Chem Biol Drug Des 2014; **83:** 1–26

Review

Potassium Channels: Structures, Diseases, and Modulators

Chuan Tian[1,2,†], Ruixin Zhu[1,†], Lixin Zhu[3], Tianyi Qiu[1], Zhiwei Cao[1,*] and Tingguo Kang[2,*]

[1]School of Life Sciences and Technology, Tongji University, Shanghai 200092, China
[2]School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, Liaoning, 116600, China
[3]Department of Pediatrics, Digestive Diseases and Nutrition Center, The State University of New York at Buffalo, Buffalo, NY 14226, USA
**Corresponding authors: Tingguo Kang, kangtg@lnutcm.edu.cn; Zhiwei Cao, zwcao@tongji.edu.cn*
[†]These authors contributed equally to this work

Potassium channels participate in many critical biological functions and play important roles in a variety of diseases. In recent years, many significant discoveries have been made which motivate us to review these achievements. The focus of our review is mainly on three aspects. Firstly, we try to summarize the latest developments in structure determinants and regulation mechanism of all types of potassium channels. Secondly, we review some diseases induced by or related to these channels. Thirdly, both qualitative and quantitative approaches are utilized to analyze structural features of modulators of potassium channels. Our analyses further prove that modulators possess some certain natural-product scaffolds. And pharmacokinetic parameters are important properties for organic molecules. Besides, with *in silico* methods, some features that can be used to differentiate modulators are derived. There is no doubt that all these studies on potassium channels as possible pharmaceutical targets will facilitate future translational research. All the strategies developed in this review could be extended to studies on other ion channels and proteins as well.

Key words: disease, modulator, potassium channel, structure

Abbreviations: ACS, acute coronary syndrome; AD, Alzheimer's disease; ARF6, ADP-ribosylation factor 6; AUC, area under curve; BFNC, benign familial neonatal convulsions; BFNS, benign familial neonatal seizures; BK, big-conductance K channel; BRS, Brugada syndrome; CaM, calmodulin; CaM-BD, CaM-binding domain; CNG, cyclic nucleotide-gated channels; CTD, cytoplasmic domain; DLI, drug-like index; EA, episodic ataxia; EAG, ether-a-go-go-related gene; EP, equilibrium potential; hERG, human ether-à-go-go-related gene; IK channels, intermediate-conductance K channels; Ikr, rapid delayed rectifier K current; Iks, slow delayed rectifier K current; Ito, cardiac transient outward K current; JLNS, Jervell and Lange-Nielsen syndrome; K2p channels, two-pore domain background K channels; KATP, ATP-sensitive K channels; Kca channels, calcium-activated K channels; Kir channels, inward rectifying K channels; Kv channels, voltage-gated K channels; LQTS, long QT syndrome; PD, pore domain; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinases A; PKC, protein kinases C; PNDM, permanent neonatal diabetes mellitus; RCK, regulator of K conductance; RWS, Romano-Ward syndrome; SIDS, sudden infant death syndrome; SK channels, small-conductance K channels; SNP, single nucleotide polymorphism; SQTS, short QT syndrome; SUMO, small ubiquitin-like modifier; TM, transmembrane; SUR, sulfonylurea receptors; VSDs, voltage-sensing domains.

Potassium channels are a diverse family of membrane proteins in both excitable and non-excitable cells. The human genome carries more than 90 genes coding for principal subunit of potassium channels. The huge number of this superfamily brought about a large quantity of studies concerning structural analysis, gating mechanism, induced diseases, and therapeutic drugs since 20 years ago (1–7). Especially in recent years, studies on this membrane protein family dramatically increased. Structural and functional analyses of different potassium channels have been reported continually, which contribute to deep understanding molecular mechanism of potassium channels ion selectivity, conduction and gating. These findings include aspects as follows: K channels in complex with diverse ligands such as toxin or intrinsic ligands have expanded; increasing polymers and proteins with lipid membrane (8) have been crystallized; some functional domains contributing to gating or some potential regulation are obtained; and lastly, crystal structure of *Homo sapiens* protein have made structural analysis more valuable and loser to the real (9–21). The physiological feature makes potassium channel serving as therapeutic target for numerous disorders. The dysfunction of a subfamily or even a subtype of potassium channels might induce some serious diseases such as Alzheimer, Parkinson's disease, and so on. Increasing studies have been explaining the mechanism of these diseases, and these studies dig deep into the molecular level (22–30). The progress in studying diseases facilitates the development of therapeutics. Modulators of potassium channels such as openers or blockers have also been discovered through chemical synthesis, virtual screening or a combination of both of these. These

 doi: 10.1111/cbdd.12237

modulators block or activate potassium channels and in turn ameliorate the pathological state (31–38). Although a few reviews emerge for the past few years (30,39–43), most of them summarize only one type of potassium channels or review only one or two aspects such as physiology or pathology of potassium channels. Here, we are trying to systematically review recent achievements concerning all types of potassium channels from structures, diseases, and modulators aspects.

Structure of Potassium Channels

Potassium channels contain principal subunits (often called α-subunits), which determine the structure of the channel, and auxiliary subunits (often called β-subunits), which can modify the properties of the channel. Most of the known principal subunits express in heterologous expression systems as functional homo-multimeric channel complexes. Some other principal subunits co-assemble with auxiliary proteins for expression of functional channels. Potassium channel α-subunits fall into at least eight families based on predicted structural and functional similarities (44). Three of these families [Kv, ether-a-go-go-related gene (EAG), and KQT] share a common motif of six transmembrane (TM) domains and are primarily gated by voltage. Two other families, CNG and SK/IK, also contain this motif but are gated by cyclic nucleotides and calcium, respectively. The three other families of potassium channel α-subunits have distinct patterns of TM domains. Slo family potassium channels (BK channel) have seven TM domains (45) and are gated by both voltage and calcium or pH (46). Kir channels belong to a structural family containing two TM domains. An eighth functionally diverse family K2p channels contains two tandem repeats of this inward-rectifier motif. Thus, these families could be mainly divided into three groups termed as voltage-gated six TM potassium channels (Kv channels), calcium-activated six/seven TM potassium channels (Kca channels), and two TM potassium channels, respectively. Structure and regulation of the channels aforementioned will be briefly outlined in the following sections.

Additionally, after systematic literatures reviewing, we put related information on potassium channels in supplementary table (Table S1). This table comprises of gene and protein names, organ distribution and modulators, aims mainly to scientists who are interested in finding possible molecular correlations in their studies. The gene names *KCNA* to *KCNV* and the protein names Kv, Kca, Kir, K2p are under a systematic nomenclature accepted by the Human Genome Organization (HUGO).

Voltage-gated six transmembrane potassium channels

Voltage-gated potassium channels (Kv channels) are the largest group in potassium channel family, which in humans are encoded by 40 genes and are divided into 12 subfamilies. These include Kv1 (*KCNA*), Kv2 (*KCNB*), Kv3 (*KCNC*), Kv4 (*KCND*), Kv7 (*KCNQ*, also named *KQT*), Kv10, Kv11 (*KCNH*, also named *EAG*) and Kv12. Kv5, Kv6, Kv8, and Kv9 channels are not functional alone; they co-assemble with Kv2 subunits and modify their function. These family members share six TM and are gated by voltage. Similar to the Kv channel that was first cloned, the *Drosophila Shaker* channel (47), all mammalian Kv channels consist of four α-subunits, each containing six TM α-helical segments, S1–S6, and a P-loop, which are arranged circumferentially around a central pore as homo-tetramers or hetero-tetramers (Figure 1). The pore domain (PD) represents a tetramer of two membrane-spanning α helixes that are connected with each other via a P-loop, which is responsible for potassium ion selectivity. The PD contains a channel gate, which controls ion permeation (48–50). The structure of the gate is a bundle of

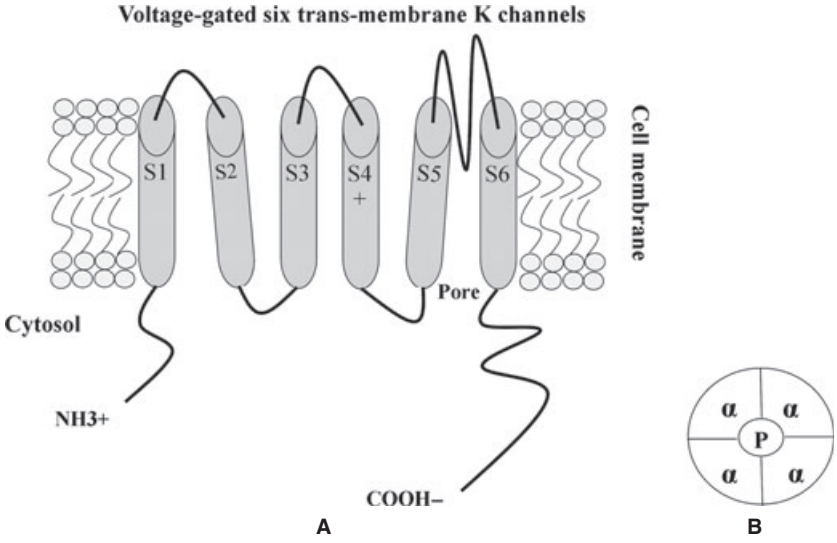

Figure 1: (A) Schematic representation of the transmembrane (TM) topology of Kv channel α-subunits containing six TM segments with the pore region formed by S5 and S6 segments. (B) Structure of the tetrameric assembly of the Kv channel.

overcrossing α helixes at the cytoplasmic entryway of the channel pore. These α helices correspond to the S6 helix of Kv channels (51,52). In Kv channels, the pore is covalently linked to four specialized membrane-embedded peripheral functional modules, voltage-sensing domains (VSDs), which are comprised of S1–S4 membrane-spanning segments, with four positively charged arginine residues in the S4 helix (53,54). Voltage-sensing domains are connected to the PD by a linker, known as S4–S5 linker at the cytoplasmic side of the membrane. The VSD-PD assembly represents an exquisite molecular electromechanical coupling device, which converts potential energy of the membrane electric field into the mechanical work needed to control the selective permeation of potassium ions (55).

Several functional studies indicate that concordance between the S4–S5 linker and distal S6 region of the PD is important for transmission of conformational changes from VSDs to PD (12,18,56–60). How the conformational changes originating in VSDs are transferred to the PD and how they influence the functional state of the channel gate remains unclear (61). The α-subunits can hetero-multimerize relatively freely, resulting in a wide range of possible channel tetramers with different biophysical and pharmacological properties. The properties of Kv channel α -subunit can be further modified by association with intracellular β-subunits (62,63). In the central nervous system of higher organisms, Kv1 and Kv4 families form an association with cytoplasmic proteins known as β-subunits (64). For example, Kv1 channels interact through their amino-terminal tetramerization domain with Kv β1–3 proteins, which form a second symmetrical tetramer on the intracellular surface of the channel and modify the gating of the α-subunits. In addition to this 'mixing and matching' of α- and β-subunits, Kv channel properties can be further modified by phosphorylation, dephosphorylation, ubiquitylation, sumoylation, and palmitoylation (65). Recent crystallization experiments of a functional channel in a membrane environment at high resolution establish an unprecedented connection between channel structure and function. It is not only the first structure of a channel obtained by crystallization of the protein in its native environment: a lipid bilayer; but also further implicate how the sensor and pore modules interact with each other (21).

Besides, computational approaches like molecular dynamics (MD) simulations combined with homology modeling also identify potential interactions between VSDs and PD in a human EAG (*hERG*, EAG) channel model (66). Using all-atom MD simulations, it is also shown how a Kv channel switches between activated and deactivated states. On deactivation, pore hydrophobic collapse rapidly halts ion flow. Subsequent VSD relaxation, including inward, 15-Å S4-helix motion, completes the transition. On activation, outward S4 motion tightens the VSD–pore linker, perturbing linker–S6-helix packing (67). Except for the regulation mechanism, molecular determinants like interaction between K channels were also studied by utilizing MD simulations in recent years. MD studies suggest a stable interaction of the *KCNE1* TM α-helix with the PD S5/S6 and part of the voltage sensor domain S4 of *KCNQ1* in a putative preopen channel state. Formation of this state may induce slow activation gating, the pivotal characteristic of native cardiac I_{ks} channels (68).

Agents that modulate Kv channels can be broadly divided into three chemical categories: metal ions, organic small molecules (molecular weight 200–500 da), and venom peptides (molecular weight 3–6 kda; 10,13). These substances affect Kv channel function by blocking the ion-conducting pore from the external or internal side, or modifying channel gating through binding to the voltage sensor domain or auxiliary subunits. Peptide toxins typically bind either to the outer vestibule or the voltage sensor of Kv channels. By contrast, small molecules block Kv channels by physically occluding the inner pore. Another interesting mechanism of action for channels with β-subunits is the so-called disinactivators that disrupt the interaction between α- and β-subunits and thereby modify channel behavior (69,70).

Calcium-activated six/seven transmembrane potassium channels

This family of ion channels is, for the most part, activated by intracellular Ca^{2+} and contains eight members, which are big-conductance Kca1.1 (BK, slo1); small-conductance Kca2.1 (SK1), Kca2.2 (SK2), Kca2.3 (SK3); intermediate-conductance Kca3.1 (IK, SK4); and other subfamilies Kca4.1 (Slo2.2), Kca4.2 (Slo2.1), Kca5.1 (Slo3). However, some of these channels (Kca4 and Kca5 channels) are responsive to intracellular Na^+ and Cl^-. The Kca1 family is both Ca^{2+} and voltage activated. The Kca channel α-subunits have six TM segments similar to the Kv, except for Kca1, in which the N-terminus makes a seventh pass across the membrane to end up outside the cell.

Big-conductance K channels contain four identical subunits, each comprising seven TM segments and a large intracellular C-terminus. Transmembrane segments S0–S4 form the voltage sensor; S5, S6, and the intervening amino acids form the pore and selectivity filter; and the C-terminus forms the large cytoplasmic domain (CTD; 45). The CTD structure is less certain (71). Voltage sensor of BK channels resembles the regions of other voltage-dependent K channels, but BK channels possess a unique TM helix referred to as S0 at their N-terminus, which is absent in other members of the voltage-gated channel superfamily. Recently, S0 was found to pack close to TM segments S3 and S4, which are important components of the BK voltage-sensing apparatus. Evidence shows that the structural transitions resolved from the S0 region exhibited voltage dependence similar to that of charge-bearing TM domains S2 and S4. Thus, S0 acts as a pivot component against which the voltage-sensitive S4 moves upon

depolarization to facilitate channel activation (72). The BK C-terminus was proposed to contain two regulator of K conductance (RCK) domains (73), shown in blue (RCK1) and red (RCK2; Figure 2).

Big-conductance K channels have an unusually high single-channel conductance, but their most important physiological property is dual regulation through membrane voltage and intracellular Ca^{2+}. Depolarization of the membrane voltage and increased intracellular Ca^{2+} levels both cause BK channels to open (74). Mutagenesis studies have identified the putative primary Ca^{2+} binding site within the RCK2, which comprises a stretch of aspartic amino acids referred to as the 'Ca^{2+} bowl'. A major focus of research on the BK channel has been aimed at defining the region of Ca^{2+} binding (74). Mutations within the Ca^{2+} bowl could influence Ca^{2+} activation. Deletions and point mutations within the Ca^{2+} bowl sequence, DQDDDDDPD, rendered the channel less sensitive to Ca^{2+} and caused a shift in the conductance-voltage curve to more depolarized membrane voltages (75–77). In the CTD, the Ca^{2+} bowl is located between the last two β strands, βO and βP of the RCK2. Thus, the Ca^{2+} bowl is an integral structural element of RCK2. Three distinct regions of the structure tend to affect BK channel function. One is surrounding the Ca^{2+} bowl (78); the second scatter in the helix-turn-helix connector, which forms a large surface on the flexible interface (79–81); and the third cluster on the N-terminus of RCK1 (82–84). These support that the pore is controlled directly by Ca^{2+} through the connection of S6 to the gating ring and directly by voltage through the S4–S5 linker and voltage sensors. Big-conductance K channels are regulated by a multiplicity of signals. The prevailing view is that different BK gating mechanisms converge to determine channel opening and that these gating mechanisms are allosterically coupled. In most instances, the pore-forming α-subunit of BK is associated with one of four alternative β-subunits that appear to target specific gating mechanisms to regulate the channel activity. In particular, β1 stabilizes the active configuration of the BK voltage sensor having a large effect on BK Ca^{2+} sensitivity (85).

Non-genomic modulation of BK activity by steroids is increasingly recognized, but the precise location of steroid action remains unknown. By MD simulations, the steroid interaction site from two regions in BK β1 TM domain 2 proposed is identified: the outer site includes L157, L158, and T165, whereas the inner site includes T169, L172, and L173. Combining mutagenesis and patch-clamping data, it is suggested that TM domain 2 outer site does not contribute to steroid action, while T169, L172, and L173 provide the interaction area for cholane steroid activation of BK channels (86).

Big-conductance K channels were the first Ca^{2+}-dependent K channels to be identified in single-channel recordings (87,88). Three members of the small-conductance Ca^{2+}-activated K channel (SK channels) family, SK1 (Kca2.1), SK2 (Kca2.2) and SK3 (Kca2.3), were cloned in 1996 by Adelman and colleagues (4), shortly followed by cloning of the intermediate-conductance Ca^{2+}-activated K channel (IK, SK4, Kca3.1; 89–93). The intermediate-conductance Ca^{2+}-activated K channel (IK channel) is structurally and functionally similar to an SK channel and is part of the same gene family. SK-channel subunits share the serpentine TM topology of voltage-gated K channels, with six TM domains and cytosolic N- and C-termini. The fourth TM domain is decorated with positively charged residues and composes the voltage sensor. Unlike Kv channels, the SK channels retain only two of the seven positively charged amino acids that are found in the S4 segment of Kv channels, and only one of these residues corresponds to the four arginine residues that carry the gating charges in Kv channels (94).

