Structures Illuminate Cardiac Ion Channel Functions in Health and in Long QT Syndrome

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The cardiac action potential is critical to proper heart function. Beginning with the activation of “pacemaker” cells, the action potential propagates through the atria and into the ventricles in a unidirectional waveform of excitation and relaxation, resulting in the coordinated expansion and contraction of heart tissue (Nerbonne and Kass, 2005). The action potential is governed by an intricate series of ion channel activities (Grant, 2009), including those of the KCNQ1 (K\(_\text{v}7.1\)) and hERG (KCNH2, K\(_\text{v}11.1\)) potassium channels and the SCN5A (Na\(_\text{v}1.5\)) sodium channel. Mutations in these three channels are the most frequent cause of congenital long QT syndrome (LQTS), a cardiac arrhythmia disorder that is one of the primary causes of sudden arrhythmic death syndrome (SADS) (Skinner et al., 2019).
KCNQ1, hERG, and SCN5A each play a distinct role in generating the cardiac action potential (Figure 1), consequently producing distinct LQTS forms when mutated (Skinner et al., 2019). The initial upstroke is governed primarily by SCN5A, producing the $I_{Na}$ current that amplifies membrane depolarization and propagates the action potential (Moss and Kass, 2005; Skinner et al., 2019). Mutation in SCN5A causes LQTS type 3 (LQT3). hERG and the KCNQ1–KCNE1 complex produce the rapid ($I_{K_r}$) and slow ($I_{K_s}$) delayed rectifier currents (Figures 1B, C), respectively. KCNQ1 mutation causes LQT1, and hERG, LQT2 (Skinner et al., 2019). Additional ion channels and transporters also shape the cardiac action potential (Skinner et al., 2019), among them $Ca_{n1.2}$, NCX1, and Kir2.1. The roles of these channels in the cardiac action potential and cardiac arrhythmias have been reviewed elsewhere (Bidaud and Lory, 2011; Hancox et al., 2018; Meza et al., 2018; Vaidyanathan et al., 2018; Li et al., 2019). These channels are not typically causative for LQTS and will not be discussed here.

Structurally, the KCNQ1, hERG, and SCN5A channels belong to the voltage-gated ion channel superfamily and share a general transmembrane topology (Bezanilla, 2005; Dehghani-Samani et al., 2019). The transmembrane channel domain is composed of six helices per subunit in hERG and KCNQ1 (Figures 2A, B) or a monomeric tetrad repeat of linked 6-helix domains for SCN5A, each repeat exhibiting varying sequences, lengths, and tertiary folds (Figures 2C, D). The assembled channel is tetrameric (pseudo-tetrameric for SCN5A), with each channel domain composed of four voltage sensing domains (VSDs) surrounding a central pore domain (PD) (Figure 2D). The VSD is comprised of the first four transmembrane helices (S1–S4) preceded by a small amphipathic helix—S0—in both KCNQ1 and SCN5A (Jiang et al., 2020; Sun and Mackinnon, 2020). The PD is formed by the tetramerization of S5 and S6 helices from each subunit/repeat (Figure 2D). A pore loop between S5 and S6 contains the selectivity filter (SF) that confers ion specificity. A linker helix between helices S4 and S5, termed the S4–S5 linker, connects the VSD to the PD (Bezanilla, 2005; Dehghani-Samani et al., 2019) (Figures 2A–C).

Given these similarities in channel topology and components, how is it that KCNQ1, hERG, and SCN5A perform such distinct functions, and produce phenotypically distinct forms of LQTS? To explore this question, a number of high-resolution ion channel structures, including cryo-electron microscopy (cryo-EM) structures of frog and human KCNQ1 (Sun and Mackinnon, 2020) and of human hERG (Wang and Mackinnon, 2017), as well as the structures of the rat homolog of SCN5A (Jiang et al., 2020) and human Na$_1$ isoforms Nav1.4 and 1.7 (Pan et al., 2018; Shen et al., 2019; Xu et al., 2019), have been determined. Moreover, the frog KCNQ1 structure has been used to develop what is likely a reliable homology model for the human KCNQ1 channel in resting and fully active conformations (Kuenze et al., 2019). A homology model of human SCN5A in the resting state has also been devised (Kroncke et al., 2019). These structures and structural models reveal critical differences in the atomic details of KCNQ1, hERG, and SCN5A structures associated with their distinct functions and disease phenotypes. Notably, the subunits of KCNQ1 undergo domain swapping, with a similar arrangement observed in SCN5A but not in the hERG channel (Figure 2D). The monomeric sequence of SCN5A causes the channel to adopt an asymmetric three-dimensional fold, in contrast to the inherent symmetry of tetrameric hERG and KCNQ1 (Figure 2D). Additionally, the C-terminal domains contain distinct folds and mediate unique regulatory functions. These and other structural differences contribute to the varying properties of these three channels and to their distinct roles in the cardiac action potential.

The aim of this review is to compare and contrast the KCNQ1, hERG, and SCN5A channels using available structures and structural models as a guide. Through this lens, channel gating, regulation, LQTS mechanisms, and pharmacology will be discussed, in order to explore the molecular basis of these unique properties.
STRUCTURAL MECHANISMS OF CHANNEL GATING

KCNQ1, hERG, and SCN5A undergo conformational changes in response to changes in membrane potential that result in channel opening or closing. These responses confer specific gating properties including activation, deactivation, inactivation, and recovery from inactivation (Hosseini, 2018; Zhang et al., 2018). In activation, protein conformational changes result in channel pore opening from a resting state, while deactivation entails a return to the resting state (Zhang et al., 2018). Inactivation confers a third channel state distinct from the activated and resting states which inhibits current flow prior to full deactivation (Zhang et al., 2018). While all three channels share common structural elements that are responsible for producing these states, there are also elements that give rise to specific gating properties in each channel, as discussed below.

**Activation and Ion Conduction**

**KCNQ1**

Voltage-gated channels contain up to six positively-charged basic residues in the S4 helix, called gating charges, which move in the electric field of the membrane in response to voltage (Jiang et al., 2003). Gating charges are numbered according to their position in the S4 helix, from the extracellular to the intracellular side. In KCNQ1, S4 contains four arginine (R) gating charges that are conserved in other KV channels and confer voltage sensitivity. However, in KCNQ1 the canonical R3 is replaced by a neutral glutamine (Q234, Q3), and the fifth gating charge (K5 in Shaker) is replaced by histidine (H241, H5), which in the membrane

**hERG**

**SCN5A**

FIGURE 2 | Overall topology of KCNQ1, hERG, and SCN5A channels. (A) KCNQ1 topology. Transmembrane domain alpha helices are labeled S0–S6. PH indicates the pore helix. SF denotes the selectivity filter. S2–S3 indicates the S2–S3 linker. S4–S5 denotes the S4–S5 linker. Cytosolic alpha helices are labeled HA-HD. (B) hERG topology. PAS denotes the Per-ARNT-Sim domain. CNBHHD indicates the C-terminal cyclic nucleotide-binding homology domain. (C) SCN5A topology. CTD indicates the C-terminal domain. (D) Top views of the human KCNQ1 (PDB ID: 6UZZ) (Sun and Mackinnon, 2020), human hERG (PDB ID: 5VA1) (Wang and Mackinnon, 2017), and rat SCN5A (PDB ID: 6UZ3) (Jiang et al., 2020) channels, respectively. Outer voltage-sensing domains (VSD) and the central pore domain (PD) are labeled.
environment is expected to be neutral at physiological pH (Figures 3A, B). Due to these substitutions at positions 3 and 5, the S4 helix of KCNQ1 has a lower net positive charge (+4) than Shaker-class K⁺ channels, such as Kv1.2 (+6). The lower net positive charge may explain why KCNQ1 S4 mutations that result in charge loss or reversal (Panaghie and Abbott, 2007; Wu et al., 2010b) result in constitutive channel activity.

Voltage-gated channels also feature a charge transfer center (CTC), formed by a bulky aromatic ring and two negatively charged residues, that facilitates S4 movement (Tao et al., 2010). The KCNQ1 CTC consists of E170 (E2) and F167 on S2, and D202 on S3, which work with E160 (E1) to define the S4 position. During activation, S4 moves towards the extracellular side of the membrane (Nakajo and Kubo, 2007; Rocheleau and Kobertz, 2008; Osteen et al., 2010; Ruscic et al., 2013; Barro-Soria et al., 2014; Nakajo and Kubo, 2014; Barro-Soria et al., 2017) through interactions between basic gating charges and E1 and E2 in the CTC (Figure 3A). These interactions change during the course

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**FIGURE 3** The voltage sensors of KCNQ1, hERG, and SCN5A. (A) Structure of the VSD from human KCNQ1 (left) (PDB: 6UZZ) (Sun and Mackinnon, 2020) and hERG (right) (PDB: 5VA2) (Wang and Mackinnon, 2017) in putative activated conformations. Basic residues on S4 (labeled in blue), acidic residues on S2 and S3 (labeled in red), and the phenylalanine residue in the gating charge transfer center (purple) are shown as sticks. The first four basic residues on S4 (R1–R4) in KCNQ1 and the first three (K1–R3) in hERG are located above the charge transfer center. (B) Multiple sequence alignment of VSDs I-IV of SCN5A with the VSDs of human KCNQ1 and hERG. Basic residues in S4 implicated with voltage sensing are colored blue, and acidic or polar residues in S1–S3 suggested to interact with S4 gating charges are colored red. The conserved aromatic residue at the gating charge transfer center in S2 is colored purple. Another tryptophan residue in S3, conserved in the VSDs of Na⁺ channels and also present in hERG, is colored green.
of activation, permitting S4 translocation. E1 interacts with R1 (R228) or R4 (R237) in the resting and activated states of the VSD, respectively (Wu et al., 2010a). S4 motion occurs in two distinct steps, transitioning through a stable intermediate before reaching the activated state (Wu et al., 2010a; Barro-Soria et al., 2014; Zaydman et al., 2014). The intermediate state features salt bridge interactions between E1 and R2, distinct from the resting and activated VSD states, according to the recently-determined structure of the intermediate state KCNQ1 VSD (Taylor et al., 2020). Interestingly, the pore of KCNQ1 opens in both the intermediate state (IO) and fully activated (AO) states (Zaydman et al., 2014). These two open states possess distinct channel properties with differing opening probabilities and pharmacology (Hou et al., 2017), and have distinct pore structures (Zaydman et al., 2014). Importantly, ion conductance when the VSD is in either the intermediate or activated state appears to be unique to KCNQ1. However, formation of the KCNQ1–KCNE1 complex eliminates the conductance associated with the VSD intermediate state (Zaydman et al., 2014), such that IKs reflects only the fully activated state.

The ion conduction pathway in KCNQ1 is lined by the four S6 helices, with the SF on the extracellular side of the pore. Mutations in S6 cause changes in current amplitude and voltage dependence of activation (Wang et al., 1999; Seebohm et al., 2005; Panaghie et al., 2006; Hoosien et al., 2013). Comparison of S6 in the closed pore structure of human KCNQ1 (Sun and Mackinnon, 2020) with that of the open channel demonstrates that channel opening results from bending of S6 so that the cytosolic ends of the four S6 segments swing away from the central axis, enlarging the diameter of the pore to allow diffusion of K⁺ into the central cavity. The hinge responsible for this bending motion in S6 is the P343–A344–G345 (PAG) motif, which corresponds to PVP in Shaker K⁺ channels (Labro and Snyders, 2012). Additionally, A336 may also be important in the motion of the activation gate, as mutations at this position alter the voltage dependence of activation (Seebohm et al., 2006).

