic pore region of the channel resulted in loss of dronedarone inhibition, suggesting that this site is involved in binding the drug to mediate its effect.

Recently, a selective $I_{K_1}$ agonist was identified.\textsuperscript{101} Zacopride is a potent 5-HT$_3$ receptor antagonist and 5-HT$_4$ receptor agonist, commonly used as a gastrointestinal prokinetic agent. Application of zacopride to isolated rat cardiomyocytes revealed dose-dependent activation, resulting in hyperpolarization of the resting membrane potential and shortening of the AP. Interestingly, in a model of drug-induced arrhythmia, treatment with zacopride protected from ventricular arrhythmias.\textsuperscript{101} This agonist effect on $I_{K_1}$ is specific to Kir2.1, as treatment of atrial myocytes resulted in no drug effect.\textsuperscript{23} Zacopride treatment of homomeric Kir2.1 channels of HEK293 cells increased current density but not homomeric Kir2.2, Kir2.3, or Kir2.1/Kir2.2, and Kir2.1/Kir2.3 heteromeric channels. Mutation of the putative PKA phosphorylation site in Kir2.1 abolished zacopride-mediated increase in $I_{K_1}$ and suggests that zacopride mediates its effect via PKA phosphorylation of Kir2.1 channels.

$I_{K_1}$ is susceptible to modulation by pharmacologic agents, which initially were identified as blocking other ion channels. Quinidine is used clinically to terminate atrial fibrillation as a Class IA antiarrhythmic drug. However, it is well documented to be a multichannel inhibitor.\textsuperscript{102} It was demonstrated that quinidine inhibited Kir2.1 by acute pore block of subunits via interactions with E224, F254, and D259 residues.\textsuperscript{103} Further investigation of other Kir2.x isoforms revealed Kir2.3 to have the highest affinity for quinidine.\textsuperscript{102} Block is achieved in a voltage-dependent manner, with residues E224, F254, D259, and E299 essential for block in Kir2.1 and corresponding residues in Kir2.3; however, only D260 was essential in Kir2.2.\textsuperscript{104} Mutational analysis of PIP$_2$ sensitivity sites revealed that high PIP$_2$ affinity resulted in low inhibition by quinidine and vice versa.\textsuperscript{104} Although Kir2.1 is the least sensitive to quinidine compared to Kir2.2 and Kir2.3, caution still should be used when prescribing this drug to avoid potential proarrhythmic effects.

Propafenone, another Vaughan-Williams Class IC antiarrhythmic drug, was also shown to inhibit Kir2 channels. It has greater efficacy for Kir2.3 compared to Kir2.2 and Kir2.1, most likely related to differences in PIP$_2$ affinity.\textsuperscript{105}
Interestingly, extracellular K+ does not affect inhibition by propafenone; however, decreasing intracellular K+ results in subconductance levels in channel gating and reduced affinity for PIP2. Using mutagenesis, propafenone binds to a cytoplasmic domain located at the interface between subunits, with conserved arginine residues 228 and 260 being important for binding.105

**Clinical disease association and arrhythmogenesis related to KCNJ2 mutations**

The association of KCNJ2 mutations in arrhythmogenic disease has been recognized for more than 20 years. Clinical phenotypic disease association with KCNJ2 mutations has been described for ATS,106 short QT syndrome type 3 (SQT3),107 catecholaminergic polymorphic ventricular tachycardia (CPVT),108,109 and familial atrial fibrillation (FAF).109 Here we review the various phenotypes, broadly classified as gain of function (SQT3 and FAF) vs loss of function (ATS and CPVT), and what is known about the arrhythmia mechanism(s) for different phenotypes.

