Potassium currents in the heart: Functional roles in repolarization, arrhythmia and therapeutics

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ABSTRACT

This is the second of the two white papers from the fourth UC Davis Systems Approach to Understanding Cardiac Excitation-Contraction Coupling and Arrhythmias Symposium (March 3-4, 2016), a biannual event that brings together leading experts in different fields of cardiovascular research. The theme of the 2016 symposium was "K^+ Channels and Regulation", and the objectives of the conference were several fold: 1) to identify current knowledge gaps; 2) to understand what may go wrong in the diseased heart and why; 3) to identify possible novel therapeutic targets; and 4) to further the development of systems biology approaches to decipher the molecular mechanisms and treatment of cardiac arrhythmias.

The sessions of the Symposium focusing on the functional roles of cardiac K^+ channel in health and disease, as well as K^+ channels as therapeutic targets, were contributed by Ye Chen-Izu, Gideon Koren, James Weiss, David Paterson, David Christini, Dobromir Dobrev, Jordi Heijman, Thomas O'Hara, Crystal Ripplinger, Zhilin Qu, Jamie Vandenberg, Colleen Clancy, Isabelle Deschenes, Leighton Izu, Tamas Banyasz, Andras Varro, Heike Wulff, Eleonora Grandi, Michael Sanguinetti, Donald Bers, Jeanne Nerbonne, and Nipavan Chiamvimonvat as speakers and panel discussants. This article summarizes state-of-the-art knowledge and controversies on the functional roles of cardiac K^+ channels in normal and diseased heart. We endeavor to integrate current knowledge at multiple scales, from the single cell to the whole organ levels, and from both experimental and computational studies.

I. Diversity and functional roles of K^+ channels in cardiac repolarization: from single cell to whole organ levels

There are many types of K^+ channels in mammalian cardiac cells, the expression of which varies greatly throughout the heart, from atria to ventricles, epi- to endocardium, and from apex to base. This remarkable diversity allows for precise and differential control of resting membrane potential (RMP), action potential (AP) duration (APD), and refractoriness throughout the heart (Bartos *et al.*, 2015). Most cardiac cells express some combination of voltage-gated transient outward (I_{to}), voltage-gated delayed rectifier, inward rectifier (I_{K1}), and ligand-gated (ATP-sensitive ($I_{K,ATP}$), acetylcholine-activated (I_{KACh}), and Ca^{2+}-activated ($I_{K,Ca}$)) K^+ channels. Three delayed rectifier K^+ currents have been distinguished: ultra-rapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) (Zeng *et al.*, 1995). The structure, function, and regulation of each channel type are discussed in detail in the companion white paper by Grandi *et al*. (*J Physiol, 2016*). Here, we will highlight the diversity of K^+ channels throughout the heart and discuss how this diversity contributes to heterogeneities in repolarization and APD.

K^+ channel diversity: atrio-ventricular differences

There are several key differences between atrial and ventricular APs, with ventricular myocytes having a longer APD, a more hyperpolarized RMP, a longer plateau phase that reaches a more depolarized potential, and a faster rate of repolarization compared to atrial myocytes (Schram *et al.*, 2002). Although differential expression of Na^+ and Ca^{2+} channels, Na^+/Ca^{2+} exchanger (NCX), and gap junctions contributes to atrioventricular differences, K^+ channel diversity also plays a critical role. Most notably, I_{Kur} and $I_{K,ACh}$ (corresponding to the $K_v1.5$ and $K_{ir}3.1$:$K_{ir}3.4$ proteins, respectively) are nearly absent in ventricular myocytes, whereas both of these K^+ currents contribute significantly to the atrial AP in humans and in several animal models, including dogs, guinea pigs, and rats. (Boyle & Nerbonne, 1991;

Paulmichl *et al.*, 1991; Fedida *et al.*, 1993; Wang *et al.*, 1993; Dobrzynski *et al.*, 2001; Schram *et al.*, 2002; Gaborit *et al.*, 2007). Likewise, small-conductance Ca^{2+}-activated K^+ (SK) channels ($K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$), which underlie $I_{K,Ca}$, are predominantly expressed in the atria where they contribute to repolarization in mice and in humans (Xu *et al.*, 2003; Tuteja *et al.*, 2005). Therefore, targeting these atrial specific currents (I_{Kur}, I_{KACh}, and $I_{K,Ca}$) may represent a novel area for therapeutic intervention for atrial arrhythmias, the details of which are discussed in **Section IV**.

Ventricular myocytes exhibit a prominent I_{K1} and corresponding higher expression of $K_{ir}2.1$ (*KCNJ2*), which likely contributes to the more hyperpolarized RMP in ventricular *versus* atrial myocytes (Giles & Imaizumi, 1988; Dhamoon *et al.*, 2004; Gaborit *et al.*, 2007). The plateau phase of the AP is longer in ventricular cells due to a lower density of K^+ currents activated during the notch phase (smaller I_{to} and lack of I_{Kur}). This prolonged plateau phase allows for increased recovery of I_{Kr} from inactivation and a faster rate of repolarization in the ventricular myocytes.

Ventricular K^+ channel diversity: Transmural differences

AP characteristics and corresponding K^+ channel expression across the ventricular wall from epi- to endocardium have been well characterized in canine heart (Antzelevitch *et al.*, 1999; Antzelevitch, 2010). Three AP waveforms have been identified: epicardial, mid-myocardial (M-cells), and endocardial (**Figure 1**). The key differences between these cell types include a large 'spike and dome' or notch phase in epicardial cells, and a significantly prolonged APD in M-cells (Yan *et al.*, 1998). Epicardial cells also have a shorter APD than endocardial cells, whereas endocardial cells have a less pronounced notch phase (Sicouri & Antzelevitch, 1991). In addition to a longer baseline APD, a key feature of M-cells is a disproportionately prolonged APD in response to a slowing of rate and/or in response to

APD-prolonging agents (Antzelevitch *et al.*, 1999) (**Figure 1**). The ionic determinants of the unique features of canine M-cells have been suggested to include a smaller I_{Ks} and a larger late Na^+ current ($I_{Na,L}$) (Liu & Antzelevitch, 1995; Zygmunt *et al.*, 2001). The disproportionate prolongation of APD in canine M-cells can lead to increased transmural dispersion of repolarization and the likelihood for reentrant arrhythmias. Furthermore, a prolonged APD may also predispose M-cells to early afterdepolarizations (EADs). Thus, if present in human ventricles, M-cells may represent an important therapeutic target for the suppression of ventricular arrhythmias, especially in the setting of reduced repolarization reserve (Wilson *et al.*, 2011). There is, however, considerable disagreement on the presence and functional importance of M cells, particularly in the human heart and the functional role of M cells in contributing to the dispersion of ventricular repolarization remains a topic of active debate.

In canine ventricles, the prominent notch phase of epicardial APs has been attributed to a large I_{to} (Litovsky & Antzelevitch, 1988; Furukawa *et al.*, 1990; Nabauer *et al.*, 1996). In human and canine ventricles, $K_v4.3$ α-subunits, together with the auxiliary subunit, KChIP2, generates I_{to}. Interestingly, KChIP2, but not Kv4.3, mRNA expression is correlated with the gradient of I_{to} and prominence of the AP notch (Rosati *et al.*, 2001; Gaborit *et al.*, 2007). I_{Kr} and I_{K1} are also important for canine ventricular repolarization, although there is no clear evidence of transmural differences in these currents (Liu & Antzelevitch, 1995).

Ventricular K^+ channel diversity: RV/LV differences

The transmural gradient of APD described above exists in canine right and left ventricles (RV and LV); however, the APD is typically longer in canine LV, compared to the RV (Sicouri & Antzelevitch, 1991). The shorter APD in the RV is associated with an increased I_{to} and increased expression of both KChIP2 and $K_v4.3$ in both human and canine

hearts (Di Diego *et al.*, 1996; Volders *et al.*, 1999; Boukens *et al.*, 2009). In rodents, $K_v4.2$ α-subunits conduct I_{to} and $K_v4.2$ mRNA and protein expression are higher in the RV of rat hearts (Wickenden *et al.*, 1999). Canine RV myocytes also have increased I_{Ks} compared to LV, which correlates with increased protein expression of the accessory subunit, KCNE1, that modulates the $K_v7.1$ α-subunits that underlie I_{Ks} (Volders *et al.*, 1999; Ramakers *et al.*, 2003). No differences in other K^+ currents, including I_{Kr} or I_{K1}, have been observed between the RV and LV of canine hearts (Volders *et al.*, 1999); however, I_{K1} is higher in the LV than in the RV in guinea pig heart (Samie *et al.*, 2001; Molina *et al.*, 2016).

Ventricular K^+ channel diversity: Apico-basal differences

In the canine heart, APDs are shorter in the apex, compared to the base, of the LV and these shorter APDs correlate with increased I_{to} and I_{Ks}, as well as increased protein expression of KChIP2, $K_v7.1$ and mink (Szentadrassy *et al.*, 2005). No apico-basal differences in I_{K1} or I_{Kr} have been documented and expression of $K_{ir}2.1$ (I_{K1}), $K_v11.1$ (I_{Kr}), and MiRP1 are similar in the apex and base in the canine heart (Szentadrassy *et al.*, 2005).

Repolarization in Purkinje fibre

Purkinje fibres form specialized conduction system in the ventricles and have been shown to have unique electrophysiological properties and to play critical roles in the generation of cardiac arrhythmias (Boyden *et al.*, 2010). Specifically, canine Purkinje cells exhibit different types and densities of repolarizing K^+ currents, compared to ventricular or atrial myocytes, as well as markedly different AP profiles (Vassalle & Bocchi, 2013). Moreover, Purkinje cells display spontaneous impulse initiation, similar to pacemaker cells. It has also been shown that I_{to} is very large in canine Purkinje cells (Jeck *et al.*, 1995) and that I_{to} may play important roles in the generation of EADs in these cells (Zhao *et al.*, 2012).