Once the intracellular gate is opened, K⁺ ions move through the pore along their electrochemical gradient. The backbone carbonyl oxygens of the TIGYG motif in the SF of KCNQ1 (TVGYG in Kᵥ1.2 and KcsA) (Figure 4A) and the sidechain of T312 form four evenly spaced K⁺ binding sites (Figure 4B) that facilitate K⁺ movement (Zhou et al., 2001). The arrangement of these oxygens mimics the displaced hydration shell of K⁺, which lowers the transfer energy from the aqueous cavity at the center of the channel to the SF, allowing conduction to occur at rates near the diffusion limit (Morais-Cabral et al., 2001; Zhou et al., 2001).

hERG
The cryo-EM structure of hERG shows an open pore and activated VSDs, with the first three gating charges (K1–R3) of S4 located on the extracellular side of the CTC (Figure 3A). This is a translocation of one charge fewer than in the activated VSD of KCNQ1 (Sun and Mackinnon, 2017) and Shaker-like KV1.2–2.1 (Long et al., 2007) where four gating charges are located above the conserved phenylalanine in the CTC. This observation agrees with gating current measurements suggesting a total charge movement of only ~6 elementary charge units (movement of 1.5 positive charges per S4 helix) for hERG during activation (Zhang et al., 2004), compared to 8 to 9 (2 positive charges per helix) for KCNQ1 (Ruscic et al., 2013) and 12 to 16 (3–4 positive charges per helix) for Shaker-like Kᵥ channels (Schoppa et al., 1992; Aggarwal and Mackinnon, 1996; Seoh et al., 1996). However, the ca. 50% lower total gating charge movement for hERG relative to Shaker-like channels is not explained by differences in the number of S4 gating charges, since hERG has a total of five positively-charged residues on S4.
and Shaker has six (Pless et al., 2011b). These combined structural and functional data thus imply that S4 translocates less in hERG during activation, resulting in smaller overall VSD conformational changes.

However, the activated VSD of hERG may not be fully defined. While the position of K1–R3 above the CTC in the cryo-EM structure of hERG is consistent with a depolarized VSD, some salt bridge interactions in the VSD have suboptimal geometry, particularly with R4. This is likely due to the limited resolution of the VSD in the final map (approximately 4.5–5.5 Å) (Wang and Mackinnon, 2017), impeding unambiguous determination of sidechain conformations. While cryo-EM has proven to be a powerful structural tool, the resolution is often lower in the periphery of protein structures (Herzik et al., 2019). This can prevent accurate modeling of functional features and lead to discrepancies with experimental data. Molecular dynamics may be a useful tool in refining cryo-EM structures to mitigate these discrepancies, as is currently being carried out for the hERG structure (Khan et al., 2020).

While a structure of hERG with a closed PD has not yet been determined, we can gain insight into the conformational changes that occur during pore opening using the closed-pore structure of the closely related rat potassium voltage-gated subfamily H member 1 channel (EAG1 or KCNH1) (Whicher and Mackinnon, 2016). In hERG, the intracellular gate is likely constricted by the Q664 side chains in S6, since the radius of the cavity at Q664 is almost 6 Å in the open state hERG structure (Wang and Mackinnon, 2017), while at the corresponding position (Q476) in the EAG1 closed state structure, the pore is at its narrowest (< 1 Å) (Whicher and Mackinnon, 2016). Bending and displacement of the S6 helices is suggested by a glycine residue, located at the same position in both channels, acting as a gating hinge (G648 in hERG, G460 in EAG1).

The SF in hERG is unique among K+ channels, containing a GFG motif (Figure 4A) in place of the typical GYG motif (Long et al., 2005). The position of the phenylalanine residue in this motif is different from the corresponding tyrosine in other K+ channels of known structure (Wang and Mackinnon, 2017). This structural variation may have important implications for fast inactivation in hERG, as discussed below.

**SCN5A**

The mechanism of voltage sensing in Na+ channels is thought to be similar to that of Kv channels. The S4 helix is the key sensor of transmembrane voltage. Pairing of the positively-charged residues in S4 with polar or negatively-charged residues catalyzes S4 movement from its inward resting-state position to the outward activated state upon membrane depolarization. Recent cryo-EM structures of Na+ channels with VSDs in activated (Yan et al., 2017b; Pan et al., 2018; Pan et al., 2019; Shen et al., 2019; Jiang et al., 2020) and resting (Clairefeuille et al., 2019; Wischedt et al., 2019; Xu et al., 2019) conformations uncover a remarkable 10 to 15 Å translation of S4 across the membrane, fully consistent with the "sliding helix" model of VSD activation (Catterall, 1986). A conserved aromatic residue (tyrosine in repeat I, phenylalanine II, III, and IV) on S2 serves as the hydrophobic plug that constrains the S4 gating canal and prevents ion leak through the VSD (Jiang et al., 2020). This hydrophobic plug is mechanistically identical to the corresponding residues in the CTC of Kv channels (F167 in KCNQ1, F463 in hERG). However, in contrast to the VSD of KCNQ1 and hERG, the number of basic residues on S4 in SCN5A varies from four (repeat I) to six (repeat IV) (Jiang et al., 2020) (Figure 3B). The variation in the number of gating charges and the heterogeneous distribution of acidic and polar residues on S1–S3 between KCNQ1, hERG, and SCN5A (Figure 3B) may be responsible for their distinct voltage sensitivities and kinetics of VSD activation. In Na+ channels, the VSDs of repeats I, II, and III are mainly responsible for channel activation and pore opening, while the VSD of repeat IV is responsible for initiating and maintaining fast inactivation (Chanda and Bezanilla, 2002; Capes et al., 2013; Clairefeuille et al., 2019). This activation process has been studied in detail for the human skeletal muscle channel Na,A1.4, giving rise to the "asynchronous gating model" (Chanda and Bezanilla, 2002; Capes et al., 2013; Goldschen-Ohm et al., 2013) wherein the S4 segments of repeats I, II, and III move quickly, permitting conductance before activation of VSDIV, while S4 movement in repeat IV is slower and represents the rate-limiting step for development of and recovery from inactivation.

The structure and function of the selectivity filter in SCN5A and other Na+ channels differs fundamentally from that of Kv channels such as KCNQ1 and hERG (Figure 4). The SF gate in Na+ channels is wider to allow Na+ ions to pass in a partially hydrated state (Hille, 1971; Naylor et al., 2016). The extracellular vestibule of Na+ channels is lined by negatively-charged residues that recruit Na+ ions to the SF. Coordination by both sidechain and backbone carboxyls contribute to the Na+ permeation mechanism (Chakrabarti et al., 2013; Ullschneider et al., 2013; Naylor et al., 2016). Additionally, the SF of eukaryotic Na+ channels is formed in a pseudo-symmetric fashion by four short helix-connecting turn motifs from each subunit (Figure 4A). Four distinct residues—DEKA, one in each repeat-form the signature motif for Na+ selectivity found in all human Na+ channel pore-forming repeats (Figure 4C). The lysine residue in the DEKA motif (K1419 in SCN5A) confers selectivity for Na+ and prevents permeability of Ca2+ (Favre et al., 1996). The recent rat SCN5A structure suggests a mechanism by which K1419 contributes to this selectivity of Na+ over Ca2+, wherein lysine forms a charge delocalization network at a constriction point in the SF. Only Na+ ions, which have a compatible size and electric field strength, are able to pass through (Jiang et al., 2020).

**Electromechanical Coupling**

**KCNQ1**

In the absence of accessory subunits, KCNQ1 exhibits a constitutive current reflecting close-to-open state transitions even at very negative (~120 mV) voltages (Ma et al., 2011). Analysis of a large group of KCNQ1 mutants suggests that gating follows an allostERIC model (Ma et al., 2011). According to this model, the pore can open independently of the state of the VSD, but VSD activation increases the probability of pore opening.
Furthermore, KCNQ1 opening does not require concerted VSD movements (Osteen et al., 2012). Indeed, the VSDs appear to move independently, and pore opening can occur before all VSDs are activated (Osteen et al., 2012), consistent with an allosteric gating model.

Allosteric coupling for KCNQ1 is thought to be mediated by interactions between the VSD and the PD that translate S4 movement to channel opening and closing (Figure 5A). Studies of KCNQ1 (Boulet et al., 2007; Labro et al., 2011) and other KV channels (Lu et al., 2001; Lu et al., 2002; Long et al., 2005) have pointed to the interface between the S4–S5 linker and the C-terminal end of S6 (S6C) as one important mediator of electromechanical coupling (Figure 5A). Certain mutations in the S4–S5 linker (Labro et al., 2011) and S6C (Boulet et al., 2007) slow the opening rate and shift channel activation to more depolarized voltages, while other mutations, specifically at V254 in the S4–S5 linker and at L353 in S6C, promote a constitutively open channel. Interestingly, a V254L/L353A double mutant rescued channel closing, suggesting that the S4–S5 linker interacts with S6C to stabilize the closed state. Relocation of the S4–S5 linker during gating abolishes this interaction, releasing tension on S6 and allowing it to kink at the PAG gating hinge in a cantilever-like fashion to promote channel opening (Figure 5B). This coupling mechanism is intrinsically weak for KCNQ1, requiring modulation by auxiliary molecules (see below). Recent work has further elucidated the molecular details of S4–S5 linker relocation, revealing a two-stage mechanism involving alternative binding modes of the S4–S5 linker to the PD (Hou et al., 2020). This two-stage mechanism may apply to the majority of domain-swapped Kv channels.

hERG

The relative positioning of the VSD and PD in hERG (Wang and Mackinnon, 2017) (Figure 5A) and the EAG1 channel (Whicher and Mackinnon, 2016) suggests that the mechanism by which movements in the VSD are transduced to pore opening is different from other KV channels (Toombes and Swartz, 2016; Barros et al., 2019). This notion is supported by the finding that cutting the S4–S5 linker in hERG through separate expression of the VSD and PD fails to significantly perturb activation kinetics (Lorinczi et al., 2015). In contrast to the lever mechanism proposed for KCNQ1, lateral S4 movement in hERG toward the pore could both alter S4–S5 linker/S6 interactions and exert force through the S4–S5 linker directly onto S5. Displacement of S5 may be transmitted through the S5–S6 interface for opening or closing of the cytosolic gate (Wang and Mackinnon, 2017) (Figure 5B). A structure of hERG in the resting state may provide further insight into the molecular features of this distinct coupling mechanism.
SCN5A

Much of our knowledge about the coupling in SCN5A originates from studies of ancestral NaV channels. Comparison of locked resting state structures of the bacterial sodium channel NaVAb (Wisedchasri et al., 2019) and of the chimeric human NaV1.7–VSDU–NaVAb channel (Xu et al., 2019) with activated state structures (Yan et al., 2017b; Pan et al., 2018; Pan et al., 2019; Shen et al., 2019; Jiang et al., 2020) provide new insight into the mechanism of electromechanical coupling in NaV channels. The S4–S5 linker appears to undergo movement similar to that of KCNQ1. The linker constrains the S5 and S6 helices in the resting/closed state, with looser interactions in the activated/open state. Coupling between the VSD and PD during pore opening must involve loosening of linker/PD interactions as S4 moves outward as in KCNQ1, albeit with tighter coupling of S4 and S4–S5 linker movement. The direct connection between S4 movement and pore opening may be crucial for rapid activation of sodium channels.