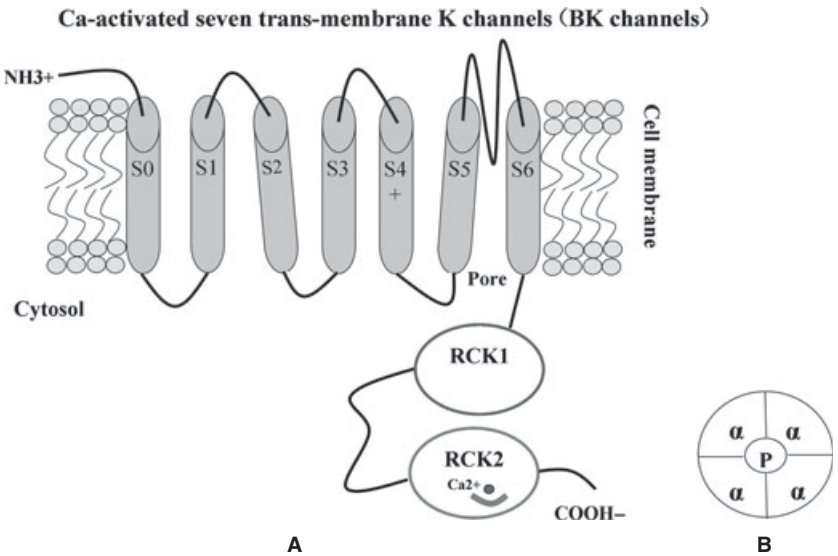

Figure 2: (A) Schematic representation of the transmembrane (TM) topology of big-conductance K channel (BK) subunits containing seven TM segments with the pore region formed by S5 and S6 segments. The two regulator of K conductance (RCK) domains are in the C-terminus, with Ca^{2+} bowl in RCK2. (B) Structure of the tetrameric assembly of the BK channel.

Unlike BK channels, SK channels have lower single-channel conductance (10–20 pS) and are almost voltage independent, as they are gated directly and solely by submicromolar concentrations of intracellular Ca^{2+} (95). Studies have shown that SK channels are highly sensitive and fast Ca^{2+} sensors, and they are ideally suited to couple intracellular Ca^{2+} levels to the membrane potential. A detailed kinetic analysis of single SK channels corroborated the results from macroscopic recordings with regard to Ca^{2+} dependence and voltage independence, and it showed that the gating of SK channels can be best described by a model with four closed and two open states, in which the transitions between different closed states are Ca^{2+}-dependent. Furthermore, SK channels can switch from a high to a low open-probability state and *vice versa* (96). The TM core of the SK-channel subunits contains the canonical K-selective signature sequence in the pore loop between TM5 and TM6. Despite their highly conservation among mammalian species, the most conserved domain among the SK-channel subunits does not reside in the TM core of the protein. Rather, it is the part of the channel C-terminal to the sixth TM domain, in the intracellular C-terminus of the subunits. The remainder is less related between different SK subunits, suggesting that these regions may impart functional and physiological distinctions. Functional SK channels assemble as homomeric tetramers (4). However, different subunits can also co-assemble into heteromeric channels both in heterologous expression systems (97) and in native tissue (98).

The activation of SK channels by Ca^{2+} resembles the binding of Ca^{2+} to Ca^{2+}-binding proteins, as they both have a fast Ca^{2+} response and a high Ca^{2+} sensitivity. However, SK-channel activation cannot be explained by Ca^{2+} binding directly to the channel, as there is no Ca^{2+}-binding motif in the primary structure of the SK subunits. Gating of SK is endowed by a constitutive interaction between the pore-forming subunits and calmodulin (CaM). Calmodulin binds to the intracellular domain immediately following the sixth TM domain, the CaM-binding domain (CaMBD), which is highly conserved across the SK family. Each subunit of the tetrameric channel is thought to bind one CaM. The binding and unbinding of Ca^{2+} to the CaM are transduced via conformational changes into channel opening and closure, respectively (99–101). Ca^{2+} gating of SK channels involves a transition from an extended tetramer of monomers to a folded dimer of dimers that rotates a region of the CaMBD, thereby translating Ca^{2+} binding into mechanical force to open the channel gate. Interestingly, in addition to the role of Ca^{2+} in gating, the association of SK channels with CaM is critical for plasma membrane expression (102). Studies showed that the observed Ca^{2+} sensitivity is conferred on the SK channel by the intimate interaction of CaM with each of the four subunits in Kca2.2. The role of CaM in SK-channel gating is generally accepted for all Kca2 and Kca3 channels and is supported by functional and biochemical evidence (96,103). Additional association of the phosphorylating kinase CK2 and dephosphorylating phosphatase PP2A on the cytoplasmic face of the protein allow for enriched Ca^{2+}-sensitivity – and thus – kinetic modulation (104; Figure 3). The SK channels show inward rectification that was initially attributed to pore block by internal divalent cations at positive membrane potentials. However, a recent report shows that inward rectification in SK channels is an intrinsic property that is mediated by three charged residues in the sixth TM domain. Importantly, these residues also contribute to setting the intrinsic open probability of SK channels in the absence of Ca^{2+}, affecting the apparent Ca^{2+} affinity for activation (105).

The three other members of this group, Kca4.1 (slo2.2), Kca4.2 (slo2.1), and Kca5.1 (slo3), are included in this group due to their structurally related features (106). Unlike the member Kca1.1, which is in fact activated by internal Ca^{2+}, none of the other members seems to be similarly Ca^{2+}-activated. In fact, for the most part, these three are insensitive to internal Ca^{2+}. Kca4.2 and Kca4.1 are

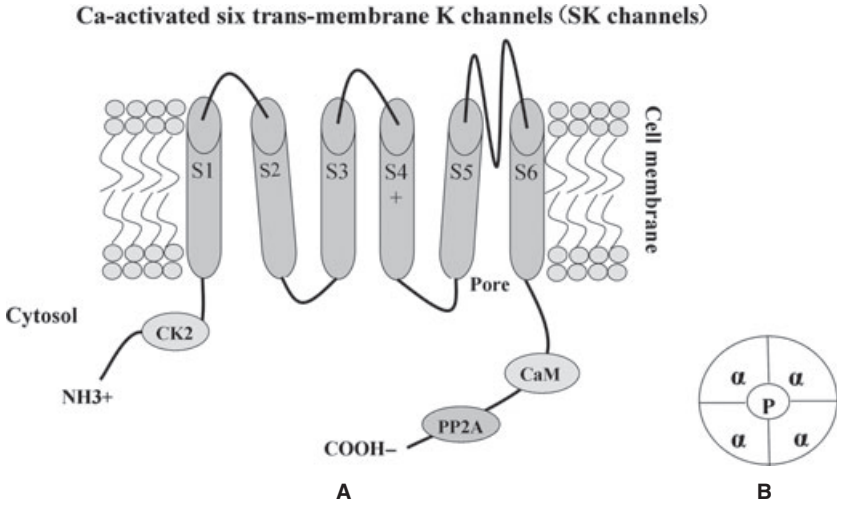

Figure 3: (A) Schematic representation of the transmembrane (TM) topology of SK-channel subunits containing six TM segments with the pore region formed by S5 and S6 segments. The regulators involve calmodulin and PP2A in the C-terminus, CK2 in the N-terminus. (B) Structure of the tetrameric assembly of the SK channel.

activated by internal Na^+ and Cl^-. Kca5.1 is activated by internal alkalization (OH^-; 46). Therefore, although they are structurally related to Kca1.1, these three channels cannot correctly be described as 'calcium-activated' channels based on functional criteria. Studies on these channels are also developing fast. Human slo3 opens upon intracellular pH increase and that its expression and functional properties are modulated by LRRC52, a testis-specific accessory subunit. The crystal structure of the human slo3 gating ring is also presented, which give insights into function of this kind of protein. However, understanding how pH affects the conformation and function of slo3 is still challenging because variations in pH can affect the protonation state of a large number of amino acid residues. But the human slo3 gating ring structure will hopefully pave the way for further experimental and computational analyses to address this important question (60).

Four transmembrane with two-pore potassium channels

K2p channels are composed of four TM domains and two pores arranged in tandem. A functional K2p channel consists of a dimer of subunits, which may heteromultimerize. The *Saccharomyces cerevisiae* TOK-1 channel (composed of eight TM segments) was the first potassium channel discovered to contain two P domains in tandem (3,107). Subsequently, subunits comprising four TM segments and two P domains in tandem have been cloned in mammals (Figure 4). Many K2p channels have a phenylalanine in the GXG motif of the selectivity filter in the second PD, instead of a tyrosine as in one-pore K channels such as Kv (108). This means that in K2p channels, the pore is predicted to have a twofold symmetry, rather than the classical fourfold arrangement. The class of mammalian K2p channel subunits now includes 16 members (Figure 5). They are subdivided into six main structural and functional classes: tandem of P domains in a weak inwardly rectifying TWIK-1, TWIK-2 and KCNK7 channels (functional expression of KCNK7 has not yet been reported); mechano-gated and arachidonic acid-activated TWIK-related TREK-1, TREK-2 and TRAAK channels; TWIK-related acid-sensitive TASK-1, TASK-3 and TASK-5 channels (functional expression of TASK-5 has not yet been reported); tandem PD halothane-inhibited THIK-1 and THIK-2 channels (functional expression of THIK-2 has not yet been reported); TWIK-related alkaline-pH-activated TALK-1, TALK-2 and TASK-2 channels; and the TWIK-related spinal cord TRESK channel, which is regulated by intracellular calcium.

Mammalian K2p channels show either a weak inward rectification, an open rectification (as for TWIK-related acid-sensitive TASK-1 channel), or an outward rectification (as for TREK-1). Some channels, such as TASK-1 and TASK-3, are constitutively open at rest, whereas other channels, including TREK-1, require physical or chemical stimulation to open. The key feature of the K2p channels is that they open over the whole voltage range and therefore qualify as leak or background K channels (109,110). Various proteins that modulate the function of K2p channels have been recently identified which makes the regulation and function of K2p more explicitly (111,112). Small ubiquitin-like modifier (SUMO) assemble in tandem with P domains in TWIK-1, but the role of sumoylation in the regulation of TWIK-1 activity is obscure (113,114). EFA6 is an exchange factor for the small G-protein ADP-ribosylation factor 6 (ARF6). ARF6 modulates endocytosis at the apical surface of epithelial cells, while EFA6 interacts with TWIK-1 only when it is bound to ARF6. The ARF6–EFA6–TWIK-1 association is probably important for TWIK-1 internalization and recycling (115). The EF hand superfamily protein p11 either promotes the expression of TASK-1 at the plasma membrane or acts as a 'retention factor' that causes localization of TASK-1 to the endoplasmic reticulum (116,117). Interaction of the scaffolding protein 14-3-3 with the C-terminal

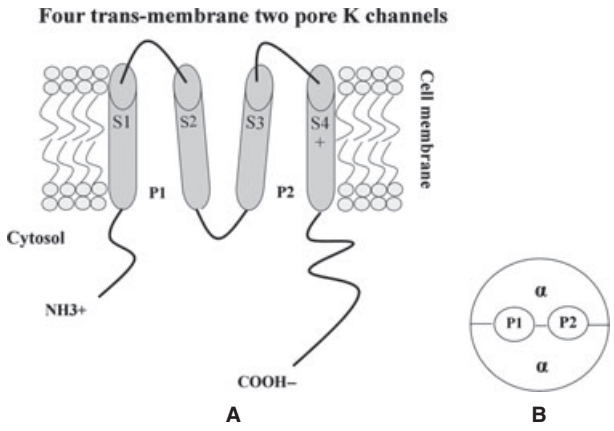

Figure 4: (A) Schematic representation of the transmembrane (TM) topology of channel subunits containing four TM segments with pore region 1 formed by S1 and S2, region 2 by S3 and S4 segments. (B) Structure of the dimer assembly of the K2P channel.

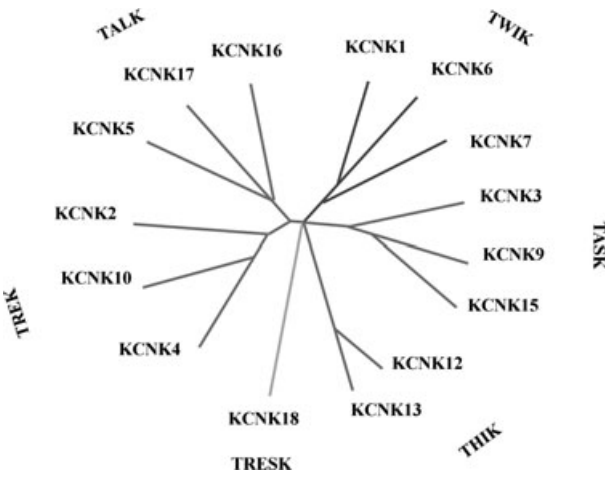

Figure 5: Classification of K2P channels.

domain of TASK-1 overcomes retention of the channel in the endoplasmic reticulum that is mediated by dibasic signals in TASK-1 binding to β-COP. In this way, 14-3-3 promotes forward transport of TASK-1 to the surface of the membrane (118). AKAP150 is also a modulator protein that interacts with TREK-1 and changes the regulatory properties of the channel (119). TASK-2 (K2P5.1) is a background K channel opened by extra- or intracellular alkalinization that plays a role in renal bicarbonate handling, central chemoreception and cell volume regulation. Recent studies, however, present results that suggest that TASK-2 is also modulated by G$\beta\gamma$ subunits of heterotrimeric G-protein. This modulation might be a novel way in which TASK-2 can be tuned to its physiological functions (120).

Atomistic models for the most studied K2p channel, TREK-1 channel indicate the nature of the direct coupling between the C-terminal domain and the membrane, which is a key structural feature underlying K2p channel function (121). A combination of molecular modeling and functional assays shows that pH-sensing histidine residues and K^+ ions mutually interact electrostatically in the confines of the extracellular ion pathway for TASK-3 (122). Concatenated channel approach with free energy analysis strongly suggests that a cycle of protonation/deprotonation of pH-sensing arginine 224 side chain gates the TASK-2 channel by electrostatically tuning the conformational stability of its selectivity filter (123).

Two transmembrane potassium channels

The eukaryotic inward-rectifying potassium channels (Kir channels) are part of an ancient class of ion channels evolved in prokaryotes (124). The 15 known mammalian Kir channels are divided into seven different families and can be further classified into four functional groups: classical Kir channels (Kir2.x) are constitutively active; G-protein-gated Kir channels (Kir3.x) are regulated by G-protein-coupled receptors; ATP-sensitive K channels (KATP) are tightly linked to cellular metabolism; and K transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x), each having their own specific expression pattern and functional characteristics (125). The primary structures of Kir channels possess a common motif of two putative membrane-spanning domains (TM1 and TM2) linked by an extracellular pore-forming region (H5) and cytoplasmic amino (NH2)- and carboxy (COOH)-terminal domains (126). We now recognize this as the basic structure that is common to all types of Kir channel. The H5 region serves as the 'ion-selectivity filter' that shares with other K-selective ion channels the signature sequence T-X-G-Y(F)-G. Subunit tetramerization, either homo- or hetero-typic, results in the formation of a functional channel. Heteromerization generally occurs between members of the same subfamily, for example, Kir2.1 can associate with any one of other Kir2.x subfamily members, namely, Kir2.2, Kir2.3, or Kir2.4 (127,128), and Kir3.1 forms heteromeric complexes with either Kir3.2, Kir3.3, or Kir3.4. An exception is where Kir4.1 assembles with Kir5.1 (129). In general, Kir channels allow more inward potassium flow at membrane potentials negative from the potassium equilibrium potential (EP) than outward flow at equivalent membrane potentials positive of EP, a characteristic known as inward rectification. Within the superfamily, strong and weak rectifiers exist. Strong rectifying channels are most apparent in excitable tissues, where they are responsible for a stable and negative resting membrane potential, and due to their strong rectification, prevent extensive potassium ion loss during action potential formation. Weak rectifiers are expressed in numerous other tissue types and organs and have a role in processes like potassium homeostasis, insulin release, and signal transduction.