β-adrenergic regulation of K^+ channels during cardiac AP

Extensive studies have shown that the cardiac K^+ channels responsible for AP repolarization are intricately regulated by β-adrenergic tone, and several K^+ channels –I_{Ks}, I_{Kr}, I_{K1}– exhibit differential sensitivity to β-adrenergic stimulation (Tromba & Cohen, 1990; Volders *et al.*, 2003; Thomas *et al.*, 2004; Harmati *et al.*, 2011). I_{Ks} is facilitated by β-adrenergic stimulation (Sanguinetti *et al.*, 1991; Marx *et al.*, 2002; Volders *et al.*, 2003; Harmati *et al.*, 2011); increased I_{Ks} contributes to the shortening of APD during rapid heart rates. When the I_{Ks} channel is defective in long QT syndrome (LQTS), a lack of adrenergic response of I_{Ks} could provide substrate for arrhythmias.

The effects of β-adrenergic stimulation on I_{Kr} have been controversial. Harmati *et al.* (Harmati *et al.*, 2011) and Heath *et al.* (Heath & Terrar, 2000) reported facilitation of I_{Kr} by isoproterenol *via* PKA and PKC pathways in canine and guinea pig ventricular myocytes. In contrast, Karle *et al.* (Karle *et al.*, 2002) reported a reduction of I_{Kr} amplitude following isoproterenol application in guinea pig ventricular myocytes and Sanguinetti *et al.* (Sanguinetti *et al.*, 1991) reported no measurable isoproterenol induced changes of I_{Kr}. Studies of the β-adrenergic stimulation on I_{K1} have also reported controversial results. Both facilitation and reduction of I_{K1} by isoproterenol have also been reported (Gadsby, 1983; Tromba & Cohen, 1990; Koumi *et al.*, 1995; Wischmeyer & Karschin, 1996; Fauconnier *et al.*, 2005; Scherer *et al.*, 2007).

Differential modulation of I_{Ks}, I_{Kr}, and I_{K1} by β-adrenergic stimulation has potentially important implications for cardiac AP repolarization and arrhythmogenesis. Banyasz *et al.* (Banyasz *et al.*, 2014) used the AP-clamp Sequential Dissection technique to directly record I_{Ks}, I_{Kr}, and I_{K1} during the AP under physiological condition (internal and external solutions matching physiological milieu with preserved Ca^{2+} homeostasis). I_{Ks}, I_{Kr}, I_{K1} were systematically measured during APs at various adrenergic states using isoproterenol in physiologically relevant concentration range (1-30 nM). Isoproterenol significantly enhanced

I_{Ks}, moderately increased I_{K1}, but slightly decreased I_{Kr} in a dose-dependent manner (**Figure 2**). By recording the three K^+ currents from the same cell, these investigators were able to dissect the relative contribution of each K^+ current to repolarization. These analyses revealed that the dominant pattern of the K^+ currents is $I_{Kr}>I_{K1}>I_{Ks}$ under physiological conditions, but that this pattern is reversed to $I_{Ks}>I_{K1}>I_{Kr}$ following ß-adrenergic stimulation (**Figure 2**). Therefore, ß-adrenergic stimulation fine-tunes the cardiac AP morphology by shifting the functional importance of the different K^+ currents in a dose-dependent manner. These findings clearly suggest an important role for sympathetic tone in determining the functional effects of K^+ channel blockers.

K^+ channel Diversity: What does it all mean?

As stated earlier, the remarkable diversity of K^+ channel expression throughout the heart allows for precise and differential control of local RMP, APD, and refractoriness. Indeed, computational AP models have shown that there are many possible combinations of ionic conductances that produce an equivalent RMP, APD and refractoriness. This is generally true of any input-output system in which the number of adjustable input parameters is large compared to the number of output constraints, leading to the concept that there are multiple "good enough solutions" that all can produce physiologically robust function (Marder & Goaillard, 2006; Weiss *et al.*, 2012). Why is this important? From an evolutionary biology standpoint, robustness is critical for any biological organism facing constantly changing environmental conditions. However, evolution also depends on the ability to adapt in response to changing environmental conditions. How, then, can robustness (the ability to maintain a stable phenotype) be compatible with adaptability (the ability to change phenotype)? A diverse range of good enough solutions among the individuals in a population resolves this paradox in the following manner: all good enough solutions confer robustness to the normal day-to-day environmental changes; however, when the environment changes in an

unusual way, some good enough solutions will adapt better than others (Marder & Goaillard, 2006; Weiss *et al.*, 2012).

As previously demonstrated by Sarkar & Sobie, one AP model may strongly depend on I_{Kr} for repolarization while the other is highly dependent on I_{Ks} (Sarkar & Sobie, 2010). Both models yield physiological APDs and Ca^{2+} transients. Now we can consider the consequences of administering an I_{Kr}-blocking drug to two individuals whose good enough solutions correspond to the two ventricular APs illustrated. The individual whose ventricular repolarization has a high dependence on I_{Kr} will exhibit much more significant APD and QT interval prolongation and consequently have a higher risk of Torsade de Pointes (TdP) than the other individual. Thus, in response to a potentially lethal perturbation, one perishes, but one survives. That is, robustness acts at the level of the individual, whereas adaptability operates at the level of the population. The good enough solutions concept underlying genetic diversity provides a compelling explanation for why the pronounced (~10-fold) heterogeneity in K^+-current amplitude between different cells (Banyasz *et al.*, 2011) is more than just random biological variability. Rather, it serves a fundamental biological role.

The "good enough solutions" concept also has major implications for mathematical modeling approaches to drug development and safety testing, in which off-target cardiac K^+ channel blocking effects are a major concern. At the present time, both non-cardiac and cardiac drugs must undergo expensive animal testing to screen for K^+ channel blocking and other effects that predispose to QT prolongation and TdP. Models which use average data to build "representative" cell models of a specific type provide a binary yes or no answer to whether a drug will cause excessive APD prolongation, and there is often disagreement between specific models (Mirams *et al.*, 2014). In biological populations, on the other hand, a I_{Kr}-blocking drug such as sotalol has a variable effect on APD and QT interval prolongation,

posing an overall risk of TdP of <5% to the population. With advances in high speed graphics processing unit (GPU)-based computation, it is becoming increasingly feasible to create populations of models to simulate the genetic and phenotypic diversity of human populations (Britton *et al.*, 2013). This approach starts with an AP model based on averaged data, and then randomly mutates the ionic conductances within appropriate experimentally-determined ranges. The mutated model is then examined to define its AP and Ca^{2+} transient properties. Mutations which cause the model to exhibit properties outside the physiologically normal range are excluded. However, mutated models still falling within the physiologically normal range are retained, generating a diverse model population incorporating a range of electrophysiological parameter values, each one representing a good enough solution for a normal cardiac AP. The extent to which the model population realistically reflects a human population can be validated against existing clinical population data, for example the incidence with which known drugs cause QT prolongation and TdP. The ultimate goal is to be able to simulate the effects of a new drug on the clinically validated model population to yield a probabilistic, rather than a binary, estimate of adverse effects. A compelling futuristic strategy for integrating population-based modeling into a three component pre-clinical drug discovery and safety testing platform has recently been outlined by Gintant *et al* (Gintant *et al.*, 2016). The first component involves automated patch clamping to characterize in detail the biophysical effects of a drug on the major human cardiac ionic channels heterologously expressed in mammalian cells; the second component simulates these effects *in silico* in human population models to estimate probability of adverse effects; and the third component, if the drug still looks promising, is to test the drug's effects in population of genetically-diverse human induced pluripotent stem cell (hiPSC)-derived cardiac myocytes, perhaps even including the intended patient's iPSC-derived cardiac myocytes.

In summary, the biological variability and genetic diversity embodied in the "good enough solutions" concept apply to all aspects of human biology, including K^+ channel diversity, and continue to play essential roles in the evolutionary success of the human race. We now have the tools to characterize the genetic diversity of human populations in unprecedented detail. The ability to incorporate this information into drug development and safety testing is an exciting new forefront with tremendous potential to become a milestone in precision medicine (please see additional discussion in **Section IV**).

II. Roles of K^+ channels in cardiac arrhythmias

Cardiac arrhythmias can be divided into those resulting in abnormally fast electrical activity (tachyarrhythmias) or abnormally slow activity (bradyarrhythmias). Conceptually, tachyarrhythmias in both atria and ventricles are mediated by abnormal impulse formation, in particular ectopic (triggered) activity, and abnormal impulse propagation, notably reentry (Rosen, 1988; Weiss *et al.*, 2015) (**Figure 3**). Ectopic activity can result from secondary depolarizations occurring either during the AP, termed early afterdepolarizations (EADs, or during diastole, termed delayed afterdepolarizations (DADs), as well as from abnormal automaticity due to enhanced diastolic depolarization in normally quiescent tissue. When occurring repeatedly at a sufficiently rapid rate, ectopic activity can produce heterogeneous "fibrillatory" conduction and sustain a tachyarrhythmia. Moreover, when ectopic activity encounters a vulnerable substrate characterized by short effective refractory periods (ERPs), large repolarization gradients and slow heterogeneous conduction, it can initiate reentry, which is considered the predominant tachyarrhythmia-maintaining mechanism. Bradyarrhythmias, on the other hand, result from reduced automaticity in the sinoatrial node (e.g., in sick sinus syndrome) or impaired atrioventricular conduction. Numerous studies have

shown that K^+-channel dysregulation, resulting from genetic defects, drug effects, or disease-related remodeling can promote all of these fundamental arrhythmia mechanisms (**Figure 3**) (Nerbonne & Kass, 2005; Schmitt *et al.*, 2014).