Comparison of the cockroach NaV PaS channel structure (Shen et al., 2017), featuring a closed pore and VSDs in distinct activation states, to other eukaryotic NaV channel structures suggests that additional structural shifts may be at work in eukaryotic NaV channels to couple VSD to PD movement. Moreover, the distinct sequence of repeats I to IV (including that of the four S4–S5 linkers) and asynchronous voltage sensor movement in eukaryotic NaV channels (Chanda and Bezanilla, 2002) suggest that distinct interactions couple the VSD of each repeat to the PD. Electromechanical coupling mechanisms may thus be more complex in SCN5A.

Inactivation

KCNQ1

Inactivation in KCNQ1 follows a mechanism distinct from canonical mechanisms (Hou et al., 2017). In the absence of KCNE1, KCNQ1 only partially inactivates, in a manner dependent on the IO and AO open states (Pusch et al., 1998). Differences in VSD-PD coupling between these two states appear to contribute to inactivation. The AO state has a lower coupling efficiency than IO, producing a lower open probability. Transition from IO to AO thus results in partial inactivation because the channel is open but less conductive (Hou et al., 2017). However, association with the KCNE1 accessory β subunit removes KCNQ1 inactivation (Pusch et al., 1998; Seebohm et al., 2003c), making inactivation irrelevant to cardiac KCNQ1 function.

hERG

Inactivation plays a critical role in hERG activity in the action potential. Entry into and out of inactivation is both fast and voltage-dependent, properties that maintain the plateau of the action potential (Perry et al., 2015). Additionally, the voltage dependence of inactivation appears to be independent of that of activation (Vandenberg et al., 2006; Cheng and Claydon, 2012), indicating that activation and inactivation may operate through distinct mechanisms. Inactivation in hERG is C-type (Smith et al., 1996), occurring through structural changes in the SF (Herzberg et al., 1998; Hoshi and Armstrong, 2013). While the structural basis of C-type inactivation is not completely understood, work on hERG and other K+ channels has provided useful insight. Several residues in the KcsA SF form stabilizing hydrogen bond networks that suppress inactivation (Doyle et al., 1998; Bhate et al., 2010; Cuello et al., 2010; Vandenberg et al., 2012). While these residues are conserved in many K+ channels (Whicher and Mackinnon, 2016), they are not present in hERG. This implies that the hERG SF is more liable to collapse, leading to inactivation (Fan et al., 1999; Vandenberg et al., 2012). Indeed, in molecular dynamics simulations, several residues in the hERG SF shift in and out of the pore axis, particularly F627 in the GFG motif (Stansfeld et al., 2008). In the hERG cryo-EM structure, the orientation of F627 is offset compared to other K+ channels (Whicher and Mackinnon, 2016), and a hERG S631A mutant, which has an F627 sidechain orientation similar to other structures, does not inactivate (Wang and Mackinnon, 2017). Additionally, mutation of T432 and A443 (corresponding to S620 and S631 in hERG) in non-inactivating EAG1 to serine was sufficient to impart inactivation behavior (Ficker et al., 2001), perhaps due to reorientation of the SF to match that of hERG. These results indicate that the unique positioning of F627 is critical for hERG fast inactivation behavior. C-type inactivation may involve other rearrangements (Loots and Isacoff, 1998), including coupling the SF to motions in S1, S5, and S6 (Ferrer et al., 2011; Wang et al., 2011; Perry et al., 2013a; Perry et al., 2013b).

SCN5A

Fast inactivation in SCN5A halts inward Na+ current triggered by cardiac depolarization, permitting subsequent outward currents (e.g. Ith1, Ith2) to repolarize the cell in preparation for the next action potential (Ghovanloo et al., 2016) (Figure 6A). The S4 segment in VSDIV (S4IV) is critical for fast inactivation, along with the IFM motif in the III/IV linker (Ahern et al., 2016; Ghovanloo et al., 2016) (Figures 6B, C). Roles of individual basic sites of S4IV in NaV inactivation have been elucidated, with mutations in R1 and R2 delaying inactivation onset and mutations in R3 and R4 delaying recovery from inactivation (Nakajima et al., 2019). Furthermore, structures of an engineered human NaV1.7–NaVPaS channel with VSDIV trapped in a resting state (Clairfeuille et al., 2019) along with the structures of NaVPaS (Shen et al., 2017), electric eel (Yan et al., 2017b) and human NaV1.4 channels (Pan et al., 2018) have provided new structural insight into the mechanism of fast inactivation in NaV channels (Figure 6D). In the resting state R5 on S4IV forms an electrostatic bridge with the α1 helix of the C-terminal cytoplasmic domain (CTD), together with K7 and R8 on the S4–S5 linker. The CTD in turn binds the III/IV linker, sequestering the IFM motif (Figure 6D, left plot). VSDIV activation releases the connection between the CTD and the III/IV linker (middle and right plots in Figure 6D), permitting the IFM motif to bind to a hydrophobic pocket formed by the S4–S5 linkers and S6 helices of repeats III and IV, along with S5IV (Figures 6C, E). The insertion of the IFM motif into this pocket causes a twisting in the S6 helices that closes the gate (Yan et al., 2017b; Pan et al., 2018).
REGULATION OF CHANNEL GATING BY INTRACELLULAR DOMAINS AND AUXILIARY MOLECULES

The function of these cardiac channels is tightly regulated to produce currents that faithfully give rise to the cardiac action potential under a host of physiological conditions and prevent early or delayed contractility. We focus here on regulation through the channel cytoplasmic domains as well as regulation by auxiliary (beta) subunits and lipids. Channels are regulated through other mechanisms such as phosphorylation, but are not covered in this review.

Regulation Involving Cytoplasmic Domains KCNQ1

The C-terminal cytoplasmic domain (CTD) of KCNQ1 contains four alpha helices in lieu of the T1 tetramerization domain common to K_\text{v} channels outside the KCNQ family (Haitin and Attali, 2008). The proximal HA and HB helices form an antiparallel bundle with an IQ motif in HA and a 1-5-10 motif in HB that together enable calmodulin (CaM) binding (Yus-Najera et al., 2002). When bound, CaM prevents channel inactivation in KCNQ1, but inhibits the opening of other KCNQ family members (KCNQ2–KCNQ5) (Chang et al., 2018). While the N-lobe of CaM may be constitutively bound to calcium and the proximal HA and HB helices (Ghosh et al., 2006; Bernardo-Seisdedos et al., 2018), the C-lobe of CaM binds calcium only at higher concentrations (Bernardo-Seisdedos et al., 2018). Calcium binding by the C-lobe induces a conformational change in CaM, facilitating KCNQ1 opening (Bernardo-Seisdedos et al., 2018; Chang et al., 2018). The C-lobe interacts with HA and with a loop in the S2–S3 linker (Sun and Mackinnon, 2017). This feature may contribute to the unique mode of CaM modulation of KCNQ1.

The distal helices HC and HD of KCNQ1 form a self-assembling intersubunit coiled-coil motif that promotes channel tetramerization (Yus-Najera et al., 2002; Howard et al., 2007; Sun and Mackinnon, 2017). The distal coiled-coil domain...
is not only essential for channel tetramerization, but also for subunit specificity, permitting the exclusive formation of KCNQ1 homotetramers (Schwake et al., 2003; Haitin and Attali, 2008; Sachyani et al., 2014). In contrast, other KCNQ family proteins form heteromers (Schwake et al., 2003). Swapping the coiled-coil domain of KCNQ1 with that of KCNQ3 allows the resulting chimera to co-assemble with other KCNQ isoforms and generate channels with altered gating properties (Schwake et al., 2003; Schwake et al., 2006). Residues conferring specific KCNQ1 homotramer formation have been identified in the HD helix (Schwake et al., 2006; Wiener et al., 2008). Key interactions involve both the hydrophobic core of the assembly and exterior electrostatic interactions, with a number of the residues involved subject to LQTS-associated mutations (Howard et al., 2007; Wiener et al., 2008).

hERG
hERG channels contain an N-terminal Per-ARNT-Sim (PAS) domain, which is also present in the N-terminus of many signaling proteins (Henry and Crosson, 2011). An additional C-terminal cyclic nucleotide-binding homology domain (CNBHD) similar to cytoplasmic domains in hyperpolarization-sensitive cyclic nucleotide-gated channels is present, but without ligand-binding properties (Coddington and Trudeau, 2019). The PAS and the CNBHD appear to be critical for modulating the kinetics of slow deactivation in hERG (Gustina and Trudeau, 2011). Furthermore, slow deactivation is dependent on the direct interaction of these domains, particularly between R56 of the PAS and D803 of the CNBHD and between N12 of the N-terminal Cap (N-Cap) and E788 of the CNBHD (Ng et al., 2014; Kume et al., 2018). Interactions between the N-Cap and PAS domains with the C-linker also appear to be important for deactivation (Gustina and Trudeau, 2011; Ng et al., 2014), and the extreme N-terminus is likely essential, possibly interacting with the VSD through a patch of positively-charged residues in the N-Cap tail (Muskett et al., 2011). Indeed, the hERG cryo-EM structure corroborates the positioning of the N-Cap tail relative to the VSD (Wang and Mackinnon, 2017). The N-Cap tail may even be constitutively bound to the VSD (Moraïs Cabral et al., 1998; De La Pena et al., 2011), implying that slow deactivation could involve movement of the PAS toward the plasma membrane to alter this interaction (Barros et al., 2018).

SCN5A
Multiple structures of the SCN5A CTD in complex with regulatory factors (Wang et al., 2012; Gabelli et al., 2014; Gardill et al., 2019) have been determined, helping to elucidate how CaM (Johnson et al., 2018) and fibroblast growth factor-homologous factors (FGFhs), specifically FGF13, modulate inactivation (Liu et al., 2003; Wang et al., 2012; Musa et al., 2015; Yang et al., 2016). CaM binds the CTD in a calcium-dependent manner (Kim et al., 2004; Hovey et al., 2017; Johnson et al., 2018; Urrutia et al., 2019), altering inactivation kinetics (Yan et al., 2017a; Johnson et al., 2018) and promoting recovery from inactivation (Johnson et al., 2018). In the absence of FHF, the C-lobe of apo-CaM binds to the IQ motif located in an extended helix on the CTD, while the N-lobe binds to the preceding EF hand-like domain (EFL) (Gabelli et al., 2014). With increasing intracellular calcium, holo-CaM has stronger affinity for the SCN5A III/IV linker (Johnson et al., 2018) and the N-lobe dissociates from the EFL (Gardill et al., 2019). These calcium-dependent interactions with the III/IV linker may facilitate CaM modulation of inactivation (Hovey et al., 2017; Johnson et al., 2018). FGF13 slows both inactivation onset and inactivation recovery in SCN5A (Yang et al., 2016), opposing CaM modulation. Importantly, binding of FGF13 to the SCN5A EFL (Wang et al., 2012) prevents binding of the apo-CaM N-lobe to the same domain (Wang et al., 2012). The EFL itself also adopts different orientations when bound to FGF13 (Wang et al., 2012) or CaM (Gabelli et al., 2014). Because the EFL also binds to the III/IV linker containing the IFM inactivation motif (Shen et al., 2017; Clairfeuille et al., 2019), EFL conformational changes upon binding of CaM or FGF13 may contribute to inactivation modulation by these proteins.