The Kir channels (Kir1-7) are regulated by many factors: phosphatidylinositol-4,5-bisphosphate (PIP2), ATP, or G-proteins (130). Other factors like polyamines, kinases, pH, and Na^+ ions act cooperatively to modulate Kir channels. Inward rectification of K^+ flux results from interaction between two intracellular substances, Mg^{2+} and polyamines. Early studies led to the conclusion that rectification arises from a combination of intracellular Mg^{2+}-mediated blockage and an intrinsic activation gating process, which was due to slow polyamine unblocking (131–134). The competitive blockage of Kir channels by Mg^{2+} and polyamine is crucial for the control of the magnitude of outward current (135). A membrane-anchored phospholipid, PIP2, is essential to sustain the normal function of the majority of Kir channels (136–139; Figure 6A). In membrane patches excised from their parent cells, Kir channel activity gradually declines. This 'run-down' activity can be restored by the application of ATP to the intracellular surface of the membrane, which replenishes PIP2 via the action of lipid kinases (136). Mutation analyses suggest that PIP2 is associated with positively charged residues in the COOH termini (138,140). Intracellular or extracellular pH can regulate Kir channels, such as Kir1.1 and Kir2.3, and channels that contain Kir4.1 subunit; generally, acidic shifts of pH reduce channel activity. K channels that contain either Kir3.2 or Kir3.4 can be activated by intracellular Na^+ (141,142). ATP-sensitive potassium channels, which are made up of pore-forming Kir6.x and auxiliary sulfonylurea receptor (SUR) proteins, are inhibited by ATP and activated by intracellular nucleotide diphosphates. Phosphorylation of Kir channel subunits by protein kinases such as protein kinases A and C (PKA and PKC, respectively) can modulate K channels activity. Phosphorylation of Ser residue in Kir1.1 by PKC results in suppression of channel activity (143). Protein kinases A phosphorylates both Kir6.1 and SUR2B subunits in smooth muscle and enhances its activity (144,145). Protein–protein interactions are involved in control of Kir channel pore function as well. They include interaction between G$\beta\gamma$ and Kir3.x (146), association of SUR with Kir6.x, and binding of anchoring proteins to diverse Kir channels. Recently, a 3.5 Å resolution crystal structure of the mammalian GIRK2 (Kir3.2) channel in

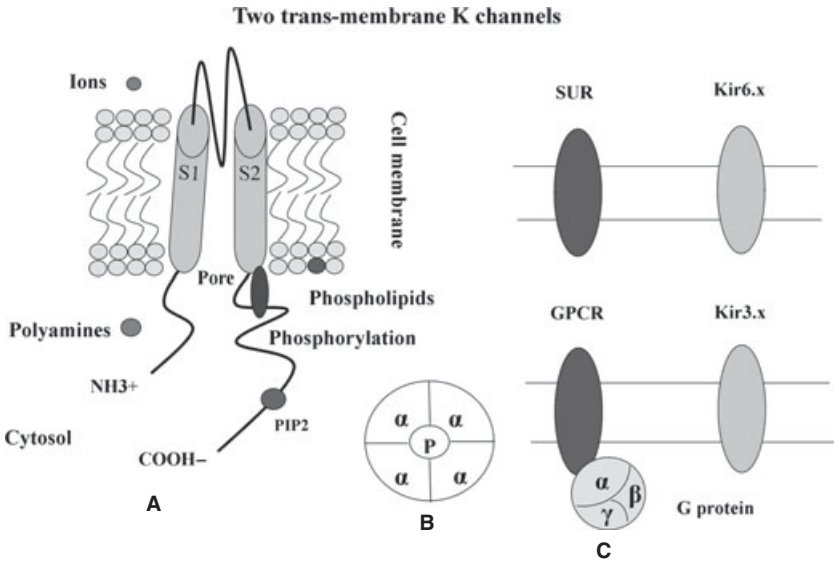

Figure 6: (A) Schematic representation of transmembrane (TM) topology of channel subunits containing two TM segments with pore formed by S1 and S2. The functions of Kir channels can be regulated by small substances. The small substances are ions such as H^+, Mg^{2+}, and Na^+ ions; polyamines; phosphatidylinositol 4,5-bisphosphate (PIP2); phosphorylation; and membrane-bound phospholipids. (B) Structure of the tetrameric assembly of the K2P channel. (C) Protein–protein interaction can also regulate function, and it involves sulfonylurea receptors (SUR), G-proteins liberated from G-protein-coupled receptors (GPCR).

complex with G$\beta\gamma$ subunits is presented. Short-range atomic and long-range electrostatic interactions stabilize four G$\beta\gamma$ subunits at the interfaces between four K channel subunits. The structure also permit a conceptual understanding of how the signaling lipid PIP2 and intracellular Na^+ ions participate in multiligand regulation of GIRK channels (147).

Modulation mechanism on Kir channels has been studied by computational methods as well. Multiscale simulations revealed a conserved binding site at the N-terminal end of the slide (M0) helix and at the interface between adjacent subunits of the channel. Polar contacts corresponded to long-lived electrostatic and H-bonding interactions between the channel and PIP2, enabling identification of key side chains (148). An emerging feature of several Kir channels is that they are regulated by cholesterol. However, the mechanism is unclear. With MD simulations, mutations of two distant Kir2.1 cytosolic residues, Leu-222 and Asn-251, were shown to form a two-way molecular switch that controls channel modulation by cholesterol and affects critical hydrogen bonding (149).

Diseases Related to Potassium Channels

Potassium channels play crucial roles in the development of many human diseases. Mutations in genes coding potassium channels lead to dysfunction in neuronal system, cardiac system, immune system, circulatory system, and some other systems. Potassium channels are broadly distributed in neuronal and cardiac systems based on the large quantity of reported K channels (150). Diseases of the neuronal, cardiac, and some other systems involving members of K channels are discussed in the following sections. We also present a table on the organ distribution of K channels (Figure 7 and Table S2). Besides, the

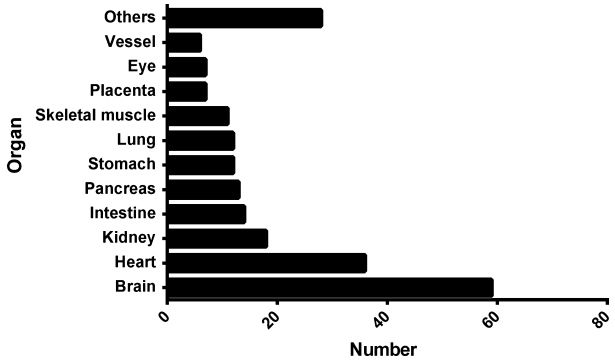

Figure 7: Organ distributions of potassium channels. The category of 'others' represents organs except for the ones shown in the abscissa.

inter-relationship of diseases, systems, organs, and K channels is provided in Table 1 and Figure 8.

Neuronal diseases

Potassium channels are critical to neurotransmission in the nervous system. Alterations in the function of these channels lead to remarkable perturbations in membrane excitability and neuronal function. Significant progress has been made in linking many neuronal disorders, such as episodic ataxia (EA), benign familial neonatal convulsions, Alzheimer's disease (AD), and so on (151).

Episodic ataxia is an autosomal dominant disorder in which the affected individuals have brief episodes of ataxia triggered by physical or emotional stress. Two types of EA are recognized including EA type 1 and EA type 2. A sufficient number of evidences have demonstrated that episodic ataxia type 1 (EA-1) is related to mutations in Kv1.1 (*KCNA1*; 12,152–156). In Kv1.1, single-point mutants

Table 1: Diseases and related potassium channels

Diseases	Systems	Organs	Potassium channels	References
Episodic ataxia	Nervous system	Cerebellum	*KCNA1*	(12,152–156)
Benign familial neonatal convulsions	Nervous system	Brain	*KCNQ2*, *KCNQ3*	(157–163)
Alzheimer's disease	Nervous system	Brain	*KCNC*, *KCNC2*, *KCNC3*, *KCNC4*, *KCNN4*	(164–167)
Parkinson's disease	Nervous system	Brain	*KCNJ2*, *KCNJ4*, *KCNJ12*, *KCNJ14*	(168–172)
Heritable long QT syndrome	Cardiovascular system	Heart	*KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, *KCNJ2*	(173–183)
Brugada syndrome	Cardiovascular system	Heart	*KCND3*	(184)
Short QT syndrome	Cardiovascular system	Heart	*KCNH2*, *KCNQ1*, *KCNJ2*	(185)
Acquired long QT syndrome	Cardiovascular system	Heart	*KCNE2*	(186–201)
Permanent neonatal diabetes mellitus	Endocrine system	Pancreas	*KCNJ11*	(202)
Type 2 diabetes mellitus	Endocrine system	Pancreas	*KCNJ15*	(203–205)
Bartter's disease	Urinary system	Kidney	*KCNJ1*	(206,207)
EAST syndrome	Urinary system and nervous system	Brain, cerebellum, kidney, inner ear	*KCNJ10*, *KCNJ16*	(211–216)

found below the channel activation gate at residue V408 are associated with human episodic ataxia type-1. They also impair channel function by accelerating decay of outward current during periods of membrane depolarization and channel opening (155).

Benign familial neonatal convulsions/seizures (BFNC/BFNS) are rare autosomal dominant generalized epilepsy of the newborn infant. They are linked to mutations in the potassium channel genes *KCNQ2* and *KCNQ3*. These encode for Kv7.2 and Kv7.3 channels, which produce an M-current that regulates the potential firing action in neurons through modulation of membrane potential (157). Sequence analysis identifies mutations in *KCNQ2* or *KCNQ3* in 60–70% of families with BFNS (158). A large sum of mutations in these two channels has been reported to cause the benign familial neonatal convulsions during last decades. These findings provide implications for diagnosis and prognosis of BFNS (159–163).

As memory loss is characteristic of AD and as potassium channels change during acquisition of memory in both mollusks and mammals, potassium channels are suggested as a possible site of AD pathology (164). Analysis of gene expression throughout the different stages of AD suggests that up-regulation of Kv3.4 (*KCNC4*) and dysregulation of Kv3.1 (*KCNC1*) alter potassium currents in neurons and leads to altered synaptic activity that may underlie the neurodegeneration observed in AD (165). In addition, further quantitative evaluation studies in murine models indicate that during postnatal development, Kv3 (Kv3.1, Kv3.2, Kv3.2, Kv3.4) transcripts and proteins showed a progressive increase in expression and reached an asymptote in adulthood, suggesting that the increase in

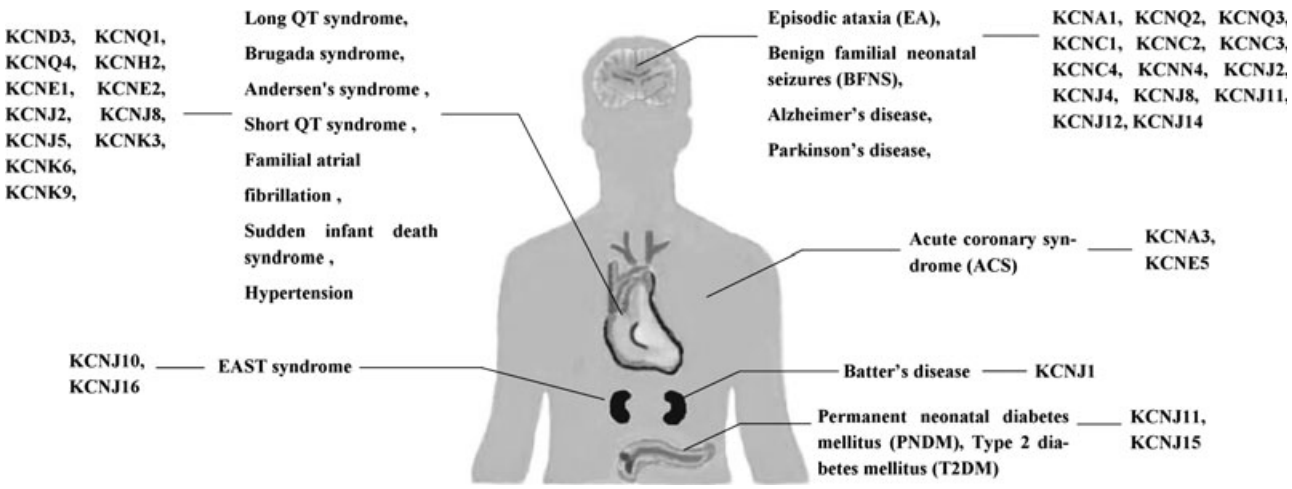

Figure 8: Diseases and related potassium channels exhibited in human being contour.

Kv3 expression during development might contribute to the maturation of the electrical activity of neurons. While in contrast, in the neocortex of aged APPPS1 mice, Kv3.1 mRNA and protein levels were significantly lower compared to wild type, suggesting that a decrease in Kv3 currents could play a role in the cognitive symptom s of Alzheimer 's disease (166). The anti-inflammatory and neuroprotective effects of KCa3.1 (KCNN4) blockade would be suitable for treating AD as well as cerebrovascular and traumatic brain injuries. It is therefore promising to test KCa3.1 blockers for AD preclinical and clinical trials (23,167).

A considerable number of potassium channels within the nervous system appear to mediate diverse cellular signaling. Recent studies on potassium channel gene expression in the basal ganglia indicate that dysfunctions of various potassium channels may be involved in the pathogenesis of Parkinson's disease. The gene expression levels of inwardly rectifying potassium channels Kir2 (Kir2.1, Kir2.2, Kir2.3, Kir2.4) were analyzed using quantitative real-time PCR among 20 PD patients with medication, 10 Parkinson's patients without medication and 16 healthy controls, respectively. The results indicate that Kir2 potassium channels may serve as a potential biomarker for screening (168). Increasing reports also suggest that KATP channels might be involved in the pathogenesis of Parkinson's disease and the blockade of neuronal KATP channels may contribute to neuroprotective effects in Parkinson's disease (169–172). Other promising targets, including Kv, SK, and K2P channels, deserve further pursuit for making comprehensive use of their novel therapeutic potential and may lead to new therapeutic strategy of Parkinson's disease.

Cardiac diseases

Because the heartbeat is so dependent on the proper movement of ions across the surface membrane, disorders of ion channels or 'channelopathies' make up a key group of cardiac diseases. Channelopathies predispose individuals to disturbances of normal cardiac rhythm or arrhythmias. Several different genetic and acquired channelopathies can cause such arrhythmias (173). Cardiac channelopathies can be mainly divided into heritable and acquired channelopathies.

Heritable channelopathies is classified into long QT syndrome (LQTS), Brugada syndrome (BRS), short QT syndrome (SQTS), and so on. LQTS is the first channelopathies found in human; the heritable LQTS has two forms, Romano-Ward syndrome (RWS) and Jervell and Lange-Nielsen syndrome (JLNS). Studies have revealed that LQTS was due to defective cardiac channels (174). It has been reported that potassium channels like *KCNQ1* (LQT1), *KCNH2* (LQT2), *KCNE1* (LQT5), *KCNE2* (LQT6), and *KCNJ2* (LQT7) are related to RWS (175–178). Long-QT-associated mutations in the K channels decrease K flux through I_{Kr} or I_{Ks} by loss-of-function or dominant-negative mechanisms (179). Because K channels are multimeric channels, the dominant-negative mutations cripple the healthy products of the wild-type allele and thus provide a ready rationale for dominant transmission. The fact that plain loss-of-function mutations also produce dominantly inherited LQTS implies, however, that two functional alleles are required for uneventful repolarization. More rarely, LQTS is inherited in an autosomal recessive manner. Such kindreds possess two dysfunctional K channel genes, which leads to a total absence of I_{Kr} or I_{Ks} (180). Individuals affected with associated deafness have mutations in the I_{Ks} genes *KCNQ1* or *KCNE1* (181). Moreover, a rare genetic disease known as Andersen's syndrome, in which LQTS is associated with multisystem pathology, has been attributed to mutations in *KCNJ2* (182). Recent structure of the C-linker/cyclic nucleotide-binding homology domain of a mosquito hERG channel reveals that the region expected to form the cyclic nucleotide-binding pocket is negatively charged and is occupied by a short β-strand, referred to as the intrinsic ligand, explaining the lack of direct regulation of ERG channels by cyclic nucleotides. Mutations in the intrinsic ligand affected hERG channel gating and LQTS mutations abolished hERG currents and altered trafficking of hERG channels, which explains the LQT phenotype (183).

Brugada syndrome is one heritable channelopathies, and emerging evidences have linked perturbations in the transient outward current (I_{to}) conducted by the *KCND3*-encoded Kv4.3 pore-forming α-subunit to BRS (184). Short QT syndrome (SQTS) is found to possess three pathogenic genes, *KCNH2*, *KCNQ1*, and *KCNJ2*, named after SQT1, SQT2, and SQT3 respectively (185).

Acquired channelopathies have yielded important insights into the pathophysiology of some acquired diseases. Heart failure represents a common, acquired form of the LQTS (186). In human heart failure, the action potential prolongation reflects selective down-regulation of two K currents, I_{to} and I_k. The down-regulation of potassium channels becomes maladaptive in the long term, predisposing the individual to after-depolarization, inhomogeneous repolarization, and ventricular tachyarrhythmia.

In addition to heart failure, acquired LQTS can also be induced by exposure to drugs that block potassium channels (187,188). Some people believed that this drug-induced LQTS might be caused by a channel mutation that alone does not cause symptoms and may potentially lead to arrhythmias in the presence of certain drugs (189). Mutations in ion channel genes may enhance drug binding and magnify the channel blockade (190). *KCNE2* is reported to underlie arrhythmias triggered by an antibiotic, but the mechanism remains uncertain (191). Approximately 10% of sudden infant death syndrome (SIDS) may stem from cardiac channelopathies, molecular and functional evidences also indicated *KCNJ8* mutations as a novel

pathogenic mechanism in SIDS (192). Hypertension is another significant cardiovascular disease that links to potassium channels, and it has been reported that *KCNJ5*, *KCNK3*, *KCNK6*, *KCNK9*, *KCNQ4*, and certain Kca channels are closely related to this disease (193–201).

Diseases in other systems

Besides neuronal and cardiac diseases, some diseases in other systems involving potassium channels are discussed in this section. Permanent neonatal diabetes mellitus (PNDM) is a rare form of diabetes diagnosed within the first 6 months of life. Heterozygous activation mutations in *KCNJ11*, encoding the Kir6.2 subunit of the KATP, which acts as a key role in insulin secretion regulation, account for about half of the cases of PNDM (202). This channel plays a pivotal role in glucose-stimulated insulin release from the pancreatic beta cell. Recent advances in genome research have enabled the identification of new genomic variations that are associated with type 2 diabetes mellitus (T2DM). The inwardly rectifying potassium channel, subfamily J, member 15 (*KCNJ15*) is identified as a new T2DM susceptibility gene (203). The mechanism of *KCNJ15* is to regulate insulin secretion and down-regulation of *KCNJ15* gives rise to increased insulin secretion both *in vitro* and *in vivo* (204,205).