The 2 clinical phenotypes associated with a gain of Kir2.1 function are SQT3 and FAF, both of which are exceedingly rare. SQT3 patients are known to have both atrial fibrillation and ventricular fibrillation, and the resting ECG has characteristic extreme abbreviation of repolarization, with QT <300 ms.107 Due to the low prevalence of these disorders, little is known about the clinical course. It has been shown experimentally that KCNJ2 gain of function dramatically shortens the AP and therefore the atrial and ventricular refractory period. Mechanistically, computer modeling has demonstrated this in turn supports the initiation and stabilization of rotors of fibrillation.110 Pharmacologic therapy logically rests on prolonging repolarization, and clinical use of hydroxychloroquine for that purpose has been reported.111

ATS is a rare (although probably underreported clinically and in the literature) disorder comprising a clinical triad of ventricular arrhythmia, periodic paralysis, and dysmorphic features.106 Due to the ubiquitous presence of Kir2.1 in excitable tissue, it is not surprising that there is a notable overlap with neurologic symptoms and skeletal muscle abnormalities occurring in ATS patients with loss of normal function mutations in KCNJ2. The majority of ATS patients harbor mutations in KCNJ2, but not all patients manifest the full clinical triad.112 This incomplete penetrance of clinical features does not seem to be mutation specific, as individual family members can present differently. Additionally, there may be some modification based on sex for some mutations.113 ECG findings of ATS patients include frequent ventricular ectopy and prominent U waves with usually normal QT intervals but long QTu intervals.114 In addition, more sustained ventricular arrhythmia associated with ATS includes polymorphic ventricular tachycardia and bidirectional ventricular tachycardia (BiVT).115 The observation of BiVT in ATS patients is interesting, as this is the signature arrhythmia for CPVT. In contrast to patients with CPVT due to RYR2 or CASQ2 mutations (CPVT1 and CPVT2, respectively) who experience arrhythmia during adrenergic surges, ATS patients can have BiVT at rest and even during sleep. There is some nuance to this finding, as some ATS patients can present with stress-/exercise-induced BiVT and polymorphic ventricular tachycardia, in a manner similar to CPVT, so the clinical syndrome of CPVT has been associated with KCNJ2 mutations. However, this phenotypic label probably is inconsistent with classically described CPVT1 or CPVT2 because the clinical course is much more benign for KCNJ2 mutation–associated CPVT phenotypes. Compared to CPVT1/CPVT2, ATS patients have a very low incidence of sudden death, as recently reviewed by Pérez-Riera et al.115 Moreover, ATS also has been categorized with long QT syndrome (LQTS) as LQT7.116,117 Recent experimental work and ongoing clinical observations call this categorization of ATS as an LQTS into question.118,119 These distinctions are important due to divergent clinical management strategies between CPVT and LQTS.

Experimental investigation has provided some insight into the mechanism for BiVT in patients with certain KCNJ2 loss-of-function mutations or loss-of-function models. Loss of Kir2 function can lead to membrane instability and therefore promote or fail to inhibit triggered activity. In a rabbit heart failure model with barium initiated to block IK1,1 loss of Kir2.1 current was linked to small increases in diastolic Ca2+, causing an imbalance in membrane voltage to intracellular Ca2+ (V_m/Cai imbalance) leading to delayed afterdepolarizations (DADs).120 Using a canine ventricular wedge model, IK1 block predisposed to DADs, and this was proposed as a mechanism for both U waves and ventricular arrhythmia in ATS.121 However, ATS has been categorized as a LQTS, and theoretically ventricular arrhythmia should be related to triggered activity of early afterdepolarizations (EADs). That said, as a strong rectifier, IK1 is negligible at AP plateau voltages, so loss of IK1 is unlikely to induce phase 2 EADs, which are classically associated with torsades de pointes from AP prolongation and phase 2 EAD vulnerability.122 We sought to address this controversy by creating a transgenic mouse model with KCNJ2 mutation from a patient in our clinic associated with stress-induced BiVT.125 Isolated myocytes demonstrated phase 3 but not phase 2 EADs in association with adrenergic-induced loss of IK1 and repolarization reserve (Figure 3).33 DADs were noted but were much less frequent than phase 3 EADs. Our findings are supported by others who have shown using mathematical modeling that inhibition of IK1 underlies initiation for phase 3 EADs.123

We suspect that, at least in our transgenic model, a hybrid of EAD and DAD conditions is necessary for phase 3 EADs, as these require adrenergic stimulation and Ca2+ loading (DAD conditions) with adrenergic-dependent loss of IK1. AP prolongation. Thus, the clinical phenotypic syndrome, although categorized as an LQTS but mimics CPVT, actually is neither one. Important clinical implications arise from this observation because beta-blockers alone likely will not adequately suppress ATS-related ventricular arrhythmia,124 and agents that block phase 3 EADs are likely to be more effective treatment methods.125 Understanding the mechanism behind ATS...
arrhythmia and methods for arrhythmia suppression are active investigations in our laboratory.