SCN5A also contains two cytoplasmic loops linking repeats I and II and repeats II and III, respectively. Unlike the III/IV linker, these loops are around 200 residues long (Pan et al., 2018) and likely disordered, based on the absence of these loops in cryo-EM structures. However, important regulatory events such as phosphorylation (Marionneau et al., 2012; Iqbal et al., 2018) and cofactor binding (Wu et al., 2008) that modulate channel properties have been identified in these loops.

**Auxiliary Beta Subunits**

**KCNQ1**
KCNQ1 co-assembles with a family of single span membrane proteins (KCNQ1–5) (Abbott, 2014), and this interaction appears to be tissue-specific. In the heart, KCNQ1 is complexed with KCNE1, and the KCNQ1-KCNE1 channel exhibits greater single-channel conductance, opening at more positive potentials, and delayed activation compared to KCNQ1 alone (Barhanin et al., 1996; Sanguinetti et al., 1996). This heteromultimeric channel also exhibits altered Rb+/K+ selectivity (Pusch et al., 2000) and loss of inactivation (Pusch et al., 1998; Pusch et al., 2000). These properties are essential for the generation of the slow delayed rectifier current (I_{Kr}) in repolarizing cardiomyocytes. In gastric parietal cells, KCNQ1 co-assembles with KCNE2 to create constitutive K+ currents essential for gastric acid secretion (Heizmann et al., 2004; Roepke et al., 2006). In intestinal epithelial cells KCNQ1 complexes with KCNE3 to allow K+ recycling for trans-epithelial chloride ion secretion (Preston et al., 2010). In contrast to KCNE1, KCNE2 and KCNE3 render KCNQ1 constitutively open (Schroeder et al., 2000; Tinel et al., 2000).

Due to the critical role of the KCNQ1-KCNE1 channel in the cardiac action potential, many research groups have probed the structural interaction between these proteins (Tapper and George, 2001; Xu et al., 2008; Chung et al., 2009; Strutz-Seebohm et al., 2011; Wang et al., 2011; Chan et al., 2012; Li et al., 2014). Combined results indicate that the transmembrane helix of KCNE1 binds in the cleft between neighboring KCNQ1...
subunits and interacts with both the VSD and PD. These binding sites overlap the binding cleft of KCNE3 (Sun and Mackinnon, 2020), indicating that KCNE1 may bind to the same site (Figure 7A). In the human KCNQ1-KCNE3 cryo-EM structure (Sun and Mackinnon, 2020) and an earlier Rosetta model of the complex (Kroncke et al., 2016), this cleft is formed by three KCNQ1 subunits (Figure 7B). KCNE3 contacts the cytoplasmic half of S5 in one subunit, the extracellular side of S6 in a second one, and along the entire length of S1 and the cytoplasmic side of S4 in a third subunit. Importantly, KCNE1 and KCNE3 produce KCNQ1 currents with different voltage dependences and gating kinetics (Figure 7C). Given the possibility of a common binding cleft, differential modulation of KCNQ1 by KCNE1 and KCNE3 may involve different sidechain interactions within this cleft.

Mutations in the KCNE1 transmembrane helix, particularly at F57, T58, and L59 (Melman et al., 2001; Melman et al., 2002), alter the ability of this subunit to modulate the function of KCNQ1. The effect of this ‘activation’ triplet on KCNQ1 gating is altered by mutations at residues S338, F339, F340, and A341 in S6 (Melman et al., 2004; Panaghi et al., 2006; Strutz-Seebohm et al., 2011; Li et al., 2014), suggesting a functional link between these residues. However, modeling studies fail to indicate direct contact between the activation triplet on KCNE1 and these S6 sites (Kang et al., 2008; Gofman et al., 2012; Xu et al., 2013), suggesting that the functional coupling may be mediated allosterically. Interestingly, KCNE1 also affects S4 movement (Rocheleau and Kobertz, 2008; Wu et al., 2010a; Nakajo and Kubo, 2014) and shifts VSD voltage-dependence of activation to more negative voltages (Osteen et al., 2010; Rusiec et al., 2013; Barro-Soria et al., 2014). A model of KCNE1 regulation has recently been proposed, in which KCNE1 alters VSD-PD coupling interactions to suppress the IO state and modulate the AO state (Zaydman et al., 2014). This model accounts for many of the observed effects of KCNE1 on KCNQ1, including KCNE1-induced inhibition of inactivation. However, this model is not structurally elaborated.

hERG

Previous studies indicate that KCNE1 (Mcdonald et al., 1997) and KCNE2 (Abbott et al., 1999) can interact with and modulate hERG function. However, whether this interaction occurs under physiological conditions is debated (Weerapura et al., 2002; Anantharam and Abbott, 2005; Abbott et al., 2007). Although KCNE1 and KCNE2 alter hERG gating kinetics (Mcdonald et al., 1997; Mazhari et al., 2001), co-expression of KCNE2 with hERG in vitro does not reproduce the native IKr current (Weerapura et al., 2002). This suggests that KCNE1 and KCNE2 may not be essential for hERG channel function or that additional factors are required for KCNE-hERG interaction in cardiac cells. The latter possibility is supported by the observation that mutations in KCNE2 may predispose patients to drug-induced LQTS (Abbott et al., 1999; Sesti et al., 2000). As hERG is particularly drug-sensitive, this finding suggests that hERG may, in fact, be modulated by KCNEs in native tissue. Additionally, a T10M mutation in KCNE2 causes arrhythmia induced by auditory stimulation, a known trigger of LQT2, (Gordon et al., 2008). Further studies are needed to resolve these conflicting results and clarify the role of KCNE proteins in regulation of hERG function.

SCN5A

In humans there are five NaV-subunit protein isoforms encoded by four genes, SCN1B-SCN4B (β1 to β4) (Detta et al., 2015; O’malley and Isom, 2015). All are expressed in the heart and have been shown to associate with SCN5A in heterologous conditions (Makita et al., 1996; Dhar Malhotra et al., 2001; Malhotra et al., 2004; Medeiros-Domingo et al., 2007; Watanabe et al., 2009; Valdivia et al., 2010), β1 to β4 are single-span transmembrane proteins containing an extracellular N-terminal immunoglobulin (ig) domain, while β1B, a splice variant of SCN1B, lacks the transmembrane domain (Brackenbury and Isom, 2011). Generally, β-subunits modulate the biophysical properties and cell surface expression
of NaV channels in heterologous cells (Calhoun and Isom, 2014). β1 and β3 interact non-covalently with NaV channels (Meadows et al., 2001), while β2 and β4 are covalently bound through cysteine bonds between the extracellular Ig domain and channel pore loops. However, modulation of SCN5A by β-subunits has been difficult to assess. Varying effects of β1 (Qu et al., 1995; Dhar Malhotra et al., 2001; Baroni et al., 2014; Zhu et al., 2017) as well as β3 (Hu et al., 2009; Valdivia et al., 2010; Wang et al., 2010) on SCN5A have been reported. The rat SCN5A structure provides a potential explanation for this difficulty, as SCN5A is missing a cysteine residue critical for covalent interaction with β2, and contains a glycosylation site that may sterically occlude β1 interaction (Jiang et al., 2020). SCN5A may not bind tightly with any β-subunit. Nevertheless, the potential importance of β-subunits in SCN5A modulation has been suggested by arrhythmia-associated mutations in all four β-subunit genes, including mutations causing Brugada syndrome (Watanabe et al., 2008; Hu et al., 2009; Hu et al., 2012), LQT3 (Medeiros-Domingo et al., 2007; Riuro et al., 2014), and atrial fibrillation (Watanabe et al., 2009; Olesen et al., 2011). However, a recent review of genetic evidence supporting these associations has disputed the clinical validity of β-subunits as monogenic causes of arrhythmia syndromes (Hosseini et al., 2018; Adler et al., 2020).

SCN5A subcellular localization also contributes importantly to channel regulation. SCN5A channels in cardiac tissue are localized at the lateral membranes and at the anchoring junction between cardiomyocytes (the intercalated disc). These expression patterns give rise to distinct sets of protein–protein interactions and biophysical properties (Lin et al., 2011; Shy et al., 2013). At the lateral membrane SCN5A interacts with the dystrophin/syntrophin multicomplex, but at the intercalated disc SCN5A interacts with ankyrin-G (Lemaillet et al., 2003; Mohler et al., 2004), which links the channel to cytoskeletal proteins such as actin and the desmosomal protein plakophilin-2 (Markara et al., 2014). Intriguingly, the functional properties of SCN5A channels between these two pools also differ: SCN5A at the lateral membrane has smaller current amplitude, distinct voltage-dependence, and slower recovery from inactivation as compared to channels in the intercalated disc (Lin et al., 2011). The functional implications of these differences are not yet clear and remain an active area of study.

**Lipid Molecules**

**KCNQ1**

KCNQ1 and other KCNQ channels are dependent on phosphatidy-4,5-bisphosphate (PIP2) for function (Loussouarn et al., 2003; Zaydman et al., 2013; Taylor and Sanders, 2017), and are inhibited upon stimulation of Gα- and G11-protein coupled receptors, which trigger phospholipase C-catalyzed PIP2 hydrolysis (Selyanko et al., 2000; Loussouarn et al., 2003; Zhang et al., 2003) (Figure 8B). Growing evidence suggests that PIP2 acts as a coupling element for KCNQ1, enhancing weak allosteric interactions between the VSD and PD (Vardanyan and Pongs, 2012; Zaydman et al., 2013; Kasimova et al., 2015; Cui, 2016). Structure-function studies, confirmed by the human KCNQ1/PIP2 structure, localize PIP2 binding to the cleft between neighboring channel subunits, with interactions involving mostly positively-charged residues in the S2–S3 linker, S4–S5 linker, and S6C (Figure 8A) (Thomas et al., 2011; Zaydman et al., 2013; Eckey et al., 2014; Chen et al., 2015; Sun and Mackinnon, 2020). This binding site seems well-suited to modulate coupling of VSD movement to the activation gate. Questions remain regarding the mechanism of PIP2 regulation of VSD-PD coupling, PIP2;KCNQ1 stoichiometry, and binding site differences between the activated and resting states.

PIP2 also binds to the CTD at a site shared with CaM (Tobelaim et al., 2017b). These two regulators may competitively regulate KCNQ1 at this site (Tobelaim et al., 2017a). Indeed, in the human KCNQ1 cryo-EM structure, CaM exhibits a nearly 180° rotation when PIP2 is bound, losing contact with the S2–S3 linker (Sun and Mackinnon, 2020). Additionally, S6 and HA in KCNQ1 form a single helix in the open channel. These structural shifts point to an interplay between PIP2 and CaM, but does not clarify the nature of this interaction, as no PIP2 density was seen at the CTD binding site in the cryo-EM structure. The details of the coordination between CaM and PIP2, and its role in channel function, remain to be elucidated.

