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GENETIC DEFECTS IN THE HOTSPOT OF INWARDLY RECTIFYING K+ (Kir) CHANNELS AND THEIR METABOLIC CONSEQUENCES: A REVIEW

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Abstract

Inwardly rectifying potassium (Kir) channels are essential for maintaining normal potassium homeostasis and the resting membrane potential. As a consequence, mutations in Kir channels cause debilitating diseases ranging from cardiac failure to renal, ocular, pancreatic, and neurological abnormalities. Structurally, Kir channels consist of two trans-membrane domains, a pore-forming loop that contains the selectivity filter and two cytoplasmic polar tails. Within the cytoplasmic structure, clusters of amino acid sequences form regulatory domains that interact with cellular metabolites to control the opening and closing of the channel. In this review, we present an overview of Kir channel function and recent progress in the characterization of selected Kir channel mutations that lie in and near a C-terminal cytoplasmic 'hotspot' domain. The resultant molecular mechanisms by which the loss or gain of channel function leads to organ failure provide potential opportunities for targeted therapeutic interventions for this important group of channelopathies.

Keywords

Inwardly rectifying potassium channel (Kir); Channelopathy; Andersen-Tawil syndrome; Bartter syndrome; DEND syndrome; EAST/SeSAME syndrome; Phosphoinositides; KCNJ; cytoplasmic bPbbb cluster; retinopathy

Introduction

In multi-cellular organisms, cells are characterized by energetically favorable gradient moving potassium from the intracellular to the extracellular environment. Inwardly rectifying potassium-selective (Kir), channels encoded by the *KCNJ* gene family are constitutively active and favor the influx of potassium more readily than its efflux from the cells, thereby maintaining potassium homeostasis. Kir channels are also known as IRK or

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KCNJ channels. Fifteen mammalian *KCNJ* gene products have been described which result in seven distinct Kir channels [1, 2]. These channels are located within the plasma membrane of most cell types, where they regulate membrane potential and potassium homeostasis (Table 1). Kir channels contribute to functions such as the repolarization of cardiac action potentials, trans-epithelial transport, and the maintainance of the voltage gradient across the cell membrane. These functions are achieved by regulating the opening and closing (i.e., gating) of Kir channels [2]. For this reason, genetic alterations in Kir channels underlie many of the hereditary ion channel diseases known as channelopathies, and which affect the function of multiple organ systems [3, 4].

Kir channel structure and the intracellular regulatory "hotspot"

High-resolution structural characterization of Kir channels predicts that their protein subunits consist of an N-terminal cytoplasmic domain followed by a trans-membrane domain, then by a pore-forming, P-loop sequence that includes the selectivity filter, followed in turn by a second trans-membrane domain, and lastly, by a C-terminal cytoplasmic domain [5, 6] (Figure 1). Four such subunits interact to form a tetramer that creates a single-pore channel. The channel may be either homo- or hetero-tetrameric [7]. A subgroup of Kir channels conduct K^+ ions into the cells most effectively (named strong inward rectifiers) whereas others more modestly facilitate the efflux of K^+ (mild inward rectifiers: Kir4.1 and Kir7.1) additionally (Figure 2). The crystal structure of a eukaryotic Kir channel (Chicken Kir 2.2) showed that in the case of the strong inward rectifiers, binding of polyvalent cations like Mg^{2+} and polyamines to concentric rings of acidic amino acids on the inner face of the pore block K^+ efflux out of the cell [6]. The cytoplasmic portion of the channel thus serves as a site for regulatory modifications that result in the opening or closing of the channel [6, 8]. Cytoplasmic sequences of Kir channels possess multiple binding sites for intracellular regulators such as H^+, Mg^{2+}, ATP, phosphoinositides, membrane cholesterol, long chain acyl Coenzyme A, polyamines, and protein kinases A and C [9–22]. Trans-Golgi trafficking and signal sequences [23] are also found primarily in the cytoplasmic distal C-terminal sequence. Several genetic mutations have been reported to affect Kir channel conductance, either through a gain-of-function or a loss-of-function, thereby affecting potassium conductance and resulting in alterations in the current-voltage relationship (Figure 2) affecting cellular physiology.