The critical role of K^+-channel dysfunction in cardiac arrhythmias is particularly evident in congenital channelopathies such as long-QT and short-QT syndromes (LQTS and SQTS, respectively), Brugada or early repolarization syndrome (BrS), and familial ("lone") atrial fibrillation (AF), all of which have been associated with an increased likelihood of tachyarrhythmias. Genetic variants in more than 15 different genes, including loss-of-function mutations in the genes, *KCNQ1* and *KCNH2*, encoding the pore-forming α-subunits, $K_v7.1$ and $K_v11.1$, of I_{Ks} and I_{Kr} channels , as well as in the genes encoding the accessory β-subunits, *KCNE1* and *KCNE2*, of I_{Ks} and I_{Kr} channels, have been associated with LQTS (Nakano & Shimizu, 2016). Given the critical role of I_{Kr} and I_{Ks} in ventricular repolarization and the aforementioned heterogeneous distribution of these channels in the heart, these mutations are expected to result in heterogeneous APD prolongation. APD prolongation provides a larger window for activation of the depolarizing L-type Ca^{2+} current (I_{Ca}), as well as the late Na^+ current ($I_{Na,L}$), thereby increasing the risk for EADs and ectopic activity. Loss-of-function mutations in I_{K1}, which controls final AP repolarization and stabilizes the RMP, have also been associated with QT prolongation (Fodstad *et al.*, 2004). In addition to prolonging APD, loss of I_{K1} can destabilize the RMP, resulting in abnormal automaticity (Miake *et al.*, 2002) or in larger DAD amplitudes in response to a given transient-inward current, thereby more readily achieving the threshold for activating Na^+ channels and triggering an abnormal AP, all of which will increase the likelihood of ectopic activity.

There is also substantial evidence for K^+-channel dysregulation in more common cardiovascular diseases. For example, down-regulation of I_{K1}, I_{Ks} and I_{to} contributes to APD

prolongation in heart failure (Li *et al.*, 2004). Similarly, excessive APD prolongation and EAD-mediated triggered activity are likely also involved in the proarrhythmic side effects of numerous drugs, which predominantly result from effects on I_{Kr}. Consequently, analysis of potential I_{Kr} blocking effects is a mandatory element during the safety screening of every drug (Heijman *et al.*, 2014a).

Gain-of-function mutations in channel subunits generating I_{K1}, I_{Kr}, I_{Ks}, and I_{to}, as well as $I_{K,ATP}$,have been associated with ventricular arrhythmias in SQTs and BrS and with familial AF (Lieve & Wilde, 2015; Brugada, 2016; Christophersen & Ellinor, 2016; Sarquella-Brugada *et al.*, 2016). Mechanistically, gain-of-function mutations in cardiac K^+ channels will decrease APD and ERP, thereby increasing the likelihood of reentry. In addition, upregulation of I_{K1} may stabilize reentrant rotors through RMP hyperpolarization, promoting arrhythmia maintenance, as has been clearly demonstrated in the mouse (Noujaim *et al.*, 2007). Similar to loss-of-function K^+ channel disorders, increases in K^+ currents have also been observed in acquired cardiovascular diseases. Activation of $I_{K,ATP}$ during the early phases of ischemia, for example, shortens ERP and increases extracellular K^+, thereby increasing the K^+ reversal potential and depolarizing the RMP, which slows conduction velocity. Both factors likely contribute to reentrant ventricular tachyarrhythmias observed under these conditions.

APD shortening is also a hallmark of AF-related electrical remodeling, likely contributing to AF maintenance and progression (Heijman *et al.*, 2014b). Upregulation of I_{K1}, I_{Ks}, as well as the two-pore K^+ channel type 3.1 ($K_{2P}3.1$) current, for example, plays a major role in AF-related APD shortening and is sufficient to offset the downregulation of other K^+ currents, such as I_{Kur} and I_{to} (Dobrev *et al.*, 2005; Caballero *et al.*, 2010; Schmidt *et al.*, 2015). Upregulation of other K^+-channels could also be involved in cardiac

arrhythmogenesis, although most are incompletely understood. For example, in AF patients, $I_{K,ACh}$ develops constitutive, acetylcholine-independent activity, contributing to the increase in total inward-rectifier K^+ current, despite reduction of agonist-induced peak $I_{K,ACh}$ (Dobrev *et al.*, 2005). The role of SK channels in atrial arrhythmias similarly remains a topic of active investigation (Xu *et al.*, 2003; Zhang *et al.*, 2015). Expression of SK channels is increased in the failing heart and Ca^{2+}-dependent activation of SK channels may shorten APD during fibrillation/defibrillation episodes, contributing to re-induction of ventricular tachyarrhythmias (Chua *et al.*, 2011). On the other hand, SK channels may constitute an important repolarization reserve and their inhibition has been associated with increased susceptibility to pacing-induced ventricular arrhythmias during hypokalemia (Chan *et al.*, 2015).

Considerable evidence, therefore, demonstrates that K^+-channel dysfunction plays a major role in cardiac arrhythmias. For most channels, both loss-of-function and gain-of-function have been associated with arrhythmias, albeit through distinct mechanisms. These findings suggest the existence of a goldilocks zone in which a balance among the different K^+ currents, as well as between repolarizing K^+ currents and depolarizing Na^+ and Ca^{2+} currents, ensures stability of cardiac electrophysiological activity. This complex interaction of ion channels, as well as the multitude of proarrhythmic mechanisms and diversity of K^+-channel remodeling under different pathophysiological conditions, makes it challenging to predict the pro- or antiarrhythmic effects of a given alteration in K^+-channel function. Of note, it has become clear that chronic modulation of K^+-channel function (e.g., in a rabbit model of LQTS type 2) can produce extensive cardiac remodeling, which may further promote cardiac arrhythmias (Terentyev *et al.*, 2014).

Differential roles of K^+ currents in arrhythmogenesis

K^+ currents affect APD by regulating the rate of repolarization. The pro-arrhythmic potential of alterations in different K^+ channel types, however, are distinct, owing to differences in channel expression and/or biophysical properties.

Delayed rectifier K^+ currents (I_{Kr} and I_{Ks}). I_{Kr} activates and inactivates rapidly (Spector *et al.*, 1996), whereas I_{Ks} activates slowly (Tristani-Firouzi & Sanguinetti, 1998). Activation of I_{Ks} is also dependent on Ca^{2+}. In guinea pig, I_{Ks} may have many closed states (Silva & Rudy, 2005), and thus, as the heart rates become slower, more channels gradually enter the more deeply closed states, and fewer channels are available for opening during the AP. At fast heart rates, the slow deactivation results in I_{Ks} accumulation between beats and increased current during the AP plateau, resulting in APD shortening. In human and canine myocytes, however, I_{Ks} is small, deactivation is fast, and I_{Ks} accumulation does not occur between beats (Virag *et al.*, 2001).

Because of marked differences in kinetics, I_{Ks} and I_{Kr} play distinct roles in regulating AP dynamics at different heart rates. As shown in experiments by Antzelevitch and colleagues (Antzelevitch *et al.*, 1991; Antzelevitch *et al.*, 1999), after I_{Kr} is blocked, the APD of the M-cells becomes very long and continues to prolong at very slow heart rates. Theoretical studies show that in the presence of prolonged APDs with reduced repolarization reserve, the rate-dependence of APD at normal or slow heart rates and the slow activation and deactivation kinetics of a K^+ current together with an inward window current of $I_{Ca,L}$ may culminate in APD alternans at normal and slow heart rates (Qu *et al.*, 2010; Qu & Chung, 2012). This may form the basis for T-wave alternans seen clinically in LQTS patients (Schwartz & Malliani, 1975).

The distinct properties and functional roles of I_{Kr} and I_{Ks} provide mechanistic insights into the clinical presentation of different LQTS types (Schwartz, 2006). In LQT2 and LQT3, arrhythmias tend to occur during bradycardia or after a pause (Viswanathan & Rudy, 1999;

Viskin *et al.*, 2000; Clancy & Rudy, 2002), whereas in LQT1, arrhythmias are often exercise-induced (tachycardia-related). During slow heart rates, there are fewer I_{Ks} channels available for opening. Coupled with LQT2 and LQT3, this leads to disproportionately reduced repolarization reserve during bradycardia. In LQT1, I_{Kr} and I_{to} are the main repolarizing currents, and because these currents recover quickly, bradycardia does not preferentially reduce repolarization reserve. Instead, repolarization reserve is reduced during tachycardia, when Ca^{2+} elevation causing an increase in I_{NCX} and adrenergic stimulation of $I_{Ca,L}$ is no longer counterbalanced by the adrenergic stimulation of I_{Ks}.

Transient outward K^+ current (I_{to}). A distinct feature of I_{to} is that both activation and inactivation are fast, and I_{to} is largely responsible for the spike-and-dome AP morphology in large animals. Unlike other K^+ currents, increasing I_{to} in canine ventricular myocytes first prolongs, then dramatically shortens (collapses) APD (Dong *et al.*, 2006). I_{to} also plays a somewhat unexpected role in the genesis of EADs. Recent studies in rabbit ventricular myocytes (Zhao *et al.*, 2012; Nguyen *et al.*, 2015), for example, showed that, under conditions of reduced repolarization reserve, no EADs occurred without I_{to} and that the addition of I_{to} increased the likelihood of EADs, likely reflecting an effect on the membrane voltage and $I_{Ca,L}$ re-activation.

Inward rectifier K^+ current (I_{K1}). In addition to stabilizing the RMP, I_{K1} contributes to phase 3 repolarization. Reducing I_{K1} destabilizes RMP and results in pacemaker-like activity (Miake *et al.*, 2002; Silva & Rudy, 2003) or DAD-mediated triggered activity (Schlotthauer & Bers, 2000). Due to its strong inward rectification, I_{K1} is small during the AP plateau and likely will have only a small effect on phase-2 EADs, although reducing I_{K1} may promote phase-3 EADs (Maruyama *et al.*, 2011).