Polyunsaturated fatty acids (PUFAs) also modulate KCNQ1 function (Taylor and Sanders, 2017). While PUFAs generally inhibit ion channel current (Boland and Drzewiecki, 2008), the KCNQ1–KCNE1 channel is a notable exception. The IKs current is enhanced by docosahexaenoic acid (DHA) and, to a lesser
extremity, oleic acid (Doolan et al., 2002) by shifting the conductance-vs-voltage curve (GV) to more negative voltages. Interestingly, the charge of the head group determines the direction of the (GV) shift (Figure 8B): negatively-charged DHA causes a negative shift, a neutral head group has no effect, and a positively-charged one shifts the (GV) curve to positive potentials, reducing channel function (Liin et al., 2015). Negative head group charge and a polyunsaturated acyl chain appear to be required for channel activation. The PUFA binding site in KCNQ1 appears to involve residues in the extracellular S3–S4 loop, R1 and R2 on S4, and the PD (Figure 8A), as mutations in these regions either reduce the PUFA effect or completely abolish the (GV) shift (Liin et al., 2015; Liin et al., 2018).

hERG
As with KCNQ1, the function of hERG is upregulated by PIP2 by means of increased current amplitude, a hyperpolarizing shift in voltage-dependence of activation, as well as faster activation and slower inactivation rates (Bian et al., 2001). Stimulation of Gαq-protein coupled receptors also suppresses the Iκ, current through PIP2 depletion (Bian et al., 2001; Bian et al., 2004). Binding of PIP2 to hERG likely localizes to a cluster of basic residues (R883–Q900) C-terminal to the CNBHD, as substitution of these residues to neutral or negatively-charged amino acids prevented PIP2 effects on hERG function and abolished PIP2 binding (Bian et al., 2004). Unfortunately, these residues are not resolved in the available hERG structure (Wang and Mackinnon, 2017).

SCN5A
In contrast to KCNQ1, PUFAs such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA), and α-linolenic acid (ALA) suppress Ina current in a concentration-dependent manner by shifting the voltage dependence of Ina inactivation to more hyperpolarized potentials (Kang et al., 1995; Kang et al., 1997; Leifert et al., 1999). EPA also accelerates the transition from the resting state to the inactivated state and slows recovery from inactivation (Xiao et al., 1998; Isbilen et al., 2006). However, the effect was reduced by β1 subunit expression (Xiao et al., 2000). Furthermore, N406K renders SCN5A less sensitive to inhibition by EPA, an effect strengthened by β1 subunit expression (Xiao et al., 2001). The structural basis for PUFA binding and modulation is not well understood.

CHANNEL DYSFUNCTION IN CONGENITAL LONG QT SYNDROME
Alterations in the action potential disturb impulse propagation and cause reentry (Kleber Ag, 2004), whereby the impulse re-stimulates the heart tissue that generated it. Reentry promotes cardiac arrhythmias and predisposes to sudden unexplained death (Skinner et al., 2019). LQTS is a prevalent cause of such events, with an estimated population prevalence of approximately 1:2500 (Schwartz et al., 2009).

LQTS is characterized by a prolonged rate-corrected QT interval in patient electrocardiograms (ECGs), indicative of impaired repolarization (Schwartz et al., 2012). In a large proportion of cases, QT interval prolongation is the result of either a loss-of-function in KCNQ1 or hERG, or a gain-of-function in the SCN5A channel (Moss and Kass, 2005; Schwartz et al., 2012; Earle et al., 2013; Skinner et al., 2019). Mutations in these genes confer distinct subtypes of LQTS (denoted LQT1–3, respectively), each with unique ECG features, risk factors, arrhythmia triggers, and responsiveness to β-adrenergic receptor blockers, the most common LQTS treatment (Ackerman, 2005; Moss and Kass, 2005; Skinner et al., 2019; Wallace et al., 2019). Below, the function of each ion channel in the cardiac action potential is discussed, along with how each can cause action potential dysfunction in LQTS.

Channel Function in the Cardiac Action Potential and Dysfunction in LQTS

KCNQ1
In complex with KCNE1, KCNQ1 conducts IKr, which helps shape the plateau and repolarization phases of the action potential, in part by counteracting calcium influx (Skinner et al., 2019). Due to slow activation, the KCNQ1-KCNE1 channel is only slightly open during the plateau phase, with IKr increasing slowly in a nearly linear fashion (Figure 7C) until the repolarization phase is reached and the channel becomes fully activated (Figures 1B, C) (Moss and Kass, 2005). KCNQ1-KCNE1 channels do not inactivate (Pusch et al., 1998), conducting current throughout activation and deactivation. The channel slowly deactivates, shaping the tail of the repolarization phase and mediating return to the resting potential (Moss and Kass, 2005; George, 2013; Skinner et al., 2019). Thus, loss-of-function in KCNQ1 prolongs repolarization primarily by preventing completion of the repolarization phase, broadening the tail of the T-wave (Figure 1A) and causing type 1 LQTS (LQT1). (Ackerman, 2005; Tester and Ackerman, 2014; Skinner et al., 2019; Wallace et al., 2019). The effects of a KCNQ1 mutation are exacerbated upon sympathetic activation of β-adrenergic receptors due toIKr potentiation, while resting heart rates show few perturbations (Shimizu and Antzelevitch, 1998). The QT interval is unable to shorten in response to this stimulation, making physical exertion accompanied by heightened sympathetic nervous system activity a prevalent LQT1 trigger (Schwartz et al., 2012; Bohnen et al., 2017).

hERG
hERG makes up the other primary inward rectifier current IKr, which acts in both the plateau and the repolarization phase (Figures 1B, C) (Moss and Kass, 2005; Skinner et al., 2019). As the channel activates, rapid entry into and out of inactivation (due to SF fluctuations) creates a persistent current through the plateau phase until slow deactivation, regulated by the PAS and CNBHID domains, closes the channel during the repolarization phase. While both hERG and KCNQ1 are conductive during the plateau and repolarization phases, hERG has a higher unitary conductance than KCNQ1 in both phases (Moss and Kass, 2005;2008).
The resulting I_{Ks} current thus supplies a greater proportion of the overall I_{Na} current (Cheng and Kodama, 2004), with loss-of-function in hERG (LQT2) correlating with a lower T-wave amplitude in contrast to the normal T-wave amplitude associated with LQT1. Additionally, LQT2 is often triggered at rest (Skinner et al., 2019), consistent with the greater hERG contribution to I_{Ks}. However, while I_{Ks} is usually more prominent (Cheng and Kodama, 2004), the relative density of I_{Ks} and I_{Ki} vary by ventricular cell type (Liu and Antzelevitch, 1995; Viswanathan et al., 1999).

**SCN5A**

SCN5A produces the I_{Na} current that shapes the initial upstroke of the cardiac action potential (Skinner et al., 2019), initiating depolarization. SCN5A is rapidly activated through asynchronous motion of the VSD’s in repeats I-III coupled to pore opening, then quickly inactivates through interaction of the IFM motif with the channel, resulting in a brief inward spike of sodium current that depolarizes the cell (Ghovanloo et al., 2016) (Figures 1B, C). Unlike KCNQ1 and hERG, LQT3 is conferred by SCN5A gain-of-function mutations that cause a “leaky” inward sodium current (Wilde and Amin, 2018; Skinner et al., 2019). The functional I_{Ks} and I_{Ki} outward currents are unable to compensate for the persistent inward I_{Na} current, prolonging entry into and completion of the repolarization phase and giving rise to LQT3. As in LQT2, LQT3-related arrhythmias occur most often at rest, rather than in response to stress or exercise (Schwartz et al., 2012). It should be noted that while loss-of-function mutations for SCN5A are not relevant to LQTS, they are prevalent in other channelopathies, particularly Brugada syndrome (Wilde and Amin, 2018; Skinner et al., 2019).

**Molecular Mechanisms of LQTS**

Mutations in KCNQ1, hERG, and SCN5A can lead to LQTS by perturbing channel function through distinct molecular mechanisms. Structural studies of these channels can help tease apart these molecular mechanisms for a more detailed understanding of LQTS. We have mapped LQTS mutations identified as pathogenic according to ClinVar (Harrison et al., 2016) and HGMD (Stenson et al., 2012) databases onto the human KCNQ1 (Sun and Mackinnon, 2020) and hERG structures (Wang and Mackinnon, 2017), as well as a recent model of SCN5A (Kroncke et al., 2019) and the rat SCN5A homolog (Jiang et al., 2020) (Figures 9–11). The list of curated mutations is found in Table 1: 261 mutations in KCNQ1, 320 mutations in hERG, and 122 mutations in SCN5A. Examination of these mutation sites provides some insight into the channel mechanisms that may be commonly disrupted in LQT1–3.

**KCNQ1**

KCNQ1 mutations studied to date are highly varied in molecular effects, with the potential to induce defects in channel stability, trafficking, electrophysiology, or all three (Chen et al., 2011; Heijman et al., 2012; Wu et al., 2016; Bohnen et al., 2017). So far, a strong disposition for either expression or functional defects has not been elucidated. However, analysis of mutation sites points to regions of increased pathological risk and provides hints regarding prevalent mechanisms.

LQT1-associated mutations are found in every domain of the protein (Figure 9A), particularly in the transmembrane channel domain (Shimizu et al., 2004; Moss et al., 2007; Kapa et al., 2009). Mutations in the SF, (Figure 9A, inset), VSD (Figure 9B), and the S4–S5 linker (Figure 9C) are especially prominent.

Mutations in and around the SF (Figure 9A, inset) point to a potential ion permeation defect, as conformational changes may prevent potassium ion permeation and thus decrease the effective I_{Ks} current (Choveau and Shapiro, 2012), as noted in several SF mutations (Ikra et al., 2008; Thomas et al., 2010; Burgess et al., 2012; Chen et al., 2019). Molecular dynamics simulations of analogous mutations in KcsA indicated that mutations in the SF-interacting residues (including T322M, T322A, or G325R) may disrupt the organization of backbone atoms in the SF, abolishing K+ ion coordination and producing a dominant-negative effect on channel current (Burgess et al., 2012).

VSD mutations are more common in KCNQ1 compared to hERG and SCN5A (see Figures 9B, 10B, and 11A), indicating that the VSD is more important in LQT1. Analysis of LQTS mutations located in the VSD (Huang et al., 2018; Vanoye et al., 2018) indicate that loss of channel function was most commonly a consequence of mutation-induced destabilization of the channel, resulting in mistrafficking and lower surface expression. These results strongly correlated with Rosetta energy calculations of VSD mutants (Kuenze et al., 2019), supporting the importance of VSD destabilization as a LQT1 mechanism. A number of the deleterious mutations were found in the S0 helix and S0-contacting regions of the VSD. Among the 50 VSD mutations studied, the S0 mutations had the largest energetic destabilizations consistent with the large number of contacts formed between S0 and other regions of the VSD. (Huang et al., 2018; Kuenze et al., 2019). However, these studies also identified variants that did not exhibit folding and trafficking defects, but were either non-functional (low conductance) or dysfunctional, with modified gating, again pointing to a complex spectrum of LQT1 disease mechanisms.