Phosphoinositides, e.g. PIP_2, are important regulators of Kir channel function [24–27]. PIP_2 is found in the cytoplasmic leaflet of the plasma membrane. The distribution of this inositol phosphate is dynamic, and is precisely controlled by lipid kinases, phospholipases and phosphatases [28]. D'Avanzo and colleagues have recently demonstrated that PIP_2 in the eukaryotic cell membrane serves as an evolutionary adaptation for the direct activation of Kir channels by PIP_2 [29]. A cluster of positively charged amino acid residues in the C-terminal cytoplasmic domain creates a site that supports an electrostatic interaction between the Kir channel and the PIP_2 head group [24] (Figure 1). This cytoplasmic 'hotspot' is defined by a cluster of basic amino acids known as the **bPbbb** cluster, wherein **b** represents a basic amino acid residue and **P** represents proline, a polar uncharged residue. This 'hotspot' is found near the inner plasma membrane leaflet at the beginning of the C-terminal cytoplasmic domain, immediately following the second trans-membrane domain (Figure 1). Although mutations in any aspect of the protein structure may result in channel dysfunction, in this review we will focus on those reported mutations that lie in or near to the bPbbb hotspot (Table 2).

The various members of the Kir family can be subdivided into three distinct groups based upon their sensitivity to PIP_2 regulation of channel function, with low (Kir3.1 and Kir6.1), intermediate (Kir1.1 and Kir7.1) and high (Kir2.1 and 4.1) sensitivity defined by

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phosphoinositide binding specificity [30]. Phosphoinositide specificity cannot be predicted by the amino acid signature of the positively charged 'hotspot', nor does phosphoinositide binding specificity determine the degree of inward rectification. For example, Kir2.1 is a strong inward rectifier whereas Kir4.1 is a weak inward rectifier, but both are highly sensitive to the regulatory effects of PIP_2 [31]. The prokaryotic bacterial Kir channel KirBac 1.1 is inhibited by PIP_2 and lacks the regulatory residues that are conserved in the transmembrane-cytoplasmic linkers of eukaryotes whose displacement upon electrostatic interaction with PIP_2 gates eukaryotic Kir channels [29]. The Kir family and its modifiers therefore provide a sensitive and specific partnership that contributes to the regulation of a number of metabolic pathways.

In this review, we will focus on the correlation between genetic alterations that lie within and around the cytoplasmic 'hotspot' cluster of positively charged residues (Figure 1), and the metabolic consequences of the resultant Kir-associated channelopathies.

Defects in Kir channel function due to mutations within the 'hotspot'

Kir1.1 (*KCNJ1*)

The *KCNJ1* gene encodes Kir1.1, also known as the Renal Outer Medullary K^+ channel (ROMK) [1, 32, 33]. Kir 1.1 is localized to kidney epithelial cells [34] and plays a crucial role in reabsorbing salt via potassium recycling in the kidney at the level of the thick ascending limb (TAL) of Henle's loop [35] (Figure 3). Kir1.1 works in conjunction with a Na^+-K^+-$2Cl^-$ co-transporter (NKCC2) to ensure proper salt and water transport between cells of the TAL and the lumen of the renal tubule. Mutations in genes encoding NKCC2 and Kir1.1 result in altered function of these ion channels and are a cause of Hyperprostaglandin E syndrome, an autosomal recessive disorder also known as Bartter syndrome (Table 2). Bartter syndrome has both neonatal and classic forms, with the classic form typically presenting in school-age children. Bartter syndrome is characterized by hypokalemic alkalosis, hyperprostaglandinuria, and hypercalciuria associated with nephrocalcinosis. The neonatal form is associated with polyhydramnios which may lead to premature birth. Affected infants suffer severe postnatal salt and water losses that can lead to life-threatening dehydration [36].

One mutation in the *KCNJ1* gene that has been associated with Bartter syndrome lies within the C-terminal cytoplasmic domain of the Kir1.1 protein, slightly upstream from the 'hotspot', and results in a nonpolar, hydrophobic Alanine at position 177 being changed to a polar Threonine residue (**A177T**). Electrophysiological analyses show a decrease in K^+ conductance by the mutant channel that is either due to disrupted channel assembly or due to altered channel conformation within the pore-forming domain [37] (Figure 3). Mutations within the 'hotspot' of the Kir1.1 channel affect PIP_2 binding but have not been associated with a disease phenotype to-date [27, 31, 38].