Importantly, because AP dynamics of a single myocyte and the spatiotemporal conduction dynamics of three dimensional cardiac tissues reflect the repertoire of ion channels expressed, altering the properties/expression of one type of channel can be proarrhythmic or antiarrhythmic, depending on the status of all of other ion channels present. As a result, the roles of individual K^+ channel types in arrhythmia generation and maintenance can only be fully understood by using multiscale and systems approaches that integrate molecular scale behaviors with tissue and organ scale behaviors (Qu & Weiss, 2015), as well as behaviors at the population scale taking into account genetic diversities and complex environmental stress. Clearly, this is one of the grand challenges facing cardiac arrhythmia research today.

III. Mechanisms of cardiac electrical remodeling in acquired disease

Regulation of cardiac K^+ channel expression both at the tissue and subcellular levels play critical roles in normal cardiac excitability. Remodeling of K^+ channel expression, which occurs in various disease states, can predispose to an increased risk of sudden cardiac death (Nass et al., 2008). Remodeling is the end result of multiple facets of cardiac disease. Several disease states provide stressors that cause an increased physical demand on the heart and higher strain on the individual myocytes of the heart. This strain becomes transduced to local signaling pathways, causing increased sympathetic tone and inflammatory activation, which stimulates altered protein expression (Armoundas *et al.*, 2001). Despite a significant amount of investigation, the precise mechanisms that underlie remodeling and drive pathogenesis remain incompletely understood and there is considerable interest in understanding the mechanisms that underlie the regulation of functional myocardial K^+ channel expression under both normal and pathological conditions.

Cells from diseased hearts often display a prolonged APD. Although increases in inward (depolarizing) (Undrovinas *et al.*, 1999; Houser *et al.*, 2000) or decreases in outward (repolarizing) (Nabauer & Kaab, 1998) currents can result in prolonged APs, remodeling of K^+ currents is, by far, the most prevalent mechanism leading to AP prolongation. Numerous studies over the last 10-20 years have documented changes in K^+ channel expression, including I_{to}, I_{Kr}, I_{Ks} and $I_{K1,}$ that occur in heart failure and AF both in animal models as well as human tissues (Nass *et al.*, 2008; Nattel *et al.*, 2008).

In AF, different changes have been described for I_{Kr} and I_{Ks} while an increase in I_{K1} appears to be the consensus (Nattel *et al.*, 2008; Yang & Nerbonne, 2016). I_{Kur} is the most prominent repolarizing current in human atrium and demonstrates pronounced remodeling in AF patients (Van Wagoner *et al.*, 1997; Van Wagoner, 2003). More recently, efforts have focused on the molecular mechanisms underlying these observations, as this is the key to determining whether it might be possible to modulate these processes for therapeutic benefit. Changes at the level of transcription, translation, assembly and biogenesis, post-translational modifications, cellular localization and degradation have all been reported (Heijman *et al.*, 2014b; Nattel, 2015; Yang & Nerbonne, 2016), but how these changes are interrelated remains to be determined.

Recent technical developments in "omics" technologies promise to be at the forefront of this field. At the gene level, next generation gene sequencing technologies have led to the identification of microRNAs and long non-coding RNAs (lncRNAs) that play critical roles in regulating transcription and translation of many genes, including ion channel genes (Greco & Condorelli, 2015; Myers *et al.*, 2015). At the protein level, new mass spectrometry methods have enabled global analyses of integrated responses rather than the analysis of individual components (Ferreira *et al.*, 2015). Protein homeostasis or "proteostasis" is another important

factor in regulating gene expression involving a highly complex interconnection of pathways that influence the fate of a protein from synthesis to degradation (Balch *et al.*, 2008). Further, it is likely that in stressed tissue, the overall cellular response may focus on maintaining certain subnetworks at the expense of others which can manifest as discrepancies between levels of transcripts, non-coding RNAs and proteins, that in turn could contribute to remodeling without necessarily maintaining a direct correlation between protein and mRNA levels (Balch *et al.*, 2008). Finally, developments in novel computer architectures have facilitated multi-scale modeling approaches to integrate details of changes at multiple molecular levels to predict outputs at the whole tissue level for the expression and distributions of ion conductances (Weiss *et al.*, 2012).

With these new levels of integration, we can now decipher not just what channels are altered, but how they change in terms of expression levels, post-translational modifications, subcellular localization and how these changes evolve over time (**Figure 4**). With such rich data sets, we will also be able to identify both the individual members, as well as networks of regulators (microRNAs, lncRNAs, transcription factors) that mediate electrical remodeling. Furthermore, we will be able to address questions such as how do related stresses result in such different outcomes, e.g., how does exercise result in adaptive hypertrophy whilst hypertension results in maladaptive remodeling. Importantly, the basis of these differences may be exploited for therapeutic benefits.

IV. Identification of novel therapeutic targets for cardiac arrhythmias

Multiple K^+ currents have been targeted for arrhythmia therapy. Unfortunately, to date, these target-specific drugs have not translated into new, safe, or effective antiarrhythmic therapy. Indeed, landmark studies of antiarrhythmic drugs have been shown to increase

mortality in patients with myocardial infarction or left ventricular dysfunction compared to placebo (CAST Investigators, 1989; Waldo *et al.*, 1996; Kober *et al.*, 2008). The key reason is the very fact that arrhythmia mechanisms are dependent on a highly complex and dynamic multi-scale system. Therefore, detailed mechanistic understanding of the molecular correlates, biophysical properties, and interdependence of cardiac ion channels at subcellular, cellular, and tissue levels are critical to the development of safe and effective antiarrhythmic drug therapy.

Atrial-specific ion channel blockers

AF is the most common sustained arrhythmia clinically (Ferrari *et al.*, 2016). Even though catheter ablation has been widely used for AF, the treatment is invasive and remains inadequate in a significant number of patients and development of new anti-arrhythmic drugs for AF is highly desirable. Because atrial and ventricular myocytes express distinct repertoires of ion channels, there has been considerable interest in developing atrial-specific ion channel blockers for atrial arrhythmias (Wettwer *et al.*, 2007; Burashnikov *et al.*, 2008; Antzelevitch & Burashnikov, 2010; Burashnikov & Antzelevitch, 2010; Schotten *et al.*, 2016; Voigt & Dobrev, 2016). However, it is critical to note that the degree of atrial selectivity of anti-arrhythmic drugs may be different in normal, compared to remodeled, hearts associated with different cardiac diseases.

$K_v1.5$, encoded by *KCNA5* (Tamkun *et al.*, 1991), underlies I_{Kur}, which is expressed in human atria, but not ventricles (Fedida *et al.*, 1993; Wang *et al.*, 1993). Blockers of I_{Kur}, AVE0118 and XEN-D0101, prolong atrial APD and ERP in animal models and in humans (Wettwer *et al.*, 2004; Schotten *et al.*, 2007; Christ *et al.*, 2008) and would be predicted to prevent AF in humans without risk of QT prolongation. However, $K_v1.5$ channels are down-regulated in chronic AF (Van Wagoner *et al.*, 1997; Van Wagoner, 2003). In addition,

blockade of I_{Kur} in human atrial tissue from patient in sinus rhythm elevates the AP plateau and shortens, rather than prolongs, APD, possibly by activating NCX in its reverse mode contributing to repolarizing current (Schotten *et al.*, 2007). Block of I_{Kur}, therefore, may provide the substrate for development of AF in healthy atria, *via* abbreviation of APD and ERP (Burashnikov & Antzelevitch, 2008). Moreover, evidence from human has shown that both loss-of-function and gain-of-function mutations in *KCN5A* are associated with early onset lone AF (Christophersen *et al.*, 2013).

Vernakalant was suggested as a potential I_{Kur} blocker for the treatment of AF. However, it is a multichannel blocker, inhibiting not only I_{Kur}, but also I_{to}, I_{Kr}, $I_{K,Ach}$, and $I_{K,ATP}$, as well as the peak and late I_{Na} (Fedida, 2007; Burashnikov *et al.*, 2008). The antifibrillatory effect of vernakalant may result from its blockade of the peak I_{Na} which increases cardiac excitation threshold, slows conduction, and creates a period of refractoriness (Burashnikov *et al.*, 2008). In addition, there are differences in the effects of vernakalant in canine and human atrial myocytes and there is a controversy about whether I_{Kur} in dog is generated by $K_v3.1$ or $K_v1.2$, rather than $K_v1.5$, as in human (Nattel *et al.*, 1999; Fedida *et al.*, 2003).

Another atrial-specific ion channel that has received considerable attention is $I_{K,ACh}$, encoded by the G-protein activated inwardly rectifying K^+ channel α-subunits, $K_{ir}3.1/K_{ir}3.4$ (Ravens *et al.*, 2013) The channels are more abundantly expressed in atrial than in ventricular myocytes (Krapivinsky *et al.*, 1995; Dobrzynski *et al.*, 2001; Schram *et al.*, 2002; Gaborit *et al.*, 2007). The current has been shown to mediate AF induced by vagal stimulation *via* activation of muscarinic M_2 receptors. $I_{K,ACh}$ hyperpolarizes the membrane potential and shortens atrial APs, contributing to maintenance of AF by promoting reentry (Kovoor *et al.*, 2001). More importantly, in chronic AF, the channels are constitutively active in the absence

of any M_2 receptor ligand (Dobrev *et al.*, 2005), suggesting $I_{K,ACh}$ should be viewed as a promising target for AF (Voigt & Dobrev, 2016).