**iPSCs and Kir2.x**

**Features of iPSC-CMs**

The advances of using iPSCs has allowed for the investigation of many human diseases in a human context without the need for invasive biopsies. Furthermore, development of patient-specific iPSC-CMs enables the investigation of inherited arrhythmia disorders such as LQTS and CPVT. Most iPSC-CMs possess several features of immature cardiomyocytes, which limits their use for modeling cellular arrhythmia mechanisms. This is due in part to immature AP properties with a depolarized resting membrane potential and spontaneous automaticity due to small $I_{K1}$ and unchecked $I_f$. 

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**Figure 3** Adrenergic-dependent phase 3 early afterdepolarizations (EADs) in R67Q+/− mice. A: Representative traces from action potential recordings (left) and calcium transients (right). B: R67Q+/− have more EADs and delayed afterdepolarizations (DADs) following adrenergic stimulation. C: Takeoff potential of EADs in R67Q+/− myocytes. D: Linear regression of takeoff potential vs peak voltage of phase 3 EADs. ISO = isoproterenol; WT = wild type. (From Reilly L, Alvarado FJ, Lang D, et al. Genetic loss of IK1 causes adrenergic-induced phase 3 early afterdepolarizations and polymorphic and bi-directional ventricular tachycardia. Circ Arrhythm Electrophysiol 2020;13:e008638.)
Spontaneous automaticity interferes with the ability to control the depolarization frequency, undermining attempts to model bradycardia-or pause-dependent arrhythmias such as torsades de pointes, the arrhythmia characteristic of atrial fibrillation. We have demonstrated that an electrically native-like myocyte-derived current (Ik1) that remains several levels below what is observed in adult cardiac myocytes.136,139

Our group has demonstrated that an electrically native-like myocyte can be achieved with the creation of IK1-enhanced iPS-CMs. We have shown that Ik1 enhancement expands the normal polarized membrane potential, allowing for recovery of inactivation for sodium channels, reflected in dV/dt values in the range of adult myocytes.9 A polarized membrane also allows for larger calcium transients compared to controls, without changes in basal calcium levels or rate of decay of the transient. Normal excitability and calcium cycling are essential cardiomyocyte properties required for disease modeling. Studies without a normal polarized membrane with physiologic IK1 hinder the accurate evaluation of pharmaceutical agents.60 These are important considerations when utilizing iPS-CMs for cardiac drug safety testing such as the Food and Drug Administration–initiated CiPA.141

Summary
Significant progress has been made toward understanding the role of Kir2.x in cardiac excitability, structure, function, and regulation, but there is still much to be learned from studying this channel. New arrhythmia mechanisms from novel mutations and new pharmaceutical agents are emerging, highlighting the importance of fully understanding the role of this channel in cardiac excitability, response to therapeutic drugs, and disease.