Mutations in the KCNQ1 S4–S5 linker and its contacting regions (Figure 9C) have the potential to perturb VSD-PD coupling and channel gating. Such effects have been demonstrated for a number of LQTS-associated mutations in the S4–S5 linker and the cytoplasmic end of S6, particularly for R243C, W248F/R, and Q357R, producing positive shifts in the voltage dependence of activation or inactivation and and/or decreased current amplitude (Franqueza et al., 1999; Boulet et al., 2006; Mousavi Nik et al., 2015). Because the S4–S5 linker is involved in both VSD/PD interactions and in binding of PIP2 (Eckey et al., 2014; Sun and Mackinnon, 2017) and KCNE1 (Kang et al., 2008; Xu et al., 2013; Cui, 2016), mutations in this region may affect gating either by weakening VSD-PD coupling or by hindering modulation by auxiliary molecules. Some LQT1 variant-induced changes in PIP2 binding at the S4–S5 linker have been noted (including R243H, R539W, and R555G) (Park et al., 2005). Due to the complex nature of electromechanical coupling
in KCNQ1 and the requirement for auxiliary subunits, a detailed structural understanding of mutation effects in this region is particularly challenging. However, the human KCNQ1, KCNQ1-KCNE3, and KCNQ1-KCNE3/PIP2 channel structures (Sun and Mackinnon, 2020) may provide guidance in experimental design to elucidate LQT1-associated coupling defects in this region.

While the majority of LQT1 mutations map to the transmembrane domain, mutations are also found in the CTD, both in the HA/HB helix bundle, where CaM binds, and in the HC and HD coiled-coil domains (Figure 9A). Mutations in HC and HD are particularly prevalent in sites of hydrophobic and electrostatic interactions that stabilize the coil, hinting at channel assembly defects (Figure 9D). Defects in CaM binding and tetramerization cause some forms of LQT1, as demonstrated for several mutations (Kanki et al., 2004; Ghosh S, 2006; Schwake et al., 2006; Wiener et al., 2008).

hERG

Loss-of-function mutations in hERG may lead to folding/trafficking defects, gating defects, or both (Anderson et al., 2014; Smith et al., 2016; Bohnen et al., 2017). However, as many as 90% of hERG loss-of-function mutations appear to cause trafficking defects, independent of which domain is mutated (Smith et al., 2016). As in KCNQ1, pore domain mutations in hERG demonstrated stronger dominant-negative effects in hERG than other regions, hinting that mutations in this region are more severe (Anderson et al., 2014).

Although hERG mutations span the entire protein, they are prominent in the SF (Figure 10A, inset), the extracellular VSD/PD interface between S1 and S5 (Figure 10C), and in the PAS and CNBHHD cytoplasmic domains (Figure 10D). Given the inherent instability of the hERG SF (Fan et al., 1999; Stansfeld et al., 2008; Vandenberg et al., 2012) and the functional necessity of the SF for fast inactivation (Herzberg et al., 1998; Hoshi and Armstrong, 2013), mutations may act by both affecting channel stability and inactivation. Inactivation defects due to LQT2-associated pore helix mutations have been identified (Zhao et al., 2009; Poulsen et al., 2015). However, mutations in LQT2 more commonly cause misfolding and/or trafficking defects. Potassium ions appear to stabilize the mature hERG channel (possibly by interacting with the SF) to promote trafficking (Wang et al., 2009; Apaja et al., 2013), with intracellular K+ depletion resulting in ER retention. G601S mimicked this phenotype (Apaja et al., 2013). G601S, a mutation in the pore loop near the SF, mimicked also impeded trafficking through enhanced chaperone association in the ER (Ficker et al., 2003), supporting the idea of SF destabilization as a cause for channel mis trafficking. A detailed understanding of mutations in the SF region requires both intensive structural and cellular analysis to uncover not only protein conformational changes upon

![Figure 9](https://example.com/figure9.png)

**FIGURE 9** | Human KCNQ1 structure with deleterious mutation sites indicated. (A) Full KCNQ1 structure (PDB: 6UZZ) (Sun and Mackinnon, 2020). Yellow sites denote locations of a single deleterious mutation, while red indicates sites with multiple mutations identified. Spheres indicate C-alpha positions of mutation sites. Subunit with LQT1 mutations sites mapped is colored green. Inset: of SF and pore helix. (B) KCNQ1 VSD. (C) S4–S5 linker (S4–S5) interaction site close-up. One subunit is colored green and the others in gray, for clarity. The PAG motif (PAG) is indicated with sticks. (D) Top: HC coiled-coil domain. Bottom: HD coiled-coil domain (PDB: 3BJ4) (Wiener et al., 2008). Residue side chains are indicated with sticks.
FIGURE 10 | hERG structure with deleterious mutation sites indicated. (A) Full hERG structure (PDB: 5VA1) (Wang and Mackinnon, 2017). Yellow sites denote locations of a single deleterious mutation, while red indicates sites with multiple mutations identified. Spheres indicate C-alpha positions of mutation sites. Subunit with LQT2 mutations sites mapped is colored green. Inset: close-up of SF and pore helix. (B) hERG VSD. (C) Close-up of the S1/S5 interface. Residues at the interface are indicated with sticks. (D) N-terminal PAS (dark gray) and C-terminal CNBHD (light gray) domain interface, cartoon representation with surface representation overlay. One subunit is green and the other gray, for clarity.

FIGURE 11 | Rat SCN5A structure with deleterious mutation sites indicated. (A) Full SCN5A structure (PDB: 6UZ3) (Jiang et al., 2020). Yellow sites denote locations of a single deleterious mutation, while red indicates sites with multiple mutations identified. Spheres indicate C-alpha positions of mutation sites. Side views of individual repeats are shown with 90° rotations between panels. In each panel, one repeat is colored green and LQT3 mutation sites for that repeat are mapped. (B) C-terminal domain with bound III/IV linker (human SCN5A model: (Kroncke 8m, 2019)). (C) Putative inactivation gate. IFM motif is indicated with sticks. (D) SCN5A constriction site. S6 helices are shown at full opacity, with the IFM motif indicated with sticks.
| Mutant | Database | Mutant | Database | Mutant | Database | Mutant | Database |
|--------|----------|--------|----------|--------|----------|--------|----------|
| A58P*  | HGMD     | T311I  | HGMD     | G6R    | HGMD     | G572R  | ClinVar, HGMD |
| S66F*  | HGMD     | T312S  | HGMD     | G6V    | HGMD     | G572S  | HGMD     |
| T96R   | HGMD     | T312I  | ClinVar, HGMD | T313N | HGMD     | G5712V | HGMD     |
| T104I  | HGMD     | I313M  | HGMD     | R20G   | HGMD     | E575Q  | HGMD     |
| Q107H  | HGMD     | G314A  | HGMD     | G9V    | HGMD     | G572S  | HGMD     |
| R109L  | HGMD     | G314D  | HGMD     | G1329S | HGMD     | R53Q   | HGMD     |
| Y111C  | ClinVar, HGMD | G314C | HGMD     | S66F   | HGMD     | E575K  | HGMD     |
| L114P  | HGMD     | Y315F  | HGMD     | T96R   | HGMD     | R582C  | ClinVar |
| E115G  | ClinVar, HGMD | G314I | HGMD     | T104I  | HGMD     | G6V    | HGMD     |
| P117L  | ClinVar, HGMD | Y315N | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| C122Y  | ClinVar, HGMD | Y315C | HGMD     | G6V    | HGMD     | G572V  | HGMD     |
| Y125G  | HGMD     | Y315H  | HGMD     | G314S  | HGMD     | R18W   | HGMD     |
| F127L  | HGMD     | Y315F  | HGMD     | G9V    | HGMD     | R53Q   | HGMD     |
| L131P  | HGMD     | Y315S  | HGMD     | G1329S | HGMD     | R53Q   | HGMD     |
| E132L  | HGMD     | Y316E  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| V133I  | HGMD     | Y316V  | HGMD     | G1329S | HGMD     | R53Q   | HGMD     |
| L134P  | ClinVar, HGMD | Y316V | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| C136F  | HGMD     | D317N  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| E146G  | HGMD     | D317G  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A150G  | HGMD     | D317Y  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| T153M  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| F157C  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| E160K  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A178P  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| G168R  | ClinVar, HGMD | D317A | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| T169R  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| T169K  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| E170G  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| V173D  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| R174P  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| H178R  | ClinVar, HGMD | D317A | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A178T  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A178P  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| K183R  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| K183M  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| Y184C  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| Y184H  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| Y184S  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| G189A  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| G189E  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A189S  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A190L  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| R190Q  | ClinVar, HGMD | D317A | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| R190L  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| R190W  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| L191P  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| R192P  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| F193L  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| R195P  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| K196T  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| P197S  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| I198V  | ClinVar, HGMD | D317A | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| S199A  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| I200N  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |
| A203F  | HGMD     | D317A  | HGMD     | T13N   | HGMD     | G572V  | HGMD     |