Several antiarrhythmic agents including azimilide, dofetilide, dronedarone, ibutilide, sotalol and terikalant block $I_{K,ACh}$ and may contribute to their efficacy in AF (Ravens *et al.*, 2013). The benzopyrane derivative NIP-142 selectively blocks $I_{K,ACh}$ and reverses the shortening effect of carbachol or adenosine on atrial APs (Matsuda *et al.*, 2006) and inhibits vagally-induced AF. The congener NIP-151 blocks $I_{K,ACh}$ with a potency that is at least four orders of magnitude higher than I_{Kr} and is highly effective in canine AF models (Hashimoto *et al.*, 2008). Although many drugs have $I_{K,ACh}$ blocking properties, selective $I_{K,ACh}$ blockade has only recently been reported using the compound NTC-801 that has been shown to be effective in AF models (Machida *et al.*, 2011).

Novel therapeutic targets

Recent studies have provided evidence for possible novel therapeutic targets for K^+ channels in the treatment of cardiac arrhythmias. Specifically, studies have demonstrated the important roles of SK channels in cardiac repolarization. Indeed, interests in cardiac SK channels are further fueled by recent studies suggesting a possible role of SK channels in lone AF (Ellinor *et al.*, 2010; Christophersen & Ellinor, 2016). Recently, three different SK channel inhibitors including UCL1684, *N*-(pyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine (ICA) (Gentles *et al.*, 2008) and NS8593 have been shown to have anti-arrhythmic effects in models of AF in rat, guinea pig, rabbit, and dog (Diness *et al.*, 2010; Diness *et al.*, 2011; Qi *et al.*, 2014). The results from these studies suggest that SK channels may represent a potential therapeutic target for the treatment of atrial arrhythmias. However, there remain major gaps in our knowledge. Blockade of SK channels in cardiac arrhythmias has been shown to be

both anti-arrhythmic (Diness *et al.*, 2010; Diness *et al.*, 2011) and proarrhythmic (Hsueh *et al.*, 2013; Wagner & Maier, 2013) in various models, possibly influenced by the state of other currents that integrate with the SK channel current to shape AP.

Combination drug therapy

One of the most commonly prescribed anti-arrhythmic drugs is amiodarone, which has been shown to block several cardiac K^+ currents, as well as I_{Na} and I_{Ca}. It is also a noncompetitive antagonist of α- and β-adrenergic receptors. Amiodarone has been demonstrated to be effective and relatively safe without inducing TdP polymorphic ventricular tachycardia that is seen as a result of QT prolongation (Zimetbaum, 2007; Singh, 2008). In addition to amiodarone, several anti-arrhythmic drugs including dronedarone, vernakalant and ranolazine have been shown to be effective clinically for AF with low incidence of proarrhythmias (Burashnikov & Antzelevitch, 2010). These anti-arrhythmic drugs inhibit I_{Na} with relatively fast kinetics, as well as block I_{Kr} and late I_{Na} (Burashnikov *et al.*, 2008). In addition, rapidly dissociating I_{Na} blockers are found to be atrial selective in contrast to the slowly dissociating blockers (Burashnikov *et al.*, 2008). A recent new line of investigation uses combinations of antiarrhythmic drugs that may allow the use of lower dosages with reduced side effects and better balance the inward and the outward currents in normalizing AP (Aguilar *et al.*, 2015; Reiffel *et al.*, 2015).

Drug-induced proarrhythmias

Drug-induced proarrhythmias can be caused by both cardiovascular and non cardiovascular drugs and can be life-threatening. This is due to drug-induced QT prolongation and TdP polymorphic ventricular tachycardia. Individual susceptibility includes pharmacokinetic risk factors and genetic predisposition. Additional risk factors include

structural heart disease and electrolyte imbalance. Drug induced proarrhythmias are discussed in more details in the companion white paper (see Grandi *et al.*, 2016).

Inherited mutations in the $K_v11.1$ (hERG) α-subunit of I_{Kr}, encoded by *KCNH2*, are linked to type 2 LQTS. In addition, life-threatening arrhythmia can also be induced by blockade of $K_v11.1$ channels in a surprisingly diverse group of drugs with vastly different chemical structures. Antiarrhythmic, antihistamine, antimicrobial, antipsychotic, and antidepressant drugs are important classes associated with risk of proarrhythmia. Indeed, effect on I_{Kr} is a common reason for drug failure in preclinical safety trials. Even though inherited LQTS and TdP can be caused by loss-of-function mutations in multiple cardiac K^+ channels, drug-induced LQTS and TdP are predominantly caused by direct blockade of $K_v11.1$ channels or disruption of $K_v11.1$ channel trafficking to the cell surface (Sanguinetti & Tristani-Firouzi, 2006; Yang *et al.*, 2014).

Previous studies have suggested that $K_v11.1$ channels have structural features that can more effectively accommodate the binding of drugs compared with other K^+ channels. Specifically, two aromatic residues (Tyr 652 and Phe 656) located in the S6 domain of the $K_v11.1$ subunit are likely important for binding of several classes of drugs. The side chains of the two residues are orientated towards the large central cavity of the channel. More importantly, these two residues are not conserved in other K_v channels in which an Ile and a Val are found in homologous positions. It was suggested that the eight aromatic side chains per channel are arranged in two concentric rings to accommodate drug-channel interactions (Sanguinetti & Tristani-Firouzi, 2006).

V. Experimental Models in the Study of Cardiac K^+ Channel Function

A variety of experimental animal models have been used over the years in studies detailing the time- and voltage-dependent properties and the physiological roles of the many K^+ channels expressed in the mammalian heart (Barry & Nerbonne, 1996). Similar to functional analysis of other types of ion channels, the mouse models began to be used increasingly in the mid-1990s to explore the molecular determinants of native myocardial K^+ channels primarily owing to the ease and speed with which molecular genetic strategies can be exploited, functional assays can be completed, and molecular mechanisms can be probed in the mouse, particularly when compared with other mammalian species. These efforts quickly led to the detailed biophysical characterization of the voltage-gated and non-voltage-gated K^+ channels expressed in mouse myocardium, as well as the identification of the genes encoding the pore-forming (α) subunits, as well as many of the accessory subunits of these channels (Nerbonne *et al.*, 2001). There were also suggestions that the mouse might be used as a model system for investigating congenital and acquired arrhythmogenic cardiovascular disease mechanisms, particularly the "ion channelopathies" (Ravens & Cerbai, 2008; Brenyo *et al.*, 2012; Abriel & Zaklyazminskaya, 2013) linked to ion channel dysfunction (London, 2001; Charpentier *et al.*, 2004), as well as for pre-clinical testing of the anti-arrhythmic efficacy and the pro-arrhythmic potential of drugs (Fabritz *et al.*, 2007).

Although there are multiple repolarizing K^+ currents in both human and mouse cardiac myocytes, there are clear differences in the properties and the molecular identities of the K^+ channels expressed (Nerbonne & Kass, 2005). The two prominent delayed rectifier K_v currents, I_{Kr} and I_{Ks}, in human ventricular myocytes, for example, are not prominent in mouse cardiac myocytes and three distinct delayed rectifier K_v currents, $I_{K,slow1}$, $I_{K,slow2}$ and I_{ss}, are expressed (Nerbonne & Kass, 2005). The genes, *KCNQ1* and *KCNH2*, that encode the K_v α subunits, $K_v7.1$ and $K_v11.1$, that generate human cardiac I_{Kr} and I_{Ks} channels, and identified as loci of

mutations in familial LQT1 and LQT2 (Ravens & Cerbai, 2008; Brenyo *et al.*, 2012; Abriel & Zaklyazminskaya, 2013) and transgenic and targeted deletion strategies in mice have been used to probe the functioning of *Kcnq1* and *Kcnh2*, as well as of the (*Kcne1*) gene, which encodes the I_{Ks} channel accessory subunit, mink (Nerbonne *et al.*, 2001). As might be expected, given that I_{Ks} and I_{Kr} are barely detectable in adult mouse cardiomyocytes, the cardiac effects of manipulating the *Kcne1*, *Kcnq1* or *Kcnh2* genes on myocardial K^+ currents and myocardial functioning in the mouse heart are quite subtle (Nerbonne *et al.*, 2001).

In several of the K_v channel transgenic and gene targeted mouse lines that have been generated, however, ECG abnormalities, including QT prolongation, as well as increased inducibility of ventricular arrhythmias, have been described and, in some cases, the observed abnormalities structurally resemble those seen in humans. Quite dramatic ECG phenotypes and spontaneous arrhythmias, for example, have been reported in some K_v channel transgenics (Nerbonne *et al.*, 2001). In the *Kcnq1*-isoform-2-expressing mouse, for example, QT prolongation, P wave abnormalities and TdP were reported. The severity of the cardiac phenotype, however, was correlated with the amount of the mutant protein expressed, observations that suggest non-specific *in vivo* cardiac effects of "over-expression" of the *Kcnq1*-isoform-2 transgene. Overall, the incidence of spontaneous and/or inducible arrhythmias, particularly lethal arrhythmias that mimic those in humans, in mice with altered K^+ channel expression is very low, observations that are interpreted as reflecting an inherent limitation of the mouse, perhaps owing to the small size of the mouse heart and the very rapid heart rate.

In spite of these limitations, important insights into the relationships between individual K^+ channel subunits and functional K^+ currents, as well as about the mechanisms underlying the electrical remodeling observed in association with alterations in the expression or

functioning of individual K^+ channel α and β subunits, have been provided through the application of *in vivo* molecular genetic strategies in the mouse. Together, these insights will guide the design, execution and interpretation of future experiments focused delineating the molecular, cellular and systemic mechanisms underlying electrical remodeling and reprogramming in the myocardium. The mouse is expected to be a widely used model system in these efforts.