Kir2.1 (*KCNJ2*)

The *KCNJ2* gene encodes the protein subunits of the Kir2.1 channel [1]. Kir 2.1 is highly expressed in cardiac and skeletal muscle in addition to neural tissue [39] (Figure 3). It plays a crucial role in determining the resting membrane potential and in controlling the duration of action potentials in excitable cells [40, 41]. In cardiac myocytes, the steep inwardly rectifying K^+-current via the Kir2.1 channel is responsible for the terminal, phase 3 repolarization of the action potential [42]. Loss of Kir2.1 current lengthens the ventricular action potential and prolongs the QT interval, putting affected individuals at risk of developing ventricular tachyarrhythmias [41]. Mutations within the 'hotspot' in the *KCNJ2* gene are associated with Andersen-Tawil syndrome (ATS) [43], a rare autosomal dominant disease [44] that is characterized by cardiac arrhythmias, periodic paralysis, short stature,

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and dysmorphic features that include cleft palate, low-set ears, and limb abnormalities (syndactyly, bradydactyly, clinodactyly) [41, 45, 46] (Figure 3). No genotype-phenotype correlations have been described between the mutations and the various clinical features of ATS.

Three loss-of-function mutations have been described in the *KCNJ2* gene. All three occur in and around the highly conserved PIP_2-binding domain and result in an amino acid with an electron dense R-group being converted to a non-polar group. The altered electrostatic interactions between the channel and PIP_2 result in abnormal channel function. Two of the mutations lie within the C-terminal cytoplasmic 'hotspot' of the Kir2.1 protein and exhibit decreased affinity of Kir2.1 for PIP_2, which may explain the loss of channel function [Table 2] [40].

KCNJ2 mutation **T192A** converts a polar, hydrophilic Threonine to a nonpolar, hydrophobic Alanine at amino acid position 192, slightly downstream from the cytoplasmic 'hotspot' [32]. Heteromeric channels (possessing both wild-type and **T192A** mutant subunits) have partial levels of Kir2.1-mediated current, whereas homomeric **T192A** Kir2.1 channels have a complete loss of function [47]. It is likely that the clinical findings in ATS in its most severe form are due to abolished Kir2.1-PIP_2 interactions and a nonfunctional channel, whereas mutations that simply result in reduced activity of Kir2.1 lead to the less severe forms of ATS [48].

When there is complete loss of Kir2.1 channel activity, an increased frequency of spontaneous action potentials is seen that is likely triggered by altered function of the Na^+/Ca^{2+} exchanger [48] (Figure 3). Reduction in potassium current in ventricular myocytes leads to spontaneous ventricular activity. The cardiac arrhythmia observed in ATS results when the cardiac action potential is prolonged due to a reduction or absence of the repolarizing current normally attributed to Kir2.1 activity [49]. In skeletal muscle, reduced Kir2.1 activity depolarizes the resting membrane potential through inactivation of Na^+ channel function and results in the periodic paralysis experienced by some individuals with *KCNJ2* mutations [49].

Kir3.1 (*KCNJ3*)

The *KCNJ3* gene encodes the Kir3.1 channel subunit [1, 50, 51]. The Kir3.1 channel is a G protein-coupled inward rectifier K^+ channel (GIRK1) [52, 53] activated by serotonin, muscarinic and opioid signaling of G protein subunits [54–59]. Kir3.1 channels form a heteromeric complex with other Kir3 channels [60] and are present in a variety of human tissues, including brain, heart, eye and muscle tissue [61–67]. Like other Kir channels, the C-terminus of Kir3 channel containing the cytoplasmic 'hotspot' is also proposed to interact with PIP_2 to regulate channel function [68], but there have been no reports of a mutation within the 'hotspot' that has been associated with a channelopathy.

Kir4.1 (*KCNJ10*)

The *KCNJ10* gene encodes the Kir4.1 channel subunit. Kir4.1 is expressed in glial cells of the central nervous system, Müller cells of the retina, and in cochlea [1, 69, 70] (Figure 3). Kir4.1 also plays a crucial role in facilitating salt reabsorption in the distal convoluted tubule of the kidney, where it has been hypothesized to recycle potassium by transporting salts down an electrochemical gradient from the tubular lumen into the cell, in combination with the sodium-potassium pump (Na^+/K^+-ATPase) [71, 72] (Figure 3).