The role of the kidney in controlling and maintaining plasma potassium levels in the normal range requires the presence and activity of renal potassium channels, and their importance has been highlighted in patients with Bartter syndrome harboring mutations in the Kir1.1 channel (206). Kir1.1 mediates potassium secretion and regulates NaCl reabsorption in the kidney. Loss-of-function mutations in this pH-sensitive potassium channel cause Bartter's disease, a familial salt-wasting nephropathy (207).

Modulation of certain T cells through potassium channels provides potential novel targets for treatment of acute coronary syndrome (ACS). Recent findings report that Kv1.3 (KCNA3) channels of peripheral CD4(+)T cell and CD28 (null)/CD28(+)T cells from ACS patients significantly increased after activation and Kv1.3-specific channel blocker could effectively abolish this effect suggesting a potential role of Kv1.3 channel blocker on plaque stabilization in ACS patients (208,209). Palmer *et al.* (210) also reports an association between rs697829, a common single nucleotide polymorphism (SNP) of *KCNE5*, and ECG measurements and survival in postacute ACS patients.

The potassium channel expressed by *KCNJ10* gene (Kir4.1) has previously demonstrated importance in retinal function in animal experiments. Recently, mutations in *KCNJ10* were recognized as pathogenic in man, causing a constellation of symptoms, including epilepsy, ataxia, sensor neural deafness and a renal tubulopathy designated as EAST syndrome (211–215). Besides, evidences also highlight the important role that Kir5.1 (*KCNJ16*) plays as a pH-sensitive regulator of salt transport in the EAST syndrome (216).

Modulators of Potassium Channels

Lots of efforts have been put into discovering modulators of potassium channels in recent decades (32,217–224). These modulators are mainly divided into peptide toxins and small molecules (225,226). Peptide toxins affect K channels by two mechanisms: firstly, toxins from scorpions, sea anemones, snakes, and cone snails bind to the outer vestibule of K channels and in most cases insert a lysine side chain into the channel pore, occluding it like a cork in a bottle (227,228). Secondly, spider toxins, such as hanatoxin, interact with the voltage sensor domain of K channels and increase the stability of the closed state (229,230). In contrast to peptide toxins (which affect K channels from the extracellular side), most small molecules bind to the inner pore, the gating hinge or the interface between the α- and β-subunit. These small molecules could be mainly classified into blockers and openers, or multitarget and specific-target. Due to the limited research on peptide modulators, we focused on nearly 70 small-molecule modulators and utilized qualitative and quantitative approaches to make analyses with respect to the following three aspects. Firstly, chemical scaffold of natural product was used to make a qualitative description. Secondly, typical pharmacokinetic parameters such as pKa, logP, and logD values were introduced to quantitatively describe them. Lastly, a quantitative method was utilized to discriminate the modulators in a systematic way. The detailed profiles are discussed as follows.

Qualitative analysis by scaffold of natural product

Natural products have long been regarded as a class of compounds with particular molecular properties and distinct structural features. Studies show that natural products occupy parts of chemical space not explored by available screening collections while at the same time largely adhering to the Lipinski's rule, also known as 'the rule of 5' (It predicts that poor absorption or permeation is more likely when there are more than five H-bond donors, 10 H-bond accepters, the molecular weight is >500 and the calculated Log P is >5; 231). The drug-like properties are important for developing natural products into marketable drugs. Many approved and clinical-trial drugs derived from natural products (232). For instance, 12 (26%) of the 46 molecular entities approved by the FDA in 2009–2010 are nature derived (233). Natural products mainly include alkaloids, peptides, phenylpropanoids, sulfur compounds, flavonoids, terpene, and triterpenoid saponins. They are basically classified according to their chemical scaffold. Each natural product has distinct structural features, for example, terpenoid is compound possessing (C5H8)n and its oxygen-containing derivatives. The structural features of natural-product scaffolds have long been widely used in

drug development and in field of medicinal chemistry (234–237).

Chemical scaffold of natural product is introduced to qualitatively study modulators. The more similar the scaffold of a modulator is to one group of natural product, the higher probability of the modulator is to be categorized into this group. In this way, all modulators were categorized according to this rule. The number and percentage of modulators in different classes were calculated (Table S3 and Figure 9) to make further comparisons. The percentage of blockers belonging to alkaloid is 85%, while for openers, it is 64%. The percentage of openers belonging to nitrile compound and amino acid is 32% and 14% respectively; while for blockers, they are both 0. These data help to explain the differences between blockers and openers in natural product scaffold to a certain extent. The percentage in peptides and sulfur compound for both blockers and openers are nearly equivalent (Figure 9A). These indicate that modulators of K channels are also similar in topology. Our results also unveil that the modulators clustered mostly in alkaloid and peptide. This may provide useful indications for novel drug design.

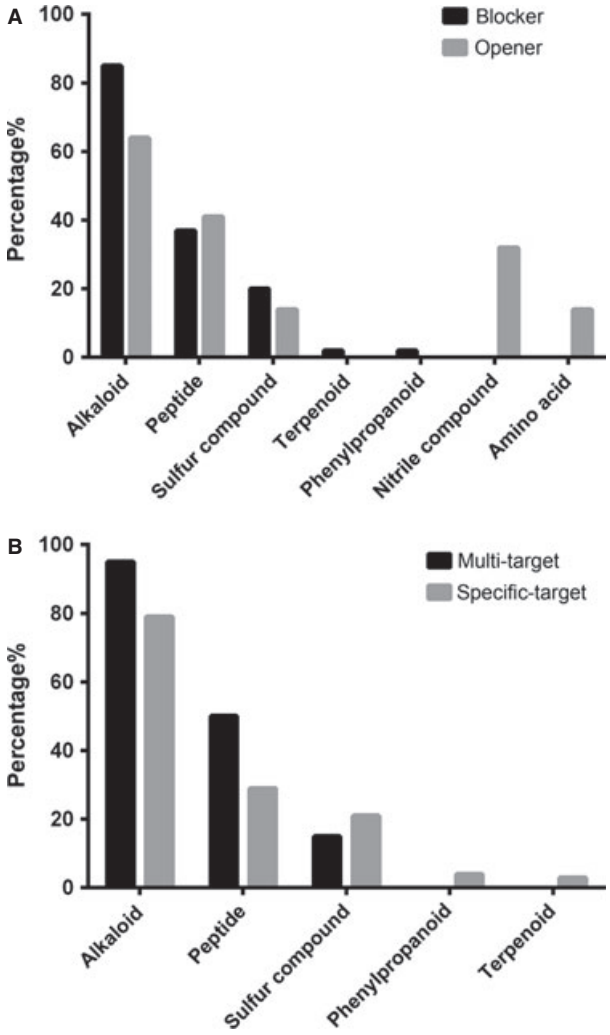

Figure 9: (A) Percentage of blockers and openers by natural product in histogram. (B) Percentage of multitarget and specific-target blockers by natural product in histogram. The percentage is of the number of modulators belonging to one certain category divided by the total number of modulators. As some modulators belong to more than one category, the sum of the percentage is beyond 100%.

Due to the limited number of reported openers, we merely subclassified the blockers into multitarget and specific target. The number and percentage of multitarget and specific-target modulators in different classes of natural product were also calculated (Table S4 and Figure 9B). Even though the difference is not significant, the percentages show that multitarget and specific-target blockers are different in alkaloid, peptide, and sulfur compound. These indicate the scaffold divergences to some extent. The results also reveal cluster pattern in alkaloid class for both multitarget and specific-target blockers. In combination with the clustering pattern of openers and blockers, these data further prove that most of the k channel modulators, whatever multitarget or specific target developed in recent years, derived from this class of natural product.

Quantitative analysis by pharmacokinetic parameters

After qualitatively analysis, a quantitative method was employed utilizing some relatively simple yet typical parameters. The pKa, logP, and logD are three significant parameters in pharmacokinetics. They have a profound effect on the drug-like property of a molecule. Thus, we utilized these three parameters serving as a quantitative description and made further analysis. The pKa or 'Dissociation Constant' is a measure of the strength of an acid or a base. It allows us to determine the charge on a molecule at any given pH. The logP or 'Partition Coefficient' is a measure of how well a substance partitions between lipid (oil) and water. The logD is the octanol–water distribution coefficient, which combines pKa and logP. It produces an apparent partition coefficient for any pH value. We employ these three parameters in this section of work, aiming to find a general pattern of the modulators.

Measurements of the three values are not straightforward. Experiments must be carefully performed under extremely stringent conditions to ensure the accuracy of the results. Data interpretation also takes much time and experience. Therefore, we calculate or predict part of pKa, logP and logD values when there is no experimental data. Two-dimensional descriptor 'logP(o/w)' in MOE (238) was used to calculate logP value. The online calculator 'SPARC' (239) was employed to calculate pKa and logD values.

Although experimental data of some molecules could be obtained, we still utilized calculated data to ensure the

consistency of comparison. With the obtained data, we hope to seek for differences between blockers and openers, and between multitarget and specific-target blockers. As the variables do not exhibit normal distribution, Wilcoxon's rank-sum test was used to find the difference. The results show that except for the P value for the logP between openers and blockers (first row, second column in Figure 10) which is 0.0149, indicating statistical significance, the other five P values are all higher than 0.05, indicating the differences are not significant. This indicates that the three pharmacokinetic parameters are relatively too simple in describing molecules quantitatively, although they are quite important properties for drugs individually. Therefore, we had better employ a more comprehensive and systematic method to address this problem.

Quantitative analysis by descriptors

We utilized descriptors named drug-like index (DLI) to make further quantitative analysis. Drug-like index is developed by Jun Xu in 2000, which is useful in ranking compounds from a structural perspective (240). Drug-like index contains 28 features including hydrogen bond donor, hydrogen bond acceptor, the number of non-H polar bonds and so on to define an organic molecule in multiple aspects.

In this section, descriptors were adopted together with logistic regression to make a more systematic analysis on modulators. We selected top three features from the 28 features through the forward search algorithm in machine learning. Further, we made analysis based on these three features. The blockers and openers could be clearly separated into two clusters, so are the multitarget and specific-target modulators (Figure 11). Receiver operating characteristic (ROC) curves were also plotted (Figure 12): the red line represents the model built by selected features. We further calculated area under curve (AUC) value. The model shows a good prediction ability achieving an AUC value larger than 0.9. For blockers and openers, the AUC value is 0.9644; while for multitarget and specific-target modulators, it is 0.9301. The results indicate that the features we selected can be used to differentiate modulators. They also demonstrate that a more systematic method like multidimensional descriptors together with logistic regression could define molecules in a more accurate and comprehensive way.

To differentiate blockers and openers, the selected three features are DLI3, DLI4, and DLI13. It suggests that molecular cyclized degree, the number of non-H rotating bonds and the number of 2-degree cyclic atoms (the cyclic atom without non-H atom substitution is a 2-degree cyclic atom) are significant in differentiating

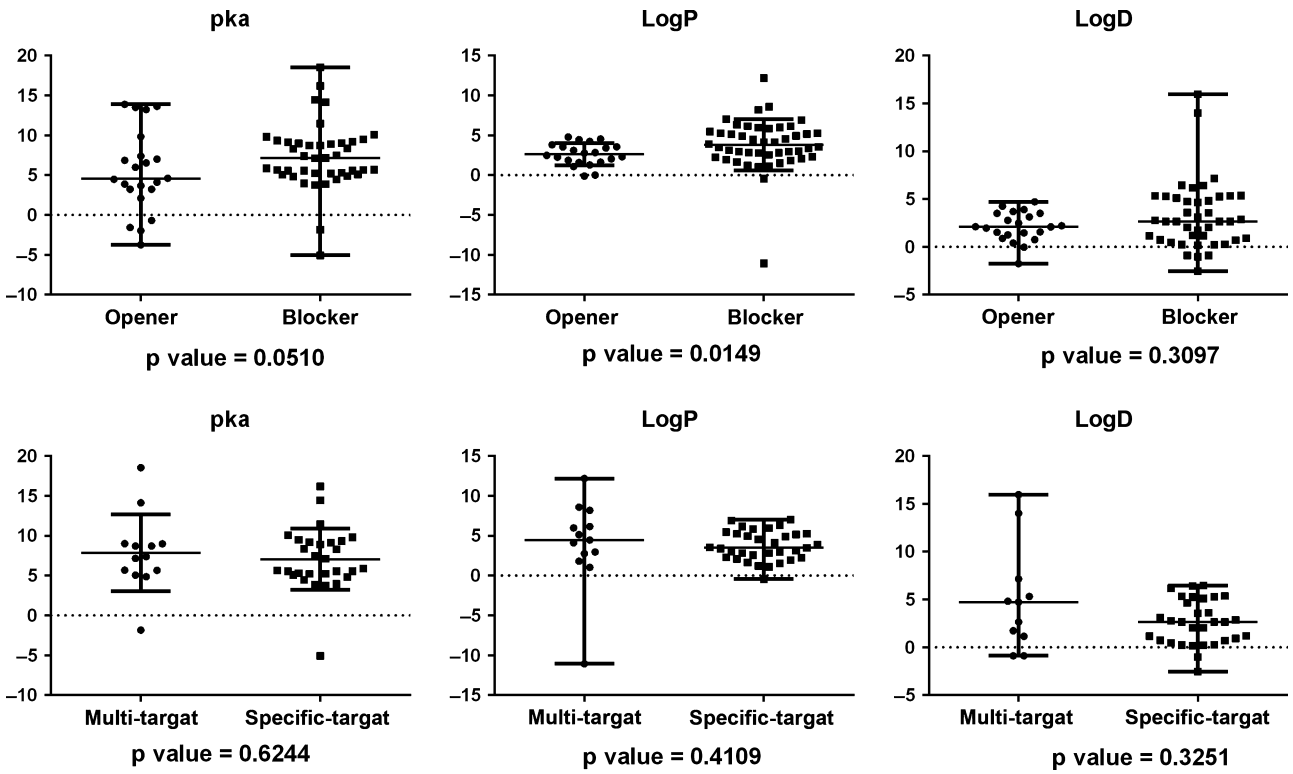

Figure 10: Wilcoxon's rank-sum tests on blockers and openers; multitarget and specific-target blockers. The *x*-axis stands for the type of molecules, while the *y*-axis stands for the pharmacokinetic parameters.

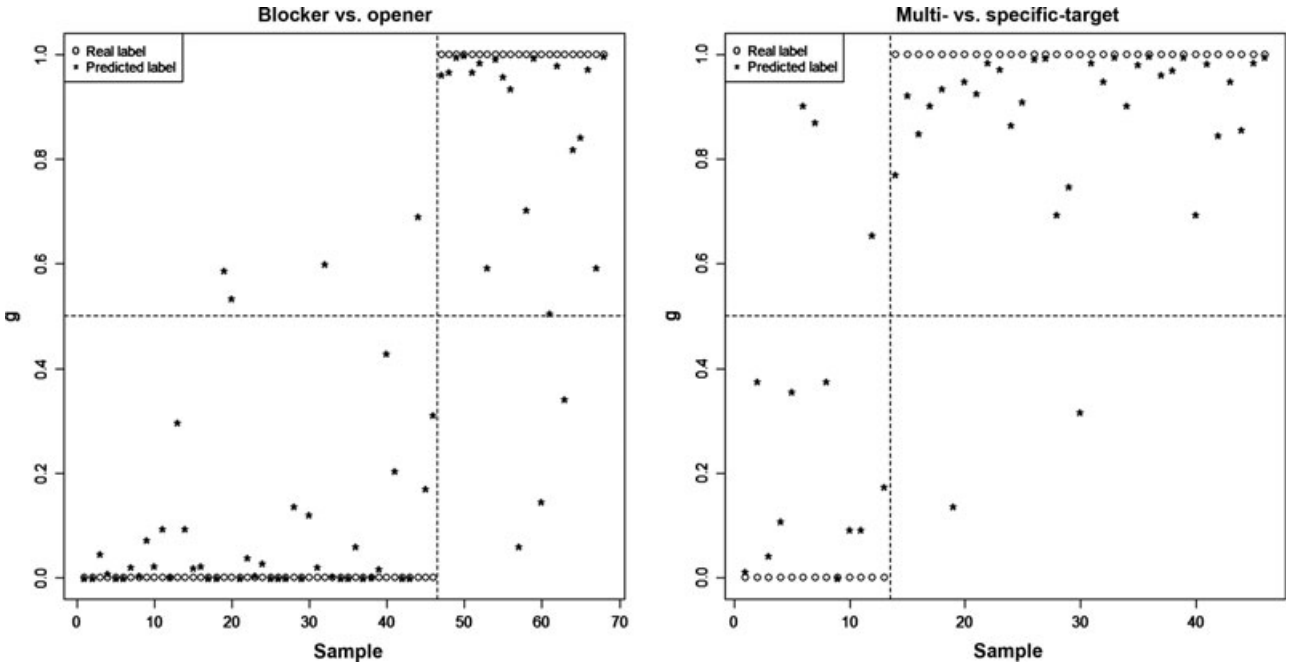

Figure 11: Logistic regression analysis. The *x*-axis stands for samples. For the figure on the left, openers are from 0 to 22, while blockers are from 23 to 68. For the figure on the right, multitarget blockers are from 0 to 13, while specific-target blockers are from 14 to 46. The *y*-axis stands for the g in the equation specified below:

$$z = \theta^T x \tag{1}$$

$$g(z) = \frac{1}{1 + e^{-z}} \tag{2}$$

Logistic regression is applied which combines linear regression (1) with sigmoid function (2). The 'o' is real label, while the '*' is predicted label by the selected three features. The horizontal and perpendicular red lines separate the regions into four parts; the two sets of samples are mainly distributed in the left down and right up parts.