References
1. Moher D, Liberati A, Tetzlaff J, Altman DG, Group TP. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement. PLoS Med 2009;6:1.e1000097.
2. Nichols CG, Makhina EN, Pearson WL, Sha Q, Lopatin AN. Inward rectification and implications for cardiac excitability. Circ Res 1996;78:1–7.
3. Krapivinsky G, Gordon EA, Wickman K, Velimirović B, Krapivinsky L, Clapham DE. The G-protein-gated atrial K+ channel IK1 is a heteromultimer of two inwardly rectifying K+ channel proteins. Nature 1995;374:135–141.
4. Nichols CG, Lopatin AN. Inward rectifier potassium channels. Annu Rev Physiol 1997;59:171–191.
5. Kubo Y, Baldwin TJ, Jan YN, Jan LY. Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature 1993;362:127–133.
6. Anumonwo JMB, Lopatin AN. Cardiac strong inward rectifier potassium channels. J Mol Cell Cardiol 2010;48:45–54.
7. Li X, Ortega B, Kim B, Wellin PA. A common signal path drives AP-1 protein-dependent Golgi export of inwardly rectifying potassium channels. J Biol Chem 2016;291:14963–14972.
8. Muscher D, Peper K. Two components of inward current in myocardial muscle fibers. Pflügers Archiv 1969:307:190–203.
9. Vaidyanathan R, Markandeya YS, Kamp TJ, Makielcki JC, January CT, Eckhardt LL. IK1-enhanced human-induced pluripotent stem cell-derived cardiomyocytes: an improved cardiomyocyte model to investigate inherited arrhythmia syndromes. Am J Physiol Heart Circ Physiol 2016;310:H1611–H1621.
10. Huang C-L, Feng S, Hilgemann DW. Direct activation of inward rectifier potassium channels by IP3 and its stabilization by Gβγ7. Nature 1998;391:35882.
11. Fan Z, Makielcki JC. Anionic phospholipids activate ATP-sensitive potassium channels. The J Biol Chem 2007;282:27322–27328.
12. Hansen SB, Tao X, MacKinnon R. Structural basis of IP32 activation of the classical inward rectifier K+ channel Kir2.2. Nature 2013;477:495–498.
13. Ballester LY, Vanoye CG, George AJ, Jr. Exaggerated Mg2+ inhibition of KCN2 as a consequence of reduced IP2P2 sensitivity in Andersen syndrome. Channels 2007;1:209–217.
14. Lopes CMB, Zhang H, Rohacs T, Jin T, Yang J, Logothetis DE. Alterations in conserved Kir2 channel-IP3 interactions underlie channelopathies. Neuron 2002;34:933–944.
15. Liu GX, Derct C, Schlichter C, et al. Comparison of cloned Kir2 channels with native inward rectifier K+ channels from guinea-pig cardiomyocytes. J Physiol 2001;512:115–126.
16. Vaidyanathan R, Taffet SM, Vikstrom KL, Anumonwo JMB. Regulation of cardiac inward rectifier potassium current (IK1) by synapase-associated protein-97. J Biol Chem 2010;285:28000–28009.
17. Preissig-Müller R, Schlichter C, George T, et al. Heteromeration of Kir2.x potassium channels contributes to the phenotype of Andersen’s syndrome. Proc Natl Acad Sci U S A 2002;99:7774–7779.
18. Vaidyanathan R, Ert HV, Haq KT, et al. Inward rectifier potassium channels (Kir.x.s) and caveolin-3 domain-specific interaction. Circ Arrhythm Electrophysiol 2018;11:005800.
19. Wang Z, Yue L, White M, Pelletier G, Nattel S. Differential distribution of inward rectifier potassium channels in rabbit atrial and ventricular muscle transcripts in human atrium versus ventricle. Circulation 1998;98:2422–2428.
20. Melnyk P, Zhang L, Shrier A, Nattel S. Differential distribution of Kir2.1 and Kir2.3 subunits in canine atrium and ventricle. Am J Physiol Heart Circ Physiol 2002;283:H1123–H1133.
21. Dhamoon AS, Pandit SV, Sarmast F, et al. Unique Kir2.x properties determine regional and species differences in the cardiac inward rectifier K+ current. Circ Res 2006;94:1332–1339.
22. Du X, Zhang H, Lopes C, Mirthshali T, Rohacs T, Logothetis DE. Characteristic interactions with phosphatidylinositol 4,5-bisphosphate determine regulation of Kir channels by diverse modulators. J Biol Chem 2004;279:37271–37281.
23. Zhang L, Liu Q, Liu C, et al. Zacopride selectively activates the Kir2.1 channel via a PKA signaling pathway in rat cardiomyocytes. Sci China Life Sci 2013;56:788–796.
24. Vega AL, Tester DJ, Ackerman MJ, Makielcki JC. Protein kinase A-dependent biochemical phenotype for V227EC-KCNJ2 mutation in catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol 2009;2:540–547.
25. Kalscheur MM, Vaidyanathan R, Orland KM, et al. KCNJ2 mutation causes an adrenergic-dependent rectification abnormality with calcium sensitivity and ventricular arrhythmia. Heart Rhythm 2014;11:885–894.
26. Fukler B, Brändle U, Glowatzki E, Zrenner H-P, Ruppersberg JP. Kir2.1 inward rectifier K+ channels are regulated independently by protein kinases and ATP hydrolysis. Nature 1994;313:1413–1420.
27. Wible BA, Biasi MD, Majumder K, Taglialatela M, Brown AM. Cloning and functional expression of an inwardly rectifying K+ channel from human atrium. Circ Res 1995;76:343–350.
28. Ashen MD, O’Rourke B, Kluge KA, Johns DC, Tomaselli GF. Inward rectifier K+ channel from human heart and brain: cloning and stable expression in a human cell line. Am J Physiol Heart Circ Physiol 1995;268:H506–H511.
29. Zaritsky J, Redell JB, Tempel BL, Schwartz TL. The consequences of disrupting cardiac inwardly rectifying K+ current (Ik1) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. J Physiol 2001;533:697–710.
30. Wischmeyer E, Karschin A. Receptor stimulation causes slow inhibition of IRK1 inward rectifying K+ channels by beta and alpha adrenergic blockers. Biophys J 1990;57:335–339.
31. Kalscheur MM, Vaidyanathan R, Orland KM, et al. KCNJ2 mutation causes an adrenergic-dependent rectification abnormality with calcium sensitivity and ventricular arrhythmia. Circ Res 1995;76:343–350.
32. Zrenner H-P, Glower A, et al. Heteromerization of Kir2.x channels are regulated independently by protein kinases and ATP hydrolysis. Nature 1994;313:1413–1420.
56. Sabirov RZ, Tominaga T, Miwa A, Okada Y, Oiki S. A conserved arginine residue in Kir2.3 contributes to inward rectification in cationic channel block. J Biol Chem 2009;284:11316–11327.