The tissue distribution of Kir4.1 explains why loss-of-function mutations in *KCNJ10* have been associated with the autosomal recessive SeSAME syndrome (Seizures, Sensorineural deafness, Ataxia, Mental retardation, and Electrolyte imbalance), also known as EAST

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syndrome (Epilepsy, Ataxia, Sensorineural deafness, and Tubulopathy)) [73–75] (Table 2). Loss of Kir4.1 channel function in the brain or spinal cord induces astrocyte depolarization, loss of K^+ clearance, and a reduced seizure threshold as seen in the case of reactive gliosis (Figure 3) [76, 77]. In contrast, epithelial transport abnormalities occur when Kir4.1 function is lost in the kidney or in the cochlea. Loss of cochlear function contributes to the generation of an abnormal endocochlear potential and consequent hearing loss [78]. Loss of Kir4.1 function in the kidney leads to abnormal salt reabsorption in the distal convoluted tubule which leads to serum electrolyte abnormalities [74]. Several members of the Kir channel family are expressed in the retina [79], and although there have been no clinically significant vision abnormalities described, the mutations associated with SeSAME/EAST syndrome do result in altered retinal physiology [80].

Two SeSAME/EAST-associated mutations have been described in the *KCNJ10* gene that are found immediately upstream of the C-terminal cytoplasmic 'hotspot' (Table 2). A third mutation lies directly within the 'hotspot' and results in a polar threonine at position 164 changing to a hydrophobic isoleucine (**T164I**). The isoleucine substitution prevents the formation of a hydrogen bond with the lysine residue at amino acid position 67 that likely controls both the pH and PIP_2 gating [81]. The loss of the hydrogen bond alters the channel response to pH, and this results in a loss of channel function as demonstrated by patch-clamp electrophysiology [72, 75, 82–84]. Another hotspot mutation is located within the second transmembrane domain (**A167V**) and is also linked with defects in channel gating [82]. Expression of this mutation leads to the autosomal recessive finding of decreased Kir channel current when compared to the wild type [72, 75, 82–84]. Cells expressing hotspot mutation **R175Q** have impaired channel function as measured by reduced current, negligible inward rectification, channel-open probabilities in the 10–15% range, and reduced pH and PIP_2 sensitivity [73].

Kir5.1 (*KCNJ16*)

The *KCNJ16* gene encodes the inwardly rectifying potassium channel 5.1 (Kir5.1) [1]. Kir5.1 channels influence the function of Kir4.1 (as in kidney) [85–87] or Kir4.2 [88] channels. Mutations in Kir5.1 likely regulate the overall K^+ conductance by affecting other Kir subunits with which they assemble [89]. No specific mutations in the hotspot region have been described that are associated with a channelopathy.

Kir6.2 (*KCNJ11*)

The *KCNJ11* gene encodes the Kir6.2 channel, also known as an ATP-sensitive potassium channel (K_{ATP}) [1, 2]. Kir6.2 regulates electrical signaling in a variety of cell types, including brain, heart, skeletal muscle, and the pancreas [2, 90], and acts by coupling K^+ movement with various aspects of cellular metabolic activity [2] (Figure 3). K_{ATP} channels are made up of four pore-forming Kir6.2 subunits and four sulfonylurea receptor (SUR) subunits which respond to the absolute concentrations of ATP and ADP in the cell [91]. Kir6.2 channels are activated by ADP and are inhibited by ATP [2].

In the brain, Kir6.2 responds to the serum glucose concentration and contributes to mechanisms that protect against seizures. In skeletal muscle, it influences muscular tone. Kir6.2 protects against ischemic stress in the heart [92]. In pancreatic beta cells, Kir6.2 channels trigger insulin secretion when high levels of glucose in the blood increases the intracellular ATP concentration. The increased concentration of ATP inhibits K_{ATP} channel activity which stimulates an increase in cytosolic Ca^{2+} leading to the release of insulin [93]. In contrast, when glucose levels are low, ADP concentrations increase, leading to Kir6.2 channel activation resulting in a decrease in cytosolic Ca^{2+} and an inhibition of insulin release.