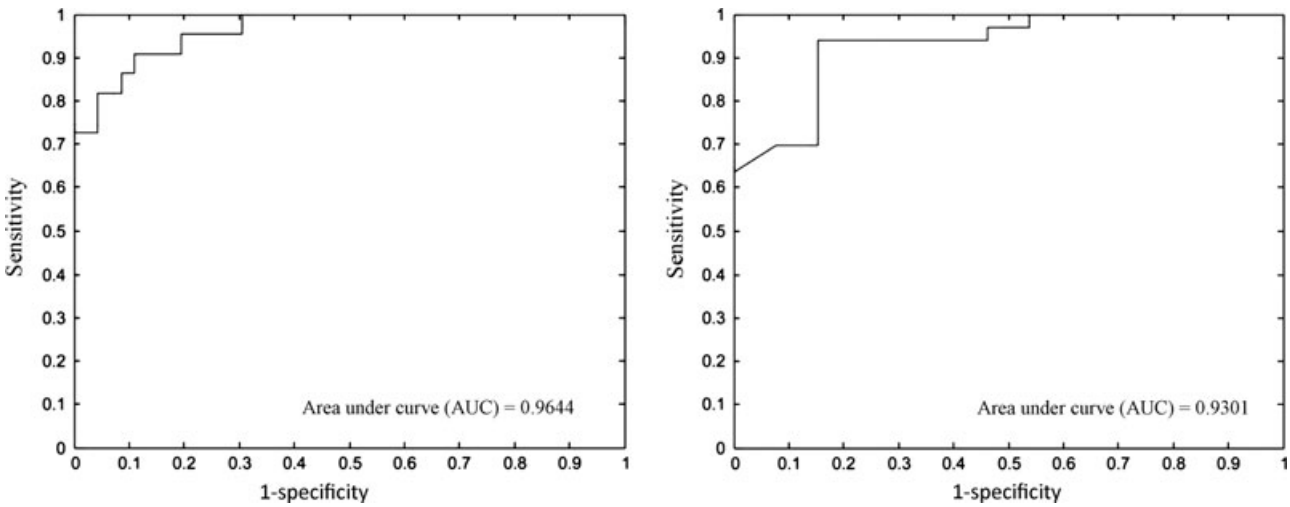

Figure 12: Receiver operating characteristic curves for logistic regression analysis. The area under curve for openers and blockers (figure on the left) is 0.9644. While for multitarget and specific-target modulators (figure on the right), it is 0.9301.

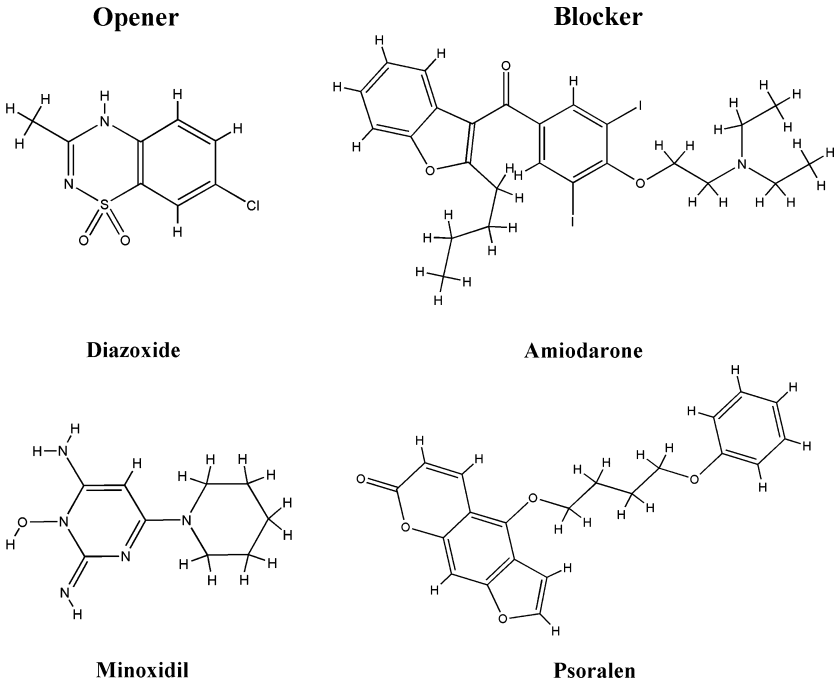

Figure 13: Openers and blockers of potassium channel. Shown are two openers and two blockers. The four modulators are among the best samples in logistic regression analysis for openers and blockers. DLI3 stands for the molecular cyclized degree; DLI4 stands for the number of non-H rotating bonds; DLI13 stands for the number of 2-degree cyclic atoms (the cyclic atom without non-H atom substitution is a 2-degree cyclic atom).

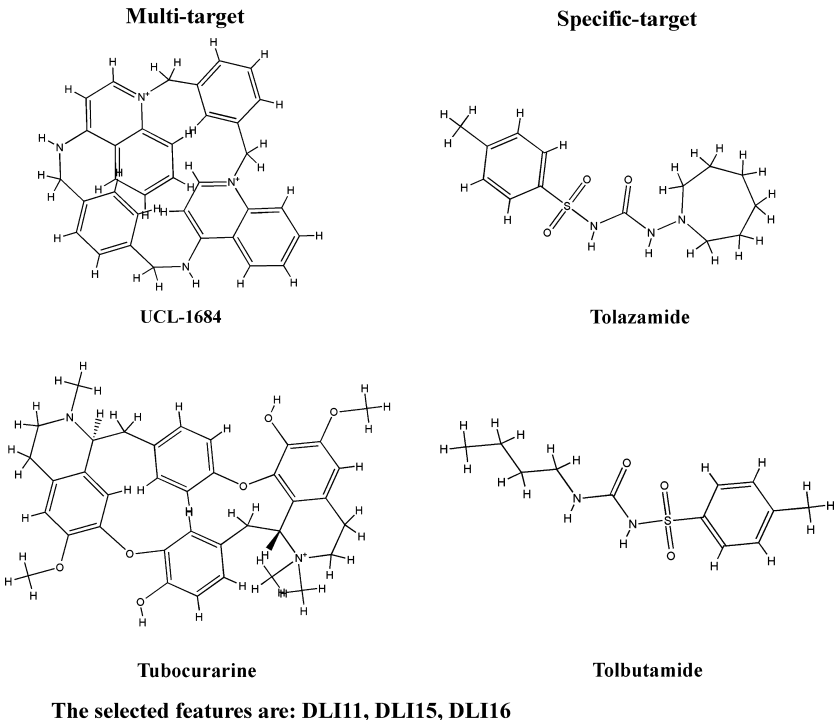

Figure 14: Multitarget and specific-target modulators of potassium channel. Shown are two multitarget and two specific-target modulators. The four modulators are among the best samples in logistic regression analysis for multitarget and specific-target modulators. DLI11 stands for the number of N atoms and O atoms; DLI15 stands for the number of 3-degree cyclic atoms (the cyclic atom with one non-H atom substitution is a 3-degree cyclic atom); DLI16 stands for the number 1-level bonding pattern.

blockers and openers (240). Similarly, to differentiate multitarget and specific-target blockers, the selected features are DLI11, DLI15, and DLI16. It suggests that the number of N atoms and O atoms, the number of 3-degree cyclic atoms (the cyclic atom with one non-H atom substitution is a 3-degree cyclic atom), and the number of 1-level bonding pattern are significant in differentiating multitarget and specific-target blockers. Examples of some typical modulators are displayed in Figures 13 and 14.

Conclusions and Future Directions

We provide an overview of potassium channels concerning structure, diseases, and modulators. Structural and functional features on the basis of membrane topology are discussed. Relations between potassium channels and diseases are reviewed. In the last part, we also perform some *in silico* analysis on modulators based on their topological structure. Both natural-product scaffold and pharmacokinetic parameters are significant factors when discussing certain features of modulators. The features that are important in differentiating openers and blockers, multitarget, and specific-target blockers are also selected by quantitative approaches. Additionally, an informative supplementary table (Table S1) with gene and protein names, organ distribution, modulators (including name, structure, CAS number and type), and references is provided. Our work not only provides relatively integrated information on K channels, but also offers some useful indications for future drug design.

Experimental techniques for the identification of membrane proteins have greatly advanced in the last decades and offer us great opportunities in systematically studying membrane proteins. Recent advances in structural biology underlying mechanisms of channel gating have strengthened our knowledge about how potassium channels can be interconvertible between conductive and nonconductive states (241). An alternative and unbiased approach, mass spectrometry (MS)-based proteomic analysis, is also matured. The application of proteomic approaches to identify the components of native neuronal (and cardiac) Kv4 channel complexes has revealed greater complexity than anticipated (242). The potential power of these methodologies in identifying the channels, as well as post-translational modifications of channel components, is already very clear (243–245). Except for the advances in experiment, the developments in high-performance calculation techniques have also enhanced our knowledge into microscopic level of the working mechanism (246–248). MD simulations are an invaluable tool for studying the structural and functional properties of complex biological membrane (249). In combination with homology modeling and associated calculations, MD simulations provide a powerful approach in understanding structure/function relationships in ion channels. The wide distribution of some potassium channels makes them significant and valid targets for several pathologies. On the other hand, the ubiquitous nature is a relevant drawback because of the side-effects related to the lack of selectivity (250). Another case is that some specific organs such as heart contain numerous subtypes of one specific potassium channel, but no successful selective blockers have been developed yet (251–253). These facts indicate that organ selective and subtype selective modulators might pave the way to the development of innovative and effective modulators for clinical use. Development of novel selective modulators for this family of membrane protein remains a challenge for the future. With the advances of both experimental and computational approaches, an increasing number of ion channels would be identified and deeper mechanism would be clarified. All the strategies for potassium channel analysis developed in our work could be extended to other studies on proteins. This will facilitate future translational research on this kind of membrane protein and will help expand our understanding toward ion channel and other kinds of membrane proteins.

Acknowledgments

This work was supported in part by grants from National Natural Science Foundation of China (31200986, 30973611), Research Fund for the Doctoral Program of Higher Education of China (20100072120050), The Fundamental Research Funds for the Central Universities (2000219083), and TCM Modernization of Shanghai (09dZ1972800).

Conflict of Interest

There are no conflicts of interest.

References

1. Grover G.J., Garlid K.D. (2000) ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. J Mol Cell Cardiol;32:677–695.
2. Jentsch T.J. (2000) Neuronal KCNQ potassium channels: physiology and role in disease. Nat Rev Neurosci;1:21–30.
3. Ketchum K.A., Joiner W.J., Sellers A.J., Kaczmarek L.K., Goldstein S.A. (1995) A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. Nature;376:690–695.
4. Kohler M., Hirschberg B., Bond C.T., Kinzie J.M., Marrion N.V., Maylie J., Adelman J.P. (1996) Small-conductance, calcium-activated potassium channels from mammalian brain. Science;273:1709–1714.
5. Neyroud N., Tesson F., Denjoy I., Leibovici M., Donger C., Barhanin J., Faure S., Gary F., Coumel P., Petit C., Schwartz K., Guicheney P. (1997) A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nat Genet;15:186–189.
6. Nichols C.G., Lopatin A.N. (1997) Inward rectifier potassium channels. Annu Rev Physiol;59:171–191.
7. Rettig J., Heinemann S.H., Wunder F., Lorra C., Parcej D.N., Dolly J.O., Pongs O. (1994) Inactivation properties of voltage-gated K+ channels altered by presence of beta-subunit. Nature;369:289–294.
8. Weingarth M., Prokofyev A., van der Cruijsen E.A.W., Nand D., Bonvin A.M.J.J., Pongs O., Baldus M.

(2013) Structural determinants of specific lipid binding to potassium channels. J Am Chem Soc;135:3983–3988.
9. Brohawn S.G., Campbell E.B., MacKinnon R. (2013) Domain-swapped chain connectivity and gated membrane access in a Fab-mediated crystal of the human TRAAK K+ channel. Proc Natl Acad Sci USA;110:2129–2134.
10. Goodchild S.J., Xu H., Es-Salah-Lamoureux Z., Ahern C.A., Fedida D. (2012) Basis for allosteric open-state stabilization of voltage-gated potassium channels by intracellular cations. J Gen Physiol;140:495–511.
11. Horrigan F.T. (2012) Perspectives on: conformational coupling in ion channels: conformational coupling in BK potassium channels. J Gen Physiol;140:625–634.
12. Jan L.Y., Jan Y.N. (2012) Voltage-gated potassium channels and the diversity of electrical signalling. J Physiol;590:2591–2599.
13. Kruse M., Hammond G.R., Hille B. (2012) Regulation of voltage-gated potassium channels by PI(4,5)P2. J Gen Physiol;140:189–205.
14. McCoy J.G., Nimigean C.M. (2012) Structural correlates of selectivity and inactivation in potassium channels. Biochim Biophys Acta;1818:272–285.
15. Miller A.N., Long S.B. (2012) Crystal structure of the human two-pore domain potassium channel K2P1. Science;335:432–436.
16. Plant L.D., Zuniga L., Araki D., Marks J.D., Goldstein S.A. (2012) SUMOylation silences heterodimeric TASK potassium channels containing K2P1 subunits in cerebellar granule neurons. Sci Signal;5:ra84.
17. Schmidt D., del Marmol J., MacKinnon R. (2012) Mechanistic basis for low threshold mechanosensitivity in voltage-dependent K+ channels. Proc Natl Acad Sci USA;109:10352–10357.
18. Tao X., Lee A., Limapichat W., Dougherty D.A., MacKinnon R. (2010) A gating charge transfer center in voltage sensors. Science;328:67–73.
19. Telezhkin V., Brown D.A., Gibb A.J. (2012) Distinct subunit contributions to the activation of M-type potassium channels by PI(4,5)P2. J Gen Physiol;140:41–53.
20. Yuan P., Leonetti M.D., Hsiung Y., MacKinnon R. (2012) Open structure of the Ca^{2+} gating ring in the high-conductance Ca^{2+}-activated K+ channel. Nature;481:94–97.
21. Santos J.S., Asmar-Rovira G.A., Han G.W., Liu W., Syeda R., Cherezov V., Baker K.A., Stevens R.C., Montal M. (2012) Crystal structure of a voltage-gated K+ channel pore module in a closed state in lipid membranes. J Biol Chem;287:43063–43070.
22. Gui L., LaGrange L.P., Larson R.A., Gu M., Zhu J., Chen Q.H. (2012) Role of small conductance calcium-activated potassium channels expressed in PVN in regulating sympathetic nerve activity and arterial blood pressure in rats. Am J Physiol Regul Integr Comp Physiol;303:R301–R310.
23. Jenkins D.P., Maezawa I., Wulff H., Jin L.W. (2012) Microglial Kv1.3 channels as a potential target for Alzheimer's disease. Int J Alzheimers Dis;2012: Article ID 868972.
24. Kasianowicz J.J. (2012) Introduction to ion channels and disease. Chem Rev;112:6215–6217.
25. Lam J., Wulff H. (2011) The lymphocyte potassium channels Kv1.3 and KCa3.1 as targets for immunosuppression. Drug Dev Res;72:573–584.
26. Li X.N., Herrington J., Petrov A., Ge L., Eiermann G., Xiong Y., Jensen M.V. *et al.* (2013) The role of voltage-gated potassium channels Kv2.1 and Kv2.2 in the regulation of insulin and somatostatin release from pancreatic islets. J Pharmacol Exp Ther;344:407–416.
27. Srivastava R., Aslam M., Kalluri S.R., Schirmer L., Buck D., Tackenberg B., Rothhammer V., Chan A., Gold R., Berthele A., Bennett J.L., Korn T., Hemmer B. (2012) Potassium Channel KIR4.1 as an Immune Target in Multiple Sclerosis. New Engl J Med;367:115–123.
28. Walters A.M., Porter G.A., Brookes P.S. (2012) Mitochondria as a drug target in ischemic heart disease and cardiomyopathy. Circ Res;111:1222–1236.
29. Wulff H., Kohler R. (2013) Endothelial small-conductance and intermediate-conductance KCa channels: an update on their pharmacology and usefulness as cardiovascular targets. J Cardiovasc Pharm;61:102–112.
30. Wulff H., Castle N.A., Pardo L.A. (2009) Voltage-gated potassium channels as therapeutic targets. Nat Rev Drug Discov;8:982–1001.
31. Bodendiek S.B., Mahieux C., Hansel W., Wulff H. (2009) 4-Phenoxybutoxy-substituted heterocycles – A structure-activity relationship study of blockers of the lymphocyte potassium channel Kv1.3. Eur J Med Chem;44:1838–1852.
32. Chen Z.Y., Hu Y.T., Yang W.S., He Y.W., Feng J., Wang B., Zhao R.M, Ding J.P., Cao Z.J., Li W.X., Wu Y.L. (2012) Hg1, novel peptide inhibitor specific for Kv1.3 channels from first scorpion Kunitz-type potassium channel toxin family. J Biol Chem;287:13813–13821.
33. Cheng H., Zhang Y., Du C., Dempsey C.E., Hancox J.C. (2012) High potency inhibition of hERG potassium channels by the sodium-calcium exchange inhibitor KB-R7943. Br J Pharmacol;165:2260–2273.
34. Jenkins D.P., Strobaek D., Hougaard C., Jensen M.L., Hummel R., Sorensen U.S., Christophersen P., Wulff H. (2011) Negative gating modulation by (R)-N-(benzimidazol-2-yl)-1,2,3,4-tetrahydro-1-naphthylamine (NS8593) depends on residues in the inner pore vestibule: pharmacological evidence of deep-pore gating of K(Ca)2 channels. Mol Pharmacol;79:899–909.
35. Kroigaard C., Dalsgaard T., Nielsen G., Laursen B.E., Pilegaard H., Kohler R., Simonsen U. (2012) Activation of endothelial and epithelial KCa2.3 calcium-activated potassium channels by NS309 relaxes human small