57. Gozlan Y, Koval J, Sutcliffe MJ. The selectivity filter of Kir2.1 channels: differential contribution by two distinct residues. J Physiol 2001;531:465–466.

58. Yang L, Frindt G, Palmer LG. Magnesium modulates ROMK channel–mediated potassium secretion. J Am Soc Nephrol 2010;21:209–2116.

59. Li J, Xie X, Liu J, et al. Lack of negatively charged residues at the external mouth of Kir2.2 channels enables the voltage-dependent block by external Mg2+. PLoS One 2014;9:13712.

60. Sackin H, Nanazashvili M, Li H, Palmer LG, Yang L. Modulation of Kir1.1 inactivation by extracellular Ca2+ and Mg2+. Biophys J 2011;100:1207–1215.

61. Owen JM, Quinn CC, Leach R, Findlay JBC, Boyett MR. Effect of extracellular cations on the inward rectifying Kir 2.1 currents. J Physiol 1999;64:471–488.

62. Yang L, Frindt G, Palmer LG. Magnesium modulates ROMK channel–mediated potassium secretion. J Am Soc Nephrol 2010;21:209–2116.

63. Li J, Xie X, Liu J, et al. Lack of negatively charged residues at the external mouth of Kir2.2 channels enables the voltage-dependent block by external Mg2+. PLoS One 2014;9:13712.

64. Matsuda H, dos S Cruz J. Voltage-dependent block by internal Ca2+ ions of inwardly rectifying Kir 2 channels in guinea-pig ventricular cells. J Physiol 1994;475:295–311.

65. Zara A, Rocchetti M, Brioschi A, Cantadori A, Ferroni A. Dynamic Ca2+–induced inward rectification of Kir2.1 during the ventricular action potential. Circ Res 1998;82:947–956.

66. Mazzanti M, DiFrancesco D. Intraocular Ca2+ modulates K+–inward rectification in cardiac myocytes. Pflugers Arch 1989;413:322–324.

67. Lopatin AN, Shantz LM, Mackintosh CA, Nichols CG, Pegg AE. Modulation of potassium channels in the hearts of transgenic and mutant mice with altered polypeptide biosynthesis. J Mol Cell Cardiol 2000;32:2007–2024.

68. Hehir J, Lopatin AN. Differential polypeptide sensitivity in inwardly rectifying Kir2 potassium channels. J Physiol 2006;571:287–302.

69. Lopatin AN, Makhina EN, Nichols CG. The mechanism of inward rectification of potassium channels: “long-pore plugging” by cytoplasmic polypeptides. J Gen Physiol 1995;106:923–955.