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Abnormalities in insulin secretion that are present in the newborn period are associated with defects in Kir6.2 function. The mutations fall within two functional categories: 1) loss of channel function, and 2) abnormal biosynthesis or trafficking of Kir6.2 channels resulting in absent or reduced expression of the channel at the cellular membrane [94]. In other instances, gain-of-function mutations lead to Permanent or Transient Neonatal Diabetes Mellitus (PNDM or TNDM). Developmental delay and Epileptic episodes in association with Neonatal Diabetes (DEND syndrome) occur when Kir6.2 channels become less responsive to ATP inhibition, resulting in persistent hyperpolarization and decreased insulin secretion. DEND syndrome can also be the result of altered channel biosynthesis which, in the absence of ATP inhibition, increases the stability of the open state [95]. Five *de novo* heterozygous activating mutations have been described that occur near, and a sixth mutation is found within, the C-terminal cytoplasmic 'hotspot' of the Kir6.2 protein (Table 2). Genotype-phenotype correlations have shown that two of these mutations are linked to DEND syndrome (C166F, I167L) three of the mutations are associated with PNDM (K170R, K170N, R176C), and one mutation is associated with TNDM (E179A) (Table 2). The effect of these mutations lies in stark contrast to the effect of mutations located distal to the hotspot region (Y12X and L147P) that result in excessive insulin secretion and have been associated with Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI, nesidioblastosis) [4].

The **C166F** mutation in the *KCNJ11* gene is associated with DEND syndrome and results in the substitution of a polar, hydrophilic cysteine residue at amino acid position 166 with a nonpolar, aromatic phenylalanine [96]. The Kir6.2 **C166F** mutant channel has a marked increase in the probability of being in the open state, as well as a reduced sensitivity to ATP [96]. The proband exhibited severe intrauterine growth retardation, postnatal feeding problems, and at 3 months of age was diagnosed with diabetes mellitus which was confirmed by the presence of polyuria, polydipsia, hyperglycemia and ketosis. In addition, the proband experienced seizures, hypsarrhythmia, neurologic deterioration, diffuse hypotonia and had dysmorphic features.

The **I167L** mutation is another cause of DEND syndrome and results in the substitution of a nonpolar isoleucine with a hydrophobic leucine residue [97]. In this instance, the proband exhibited persistent hyperglycemia within hours after birth and had seizures by two weeks of age. By 3.5 years of age, the child was severely delayed, with a developmental age of 6 months. The **I167L** mutation increases the probability of the channel being in the open state, thereby indirectly reducing ATP inhibition which may impact normal pore gating [97].

Other mutations in or near to the 'hotspot' region in the *KCNJ11* gene also lead to Permanent Neonatal Diabetes Mellitus (PNDM). Barbetti and colleagues studied two individuals with mutations in this region, both at residue 170, that are associated with PNDM [54] (Table 2). The first mutation, **K170R**, alters the basic lysine residue to a positively charged basic arginine. The second mutation, **K170N**, converts the basic lysine residue to a polar, hydrophilic asparagine [98]. Both probands were diagnosed with neonatal diabetes and ketoacidosis prior to 3 months of age. Although functional assays have not been performed on either the **K170R** or **K170N** mutant channels, it is known that a **K170C** mutation results in a nonfunctional Kir6.2 channel [98].

A third KCNJ11 mutation of interest lies directly within the 'hotspot' region and results in the substitution of a basic arginine with a cysteine residue at amino acid position 176 (**R176C**) [99]. Residue 176 is involved in PIP_2 binding to the K_{ATP} channel and induction of channel opening. When PIP_2 binding to the channel is decreased, it leads to channel closure and the absence of Kir6.2 activity [100–102]. The proband was diagnosed with Type 1 diabetes mellitus [99].

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Transient neonatal diabetes (TNDM) typically undergoes remission during infancy, with a potential for relapse in early childhood and adolescence. Most TNDM cases result from defects in imprinting at chromosome 6q24, but surprisingly mutations in the *KCNJ11* gene (which is located at 11p15.1) can also lead to this transient disease [103]. TNDM mutation **E179A** results in a hydrophilic glutamic acid residue being changed to a hydrophobic alanine at amino acid position 179. The **E179A** proband exhibited reduced birth weight, diabetes diagnosed within the first few months of life, and the remission of diabetes during infancy. Although functional assays have not been completed for this Kir6.2 mutation, the clinical manifestations suggest that mutant Kir6.2 channels in pancreatic beta-cells result in altered release of insulin [103] (Figure 3).