pulmonary arteries and bronchioles. Br J Pharmacol;167:37–47.
36. Minieri L., Pivonkova H., Caprini M., Harantova L., Anderova M., Ferroni S. (2013) The inhibitor of volume-regulated anion channels DCPIB activates TREK potassium channels in cultured astrocytes. Br J Pharmacol;168:1240–1254.
37. Sankaranarayanan A., Raman G., Busch C., Schultz T., Zimin P.I., Hoyer J., Köhler R., Wulff H. (2010) Naphtho[1,2-d]thiazol-2-ylamine (SKA-31), a new activator of KCa2 and KCa3.1 potassium channels, potentiates the endothelium-derived hyperpolarizing factor response and lowers blood pressure. Abstr Pap Am Chem Soc;239:281–295.
38. Wulff H. (2010) Spiro azepane-oxazolidinones as Kv1.3 potassium channel blockers: WO2010066840. Expert Opin Ther Pat;20:1759–1765.
39. Es-Salah-Lamoureux Z., Steele D.F., Fedida D. (2010) Research into the therapeutic roles of two-pore-domain potassium channels. Trends Pharmacol Sci;31:587–595.
40. Gonzalez C., Baez-Nieto D., Valencia I., Oyarzun I., Rojas P., Naranjo D., Latorre R. (2012) K+ channels: function-structural overview. Compr Physiol;2:2087–2149.
41. Hibino H., Inanobe A., Furutani K., Murakami S., Findlay I., Kurachi Y. (2010) Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev;90:291–366.
42. Vardanyan V., Pongs O. (2012) Coupling of voltage-sensors to the channel pore: a comparative view. Front Pharmacol;3:145.
43. Wei A.D., Gutman G.A., Aldrich R., Chandy K.G., Grissmer S., Wulff H. (2005) International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. Pharmacol Rev;57:463–472.
44. Wei A., Jegla T., Salkoff L. (1996) Eight potassium channel families revealed by the C. elegans genome project. Neuropharmacology;35:805–829.
45. Meera P., Wallner M., Song M., Toro L. (1997) Large conductance voltage- and calcium-dependent K+ channel, a distinct member of voltage-dependent ion channels with seven N-terminal transmembrane segments (S0-S6), an extracellular N terminus, and an intracellular (S9-S10) C terminus. Proc Natl Acad Sci USA;94:14066–14071.
46. Schreiber M., Wei A., Yuan A., Gaut J., Saito M., Salkoff L. (1998) Slo3, a novel pH-sensitive K+ channel from mammalian spermatocytes. J Biol Chem;273:3509–3516.
47. Papazian D.M., Schwarz T.L., Tempel B.L., Jan Y.N., Jan L.Y. (1987) Cloning of genomic and complementary DNA from Shaker, a putative potassium channel gene from Drosophila. Science;237:749–753.
48. del Camino D., Kanevsky M., Yellen G. (2005) Status of the intracellular gate in the activated-not-open state of shaker K+ channels. J Gen Physiol;126:419–428.
49. del Camino D., Yellen G. (2001) Tight steric closure at the intracellular activation gate of a voltage-gated K(+) channel. Neuron;32:649–656.
50. Swartz K.J. (2004) Opening the gate in potassium channels. Nat Struct Mol Biol;11:499–501.
51. Doyle D.A., Morais Cabral J., Pfuetzner R.A., Kuo A., Gulbis J.M., Cohen S.L., Chait B.T., MacKinnon R. (1998) The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science;280:69–77.
52. Holmgren M., Shin K.S., Yellen G. (1998) The activation gate of a voltage-gated K+ channel can be trapped in the open state by an intersubunit metal bridge. Neuron;21:617–621.
53. Long S.B., Campbell E.B., Mackinnon R. (2005) Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. Science;309:897–903.
54. Long S.B., Campbell E.B., Mackinnon R. (2005) Voltage sensor of Kv1.2: structural basis of electromechanical coupling. Science;309:903–908.
55. Schow E.V., Freites J.A., Nizkorodov A., White S.H., Tobias D.J. (2012) Coupling between the voltage-sensing and pore domains in a voltage-gated potassium channel. Biochim Biophys Acta;1818:1726–1736.
56. Chen J., Mitcheson J.S., Tristani-Firouzi M., Lin M., Sanguinetti M.C. (2001) The S4-S5 linker couples voltage sensing and activation of pacemaker channels. Proc Natl Acad Sci USA;98:11277–11282.
57. Lu Z., Klem A.M., Ramu Y. (2001) Ion conduction pore is conserved among potassium channels. Nature;413:809–813.
58. Lee S.-Y., Banerjee A., MacKinnon R. (2009) Two separate interfaces between the voltage sensor and pore are required for the function of voltage-dependent K(+) channels. PLoS Biol;7:e47.
59. Choveau F.S., Rodriguez N., Abderemane Ali F., Labro A.J., Rose T., Dahimene S., Boudin H., Le Hénaff C., Escande D., Snyders D.J., Charpentier F., Mérot J., Baró I., Loussouarn G. (2011) KCNQ1 channels voltage dependence through a voltage-dependent binding of the S4-S5 linker to the pore domain. J Biol Chem;286:707–716.
60. Leonetti M.D., Yuan P., Hsiung Y., MacKinnon R. (2012) Functional and structural analysis of the human SLO3 pH- and voltage-gated K+ channel. Proc Natl Acad Sci USA;109:19274–19279.
61. Arrigoni C., Schroeder I., Romani G., Van Etten J.L., Thiel G., Moroni A. (2013) The voltage-sensing domain of a phosphatase gates the pore of a potassium channel. J Gen Physiol;141:389–395.
62. Barros F., Dominguez P., de la Pena P. (2012) Cytoplasmic domains and voltage-dependent potassium channel gating. Front Pharmacol;3:49.
63. Kearney J. (2013) Voltage-gated ion channel accessory subunits: sodium, potassium, or both? Epilepsy Curr;13:30–31.
64. Nakahira K., Shi G., Rhodes K.J., Trimmer J.S. (1996) Selective interaction of voltage-gated K+ channel

beta-subunits with alpha-subunits. J Biol Chem;271:7084–7089.
65. Wulff H., Zhorov B.S. (2008) K+ channel modulators for the treatment of neurological disorders and autoimmune diseases. Chem Rev;108:1744–1773.
66. Colenso C.K., Sessions R.B., Zhang Y.H., Hancox J.C., Dempsey C.E. (2013) Interactions between voltage sensor and pore domains in a hERG K(+) channel model from molecular simulations and the effects of a voltage sensor mutation. J Chem Inf Model;53:1358–1370.
67. Jensen M.O., Jogini V., Borhani D.W., Leffler A.E., Dror R.O., Shaw D.E. (2012) Mechanism of voltage gating in potassium channels. Science;336:229–233.
68. Strutz-Seebohm N., Pusch M., Wolf S., Stoll R., Tapken D., Gerwert K., Attali B., Seebohm G. (2011) Structural basis of slow activation gating in the cardiac I Ks channel complex. Cell Physiol Biochem;27:443–452.
69. Zhang Z.-H., Rhodes K.J., Childers W.E., Argentieri T.M., Wang Q. (2004) Disinactivation of N-type inactivation of voltage-gated K channels by an erbstatin analogue. J Biol Chem;279:29226–29230.
70. Lu Q., Peevey J., Jow F., Monaghan M.M., Mendoza G., Zhang H., Wu J., Kim C.Y., Bicksler J., Greenblatt L., Lin S.S., Childers W., Bowlby M.R. (2008) Disruption of Kv1.1 N-type inactivation by novel small molecule inhibitors (disinactivators). Bioorg Med Chem;16:3067–3075.
71. Yuan P., Leonetti M.D., Hsiung Y.C., MacKinnon R. (2012) Open structure of the Ca^{2+} gating ring in the high-conductance Ca^{2+}-activated K+ channel. Nature;481:94–U105.
72. Pantazis A., Kohanteb A.P., Olcese R. (2010) Relative motion of transmembrane segments S0 and S4 during voltage sensor activation in the human BK(Ca) channel. J Gen Physiol;136:645–657.
73. Jiang Y., Pico A., Cadene M., Chait B.T., MacKinnon R. (2001) Structure of the RCK domain from the E. coli K+ channel and demonstration of its presence in the human BK channel. Neuron;29:593–601.
74. Latorre R., Brauchi S. (2006) Large conductance Ca^{2+}-activated K+ (BK) channel: activation by Ca^{2+} and voltage. Biol Res;39:385–401.
75. Schreiber M., Salkoff L. (1997) A novel calcium-sensing domain in the BK channel. Biophys J;73:1355–1363.
76. Aydin M., Wang H.Z., Zhang X., Chua R., Downing K., Melman A., DiSanto M.E. (2012) Large-conductance calcium-activated potassium channel activity, as determined by whole-cell patch clamp recording, is decreased in urinary bladder smooth muscle cells from male rats with partial urethral obstruction. BJU Int;110:E402–E408.
77. Kim J.M., Beyer R., Morales M., Chen S., Liu L.Q., Duncan R.K. (2010) Expression of BK-type calcium-activated potassium channel splice variants during chick cochlear development. J Comp Neurol;518:2554–2569.
78. Bao L., Kaldany C., Holmstrand E.C., Cox D.H. (2004) Mapping the BKCa channel's "Ca^{2+} bowl": side-chains essential for Ca^{2+} sensing. J Gen Physiol;123:475–489.
79. Bao L., Rapin A.M., Holmstrand E.C., Cox D.H. (2002) Elimination of the BK(Ca) channel's high-affinity $Ca(^{2+})$ sensitivity. J Gen Physiol;120:173–189.
80. Kim H.-J., Lim H.-H., Rho S.-H., Bao L., Lee J.-H., Cox D.H., Kim do H., Park C.S. (2008) Modulation of the conductance-voltage relationship of the BK Ca channel by mutations at the putative flexible interface between two RCK domains. Biophys J;94:446–456.
81. Santarelli L.C., Wassef R., Heinemann S.H., Hoshi T. (2006) Three methionine residues located within the regulator of conductance for K+ (RCK) domains confer oxidative sensitivity to large-conductance Ca^{2+}-activated K+ channels. J Physiol;571:329–348.
82. Xia X.-M., Zeng X., Lingle C.J. (2002) Multiple regulatory sites in large-conductance calcium-activated potassium channels. Nature;418:880–884.
83. Hou S., Xu R., Heinemann S.H., Hoshi T. (2008) Reciprocal regulation of the Ca^{2+} and H+ sensitivity in the SLO1 BK channel conferred by the RCK1 domain. Nat Struct Mol Biol;15:403–410.
84. Yang H., Shi J., Zhang G., Yang J., Delaloye K., Cui J. (2008) Activation of Slo1 BK channels by Mg^{2+} coordinated between the voltage sensor and RCK1 domains. Nat Struct Mol Biol;15:1152–1159.
85. Contreras G.F., Neely A., Alvarez O., Gonzalez C., Latorre R. (2012) Modulation of BK channel voltage gating by different auxiliary beta subunits. Proc Natl Acad Sci USA;109:18991–18996.
86. Bukiya A.N., Singh A.K., Parrill A.L., Dopico A.M. (2011) The steroid interaction site in transmembrane domain 2 of the large conductance, voltage- and calcium-gated potassium (BK) channel accessory beta1 subunit. Proc Natl Acad Sci USA;108:20207–20212.
87. Marty A. (1981) Ca-dependent K channels with large unitary conductance in chromaffin cell membranes. Nature;291:497–500.
88. Pallotta B.S., Magleby K.L., Barrett J.N. (1981) Single channel recordings of Ca^{2+}-activated K+ currents in rat muscle cell culture. Nature;293:471–474.
89. Joiner W.J., Wang L.Y., Tang M.D., Kaczmarek L.K. (1997) hSK4, a member of a novel subfamily of calcium-activated potassium channels. Proc Natl Acad Sci USA;94:11013–11018.
90. Logsdon N.J., Kang J., Togo J.A., Christian E.P., Aiyar J. (1997) A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. J Biol Chem;272:32723–32726.
91. Ishii T.M., Silvia C., Hirschberg B., Bond C.T., Adelman J.P., Maylie J. (1997) A human intermediate conductance calcium-activated potassium channel. Proc Natl Acad Sci USA;94:11651–11656.

92. Li M.L., Li T., Lei M., Tan X.Q., Yang Y., Liu T.P., Pei J., Zeng X.R. (2011) Increased small conductance calcium-activated potassium channel (SK2 channel) current in atrial myocytes of patients with persistent atrial fibrillation. Zhonghua Xin Xue Guan Bing Za Zhi;39:147–151.
93. Shao Z., Makinde T.O., Agrawal D.K. (2011) Calcium-activated potassium channel KCa3.1 in lung dendritic cell migration. Am J Respir Cell Mol Biol;45:962–968.
94. Stocker M. (2004) Ca(2+)-activated K+ channels: molecular determinants and function of the SK family. Nat Rev Neurosci;5:758–770.
95. Faber E.S.L., Sah P. (2003) Calcium-activated potassium channels: multiple contributions to neuronal function. Neuroscientist;9:181–194.
96. Hirschberg B., Maylie J., Adelman J.P., Marrion N.V. (1998) Gating of recombinant small-conductance Ca-activated K+ channels by calcium. J Gen Physiol;111:565–581.
97. Monaghan A.S., Benton D.C.H., Bahia P.K., Hosseini R., Shah Y.A., Haylett D.G., Moss G.W. (2004) The SK3 subunit of small conductance Ca^{2+}-activated K+ channels interacts with both SK1 and SK2 subunits in a heterologous expression system. J Biol Chem;279:1003–1009.
98. Strassmaier T., Bond C.T., Sailer C.A., Knaus H.-G., Maylie J., Adelman J.P. (2005) A novel isoform of SK2 assembles with other SK subunits in mouse brain. J Biol Chem;280:21231–21236.
99. Xia X.M., Fakler B., Rivard A., Wayman G., Johnson-Pais T., Keen J.E., Ishii T., Hirschberg B., Bond C.T., Lutsenko S., Maylie J., Adelman J.P. (1998) Mechanism of calcium gating in small-conductance calcium-activated potassium channels. Nature;395:503–507.
100. Li W., Aldrich R.W. (2009) Activation of the SK potassium channel-calmodulin complex by nanomolar concentrations of terbium. Proc Natl Acad Sci USA;106:1075–1080.
101. Schumacher M.A., Crum M., Miller M.C. (2004) Crystal structures of apocalmodulin and an apocalmodulin/SK potassium channel gating domain complex. Structure;12:849–860.
102. Lee W.-S., Ngo-Anh T.J., Bruening-Wright A., Maylie J., Adelman J.P. (2003) Small conductance Ca^{2+}-activated K+ channels and calmodulin: cell surface expression and gating. J Biol Chem;278:25940–25946.
103. Keen J.E., Khawaled R., Farrens D.L., Neelands T., Rivard A., Bond C.T., Janowsky A., Fakler B., Adelman J.P., Maylie J. (1999) Domains responsible for constitutive and Ca^{2+}-dependent interactions between calmodulin and small conductance Ca^{2+} activated potassium channels. J Neurosci;19:8830–8838.
104. Weatherall K.L., Goodchild S.J., Jane D.E., Marrion N.V. (2010) Small conductance calcium-activated potassium channels: from structure to function. Prog Neurobiol;91:242–255.
105. Li W., Aldrich R.W. (2011) Electrostatic influences of charged inner pore residues on the conductance and gating of small conductance Ca^{2+} activated K+ channels. Proc Natl Acad Sci USA;108:5946–5953.
106. Yuan A., Santi C.M., Wei A., Wang Z.W., Pollak K., Nonet M., Kaczmarek L., Crowder C.M., Salkoff L. (2003) The sodium-activated potassium channel is encoded by a member of the Slo gene family. Neuron;37:765–773.
107. Goldstein S.A., Price L.A., Rosenthal D.N., Pausch M.H. (1996) ORK1, a potassium-selective leak channel with two pore domains cloned from Drosophila melanogaster by expression in Saccharomyces cerevisiae. Proc Natl Acad Sci USA;93:13256–13261.
108. Lesage F., Lazdunski M. (2000) Molecular and functional properties of two-pore-domain potassium channels. Am J Physiol Renal Physiol;279:F793–F801.
109. Innamaa A., Jackson L., Asher V., Van Shalkwyk G., Warren A.Hay D., Bali A., Sowter H., Khan R. (2013) Expression and prognostic significance of the oncogenic K2P potassium channel KCNK9 (TASK-3) in ovarian carcinoma. Anticancer Res;33:1401–1408.
110. Niemeyer M.I., Cid L.P., Pena-Munzenmayer G., Sepulveda F.V. (2010) Separate gating mechanisms mediate the regulation of K2P potassium channel TASK-2 by intra- and extracellular pH. J Biol Chem;285:16467–16475.
111. Schwingshackl A., Teng B., Ghosh M., West A.N., Makena P., Gorantla V., Sinclair S.E., Waters C.M. (2012) Regulation and function of the two-pore-domain (K2P) potassium channel Trek-1 in alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol;302:L93–L102.
112. Wells G.D., Tang Q.Y., Heler R., Tompkins-MacDonald G.J., Pritchard E.N., Leys S.P., Logothetis D.E., Boland L.M. (2012) A unique alkaline pH-regulated and fatty acid-activated tandem pore domain potassium channel (K(2)P) from a marine sponge. J Exp Biol;215:2435–2444.
113. Feliciangeli S., Bendahhou S., Sandoz G., Gounon P., Reichold M., Warth R., Lazdunski M, Barhanin J, Lesage F. (2007) Does sumoylation control K2P1/TWIK1 background K+ channels? Cell;130:563–569.
114. Rajan S., Plant L.D., Rabin M.L., Butler M.H., Goldstein S.A.N. (2005) Sumoylation silences the plasma membrane leak K+ channel K2P1. Cell;121:37–47.
115. Decressac S., Franco M., Bendahhou S., Warth R., Knauer S., Barhanin J., Lazdunski M., Lesage F. (2004) ARF6-dependent interaction of the TWIK1 K+ channel with EFA6, a GDP/GTP exchange factor for ARF6. EMBO Rep;5:1171–1175.
116. Girard C., Tinel N., Terrenoire C., Romey G., Lazdunski M., Borsotto M. (2002) p11, an annexin II subunit, an auxiliary protein associated with the background K+ channel, TASK-1. EMBO J;21:4439–4448.
117. Renigunta V., Yuan H., Zuzarte M., Rinne S., Koch A., Wischmeyer E., Schlichthörl G., Gao Y., Karschin