Large Animal Models of Congenital and Acquired Cardiac Arrhythmias

It has long been recognized that the electrophysiological properties of the hearts of large mammals, including the types of K^+ channels expressed, more closely resemble those in the human heart, particularly compared with the mouse. Consistent with this, electrophysiological studies have detailed the distributions and the properties of the various K^+ (and other) channels expressed in cardiac cells from cat, rabbit, canine and pig heart (Nerbonne & Kass, 2005). These large animal models are also more amenable (than the mouse) to studies focused on probing conduction, propagation and arrhythmias in the intact heart, although the inability to manipulate these systems genetically compromised their utility for studying human cardiac disease mechanisms.

This barrier to progress was broken with the development of the first transgenic rabbit models of LQT1 and LQT2, produced by Koren and colleagues with the cardiac specific expression of pore mutants of the human genes *KCNQ1* (K_vLQT1-Y315S) and *KCNH2* (HERG-G628S), respectively (Brunner *et al.*, 2008). These pore mutants were shown to function in a dominant negative fashion, eliminating I_{Ks} and I_{Kr}, and resulting in AP and QT prolongation (Brunner *et al.*, 2008). Importantly and unexpectedly, the detailed electrophysiological characterization of ventricular myocytes from the transgenic K_vLQT1-Y315S and HERG-G628S rabbits revealed co-regulation of I_{Ks} and I_{Kr} (Brunner *et al.*, 2008).

In addition, the LQT2 rabbits show a high incidence of sudden death, attributed to increased dispersion of repolarization and polymorphic ventricular tachycardia. These transgenic rabbit models have enabled further studies focused on detailing arrhythmia mechanisms, as well as in efforts to explore the effects of drugs and hormones on conduction, dispersion and arrhythmia susceptibility (Ziv *et al.*, 2009; Odening *et al.*, 2010; Odening *et al.*, 2012; Odening *et al.*, 2013; Ziupa *et al.*, 2014; Kim *et al.*, 2015). More recently, a novel transgenic rabbit LQT5 model, produced by cardiac specific expression of a *KCNE1* mutant that functions as a dominant-negative, was described with markedly altered repolarization reserve attributed to changes in both I_{Ks} and I_{Kr} (Major *et al.*, 2016).

Although developing transgenic rabbit models to explore the functional effects of different mutations in the *KCNQ1*, *KCNH2*, *KCNE1* and other ion channel subunit genes to explore genotype-phenotype relations is certainly a reasonable one, a clear limitation is the time and cost involved in generating and characterizing each transgenic rabbit line. In addition, phenotypic effects might well be variable across animals owing to genetic heterogeneity, a complication that can be avoided with transgenic mice, but not rabbits. Further limitations are expected to be realized given that, in spite of the similarities in K^+ currents such as I_{Kr} and I_{Ks}, there are differences in the expression and properties of other repolarizing K^+ currents in rabbit and human cardiomyocytes (Nerbonne & Kass, 2005). Given this, it seems reasonable to suggest that there should also be increased emphasis on electrophysiological studies of human myocardial K^+ channels. Several previous reports have characterized native human cardiac K^+ currents (Iost *et al.*, 1998; Magyar *et al.*, 2000; Virag *et al.*, 2001; Jost *et al.*, 2005; O'Hara *et al.*, 2011), however, a significant number of studies is limited to atrial samples from patients undergoing open heart surgery (Van Wagoner *et al.*, 1997) and ventricular samples from explanted hearts from end-stage HF patients undergoing transplantation (Beuckelmann *et al.*, 1993; Wettwer *et al.*, 1994; Nabauer *et al.*, 1996)

reflecting the availability. One way to expand these efforts might be to acquire non-failing human hearts deemed unsuitable for transplantation for non-cardiac reasons (Glukhov *et al.*, 2010). Even with expanded efforts, sample heterogeneity, reflecting genetics, prior history, health status, as well as previous and present medications, however, will still be a complicating factor to consider when analyzing/interpreting electrophysiological data from human myocytes.

Cellular Models to Study Cardiac Arrhythmia Mechanisms

Cell-based systems have also been utilized to study the biophysical properties of genes/proteins involved in the generation of cardiac K^+ channels, as well as functional effects of mutations in K^+ channel subunit genes linked to congenital arrhythmias on channel expression, assembly, trafficking, targeting and biophysical properties. Heterologous expression systems offer several clear advantages in this regard, all of which are related to the ease and the speed with which constructs can be generated, expressed and functionally characterized. Expression systems can also be used for drug screening of potential channel blockers or activators (Haraguchi *et al.*, 2015) because of the ease with which experimental manipulations can be made and high-throughput screening methods can be applied. There are, however, also potential problems with interpreting results obtained in experiments conducted in heterologous cells for the simple reason that the detailed properties of K^+ (and other) channels depend on the cellular environment in which they are expressed owing to cell type-specific differences in RNA processing, protein-protein interactions, post-translational modifications and membrane lipid composition, etc., all of which could influence the properties of expressed K^+ channels.

An alternative cellular approach being used to detail the properties, functioning and regulation of human cardiac K^+ (and other) channels emphasizes "native" K^+ channels

expressed in human embryonic stem cell-derived cardiac myocytes (hESC-CMs) or human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) (Itzhaki *et al.*, 2011) (Blazeski *et al.*, 2012; Sallam *et al.*, 2015). The clear advantages of using hESC-CMs and hiPSC-CMs is that the cells are of human origin, sample availability is not an issue, and, in addition, these preparations are amenable to electrophysiological and molecular manipulations and can also be used for high throughput screening. In addition, hiPSC-CMs are generated from adult fibroblasts making it possible to generate patient specific hiPSC-CM lines and to study the effect(s) of identified mutations in genes encoding K^+ channels or other proteins linked to LQTS or SQTS, BrS, catecholaminergic polymorphic ventricular tachycardia, sick sinus syndrome, hypertrophic cardiomyopathy and other congenital arrhythmogenic cardiac disorders (Itzhaki *et al.*, 2011; Dirschinger *et al.*, 2012; Lahti *et al.*, 2012; Sinnecker *et al.*, 2013; Tanaka *et al.*, 2015). Importantly, the fact that these cells can be readily modified means that gene mutations can be corrected, allowing direct comparisons between the properties of cells with and without mutations, in the same genetic background (Itzhaki *et al.*, 2011; Sallam *et al.*, 2015). One major limitation of hiPSC-CMs at present is that the cells are immature both electrically and structurally and considerable effort is focused on developing methods to manipulate and improve cell maturation (Bett *et al.*, 2013; Veerman *et al.*, 2015).

VI. Computational modeling and simulation approaches to study the mechanisms of K^+ channel linked acquired cardiac arrhythmias

"To evaluate the full impact of quantitative systems pharmacology, one must remember that the mechanisms of action of most drugs are not fully understood and the origins of patient-to-patient variability in therapeutic and adverse responses are often

obscure …. And drugs that fail at late stages of clinical trials are rarely investigated further to determine the reasons for their failure.", wrote the quantitative systems pharmacology workshop group in an NIH white paper (Sorger & Allerheiligen, 2011). These statements aptly describe the driving biomedical challenges facing scientists, regulators and clinicians in trying to determine safety and efficacy of drugs.

History has shown that safety or toxicity of drug treatments cannot be observed or readily predicted *via* study of component elements alone. This is especially clear in the longstanding failure to anticipate cardiotoxicity in drug development (Roden, 2004). Cardiotoxicity is one of the most common risks for drugs in development, manifesting as prolongation of the QT interval and potential for fatal ventricular arrhythmias.

As described earlier, cardiac rhythm disturbances are most commonly a side effect from unintended block of the promiscuous drug target $K_v11.1$, the pore-forming domain of I_{Kr} in the heart. But, not all $K_v11.1$ blockers are proarrhythmic. There is an urgent need to develop new approaches for selective and sensitive prediction of how drugs with complex interactions and multiple subcellular targets will alter the ***emergent*** electrical activity in the heart. Mathematical modeling and simulation constitute some of the most promising methodologies to reveal fundamental biological principles and mechanisms, model effects of interactions between system components and predict emergent treatment effects. There are no reasonable, efficient and cost-effective experimental alternatives that can achieve these goals. Application of new computational and simulation methods may in the next decade usher in an era that allows for integration of data in physiological networks to reveal emergent drug effects at the cellular, tissue and organ levels and to facilitate prediction and development of safer therapeutic interventions. Quantitative systems pharmacology approaches are currently

being developed in conjunction with high efficiency computational processes for probing the mechanisms of action of prototypical drugs in the setting of cardiac electrical disorders.

While the crystal structure of the K_v11.1 channel is not yet available, multiple *in silico* approaches including homology modeling, de-novo protein design and molecular dynamics simulations have been undertaken to link structural determinants of K_v11.1 to its function (Stary *et al.*, 2010). Sequence conservation is substantial between K_v11.1 and other K_v channels with known structures and new studies suggest that computer-based homology models based on solved K_V based on the available crystal structures of KcsA, MthK, K_v1.2 and K_vAP pore domains can successfully be utilized to study the human K^+ channels (Mitcheson *et al.*, 2000; Perry *et al.*, 2004; Stansfeld *et al.*, 2007). The predictions from molecular modeling studies based bacterial KcsA and MthK (Perry *et al.*, 2004; Stansfeld *et al.*, 2007) channels or the mammalian channel K_v1.2 (Durdagi *et al.*, 2010; Stary *et al.*, 2010) concur with the experimental findings that have revealed two key residues responsible for drug stabilization in the K_v11.1 cavity, e.g. Y652 and F656 (Lees-Miller *et al.*, 2000; Mitcheson *et al.*, 2000). Model studies reproduced this feature in studies of K_v11.1 blockers such as dofetilide, KN-93 and other common high-affinity blockers (Durdagi *et al.*, 2011; Durdagi *et al.*, 2012).