Kir 7.1 (*KCNJ13*)

The *KCNJ13* gene encodes the Kir7.1 channel subunit which plays an important role in retinal physiology [1, 104]. Kir7.1 channels are mildly inwardly-rectifying [105, 106] and are expressed in multiple tissues, including kidney, intestine, stomach, thyroid, spinal cord, brain, and eye [107] (Figure 3). Partnered with NKCC transporters and the Na^+-K^+-ATPase in the retinal pigment epithelium (RPE) apical membrane [108], the Kir7.1 channels help to maintain the electrical potential necessary for driving trans-epithelial fluid transport [109, 110] (Figure 3). The apical aspects of RPE cells have an abundance of Kir7.1 and interdigitate with, and help to maintain potassium homeostasis around the photoreceptor outer segments (POS). Tight regulation of these channels by membrane PIP_2 [111] may contribute to the light response that is mediated by RPE cells. For example, receptor-activated (P2Y) depletion of PIP_2 reduces Kir7.1 channel activity in the apical membrane. The same signaling pathway also increases intracellular Ca^{2+} concentration through the activation of IP3 mediated release. A rise in intracellular Ca^{2+} concentration leads to the activation of the basal membrane Cl^- conductance and results in a net depolarization of the RPE cell. This effect is typically recorded as a delayed light response originating within the RPE cell [110]. Thus, regulation of Kir7.1 channel function in the apical membrane is coupled to basal membrane conductance, and influences RPE cell physiology.

The **R162W** mutation within the **bPbbb** 'hotspot' in the *KCNJ13* gene converts a basic arginine residue to a bulky tryptophan at Kir7.1 amino acid position 162 and is associated with Snowflake Vitreoretinal Degeneration (SVD) [112, 113] (Table 2). In the rat, a similar mutation affecting Kir7.1 results in a non-selective leaky channel and might thereby lead to premature depolarization of the RPE cells [66]. The resultant lack of regulation of transport across the RPE may contribute to the deposition of cellular metabolites as debris on the retina that is visible on the clinical fundus examination of SVD patients. Our group has recently demonstrated that a human Kir7.1 mutant clone is non-functional when is ectopically expressed in a heterologous expression system. Co-expression with the wild type Kir7.1 subunit revealed that the mutant Kir7.1 subunit has a dominant-negative effect on the heteromeric Kir7.1 channel function (Pattnaik and Pillers, unpublished results).

Another loss-of-function homozygous mutation in the hotspot domain, **R166X,** was recently identified in a patient suffering from Leber Congenital Amaurosis (LCA) [114] (Table 2). **R166X** results in an early stop codon and thereby produces a truncated Kir7.1 protein lacking most of the cytoplasmic C-terminal sequence. The C-terminal sequence is critical for the membrane trafficking of the translated protein [71]. The truncated protein likely does not successfully localize to the RPE membrane domain where Kir channels normally mediate potassium influx.

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Summary

Deregulation of Kir channel function may be one of the earliest cellular events that lead to the complex multi-organ findings that are associated with Kir channelopathies. Kir channels are typically comprised of either homo- or heterotetrameric structures whose function is highly sensitive to genetic alterations in the cytoplasmic domain. Although mutations in any domain may influence channel function, in this review we have focused solely on those mutations that lie within or near to the **bPbbb** cluster hotspot. The importance of the 'hotspot' is that it plays a role as a receptor in Kir channel regulation by intracellular metabolites, such as PIP_2. The C-terminal cytoplasmic 'hotspot' domain is critical to normal Kir channel function and thereby, mutations in this region lead to altered organ function. Given the contribution of the cytoplasmic 'hotspot' to Kir channel regulation, any interactions between the channel and its metabolic regulators could be important targets for the development of novel therapeutic interventions for Kir channelopathies.

Highlights

- Inwardly rectifying potassium (Kir) channels are ubiquitous.
- They control cellular events from neurotransmitter release to epithelial transport.
- We will focus on genetic and molecular understanding of various disease-related Kir channel mutations.
- These mutations are positioned within a regulatory cytoplasmic "hotspot".
- Various metabolic regulation pathways provide insight into phenotypic heterogeneity

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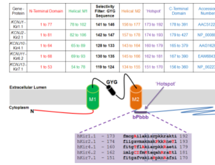

Figure 1. Kir channel topology

The predicted amino acid positions of the cytoplasmic, trans-membrane, and extracellular domains of the selected Kir channel subunits. The membrane topology illustrating the localization of two cytoplasmic (N and C terminal), two trnasmembrane (M1 and M2), and extracellular GYG selectivity loop with reference to plasma-membrane is represented. The cytoplasmic ‘hotspot’ is highlighted with sequence homology amongst human Kir channels compared. Highlighted aminoacids within and nearby the ‘hotspot’ are shown that represent disease-causing mutations.