A., Jacob R., Schwappach B., Daut J., Preisig-Müller R. (2006) The retention factor p11 confers an endoplasmic reticulum-localization signal to the potassium channel TASK-1. Traffic;7:168–181.
118. O'Connell A.D., Morton M.J., Hunter M. (2002) Two-pore domain K+ channels-molecular sensors. Biochim Biophys Acta;1566:152–161.
119. Sandoz G., Thummler S., Duprat F., Feliciangeli S., Vinh J., Escoubas P., Guy N., Lazdunski M., Lesage F. (2006) AKAP150, a switch to convert mechano-, pH- and arachidonic acid-sensitive TREK K(+) channels into open leak channels. EMBO J;25:5864–5872.
120. Anazco C., Pena-Munzenmayer G., Araya C., Cid L.P., Sepulveda F.V., Niemeyer M.I. (2013) G protein modulation of K potassium channel TASK-2: a role of basic residues in the C terminus domain. Pflugers Arch; doi: 10.1007/s00424-013-1314-0.
121. Treptow W., Klein M.L. (2010) The membrane-bound state of K2P potassium channels. J Am Chem Soc;132:8145–8151.
122. Gonzalez W., Zuniga L., Cid L.P., Arevalo B., Niemeyer M.I., Sepulveda F.V. (2013) An extracellular ion pathway plays a central role in the cooperative gating of a K(2P) K+ channel by extracellular pH. J Biol Chem;288:5984–5991.
123. Zuniga L., Marquez V., Gonzalez-Nilo F.D., Chipot C., Cid L.P., Sepulveda F.V., Niemeyer M.I. (2011) Gating of a pH-sensitive K(2P) potassium channel by an electrostatic effect of basic sensor residues on the selectivity filter. PLoS One;6:e16141.
124. Durell S.R., Guy H.R. (2001) A family of putative Kir potassium channels in prokaryotes. BMC Evol Biol;1:14.
125. Dahal G.R., Rawson J., Gassaway B., Kwok B., Tong Y., Ptacek L.J., Bates E. (2012) An inwardly rectifying K+ channel is required for patterning. Development;139:3653–3664.
126. Heginbotham L., Lu Z., Abramson T., MacKinnon R. (1994) Mutations in the K+ channel signature sequence. Biophys J;66:1061–1067.
127. Preisig-Muller R., Schlichthorl G., Goerge T., Heinen S., Bruggemann A., Rajan S., Derst C., Veh R.W., Daut J. (2002) Heteromerization of Kir2.x potassium channels contributes to the phenotype of Andersen's syndrome. Proc Natl Acad Sci USA;99:7774–7779.
128. Schram G., Melnyk P., Pourrier M., Wang Z., Nattel S. (2002) Kir2.4 and Kir2.1 K(+) channel subunits coassemble: a potential new contributor to inward rectifier current heterogeneity. J Physiol;544:337–349.
129. Tang X., Schmidt T.M., Perez-Leighton C.E., Kofuji P. (2010) Inwardly rectifying potassium channel Kir4.1 is responsible for the native inward potassium conductance of satellite glial cells in sensory ganglia. Neuroscience;166:397–407.
130. Kloukina V., Herzer S., Karlsson N., Perez M., Daraio T., Meister B. (2012) G-protein-gated inwardly rectifying K+ channel 4 (GIRK4) immunoreactivity in chemically defined neurons of the hypothalamic arcuate nucleus that control body weight. J Chem Neuroanat;44:14–23.
131. Ishihara K., Mitsuiye T., Noma A., Takano M. (1989) The Mg^{2+} block and intrinsic gating underlying inward rectification of the K+ current in guinea-pig cardiac myocytes. J Physiol;419:297–320.
132. Kurachi Y. (1985) Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig heart. J Physiol;366:365–385.
133. Liu J.H., Bijlenga P., Fischer-Lougheed J., Occhiodoro T., Kaelin A., Bader C.R., Bernheim L. (1998) Role of an inward rectifier K+ current and of hyperpolarization in human myoblast fusion. J Physiol;510(Pt 2):467–476.
134. Lopatin A.N., Makhina E.N., Nichols C.G. (1995) The mechanism of inward rectification of potassium channels: "long-pore plugging" by cytoplasmic polyamines. J Gen Physiol;106:923–955.
135. Yamashita T., Horio Y., Yamada M., Takahashi N., Kondo C., Kurachi Y. (1996) Competition between Mg^{2+} and spermine for a cloned IRK2 channel expressed in a human cell line. J Physiol;493(Pt 1):143–156.
136. Hilgemann D.W., Ball R. (1996) Regulation of cardiac Na+, Ca^{2+} exchange and KATP potassium channels by PIP2. Science;273:956–959.
137. Hilgemann D.W., Feng S., Nasuhoglu C. (2001) The complex and intriguing lives of PIP2 with ion channels and transporters. Sci STKE;2001:re19.
138. Huang C.L., Feng S., Hilgemann D.W. (1998) Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by Gbetagamma. Nature;391:803–806.
139. Takano M., Kuratomi S. (2003) Regulation of cardiac inwardly rectifying potassium channels by membrane lipid metabolism. Prog Biophys Mol Biol;81:67–79.
140. Lopes C.M., Zhang H., Rohacs T., Jin T., Yang J., Logothetis D.E. (2002) Alterations in conserved Kir channel-PIP2 interactions underlie channelopathies. Neuron;34:933–944.
141. Ho I.H., Murrell-Lagnado R.D. (1999) Molecular determinants for sodium-dependent activation of G protein-gated K+ channels. J Biol Chem;274:8639–8648.
142. Petit-Jacques J., Sui J.L., Logothetis D.E. (1999) Synergistic activation of G protein-gated inwardly rectifying potassium channels by the betagamma subunits of G proteins and Na(+) and Mg(2+) ions. J Gen Physiol;114:673–684.
143. Lin D., Sterling H., Lerea K.M., Giebisch G., Wang W.H. (2002) Protein kinase C (PKC)-induced phosphorylation of ROMK1 is essential for the surface expression of ROMK1 channels. J Biol Chem;277:44278–44284.
144. Quinn K.V., Giblin J.P., Tinker A. (2004) Multisite phosphorylation mechanism for protein kinase A acti-

vation of the smooth muscle ATP-sensitive K+ channel. Circ Res;94:1359–1366.
145. Shi Y., Wu Z., Cui N., Shi W., Yang Y., Zhang X., Rojas A., Ha B.T., Jiang C. (2007) PKA phosphorylation of SUR2B subunit underscores vascular KATP channel activation by beta-adrenergic receptors. Am J Physiol Regul Integr Comp Physiol;293:R1205–R1214.
146. Mase Y., Yokogawa M., Osawa M., Shimada I. (2012) Structural basis for modulation of gating property of G protein-gated inwardly rectifying potassium ion channel (GIRK) by i/o-family G protein alpha subunit (Galphai/o). J Biol Chem;287:19537–19549.
147. Whorton M.R., MacKinnon R. (2013) X-ray structure of the mammalian GIRK2-betagamma G-protein complex. Nature;498:190–197.
148. Stansfeld P.J., Hopkinson R., Ashcroft F.M., Sansom M.S. (2009) PIP(2)-binding site in Kir channels: definition by multiscale biomolecular simulations. Biochemistry;48:10926–10933.
149. Rosenhouse-Dantsker A., Noskov S., Han H.Z., Adney S.K., Tang Q.Y., Rodriguez-Menchaca A.A., Kowalsky G.B., Petrou V.I., Osborn C.V., Logothetis D.E., Levitan I. (2012) Distant cytosolic residues mediate a two-way molecular switch that controls the modulation of inwardly rectifying potassium (Kir) channels by cholesterol and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P-2). J Biol Chem;287:40266–40278.
150. Malinowska D.H., Sherry A.M., Tewari K.P., Cuppoletti J. (2004) Gastric parietal cell secretory membrane contains PKA- and acid-activated Kir2.1 K+ channels. Am J Physiol Cell Physiol;286:C495–C506.
151. Shieh C.C., Coghlan M., Sullivan J.P., Gopalakrishnan M. (2000) Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol Rev;52:557–594.
152. Zhu J., Alsaber R., Zhao J., Ribeiro-Hurley E., Thornhill W.B. (2012) Characterization of the Kv1.1 I262T and S342I mutations associated with episodic ataxia 1 with distinct phenotypes. Arch Biochem Biophys;524:99–105.
153. Ishida S., Sakamoto Y., Nishio T., Baulac S., Kuwamura M., Ohno Y., Takizawa A., Kaneko S., Serikawa T., Mashimo T. (2012) Kcna1-mutant rats dominantly display myokymia, neuromyotonia and spontaneous epileptic seizures. Brain Res;1435:154–166.
154. Baloh R.W. (2012) Episodic ataxias 1 and 2. Handb Clin Neurol;103:595–602.
155. Peters C.J., Werry D., Gill H.S., Accili E.A., Fedida D. (2011) Mechanism of accelerated current decay caused by an episodic ataxia type-1-associated mutant in a potassium channel pore. J Neurosci;31:17449–17459.
156. Tomlinson S.E., Tan S.V., Kullmann D.M., Griggs R.C., Burke D., Hanna M.G., Bostock H. (2010) Nerve excitability studies characterize Kv1.1 fast potassium channel dysfunction in patients with episodic ataxia type 1. Brain;133:3530–3540.
157. Volkers L., Rook M.B., Das J.H.G., Verbeek N.E., Groenewegen W.A., van Kempen M.J.A., Lindhout D., Koeleman B.P. (2009) Functional analysis of novel KCNQ2 mutations found in patients with Benign Familial Neonatal Convulsions. Neurosci Lett;462:24–29.
158. Bellini G., Miceli F., Soldovieri M.V., Miraglia del Giudice E., Pascotto A., Taglialatela M. (editors) (1993–2013) GeneReviews™ [Internet]. Seattle, WA: University of Washington, Seattle. GeneReviews Authors. http://www.ncbi.nlm.nih.gov/books/NBK132723/.
159. Goldberg-Stern H., Kaufmann R., Kivity S., Afawi Z., Heron S.E. (2009) Novel mutation in KCNQ2 causing benign familial neonatal seizures. Pediatr Neurol;41:367–370.
160. Blumkin L., Suls A., Deconinck T., De Jonghe P., Linder I., Kivity S., Dabby R., Leshinsky-Silver E., Lev D., Lerman-Sagie T. (2012) Neonatal seizures associated with a severe neonatal myoclonus like dyskinesia due to a familial KCNQ2 gene mutation. Eur J Paediatr Neurol;16:356–360.
161. Sugiura Y., Nakatsu F., Hiroyasu K., Ishii A., Hirose S., Okada M., Jibiki I., Ohno H., Kaneko S., Ugawa Y. (2009) Lack of potassium current in W309R mutant KCNQ3 channel causing benign familial neonatal convulsions (BFNC). Epilepsy Res;84:82–85.
162. Li N., Li H., Jiang H., Shen L., Yan X., Guo J., Song Y. *et al.* (2010) Mutation detection in candidate genes for benign familial infantile seizures on a novel locus. Int J Neurosci;120:217–221.
163. Yum M.-S., Ko T.-S., Yoo H.-W. (2010) The first Korean case of KCNQ2 mutation in a family with benign familial neonatal convulsions. J Korean Med Sci;25:324–326.
164. Etcheberrigaray R., Ito E., Oka K., Tofel-Grehl B., Gibson G.E., Alkon D.L. (1993) Potassium channel dysfunction in fibroblasts identifies patients with Alzheimer disease. Proc Natl Acad Sci USA;90:8209–8213.
165. Angulo E., Noe V., Casado V., Mallol J., Gomez-Isla T., Lluis C., Ferrer I., Ciudad C.J., Franco R. (2004) Up-regulation of the Kv3.4 potassium channel subunit in early stages of Alzheimer's disease. J Neurochem;91:547–557.
166. Boda E., Hoxha E., Pini A., Montarolo F., Tempia F. (2012) Brain expression of Kv3 subunits during development, adulthood and aging and in a murine model of Alzheimer's disease. J Mol Neurosci;46:606–615.
167. Maezawa I., Jenkins D.P., Jin B.E., Wulff H. (2012) Microglial KCa3.1 channels as a potential therapeutic target for Alzheimer's disease. Int J Alzheimers Dis;2012:868972.
168. Gui Y.-X., Wan Y., Xiao Q., Wang Y., Wang G., Chen S.-D. (2011) Verification of expressions of Kir2 as potential peripheral biomarkers in lymphocytes from patients with Parkinson's disease. Neurosci Lett;505:104–108.

169. Deutch A.Y., Winder D.G. (2006) A channel to neurodegeneration. Nat Med;12:17–18.
170. Wang S., Hu L.-F., Yang Y., Ding J.-H., Hu G. (2005) Studies of ATP-sensitive potassium channels on 6-hydroxydopamine and haloperidol rat models of Parkinson's disease: implications for treating Parkinson's disease? Neuropharmacology;48:984–992.
171. Wu C., Yang K., Liu Q., Wakui M., Jin G.-Z., Zhen X., Wu J. (2010) Tetrahydroberberine blocks ATP-sensitive potassium channels in dopamine neurons acutely-dissociated from rat substantia nigra pars compacta. Neuropharmacology;59:567–572.
172. Sander S.E., Lemm C., Lange N., Hamann M., Richter A. (2012) Retigabine, a K(V)7 (KCNQ) potassium channel opener, attenuates L-DOPA-induced dyskinesias in 6-OHDA-lesioned rats. Neuropharmacology;62:1052–1061.
173. Marban E. (2002) Cardiac channelopathies. Nature;415:213–218.
174. Curran M.E., Splawski I., Timothy K.W., Vincent G.M., Green E.D., Keating M.T. (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell;80:795–803.
175. Brugada R., Hong K., Dumaine R., Cordeiro J., Gaita F., Borggrefe M., Menendez T.M. *et al*. (2004) Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation;109:30–35.
176. Chen Y.H., Xu S.J., Bendahhou S., Wang X.L., Wang Y., Xu W.Y., Jin H.W. *et al*. (2003) KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science;299:251–254.
177. Kraushaar U., Meyer T., Hess D., Gepstein L., Mummery C.L., Braam S.R., Guenther E. (2012) Cardiac safety pharmacology: from human ether-a-gogo related gene channel block towards induced pluripotent stem cell based disease models. Expert Opin Drug Saf;11:285–298.
178. Martin C.A., Matthews G.D., Huang C.L. (2012) Sudden cardiac death and inherited channelopathy: the basic electrophysiology of the myocyte and myocardium in ion channel disease. Heart;98:536–543.
179. Hoppe U.C., Marban E., Johns D.C. (2001) Distinct gene-specific mechanisms of arrhythmia revealed by cardiac gene transfer of two long QT disease genes, HERG and KCNE1. Proc Natl Acad Sci USA;98:5335–5340.
180. Splawski I., Shen J., Timothy K.W., Lehmann M.H., Priori S., Robinson J.L., Moss A.J., Schwartz P.J., Towbin J.A., Vincent G.M., Keating M.T. (2000) Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation;102:1178–1185.
181. Keating M.T., Sanguinetti M.C. (2001) Molecular and cellular mechanisms of cardiac arrhythmias. Cell;104:569–580.
182. Plaster N.M., Tawil R., Tristani-Firouzi M., Canun S., Bendahhou S., Tsunoda A., Donaldson M.R. *et al*. (2001) Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. Cell;105:511–519.
183. Brelidze T.I., DiMaio F., Trudeau M.C., Zagotta W.N. (2013) Structure of the C-Terminal region of an ERG channel and functional implications for long-QT syndrome. Biophys J;104:356a-a.
184. Giudicessi J.R., Ye D., Kritzberger C.J., Nesterenko V.V., Tester D.J., Antzelevitch C., Ackerman M.J. (2012) Novel mutations in the KCND3-encoded Kv4.3 K+ channel associated with autopsy-negative sudden unexplained death. Hum Mutat;33:989–997.
185. Bellocq C., van Ginneken A.C., Bezzina C.R., Alders M., Escande D., Mannens M.M., Baró I., Wilde A.A. (2004) Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. Circulation;109:2394–2397.
186. Marban E. (1999) Heart failure: the electrophysiologic connection. J Cardiovasc Electrophysiol;10:1425–1428.
187. Camm A.J., Janse M.J., Roden D.M., Rosen M.R., Cinca J., Cobbe S.M. (2000) Congenital and acquired long QT syndrome. Eur Heart J;21:1232–1237.
188. Sanguinetti M.C., Jiang C., Curran M.E., Keating M.T. (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell;81:299–307.
189. Roden D.M., Spooner P.M. (1999) Inherited long QT syndromes: a paradigm for understanding arrhythmogenesis. J Cardiovasc Electrophysiol;10:1664–1683.
190. Roden D.M. (2001) Pharmacogenetics and drug-induced arrhythmias. Cardiovasc Res;50:224–231.
191. Mazhari R., Greenstein J.L., Winslow R.L., Marban E., Nuss H.B. (2001) Molecular interactions between two long-QT syndrome gene products, HERG and KCNE2, rationalized by in vitro and in silico analysis. Circ Res;89:33–38.
192. Tester D.J., Tan B.H., Medeiros-Domingo A., Song C., Makielski J.C., Ackerman M.J. (2011) Loss-of-function mutations in the KCNJ8-encoded Kir6.1 K (ATP) channel and sudden infant death syndrome. Circ Cardiovasc Genet;4:510–515.
193. Charmandari E., Sertedaki A., Kino T., Merakou C., Hoffman D.A., Hatch M.M., Hurt D.E., Lin L., Xekouki P., Stratakis C.A., Chrousos G.P. (2012) A novel point mutation in the KCNJ5 gene causing primary hyperaldosteronism and early-onset autosomal dominant hypertension. J Clin Endocrinol Metab;97:E1532–E1539.
194. Jepps T.A., Chadha P.S., Davis A.J., Harhun M.I., Cockerill G.W., Olesen S.P., Hansen R.S., Greenwood I.A. (2011) Downregulation of Kv7.4 channel activity in primary and secondary hypertension. Circulation;124:602–611.
195. Guagliardo N.A., Yao J., Hu C., Schertz E.M., Tyson D.A., Carey R.M., Bayliss D.A., Barrett P.Q. (2012) TASK-3 channel deletion in mice recapitulates low-