(Continued)
| Mutant | Database | Mutant | Database | Mutant | Database | Mutant | Database |
|--------|----------|--------|----------|--------|----------|--------|----------|
| I204M  | HGMD     | R204P  | HGMD     | A204V  | HGMD     | F204V  | HGMD     |
| I204F  | HGMD     | R204W  | HGMD     | A204I  | HGMD     | F204L  | HGMD     |
| Y205M  | ClinVar, HGMD | G205D | HGMD     | A205L  | HGMD     | F205L  | HGMD     |
| S209F  | HGMD     | A217T  | HGMD     | A217V  | HGMD     | F217I  | HGMD     |
| K218E  | HGMD     | A220D  | HGMD     | A220V  | HGMD     | F220L  | HGMD     |
| T224M  | HGMD     | S237P  | HGMD     | L237P  | HGMD     | S237L  | HGMD     |
| S225L  | HGMD     | W239S  | HGMD     | A239I  | HGMD     | G239A  | HGMD     |
| A226V  | HGMD     | W239S  | HGMD     | A239I  | HGMD     | G239A  | HGMD     |
| I227L  | HGMD     | R240G  | HGMD     | E240K  | HGMD     | D240H  | HGMD     |
| G229D  | HGMD     | R240W  | HGMD     | E240K  | HGMD     | D240H  | HGMD     |
| R231C  | HGMD     | E243K  | HGMD     | E243K  | HGMD     | D243E  | HGMD     |
| R231H  | ClinVar, HGMD | S246F | HGMD     | I246M  | HGMD     | D246V  | HGMD     |
| I235N  | ClinVar, HGMD | S250Y | HGMD     | F250L  | HGMD     | Y250H  | HGMD     |
| L238R  | HGMD     | T250I  | HGMD     | S250P  | HGMD     | Y250H  | HGMD     |
| L238P  | HGMD     | W252R  | HGMD     | R252C  | HGMD     | Y252H  | HGMD     |
| L239P  | HGMD     | Y255S  | ClinVar  | R255S  | HGMD     | Y255H  | HGMD     |
| V241G  | HGMD     | K262T* | HGMD     | L262V  | HGMD     | Y262C  | HGMD     |
| D242N  | HGMD     | T264M  | HGMD     | L264P  | HGMD     | Y264H  | HGMD     |
| D242Y  | HGMD     | D264E* | HGMD     | L264P  | HGMD     | Y264H  | HGMD     |
| R243C  | ClinVar, HGMD | H265Y  | HGMD     | L265P  | HGMD     | Y265H  | HGMD     |
| R243F  | ClinVar, HGMD | R266A  | HGMD     | L266P  | HGMD     | Y266H  | HGMD     |
| G245V  | HGMD     | T268M* | HGMD     | L268V  | HGMD     | Y268H  | HGMD     |
| W249C  | HGMD     | I270S  | HGMD     | L270P  | HGMD     | Y270H  | HGMD     |
| L250H  | HGMD     | S272P  | HGMD     | S272Y  | HGMD     | F272L  | HGMD     |
| L250P  | HGMD     | S272Y  | HGMD     | S272Y  | HGMD     | F272L  | HGMD     |
| L251Q  | HGMD     | V275F  | HGMD     | K275E  | HGMD     | E275D  | HGMD     |
| L251P  | HGMD     | V275F  | HGMD     | K275E  | HGMD     | E275D  | HGMD     |
| L252D  | HGMD     | A279T  | HGMD     | A279T  | HGMD     | A279T  | HGMD     |
| S253C  | ClinVar  | R281W  | ClinVar, HGMD | R281W | HGMD     | A281I  | HGMD     |
| V254M  | ClinVar, HGMD | E282K  | HGMD     | L282P  | HGMD     | Y282H  | HGMD     |
| V254L  | HGMD     | S284L  | HGMD     | L284V  | HGMD     | Y284H  | HGMD     |
| H258N  | HGMD     | S284L  | HGMD     | L284V  | HGMD     | Y284H  | HGMD     |
| H258P  | HGMD     | S284L  | HGMD     | L284V  | HGMD     | Y284H  | HGMD     |
| H258R  | ClinVar, HGMD | V285F  | HGMD     | L285P  | HGMD     | Y285H  | HGMD     |
| R259C  | ClinVar, HGMD | S288Y  | ClinVar, HGMD | R288Q | HGMD     | A288T  | HGMD     |
| R259L  | ClinVar, HGMD | S288Y  | ClinVar, HGMD | R288Q | HGMD     | A288T  | HGMD     |
| R259H  | HGMD     | S288Y  | HGMD     | L288P  | HGMD     | Y288H  | HGMD     |
| E261Q  | HGMD     | S290T  | HGMD     | L290P  | HGMD     | Y290H  | HGMD     |
| E261K  | HGMD     | S290T  | HGMD     | L290P  | HGMD     | Y290H  | HGMD     |
| E261V  | HGMD     | S290T  | HGMD     | L290P  | HGMD     | Y290H  | HGMD     |
| L262V  | HGMD     | S290T  | HGMD     | L290P  | HGMD     | Y290H  | HGMD     |
| H265I  | ClinVar, HGMD | L293P  | HGMD     | L293P  | HGMD     | Y293H  | HGMD     |
| L268F  | HGMD     | S306F  | HGMD     | L306P  | HGMD     | Y306H  | HGMD     |
| G269F  | HGMD     | S306F  | HGMD     | L306P  | HGMD     | Y306H  | HGMD     |
| G269D  | ClinVar, HGMD | I307F  | HGMD     | L307P  | HGMD     | Y307H  | HGMD     |
| G272D  | ClinVar  | I307F  | HGMD     | L307P  | HGMD     | Y307H  | HGMD     |
| G272V  | HGMD     | I307F  | HGMD     | L307P  | HGMD     | Y307H  | HGMD     |
| L273F  | ClinVar, HGMD | G318A  | ClinVar, HGMD | G318A | HGMD     | A318T  | HGMD     |
| L273R  | HGMD     | G318A  | HGMD     | L318P  | HGMD     | Y318H  | HGMD     |
| F275S  | HGMD     | K326E* | HGMD     | L326P  | HGMD     | Y326H  | HGMD     |
| S277L  | ClinVar, HGMD | S331L* | HGMD     | L331P  | HGMD     | Y331H  | HGMD     |
| S277P  | HGMD     | F333L* | HGMD     | L333P  | HGMD     | Y333H  | HGMD     |
| S277W  | HGMD     | R333C* | HGMD     | L333P  | HGMD     | Y333H  | HGMD     |
| Y281C  | HGMD     | N348D  | HGMD     | L348P  | HGMD     | Y348H  | HGMD     |
| L282P  | HGMD     | N348D  | HGMD     | L348P  | HGMD     | Y348H  | HGMD     |
| A283T  | HGMD     | T357M  | ClinVar, HGMD | T357M | HGMD     | Y357H  | HGMD     |
| A302T  | HGMD     | G368D  | ClinVar, HGMD | G368D | HGMD     | A368T  | HGMD     |
| L303P  | HGMD     | A368T  | HGMD     | A368T  | HGMD     | A368T  | HGMD     |
| W304R  | HGMD     | R391C  | HGMD     | A391T  | ClinVar, HGMD | D676Y | HGMD     |

(Continued)
mutation, but also cellular quality control responses to these changes.

The VSD of hERG has fewer pathogenic mutations than that of KCNQ1 (Figure 10B), perhaps an indication that the hERG VSD has a higher folding stability. Indeed, when a set of hERG VSD mutations were assessed for membrane insertion efficiency, the majority of mutations were predicted to have no change in the free energy of insertion (Anderson et al., 2014). However, there are a surprising number of disease mutations at the extracellular interface between S1 and S5, specifically at the A422, P426, Y427, S428, H562, W563, C566, and Y569 positions (Figures 10B, C). This interface has not been studied extensively. However, we speculate that it may be important to channel stability by providing an additional contact surface between the VSD and the PD distinct from the S4–S5 linker. Destabilization of this interface may therefore lead to mistrafficking. Mutation Indeed, A422T results in ER retention but was rescued with a pharmacological chaperone (Guo et al., 2012). Other mutations in S1 alter gating properties in addition to trafficking (Anderson et al., 2014; Phan et al., 2017), indicating that gating properties may also be affected. Further studies are needed to determine the impact of mutations in this interface on folding, trafficking, and gating properties of hERG.

A number of mutations are found in the PAS and CNBHD, particularly in the PAS and at the interface between the two domains (Figures 10A, D). The structural stability of both domains impacts trafficking (Akhavan et al., 2005; Harley et al., 2012; Ke et al., 2013; Ke et al., 2014), and several trafficking-defective mutants have been characterized at the PAS/CNBHD interface (F29L, I31S, I42N, Y43C, R56Q, M124R), suggesting that occlusion of a PAS hydrophobic patch from the cytoplasm through interaction with the CNBHD is critical for proper trafficking (Ke et al., 2013). Trafficking defects due to destabilization of the PAS, the CNBHD, or their interaction may thus be a prominent mechanism for the observed defects in trafficking and gating of the mutant channels.
in LQT2. The direct interaction of these two domains has also been implicated in slow deactivation of the channel (Ng et al., 2014; Kume et al., 2018), as deactivation defects have been noted in channels with mutations at this interface (Anderson et al., 2014). Deactivation defects may be a secondary source of LQT2. Detailed structural analysis of mutation-induced destabilization of the PAS and the CNBHD as well as studies of cellular responses to PAS/CNBHD destabilization would be highly informative to our understanding of LQT2. Indeed, similar studies have already revealed differential cellular responses to LQT2 PAS mutations, depending on the severity of PAS destabilization (Foo et al., 2019).

**SCN5A**

LQT3 mutations induce channel gain-of-function in SCN5A (Wilde and Amin, 2018; Skinner et al., 2019). Accordingly, mutations that interfere with SCN5A folding and trafficking do not cause LQT3 (Bohnen et al., 2017; Wilde and Amin, 2018). The primary mechanism leading to LQT3 is rather impairment of fast inactivation (Bohnen et al., 2017; Wilde and Amin, 2018). The results of our analysis are consistent with this notion. The site where the S5 and S6 helices of repeats III and IV cross, in the binding pocket for the IFM motif, contains the majority of the mutations curated in this study (Figures 11A, C). These mutations may impact interaction of the IFM motif with other channel structures, hindering fast inactivation and generating a persistent sodium current. For instance, A1326 (A1328 in rSCN5A) and G1329 (G1331 in rSCN5A) make critical backbone interactions to help form a tight hydrophobic pocket for IFM interactions (Jiang et al., 2020). Mutations A1326P and G1329S (Table 1) may thus compromise the stability of this pocket, hindering inactivation. Additionally, S6 mutations surrounding this putative inactivation gate also map to the constriction site of the channel pore, with additional mutations in S6_{1} and S6_{II} (Figure 11D). It has been proposed that binding of the IFM motif causes a rotation in the S6 helices that closes the pore (Yan et al., 2017b). Therefore mutations in S6 may also alter inactivation by preventing helix rotation. Indeed, residues important to defining the constriction site of the Na_{1.7} pore cause LQTS upon mutation in SCN5A (L398 and Y1755) (Shen et al., 2019). A few mutations also map to the CTD of SCN5A (Figure 11B), which could alter interactions with the III/IV linker and hinder fast inactivation.

The recent structure of the rat homolog of SCN5A (Jiang et al., 2020) suggests another gain-of-function mechanism, namely that VSD mutant R225P could promote current leak through the VSD (gating pore current). Experimental evidence supporting this mechanism exists for other SCN5A (Moreau et al., 2015) and Na_{v1.4} (Sokolov et al., 2007) VSD mutations.

**STRUCTURAL BASIS FOR CHANNEL PHARMACOLOGY**

Pharmacological management of LQTS aims to minimize symptomatic arrhythmia and prevent life-threatening cardiac events. According to consensus guidelines (Zipes et al., 2006; Priori et al., 2013), treatment with β-blockers is beneficial and effective in the case of LQTS diagnosis, but should be considered only after additional diagnostics for carriers of LQTS mutations. β-blockers work by inhibiting β-adrenergic receptors, consequently slowing heart rate and preventing arrhythmias triggered by activation of the sympathetic nervous system (Bohnen et al., 2017). However, the efficacy of β-blockers is strongly genotype- and trigger-specific (Moss et al., 2000; Schwartz et al., 2001; Bohnen et al., 2017). β-blockers reduce the risk of exercise-induced arrhythmia by 78% and 71% in patients with LQT1 and LQT2, respectively, but neither are protective against cardiac events triggered by emotional arousal or during rest (Kim et al., 2010; Goldenberg et al., 2012). Patients with LQT3 tend to experience the highest breakthrough rate for cardiac events (10–15%) with β-blocker treatment (Giudicessi and Ackerman, 2013). Use of local anesthetic-type anti-arrhythmic agents (e.g. mexiletine) for clinical treatment of LQT3, however, can be accompanied by undesirable side effects (Moreno et al., 2011). The need for improved LQTS pharmacotherapy is further supported by the difficulties surrounding hERG modulation, as several clinically-used drugs can cause drug-induced LQTS by blocking hERG and suppressing I_{Kr} (Vandenbreg et al., 2012; Kalyaanamoorthy and Barakat, 2018). This section summarizes some known pharmacological agents and the modes of interaction with KCNQ1, hERG, and SCN5A. Special focus is dedicated toward unique structural features of hERG that have been implicated in the promiscuity of this channel for pro-arrhythmic drugs.

**KCNQ1**

KCNQ1 channels are targeted by several non-selective anti-arrhythmic drugs such as quinidine (Balser et al., 1991), amiodarone (Balser et al., 1991; Kamiya et al., 2001), azimilide (Busch et al., 1997), and clofilium (Yang et al., 1997). More specific KCNQ1 blockers include chromanol 293B (Bosch et al., 1998), and the benzodiazepine L-735821 (Lengyel et al., 2001). The binding site of L-735821 has been located to the SF and S6 (Seebohm et al., 2003a).