Because it is so difficult to predict how ion channel modification, studied in isolation, alters the functioning of the whole heart, computationally based modeling and simulation approaches have been developed and widely applied to link deviations in ion channel function to emergent electrical activity in cells, tissue and even simulated whole hearts. In the last twenty years, an explosion in development of sophisticated models has occurred, concomitant with improved experimental techniques that have allowed identification and characterization of numerous ion channel subtypes and their regulation in the heart from

multiple species (Romero *et al.*, 2010; Bett *et al.*, 2011; Romero *et al.*, 2011; Moreno & Clancy, 2012; Qu *et al.*, 2013; Verkerk & Wilders, 2013; Bueno-Orovio *et al.*, 2014; Glynn *et al.*, 2014; Greenstein *et al.*, 2014; Onal *et al.*, 2014; Ramirez *et al.*, 2014; Pathmanathan *et al.*, 2015) (Besse *et al.*, 2011). Sophisticated ion channel models have even been developed that account for various genetic defects that alter the behavior of ion channels. These complex ion channel representations have been incorporated into numerous cardiac model "cells" from multiple species. The cellular level models have been widely replicated and coupled, creating mathematical representations of cardiac tissue in one, two or three dimensions, with incorporation of complex anatomical heterogeneities including anisotropy, structural features and distinct cells with specifically associated electrophysiological characteristics (Trenor *et al.*, 2007; Romero *et al.*, 2009; Bers & Grandi, 2011; Niederer & Smith, 2012; Roberts *et al.*, 2012; Sugiura *et al.*, 2012; Trayanova *et al.*, 2012; Zhang *et al.*, 2012; Zhou & O'Rourke, 2012; Polakova & Sobie, 2013; Quail & Taylor, 2013; Romero *et al.*, 2013; Sato & Clancy, 2013; Tobon *et al.*, 2013; Ferrero *et al.*, 2014; Gomez *et al.*, 2014; Henriquez, 2014; Ramirez *et al.*, 2014; Trayanova & Boyle, 2014; Duncker *et al.*, 2015).

Simulation studies have revealed plausible experimentally testable mechanisms for how perturbations to ion channels and associated processes alter emergent electrical behavior at higher system scales. For example, computer simulations have revealed that the APD prolongation exerted by most mutant channels related (I_{Kr}, I_{Ks} and I_{Na}) to acquired LQTS (aLQTS) were shorter than those produced by mutations producing congenital LQTS (Itoh *et al.*, 2006). In another study, the role of the R1047L polymorphisms in *KCNH2* in dofetilide-induced TdP was investigated (Sun *et al.*, 2004). The R104L missense mutation was linked to TdP in fibrillation patients treated with dofetilide. The mutation caused a positive shift of the activation curve and slowed the activation and inactivation kinetics. Simulation of these

abnormalities resulted in prolongation of the APD, which suggests that 1047L may contribute to a higher incidence of TdP in the presence of I_{Kr} blockers (Sun *et al.*, 2004).

Investigation of drug-induced or acquired LQTS (aLQTS) resulting from $K_v11.1$ block is critical to identify individuals who are susceptible to aLQTS, which is crucial for reducing the risk of cardiac arrhythmias. Experiments have shown that the functional changes of most mutations are mild and most drug sensitivities for mutant channels are similar to that of the WT channels (Itoh *et al.*, 2006). Nevertheless, approximately 40% of patients with aLQTS have been shown to exhibit allelic variants that disrupt the function of cardiac ionic channels (Itoh *et al.*, 2006).

More recently, a systematic and comprehensive computational study has been conducted to reveal new insights into the impact of latent I_{Kr} channel kinetic dysfunction on I_{Kr} time course during the AP, susceptibility to aLQTS and the potential for adjunctive therapy with I_{Kr} channel openers (Romero *et al.*, 2014). Specifically, this study predicted the most potentially lethal combinations of kinetic anomalies and drug properties and the ideal inverse therapeutic properties of I_{Kr} channel openers that would be expected to remedy a specific defect. The simulations predicted that drugs with disparate affinities to conformation states of the I_{Kr} channel markedly enhanced the susceptibility to aLQTS, especially at slow pacing rates.

In the same study, Romero et al. simulated the M54T latent mutation in *KCNE2*, which has been related to aLQTS and arrhythmias, in the presence of dofetilide, which drastically prolonged the QT interval duration in the M54T mutation in *KCNE2* compared to wild-type. The study also predicted that application of a virtual potassium channel opener that only slows deactivation would be the ideal adjunctive therapy that could normalize the effect of dofetilide-induced AP prolongation in the presence of the M54T hMiRP1 mutation.

Simulation of the addition of the I_{Kr} activator PRP260243, which slows the deactivation and increases the current magnitude by positively shifting the inactivation curve (Perry *et al.*, 2007) was predicted to correct the APD and QT interval prolongation, but it introduced the risk of developing SQTS (Romero *et al.*, 2014).

Finally, computational modeling has also been used to yield insights into the relationship between K_v11.1 1a/1b channels, drug sensitivity, and arrhythmia proclivity (Sale *et al.*, 2008). These simulations showed that altered channel kinetics may explain reduced rectification and an increase in current during repolarization. The model also predicted that drugs that block 1a homomers of K_v11.1 are more arrhythmogenic than those that block the heteromer.

Questions/Controversies:

- Can K^+ channel diversity be exploited to target specific cell types for anti-arrhythmic therapy?
- Are M-cells present in human ventricle?
- During pathophysiological remodeling, are K^+ channel sub-types impacted similarly throughout the different regions of the heart?
- Given the proarrhythmic potential of both increased and decreased K^+-channel function, what changes in K^+ currents are adaptive and which are maladaptive in a given disease? Of note, the lack of highly selective blockers and activators for most of the myocardial K^+ channels makes it difficult to assess their specific roles in arrhythmias.
- What can we learn from the species-specific differences in the roles of K^+ channels in cardiac arrhythmias?

- Through which mechanisms are different K^+ currents co-regulated with the counteracting depolarizing currents (i.e. Ca^{2+} currents) to ensure stable electrophysiological behavior under a wide range of conditions? How are these mechanisms perturbed under diseases conditions?
- Which K^+ channels, in addition to I_{K1}, control the RMP in different regions of the heart?
- What are the (mal)adaptive responses to chronic modulation of K^+-channel expression and/or function?

Summary

A variety of cellular and animal based model systems have been, and continue to be, used in studies focused on defining the functional properties of myocardial K^+ channels and the impact of diseased-link mutations in the genes that encode K^+ channel subunits on cardiac myocyte membrane excitability and arrhythmia susceptibility. Nevertheless, the physiological roles of the various K^+ channels expressed in human heart and the cellular, molecular and systemic mechanisms linking congenital and acquired changes in K^+ expression and/or functioning to increased risk of arrhythmias and sudden death remain rather poorly understood. It seems clear that increased efforts, focused on delineating mechanisms that link changes in the expression and functioning of cardiac K^+ (and other) channels to arrhythmogenic cardiovascular disease, are needed to provide new insights. Accomplishing this will, almost certainly, require the continued development and application of multiple

model systems that allow multiscale and multidisciplinary experimental investigation and analyses.

Importantly, our understanding how disruption in cardiac K^+ channels leads to arrhythmias is continually improving due to the development and implementation of computational modeling and simulation approaches. These approaches span scales from the single atomistic ion channel scale to the high-resolution reconstruction of the heart. These new computational strategies may be applied to improve preclinical screening of compounds to detect possible proarrhythmic effects and/or predisposition from underlying genetic causes. Computer-based approaches can help to determine the mechanisms of drug actions. Expansion and development of new approaches may help to reveal why drugs that are non-specific K^+ channel blockers and interact with many targets, like amiodarone, seem to be less proarrhythmic than selective blockers like d-sotalol. Finally, models might eventually be used to guide ***therapy*** for specific clinical situations and to identify optimal "polypharmacy" to inform the common practice of clinical empirical mixing and matching of drugs to create multidrug therapeutic regimens (Yang *et al.*, 2016). Central to the success of modeling and simulation approaches for predictive safety pharmacology is the development of models that are informed and validated via tightly planned integration of experiments and simulations at every stage (Clancy *et al.*, 2016). It will be critical to demonstrate usefulness of the frameworks and to validate their utility and reproducibility. Finally, although we have endeavored to be as thorough and timely as possible in this review, this is a rapidly moving and expanding field. We have prioritized specific areas of emphasis at the expense of being comprehensive. We have, however, attempted to circumvent this limitation by including several published review articles in the references.

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Figure 1: Heterogeneity of canine ventricular repolarization and K^+ current distribution. **A,** Epicardial, M-cell, and endocardial APs from the RV and LV of the canine heart at basic cycle lengths (BCLs) of 300 and 5000 ms. Redrawn with permission from (Antzelevitch *et al.*, 1999). **B,** Relative distribution of major K^+ currents in the heart (see text for details and references).