renin essential hypertension. Hypertension;59:999–1005.
196. Jung J., Barrett P.Q., Eckert G.J., Edenberg H.J., Xuei X., Tu W., Pratt J.H. (2012) Variations in the potassium channel genes KCNK3 and KCNK9 in relation to blood pressure and aldosterone production: an exploratory study. J Clin Endocrinol Metab;97:E2160–E2167.
197. Lloyd E.E., Crossland R.F., Phillips S.C., Marrelli S.P., Reddy A.K., Taffet G.E., Hartley C.J., Bryan R.M. Jr (2011) Disruption of K(2P)6.1 produces vascular dysfunction and hypertension in mice. Hypertension;58:672–678.
198. Heran B.S., Chen J.M., Wang J.J., Wright J.M. (2012) Blood pressure lowering efficacy of potassium-sparing diuretics (that block the epithelial sodium channel) for primary hypertension. Cochrane Database Syst Rev;11:CD008167.
199. Jiang L., Zhou T., Liu H. (2012) Combined effects of the ATP-sensitive potassium channel opener pinacidil and simvastatin on pulmonary vascular remodeling in rats with monocrotaline-induced pulmonary arterial hypertension. Pharmazie;67:547–552.
200. Jin Y., Xie W.P., Wang H. (2012) [Hypoxic pulmonary hypertension and novel ATP-sensitive potassium channel opener: the new hope on the horizon]. Zhongguo Ying Yong Sheng Li Xue Za Zhi;28:510–523.
201. Penton D., Bandulik S., Schweda F., Haubs S., Tauber P., Reichold M., Cong L.D., El Wakil A., Budde T., Lesage F., Lalli E., Zennaro M.C., Warth R., Barhanin J. (2012) Task3 potassium channel gene invalidation causes low renin and salt-sensitive arterial hypertension. Endocrinology;153:4740–4748.
202. Dupont J., Pereira C., Medeira A., Duarte R., Ellard S., Sampaio L. (2012) Permanent neonatal diabetes mellitus due to KCNJ11 mutation in a Portuguese family: transition from insulin to oral sulfonylureas. J Pediatr Endocrinol Metab;25:367–370.
203. Okamoto K., Iwasaki N., Nishimura C., Doi K., Noiri E., Nakamura S. *et al.* (2010) Identification of KCNJ15 as a susceptibility gene in Asian patients with type 2 diabetes mellitus. Am J Hum Genet;86:54–64.
204. He W., Liu W., Chew C.S., Baker S.S., Baker R.D., Forte J.G., Zhu L. (2011) Acid secretion-associated translocation of KCNJ15 in gastric parietal cells. Am J Physiol Gastrointest Liver Physiol;301:G591–G600.
205. Okamoto K., Iwasaki N., Doi K., Noiri E., Iwamoto Y., Uchigata Y., Fujita T., Tokunaga K. (2012) Inhibition of glucose-stimulated insulin secretion by KCNJ15, a newly identified susceptibility gene for type 2 diabetes. Diabetes;61:1734–1741.
206. Wagner C.A. (2010) New roles for renal potassium channels. J Nephrol;23:5–8.
207. Flagg T.P., Yoo D., Sciortino C.M., Tate M., Romero M.F., Welling P.A. (2002) Molecular mechanism of a COOH-terminal gating determinant in the ROMK channel revealed by a Bartter's disease mutation. J Physiol;544:351–362.
208. Xu R., Cao M., Wu X., Wang X., Ruan L., Quan X., Lü C., He W., Zhang C. (2012) Kv1.3 channels as a potential target for immunomodulation of CD4+ CD28null T cells in patients with acute coronary syndrome. Clin Immunol;142:209–217.
209. Feng D.Y., Zhang C.T., Ma Y.X., Zhou H.L., Xu R.D., Yang X.W., Huang S., Ma J., Quan X.Q. (2009) Kv1.3 potassium channel expression changes after CD4(+) and subsets CD28(null)/CD28(+)T cells activation in peripheral blood of patients with acute coronary syndrome. Zhonghua Xin Xue Guan Bing Za Zhi;37:599–604.
210. Palmer B.R., Frampton C.M., Skelton L., Yandle T.G., Doughty R.N., Whalley G.A., Ellis C.J., Troughton R.W., Richards A.M., Cameron V.A. (2012) KCNE5 polymorphism rs697829 is associated with QT interval and survival in acute coronary syndromes patients. J Cardiovasc Electrophysiol;23:319–324.
211. Reichold M., Zdebik A.A., Lieberer E., Rapedius M., Schmidt K., Bandulik S., Sterner C. (2010) KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. Proc Natl Acad Sci USA;107:14490–14495.
212. Sala-Rabanal M., Kucheryavykh L.Y., Skatchkov S.N., Eaton M.J., Nichols C.G. (2010) Molecular mechanisms of EAST/SeSAME syndrome mutations in Kir4.1 (KCNJ10). J Biol Chem;285:36040–36048.
213. Williams D.M., Lopes C.M., Rosenhouse-Dantsker A., Connelly H.L., Matavel A., O-Uchi J., McBeath E., Gray D.A. (2010) Molecular basis of decreased Kir4.1 function in SeSAME/EAST syndrome. J Am Soc Nephrol;21:2117–2129.
214. Bandulik S., Schmidt K., Bockenhauer D., Zdebik A.A., Humberg E., Kleta R., Warth R., Reichold M. (2011) The salt-wasting phenotype of EAST syndrome, a disease with multifaceted symptoms linked to the KCNJ10 K+ channel. Pflugers Arch;461:423–435.
215. Thompson D.A., Feather S., Stanescu H.C., Freudenthal B., Zdebik A.A., Warth R., Ognjanovic M., Hulton S.A., Wassmer E., van't Hoff W., Russell-Eggitt I., Dobbie A., Sheridan E., Kleta R., Bockenhauer D. (2011) Altered electroretinograms in patients with KCNJ10 mutations and EAST syndrome. J Physiol;589:1681–1689.
216. Paulais M., Bloch-Faure M., Picard N., Jacques T., Ramakrishnan S.K., Keck M., Sohet F., Eladari D., Houillier P., Lourdel S., Teulon J., Tucker S.J. (2011) Renal phenotype in mice lacking the Kir5.1 (Kcnj16) K+ channel subunit contrasts with that observed in SeSAME/EAST syndrome. Proc Natl Acad Sci USA;108:10361–10366.
217. Choi A., Choi J.S., Yoon Y.J., Kim K.A., Joo C.K. (2009) KR-31378, a potassium-channel opener,

induces the protection of retinal ganglion cells in rat retinal ischemic models. J Pharmacol Sci;109: 511–517.
218. Diego-Garcia E., Peigneur S., Debaveye S., Gheldof E., Tytgat J., Caliskan F. (2013) Novel potassium channel blocker venom peptides from Mesobuthus gibbosus (Scorpiones: Buthidae). Toxicon;61:72–82.
219. Girodet P.O., Ozier A., Carvalho G., Ilina O., Ousova O., Gadeau A.P., Begueret H., Wulff H., Marthan R., Bradding P., Berger P. (2013) Ca(2+)-activated K(+) channel-3.1 blocker TRAM-34 attenuates airway remodeling and eosinophilia in a murine asthma model. Am J Respir Cell Mol Biol;48:212–219.
220. Juang J.H., Kuo C.H. (2010) Effects of cyclooxygenase-2 inhibitor and adenosine triphosphate-sensitive potassium channel opener in syngeneic mouse islet transplantation. Transpl Proc;42:4221–4224.
221. Lewis L.M., Bhave G., Chauder B.A., Banerjee S., Lornsen K.A., Redha R., Fallen K., Lindsley C.W., Weaver C.D., Denton J.S. (2009) High-throughput screening reveals a small-molecule inhibitor of the renal outer medullary potassium channel and Kir7.1. Mol Pharmacol;76:1094–1103.
222. Miller M.R., Zou B., Shi J., Flaherty D.P., Simpson D.S., Yao T., Maki B.E., Day V.W., Douglas J.T., Wu M., McManus O.B., Golden J.E., Aubé J., Li M. (2010) Development of a Selective Chemical Inhibitor for the Two-Pore Potassium Channel, KCNK9. Probe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda, MD: National Center for Biotechnology Information (US).
223. Pan Z., Huang J., Cui W., Long C., Zhang Y., Wang H. (2010) Targeting hypertension with a new adenosine triphosphate-sensitive potassium channel opener iptakalim. J Cardiovasc Pharm;56:215–228.
224. Sudo H., Yogo K., Ishizuka N., Otsuka H., Horie S., Saito K. (2008) Nicorandil, a potassium channel opener and nitric oxide donor, improves the frequent urination without changing the blood pressure in rats with partial bladder outlet obstruction. Biol Pharm Bull;31:2079–2082.
225. Moczydlowski E., Lucchesi K., Ravindran A. (1988) An emerging pharmacology of peptide toxins targeted against potassium channels. J Membr Biol;105:95–111.
226. Robertson D.W., Steinberg M.I. (1990) Potassium channel modulators: scientific applications and therapeutic promise. J Med Chem;33:1529–1541.
227. MacKinnon R., Miller C. (1989) Mutant potassium channels with altered binding of charybdotoxin, a pore-blocking peptide inhibitor. Science;245:1382–1385.
228. MacKinnon R., Heginbotham L., Abramson T. (1990) Mapping the receptor site for charybdotoxin, a pore-blocking potassium channel inhibitor. Neuron;5:767–771.
229. Swartz K.J., MacKinnon R. (1997) Hanatoxin modifies the gating of a voltage-dependent K+ channel through multiple binding sites. Neuron;18:665–673.
230. Swartz K.J. (2007) Tarantula toxins interacting with voltage sensors in potassium channels. Toxicon;49:213–230.
231. Lipinski C.A., Lombardo F., Dominy B.W., Feeney P.J. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev;23:3–25.
232. Newman D.J., Cragg G.M. (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod;70:461–477.
233. Zhu F., Qin C., Tao L., Liu X., Shi Z., Ma X., Jia J., Tan Y., Cui C., Lin J., Tan C., Jiang Y., Chen Y. (2011) Clustered patterns of species origins of nature-derived drugs and clues for future bioprospecting. Proc Natl Acad Sci USA;108:12943–12948.
234. Barnes E.C., Choomuenwai V., Andrews K.T., Quinn R.J., Davis R.A. (2012) Design and synthesis of screening libraries based on the muurolane natural product scaffold. Org Biomol Chem;10:4015–4023.
235. Sharma U., Srivastava S., Prakesch M., Sharma M., Leek D.M., Arya P. (2006) Part 2: building diverse natural-product-like architectures from a tetrahydroaminoquinoline scaffold. Modular solution- and solid-phase approaches for use in high-throughput generation of chemical probes. J Comb Chem;8: 735–761.
236. Messer R., Schmitz A., Moesch L., Haner R. (2004) Elaboration of D-(-)-ribose into a tricyclic, natural product-like scaffold. J Org Chem;69:8558–8560.
237. Kulkarni B.A., Roth G.P., Lobkovsky E., Porco J.A. Jr (2002) Combinatorial synthesis of natural product-like molecules using a first-generation spiroketal scaffold. J Comb Chem;4:56–72.
238. Molecular Operation Environment (2008) Molecular Operation Environment. Montreal, QC, Canada: Chemical Computing Group Inc.
239. Hilal S.H., Karickhoff S.W., Carreira L.A. (1995) A rigorous test for SPARC's chemical reactivity models: estimation of more than 4300 ionization pK(a)s. Quant Struct-Act Rel;14:348–355.
240. Xu J., Stevenson J. (2000) Drug-like index: a new approach to measure drug-like compounds and their diversity. J Chem Inf Comput Sci;40:1177–1187.
241. Raja M., Olrichs N.K., Vales E., Schrempf H. (2012) Transferring knowledge towards understanding the pore stabilizing variations in K(+) channels: pore stability in K(+) channels. J Bioenerg Biomembr;44:199–205.
242. Marionneau C., Townsend R.R., Nerbonne J.M. (2011) Proteomic analysis highlights the molecular complexities of native Kv4 channel macromolecular complexes. Semin Cell Dev Biol;22:145–152.
243. Nadal M.S., Ozaita A., Amarillo Y., Vega-Saenz de Miera E., Ma Y., Mo W., Goldberg E.M., Misumi Y., Ikehara Y., Neubert T.A., Rudy B. (2003) The CD26-related dipeptidyl aminopeptidase-like protein DPPX

is a critical component of neuronal A-type K+ channels. Neuron;37:449–461.
244. Park K.S., Yang J.W., Seikel E., Trimmer J.S. (2008) Potassium channel phosphorylation in excitable cells: providing dynamic functional variability to a diverse family of ion channels. Physiology (Bethesda);23:49–57.
245. Sandoz G., Lesage F. (2008) Protein complex analysis of native brain potassium channels by proteomics. Methods Mol Biol;491:113–123.
246. Huang Q., Jin H.X., Liu Q., Wu Q., Kang H., Cao Z.W., Zhu R. (2012) Proteochemometric modeling of the bioactivity spectra of HIV-1 protease inhibitors by introducing protein-ligand interaction fingerprint. PLoS One;7:e41698. doi: 10.1371/journal.pone.0041698.
247. Kang H., Sheng Z., Zhu R.X., Huang Q., Liu Q., Cao Z.W. (2012) Virtual drug screen schema based on multiview similarity integration and ranking aggregation. J Chem Inf Model;52:834–843.
248. Wu D.F., Huang Q., Zhang Y.D., Zhang Q.C., Liu Q., Gao J., Cao Z., Zhu R. (2012) Screening of selective histone deacetylase inhibitors by proteochemometric modeling. BMC Bioinformatics; doi: 10.1186/1471-2105-13-212.
249. Wu Q., Kang H., Tian C., Huang Q., Zhu R.X. (2013) Binding mechanism of inhibitors to CDK5/p25 complex: free energy calculation and ranking aggregation analysis. Mol Inform;32:251–260.
250. Rapposelli S. (2011) Novel adenosine 5′-triphosphate-sensitive potassium channel ligands: a patent overview (2005–2010). Expert Opin Ther Pat;21:355–379.
251. Gaborit N., Le Bouter S., Szuts V., Varro A., Escande D., Nattel S., Demolombe S. (2007) Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. J Physiol;582:675–693.
252. Tanaka H., Namekata I., Hamaguchi S., Kawamura T., Masuda H., Tanaka Y., Iida-Tanaka N., Takahara A. (2010) Effect of NIP-142 on potassium channel alpha-subunits Kv1.5, Kv4.2 and Kv4.3, and mouse atrial repolarization. Biol Pharm Bull;33:138–141.
253. Wang Y., Xu H., Kumar R., Tipparaju S.M., Wagner M.B., Joyner R.W. (2003) Differences in transient outward current properties between neonatal and adult human atrial myocytes. J Mol Cell Cardiol;35:1083–1092.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1–S18. Chemical structure of K channel modulators (divided into openers, blockers, multitarget blockers and specific-target blockers) categorized by natural-product scaffold.

Table S1. Potassium channels and related information on genes, proteins, organs, and modulators.

Table S2. Number of potassium channels in different organs.

Table S3. Classification with chemical scaffold of natural product for blockers and openers.

Table S4. Classification with chemical scaffold of natural product for multitarget and specific-target blockers.