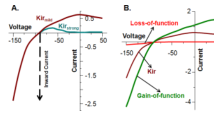

Figure 2. Inward rectification properties of Kir channels

Current amplitude in response to membrane voltage is shown by representative current-voltage (I–V) relationships of Kir channels with both strong inward rectifiers (A. aqua trace), or mild inward rectifiers illustrated (A. dark red trace). Current in the negative direction (inward current) is indicated by a downward arrow and current in the positive direction is the outward current. For strong inward rectifiers, the outward current is completely blocked by intracellular factors affecting the I–V relationship as compared to the persistent outward current demonstrated by mild inward rectifiers. B) I–V relationship model of a mildly inward rectifier channel (B. dark red trace, as in A.) showing predicted changes in both inward and outward current due to either a gain-of-function (B. green trace) or loss-of-function (B. red trace) due to genetic mutation(s).

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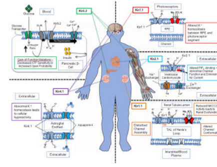

Figure 3. Tissue distribution of Kir channel subunits

The tissue-specific distribution of the Kir channels suggests that they play an important role in ion homeostasis and disease. Kir channel subunits are indicated by light blue within the membrane structure. All other possible associated channels, transporters and regulatory molecules are also shown in the membrane that controls cellular physiology. Kir channels tissue distribution along with their respective physiopathology are color-coded (Kir1.1- orange; Kir2.1- blue; Kir4.1- purple; Kir6.2- green and Kir7.1- red). Abbreviations: Kir, inwardly rectifying potassium channel; SUR, regulatory suramine subunit; ATP, adenosine tri-phosphate; ADP, adenosine di-phosphate; RPE, retinal pigment epithelium; PIP_2, phosphatidylinositol (4,5)-bisphosphate; TAL, thick ascending limb.

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Table 1

Kir gene, protein and tissue distribution.

Gene	Protein & Other Identifiers	Chromosome Location	Main Tissue Localization	References
KCNJ1	Kir1.1, ROMK, ROMK1	11q24	Kidney	34
KCNJ2	Kir2.1, HHIRK1,IRK1	17q23.1–q24.2	Heart, Skeletal Muscle	39, 40, 41
KCNJ10	Kir4.1	1q23.2	Glia (Retinal Müller cells), Kidney, Cochlea	69, 70
KCNJ11	Kir6.2, BIR	11p15.1	Beta cells, Neurons, Endocrine & Muscle cells	90
KCNJ13	Kir7.1	2q37	Retina, Small Intestine, Stomach, Kidney	107

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Table 2

Genetic correlation between Kir channel hotspot mutations and disease.

Gene-Protein	Mutation	Disease	Inheritance	References
KCNJ1- Kir1.1	Ala(A) 177 Thr(T)	Hyperprostaglandin E Syndrome/antenatal Bartter Syndrome	Autosomal Recessive - Homozygous Variant	37
KCNJ2 - Kir2.1	Pro(P) 186 Leu(L)	Andersen-Tawil Syndrome	Autosomal Dominant	45, 46
	Arg(R) 189 Ile(I)			40, 45
	Thr(T) 192 Ala(A)			47, 48
KCNJ10 -Kir4.1	Thr(T) 164 Ile(I)	SeSAME Syndrome (Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance)	Autosomal Recessive - Homozygous Variant	72
	Ala(A) 167 Val(V)		Autosomal Recessive - Compound heterozygous	72
	Arg(R) 175 Gln(Q)	EAST Syndrome (Epilepsy, Sensorineural Deafness, Tubulopathy) - same as SeSAME	Autosomal Recessive	73
KCNJ11 -Kir6.2	Cys(C) 166 Phe(F)	DEND Syndrome (Developmental Delay, Epilepsy, and Neonatal Diabetes) - Gain-of-Function mutation	Sporadic, *de novo*mutation - Heterozygous activating mutation	94
	Ile(I) 167 Leu(L)			96
	Lys(K) 170 Arg(R)	Permanent Neonatal Diabetes Mellitus (PNDM)		97
	Lys(K) 170 Asn(N)			97
	Arg(R) 176 Cys(C)			98
	Glu(E) 179 Ala(A)	Transient Neonatal Diabetes Mellitus (TNDM)		102
KCNJ13 -Kir7.1	Arg(R) 162 Trp(W)	Snowflake Vitreoretinal Degeneration	Autosomal Dominant	112
	Arg(R) 166 Trp(W)	Leber's Congenital Amaurosis	Homozygous nonsense	114