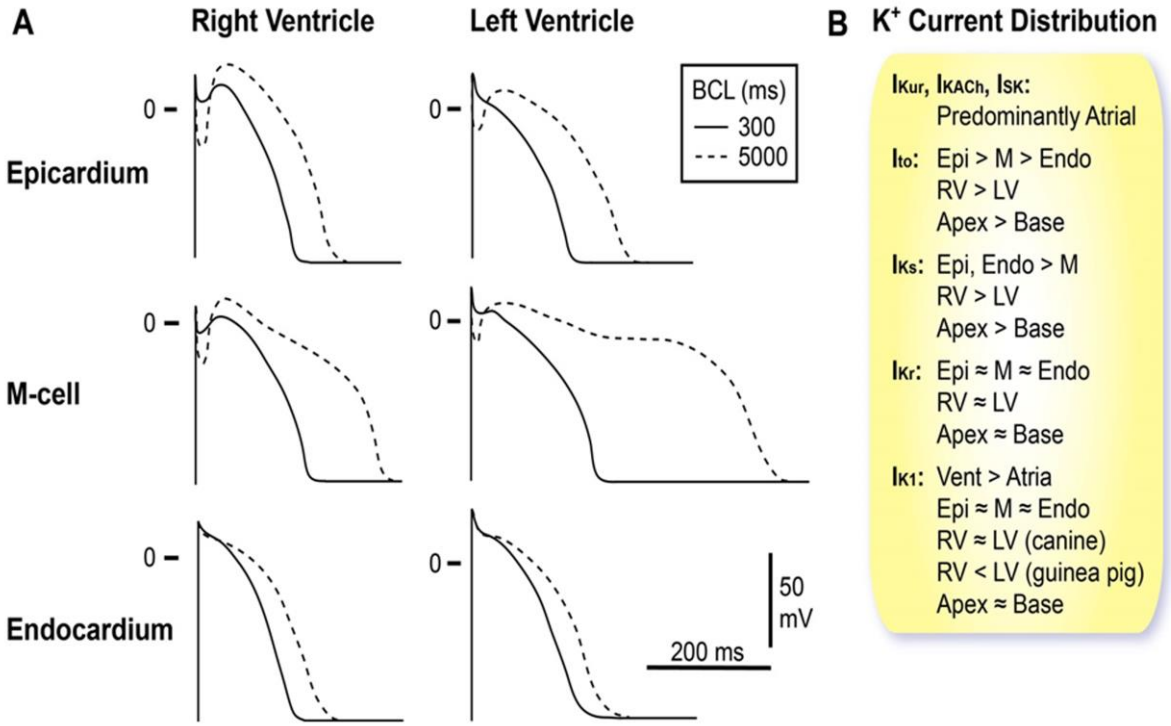

Figure 2. β-adrenergic regulation of K^+ channels during cardiac AP.
A, AP-clamp Sequential Dissection experiments were performed as followed: steady-state APs were recorded at a pacing rate of 1 Hz (upper panel), and used as the voltage command to obtain AP-clamp recordings of the (three distinct) K^+ currents in the same cell (middle panel) and the Ca^{2+} transients (lower panel) under physiological conditions. **B,** The effects of isoproterenol (ISO) on the AP waveform and the evoked K^+ currents. At 30 nM, ISO significantly enhanced I_{Ks}, moderately increased I_{K1}, but slightly decreased I_{Kr}. **C, D**, **E,** Dose-dependent effects of ISO on the peak densities of $I_{Ks,}$ I_{Kr}, I_{K1}. **F, G,** The three K^+ currents, measured in the same cell, were summed and each current was then normalized to this sum to calculate its relative contribution of each to the total K^+ influx. The normalized current values at membrane potential of +20 mV and −20 mV are shown in **F** and **G**, respectively. The ISO dose-response curves show how the relative contribution of each current shifts with various levels of β-adrenergic stimulation. **H**, Total K^+ charge movement for individual K^+ current and the sum of the currents during the AP. (Adapted from Banyasz *et al.* (Banyasz *et al.*, 2014) with permission).

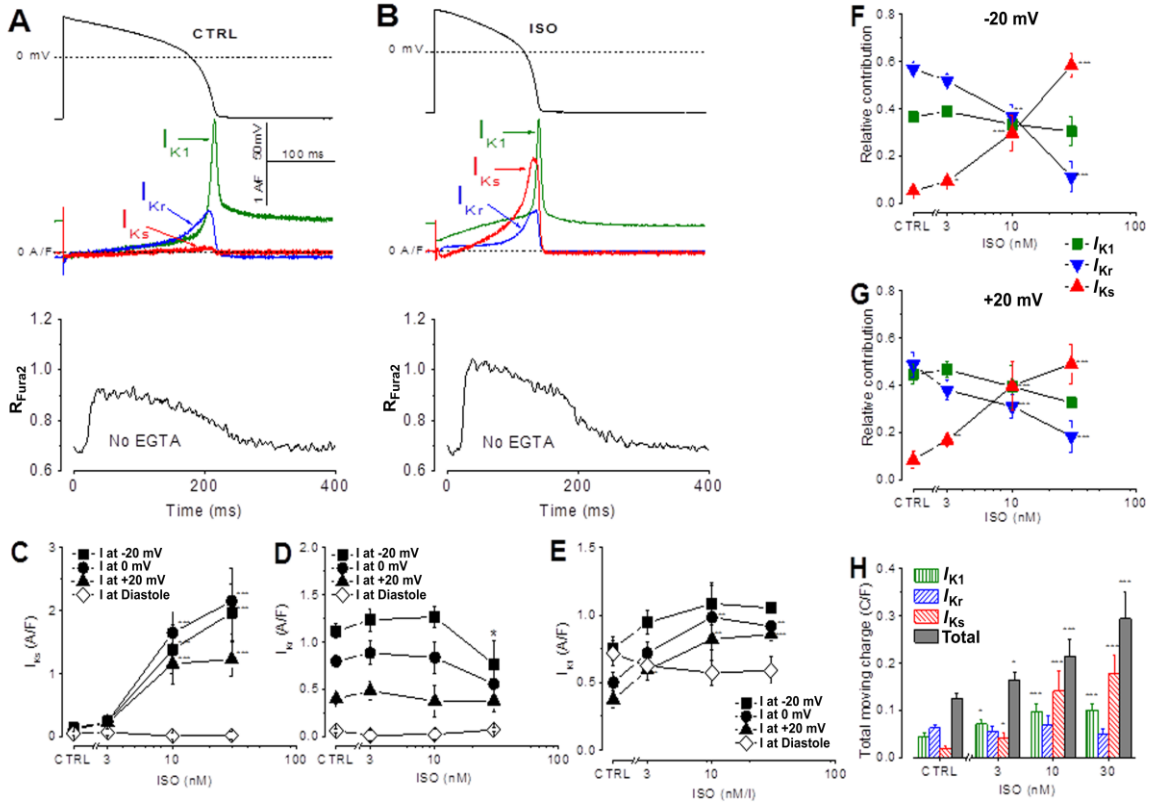

Figure 3. Schematic representation of the role of K^+ channels in inherited and acquired cardiac arrhythmias. Numerous inherited (genetic), acquired and drug-induced conditions involve alterations in a wide range of ion channels. Loss of K^+-channel function can promote ectopic activity by reducing the repolarizing current offsetting delayed afterdepolarizations (DADs) thereby increasing DAD amplitude, by promoting APD prolongation and associated early afterdepolarizations (EADs), or by accelerating phase-4 depolarization and abnormal automaticity. K^+-channel gain of function, on the other hand, produces a vulnerable substrate in which ectopic activity can initiate reentry, by shortening action potential duration (APD) and effective refractory period (ERP). Abbreviations: AF: atrial fibrillation; BrS: Brugada sydrome; ERS: early repolarization syndrome; HF: heart failure; LQTS: long-QT syndrome; SQTS: short-QT syndrome.

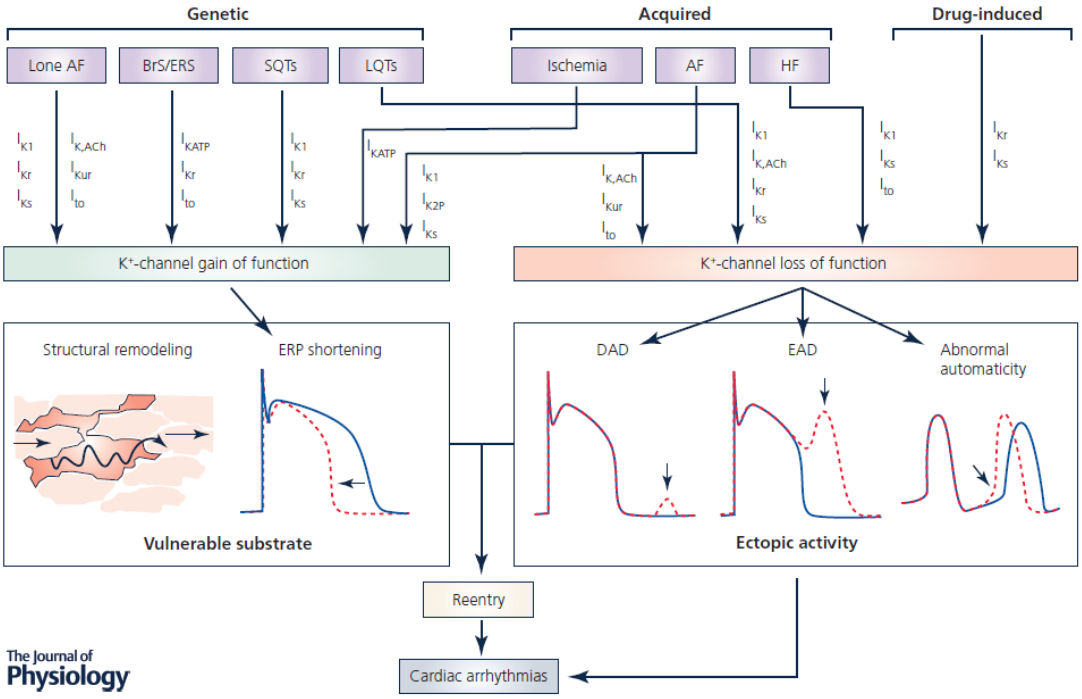

Figure 4. An integrated approach to studying K^+ channel remodeling in heart disease. Information from next generation sequencing (blue), proteomics (green) and metabolomics (red) will need to be integrated using bioinformatics and multi-scale computer modeling to establish a systems wide understanding of electrical remodeling.