Benzodiazepine R-L3 was the first KCNQ1 activator to be described. The compound acts to slow the deactivation rate and causes a hyperpolarizing shift in the voltage-dependence of channel activation (Salata et al., 1998). R-L3 appears to bind residues on S5 and S6 lining the membrane-exposed surface of the PD (Seebohm et al., 2003b) rather than to the inner channel cavity. Drug binding outside of the PD cavity was also reported for quinidine (Yang et al., 2013), which occupies a pocket between S6 and the S4–S5 linker and is postulated to elicit an allosteric channel blocking mechanism. More recently, ML277 was identified as a potent KCNQ1 agonist (Mattmann et al., 2012). ML277 has been proposed to bind to a side pocket surrounded by the S2–S3 and S4–S5 linkers on the intracellular side and helices S4 and S6 on the intramembrane lateral side (Xu et al., 2015). Binding of ML277 may selectively alter VSD/PD coupling to stabilize the AO state relative to the IO state (Hou et al., 2019).
**hERG**

hERG channels can be blocked by a wide spectrum of compounds, leading to drug-induced LQTS (Cubeddu, 2016). The list of drugs that inhibit hERG includes, among others, antibiotics (grepafloxacin (Bischoff et al., 2000)), antihistamines (astemizole (Zhou et al., 1999)), anti-arrhythmics (quinidine (Roden et al., 1986), dofetilide (Jurkiewicz and Sanguinetti, 1993)), antipsychotics (sertindole (Rampe et al., 1998)), and gastroprokinetic agents (cisapride (Vitola et al., 1998)) (Figure 12A). Several prescription drugs have been withdrawn from the market because of cardiotoxic side effects triggered by off-target interaction with hERG (Kalyaanamoorthy and Barakat, 2018). In fact, an estimated 15% of drugs still on the market may cause QT phase prolongation, and 60% of drugs in development show hERG liability (Vandenberg et al., 2017). It is now required by the US FDA that all compounds considered for advancement to clinical trials be tested for their impact on hERG channel function as part of preclinical toxicity assessments (Center for Drug Evaluation and Research, 2005a; Center for Drug Evaluation and Research, I.C.O.H., 2005b).

Considerable progress has been made in understanding the basic features of drug binding to hERG, and a plausible mechanism for promiscuity of drug binding has emerged. Pro-arrhythmic drugs commonly bind to the central inner cavity of the PD in hERG. Two pore-lining aromatic residues in S6 (Y652 and F656) are the most crucial for drug binding (Mitcheson et al., 2000), although additional residues at the end of the pore helix (T623, S624, and V625) contribute to binding for some drugs (Mitcheson et al., 2000), and mutations at F557 in the S5 helix can also affect binding (Saxena et al., 2016). The side chains of the S6 and pore helix residues point towards a putative drug binding site located in the central cavity below the SF. This cavity is slightly narrower in hERG than in the Shaker family Kv1.2–2.1 chimeric channel (Long et al., 2007). As a consequence, there is a greater negative electrostatic potential in this region (Wang and Mackinnon, 2017), which could enhance drug binding capabilities (Figure 12B). In addition, four lateral hydrophobic pockets, which are not observed in the Kv1.2–2.1 structure, extend outwards from that central constriction site, generating additional space to trap chemical moieties (Wang and Mackinnon, 2017).

Quantitative structure-activity relationship studies have identified a pharmacophore for drugs that bind to hERG (Cavalli et al., 2002). The pharmacophore consists of three centers of mass (usually aromatic rings) and an amino group that together form a flattened tetrahedron. It has been suggested that the aromatic rings could fit into one or more lateral hydrophobic pockets in hERG and/or bind by pi-pi stacking or hydrophobic interactions with Y652 and F656 (Chen et al., 2002). Likewise, the greater negative electrostatic potential in the central cavity could explain why many drugs that bind hERG, including off-targets, contain a positive charge (Fernandez et al., 2004). Additionally, a number of docking models for drug-
bound hERG, with and without this pharmacophore, are being generated (Wacker et al., 2017; Helliwell et al., 2018; Negami et al., 2019), making possible further delineation of drug binding modes within this pocket and the structural basis of their affinity for hERG.

The majority of high-affinity drugs that bind hERG off-target preferentially bind to the inactivated state rather the open state, with affinity differences ranging from 2- to 100-fold (Perrin et al., 2008). Interestingly, the related EAG1 channel (Whicher and Mackinnon, 2016) is less sensitive to inhibition by most of hERG-blocking drugs even though they share the same pore lining aromatic residues. One major difference between hERG and EAG1 is that EAG1 channels undergo minimal inactivation (Garg et al., 2012) whereas hERG channels undergo rapid and complete inactivation (Vandenbergh et al., 2012). The contribution of inactivation to high-affinity binding is further substantiated by the observation that inactivating EAG1–hERG chimeras, containing the upper half of the hERG PD, can bind drugs with almost hERG-like affinity (Herzberg et al., 1998). While cryo-EM structures have been determined for both hERG and EAG1 (Whicher and Mackinnon, 2016; Wang and Mackinnon, 2017), the structural basis of the functional and pharmacological differences requires higher resolution (Wang and Mackinnon, 2017).

**SCN5A**

Limited progress has been made in the development of SCN5A-targeted treatment strategies. Classical non-selective sodium channel blockers like the Vaughan-Williams class I antiarrhythmic drugs quinidine, lidocaine, and propafenone have only limited clinical applicability. Ranolazine is a more selective SCN5A blocker (Belardinelli et al., 2006; Zaza et al., 2008) and has been shown to attenuate action potential duration and reduce or prevent intracellular calcium overload in LQT3 models (Wu et al., 2004; Lindegerg et al., 2009). Mexiletine is a potent inhibitor of SCN5A and may be beneficial for treatment of LQT3 (Mazzanti et al., 2016), but effectiveness may depend on the baseline QT prolongation of the mutant channel (Li and Zhang, 2018).

Despite the clinical relevance of SCN5A-targeting drugs, the molecular mechanisms of promising and potentially problematic drugs are not well understood at a structural level, hindering drug design. However, key residues for apparent drug binding have been suggested within the sodium channel pore domain. Specifically, mutations of two conserved aromatic residues in S6IV, F1760 and Y1767, (Ragsdale et al., 1994; Ragsdale et al., 1996) or other S6 residues in repeats I and III (Yarov-Yarovoy et al., 2001; Yarov-Yarovoy et al., 2002) may affect drug binding. Using fluorinated phenylalanine derivatives incorporated at F1760 (Pless et al., 2011a), a strong cation–pi interaction between F1760 and the protonated amine group in class Ib anti-arrhythmic drugs was observed, defining the molecular basis for inhibition of SCN5A by this group of drugs. Additionally, mutations within the SF region can affect apparent drug binding either by enhancing slow inactivation or forming an alternative access pathway (Sunami et al., 1997; Lee et al., 2001; Tsang et al., 2005). Interactions of anti-arrhythmic and local anesthetic drugs (lidocaine, QX-314, etidocaine, flecainide, and ranolazine) docked into an inactivated state model of SCN5A (Nguyen et al., 2019) revealed several key drug binding sites in the inner pore lumen that can simultaneously accommodate up to two drug molecules. Subsequent MD simulations suggested alternative access mechanisms for drugs into the SCN5A lumen – a hydrophilic pathway through the intracellular gate and a hydrophobic pathway through a membrane-exposed fenestration between repeats III and IV.

The recent structure of rat Na1.5 (Jiang et al., 2020) has provided insight into the binding mode of the class Ic antiarrhythmic drug flecainide, which has higher affinity for the open conformation of SCN5A (Ramos and O’Leary M., 2004). Flecainide binds in the central cavity on the intracellular side of the SF. The piperidine ring of flecainide lies across the top of the central cavity with the positively-charged nitrogen pointing towards the exit of the SF and its hydrophobic edge extending towards the phenyl ring of F1762 (F1760 in human Na1.5). The trifluoroethoxy tails of flecainide extend into fenestrations formed between the pore helices of repeats I and II as well as repeats II and III, through which the central cavity is accessible from the lipid bilayer. These fenestrations represent possible entry points for flecainide and similar class Ic drugs.

**FUTURE DIRECTIONS**

The growing number of structural and functional studies of KCNQ1, hERG, and SCN5A have provided new insight into channel molecular physiology and channel dysfunction in congenital LQTS. This further motivates the study of fundamental biophysical mechanisms in ion channels. One of these mechanisms, electromechanical coupling, remains enigmatic due to a dearth of experimental resting state structures and robust methods to identify allosteric pathways, which cannot easily be discerned from ion channel structures resolved in a single conformation. However, interaction energy and MD dynamical network analysis in the Shaker K+ channel (Fernandez-Marino et al., 2018) has suggested that movements in the VSD and PD are linked by two distinct pathways – a canonical pathway through the S4–S5 linker and a hitherto unknown non-canonical pathway involving contacts at the interface between S4 and S5. Such work may provide a useful alternative approach to explore electromechanical coupling in detail.

Another major effort involves the experimental characterization of hundreds of previously uncharacterized variants of unknown significance (VUS) and LQTS mutations in KCNQ1, hERG, and SCN5A. Widespread use of whole genome and exome sequencing has provided a nearly complete catalog of common sequence variations in protein-coding genes (Lek et al., 2016), although rare sequence variants continue to be discovered. For many LQTS mutations, the molecular mechanisms responsible for impaired channel function remain poorly understood. Such mutations may cause potassium channel loss-of-function or dysfunction by promoting misfolding, mistrafficking, aggregation, or improper gating of the channel protein, as discussed above. Continued experimental testing will be required to complete functional annotation of KCNQ1, hERG, and SCN5A variants.
and to elucidate how these mutations lead to channel dysfunction. In this regard, new techniques such as high-throughput patch clamp recording (VANOYE et al., 2018; TOH et al., 2020), which allows rapid screening of ion channel variants found in patients, and the use of induced-pluripotent stem cell-derived cardiomyocytes (MORETTI et al., 2010; MA et al., 2013; TERRENOIRE et al., 2013; JOUNI et al., 2015) developed from cells of healthy or mutation-carrying patients, represent helpful tools for large-scale examination of ion channel variants.

Finally, experimentally-informed bioinformatics and modeling approaches are being developed to predict variant pathogenicity (KRONCKE et al., 2015; LI et al., 2017; KRONCKE et al., 2019). These computational algorithms can help to decrypt newly discovered VUS for which there is not enough data to determine pathogenicity. Decrypting VUS can inform medical practice and has the potential to improve LQTS therapy, both for the prevention of cardiac events in susceptible patients and as a basis for avoiding unneeded treatment in healthy patients. With improved understanding of the molecular pathophysiology and the role of mutations associated with distinct LQTS subtypes, the ultimate hope is that identifying a patient’s genotype will lead to more specific treatment considerations and the delivery of optimal care.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00550/full#supplementary-material

TABLE 1 | LQTS mutations in KCNQ1, hERG, and SCN5A.
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Brewer et al. Structures of Cardiac Ion Channels

Frontiers in Pharmacology | www.frontiersin.org May 2020 | Volume 11 | Article 55025

25
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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