70. Nie L–H, John SA, DeCor FH. Sperm block of the strong inward rectifier potassium channel Kir2.1. J Gen Physiol 2002;120:53–66.

71. Yang J, Jan YN, Jan LY. Control of rectification and permeation by residues in two distinct domains in an inward rectifier K+ channel. Neuroreport 1995;14:1047–1054.

72. Kubo Y, Murata Y. Control of rectification and permeation by two distinct sites after the second transmembrane region in Kir2.1 K+ channel. J Physiol 2001;531:645–660.

73. Stanfield PR, Davies NW, Shelton PA, et al. A single aspartate residue is involved in both intrinsic gating and blockade by Mg2+ of the inward rectifier, IRK1. J Physiol 1994;475:1215–1216.

74. Shih J, Zeng X, Hingle CJ. Spermion channels selectively activate BK channels via the Ca2++–bowl site. Proc Natl Acad Sci U S A 2012;109:E2134–E2143.

75. Matamoros M, Perez-Hernandez M, Guerrero-Serna G, et al. Nav1.5–terminal domain binding to z-syntrophin increases membrane density of human Kir2.1, Kir2.2 and Nav1.5 channels. Cardiovasc Res 2016;10:279–290.

76. Ponce-Balbuena D, Guerrero-Serna G, Valdivia CR, et al. Cardiac Kir2.1 and Nav1.5 channels traffic together to the sarclemma to control excitability. Circ Res 2018;122:1501–1516.

77. Peres-Hernandez M, Matamoros M, Alfayate S, et al. Brugada syndrome trafficking–defective Nav1.5 channels can trap cardiac Kir2.1/2.2 channels. JCI Insight 2018;3:1215–1218.

78. Hille B. Ionic channels in excitable membranes. Current problems and biophysical approaches. Biophys J 1978;22:283–294.

79. Biemann G, Vreecke J, Carmeliet E. The mechanism of the inactivation of the inward-rectifying K current during hyperpolarizing steps in guinea-pig ventricular myocytes. Pflugers Arch 1987;410:604–613.

80. Shiota T, Matsuda H, Noma A. Fast and slow blockades of the inward-rectifier K+ channel by external divalent cations in guinea-pig cardiac myocytes. Pflugers Arch 1993;422:427–435.

81. Revenue E, Jan YN, Jan LY. Contributions of a negatively charged residue in the hydrophobic domain of the IRK1 inwardly rectifying K+ channel to K+–selective permeation. Biophys J 1996;70:754–761.

82. Sabirov RZ, Tominga T, Miwa A, Okada Y, Oiki S. A conserved arginine residue in the pore region of an inward rectifier K channel (IRK1) as an external barrier for cationic blockers. J Gen Physiol 1997;110:665–677.

83. Dutt C, Leyland ML, Spencer PJ, Stanfield PR, Sculptine MJ. The selectivity filter of a potassium channel, murine Kir2.1, investigated using scanning cysteine mutagenesis. J Physiol 1998;511:25–32.

84. Sunen NB, Stanfield PR. Potentials and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. J Physiol 1978;280:169–191.

85. Shieh R–C, Chang J–C, Arreola J. Interaction of Ba2+ with the pores of the cloned inward rectifier Kir+ channels Kir2.1 expressed in Xenopus oocytes. Biochem J 1998;375:2313–2322.
138. Yoshida S, Miyagawa S, Fukushima S, et al. Maturation of cardiomyocytes derived from human induced pluripotent stem cells by soluble factors secreted from human mesenchymal stem cells. Mol Ther 2018;26:2681–2695.

139. Lewandowski J, Rozwadowska N, Kolanowski TJ, et al. The impact of in vitro cell culture duration on the maturation of human cardiomyocytes derived from induced pluripotent stem cells of myogenic origin. Cell Transplant 2018; 27:1047–1067.

140. Goversen B, van der Heyden MAG, van Veen TAB, de Boer TP. The immature electrophysiological phenotype of iPSC-CMs still hampers in vitro drug screening: special focus on IK1. Pharmacol Therapeut 2018;183:127–136.

141. Colatsky T, Fermini B, Gintant G, et al. The Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative—update on progress. J Pharmacol Toxicol 2016; 81:15–20.