Focus on Kir7.1: physiology and channelopathy

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Genetic studies have linked alterations in Kir7.1 channel to diverse pathologies. We summarize functional relevance of Kir7.1 channel in retinal pigment epithelium (RPE), regulation of channel function by various cytoplasmic metabolites, and mutations that cause channelopathies. At the apical membrane of RPE, K⁺ channels contribute to subretinal K⁺ homeostasis and support Na⁺/K⁺ pump and Na⁺/K⁺/2Cl⁻ cotransporter function by providing a pathway for K⁺ secretion. Electrophysiological studies have established that barium- and cesium-sensitive inwardly rectifying K⁺ (Kir) channels make up a major component of the RPE apical membrane K⁺ conductance. Native human RPE expresses transcripts for Kir1.1, Kir2.1, Kir2.2, Kir3.1, Kir3.4, Kir4.2, and Kir6.1, albeit at levels at least 50-fold lower than Kir7.1. Kir7.1 is structurally similar to other Kir channels, consisting of 2 transmembrane domains, a pore-forming loop that contains the selectivity filter, and 2 cytoplasmic polar tails. Within the cytoplasmic structure, clusters of amino acid sequences form regulatory domains that interact with cellular metabolites and control the opening and closing of the channel. Recent evidence indicated that intrinsic sequence motifs present in Kir7.1 control surface expression. Mutant Kir7.1 channels are associated with inherited eye pathologies such as Snowflake Vitreoretinal Degeneration (SVD) and Lebers Congenital Amaurosis (LCA16). Based on the current evidence, mutations implicated in channelopathies have the potential to be used for genetic testing to diagnose blindness due to Kir7.1.

Retinal Pigment Epithelium

The retinal pigment epithelium (RPE) is a monolayer of pigmented cells of neuro-ectodermal origin. These cells are situated between the neuroretina and the choroid. The apical membrane of the polarized RPE cells faces the photoreceptor (PR) outer segments. The basolateral membrane faces Bruch’s membrane, which separates the RPE from the fenestrated endothelium of the choriocapillaris (Figs. 1 and 2a).4,4 RPE is a hexagonally packed, tight-junction connected, single sheet of post-mitotic cells containing pigment granules. Interactions between both the RPE and the PR are essential for visual function.4,5 Without any direct vascular supply to the PR, RPE cell must manage the bi-directional flow of molecules and ions in and out of the retina. This includes removing wastes from the retina, and maintaining ionic gradients necessary for phototransduction to occur.5,8 The primary roles of the RPE are highlighted in Fig. 1: (1) Transport of nutrients, ions, and water, (2) absorption of light and protection against photo oxidation of proteins and phospholipids of the outer segments,5 (3) re-isomerization of all-trans-retinal into 11-cis-retinal, which is a central component of the visual cycle,10,11 (4) phagocytosis of shed photoreceptor membranes,12 and (5) secretion of essential elements for the morphological integrity of the retina.14,15 The involvement of various ion channels and transporters in these functions (Fig. 1) emphasizes the importance of functional membrane molecules. Furthermore, analyses of hereditary retinal degeneration reveal a strong dependence of RPE on the photoreceptors and vice versa.16

K⁺ Ions and Channels in RPE Function

In the dark, cGMP-gated cation channels are open in the PR outer segments. The influx of Na⁺ and Ca²⁺ through these channels is counterbalanced by an outflow of K⁺ at the inner segment (Fig. 2C).16 When the retina is illuminated, the cGMP-dependent cation channels in the PR are closed and the K⁺ outflow at the inner segment is severely reduced (Fig. 2D). Simultaneously, Na⁺/K⁺-ATPase at the inner segment takes up K⁺ into the PR. This reduces the K⁺ concentration in the subretinal space from 5 mM to 2 mM.17 Decrease in the subretinal K⁺ concentration results in the left shift of the K⁺ electrochemical equilibrium potential reflecting an increase in K⁺ current, in addition to the slowing of the apical Na/K⁺-ATPase.18,19 The combined effect is thus a net efflux of K⁺ into the subretinal space (Fig. 2B), and an increase of the subretinal K⁺ concentration back to normal values.20 Several different subtypes of inwardly rectifying K⁺ channel subunits have been identified in the RPE.21-25 Reverse transcription-polymerase chain reaction (RT-PCR) analysis indicated that in addition to Kir7.1, 7 other Kir channel subunits (Kir1.1, Kir2.1, Kir2.2, Kir3.1, Kir3.4, Kir4.2 and Kir6.1) are expressed in native human RPE. However, transcripts of these channels are significantly less abundant than Kir7.1 transcripts.25 While Kir channel subtypes Kir4.1 22,23 and Kir6.2,21 are detected in rat RPE, they were not detected in human RPE.25 The functional significance of the Kir subtypes beyond Kir7.1 detected in human RPE is unclear. Determining the subcellular location of the other identified Kir channel subunits may be an important key to understand their physiological function in the RPE. In a blocker sensitivity study on intact toad RPE-choroid, millimolar concentrations of
to form a complete ion channel, and functional Kir channels are
The primary structure of 2 transmembrane strands is insufficient
conductance,26 consistent with Kir7.1 properties.24 Moreover, barium were necessary to block the RPE apical membrane K
C

is common to all Kir channels.27 The H5 region serves as the ‘ion
selectivity filter’ that shows the signature sequence T-X-G-Y-G.
Unlike the Kv channels, lack of S4 helix in the Kir channels (a
region, which has been shown to control the open probability of
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barium were necessary to block the RPE apical membrane K+
conductance,26 consistent with Kir7.1 properties.24 Moreover, Snowflake Vitreoretinal Degeneration (SVD) and Leber Congenital Amaurosis (LCA) arise from mutations in Kir7.1 channel suggesting this ion channel plays an important role in RPE health and disease (Fig. 4). This underscores the importance of evaluating the basic structure, synthesis, transport signals, function, regulation, and channelpathies associated with Kir7.1 channel.

Kir7.1

Kir7.1 ion channel is the most recently identified member of the inwardly rectifying K+ channel family. It was independently identified by 3 groups in the secretory epithelial cells of the choroid plexus,27 central neuron cells28 and in the small intestine.29 The primary structure of Kir channels consists of a common motif of 2 membrane spanning domains (TM1 and TM2) linked by an extracellular pore forming region (H5) and cytoplasmic amino (NH2-) and carboxy (COOH-) terminal domains. This topology is common to all Kir channels.27 The H5 region serves as the ‘ion selectivity filter’ that shows the signature sequence T-X-G-Y-G. Unlike the Kir channels, lack of S4 helix in the Kir channels (a part of the voltage sensor region S1-S4 helices), makes them insensitive to the membrane voltage changes. Inward rectification, a defining feature of the Kir channels, is not an intrinsic function of the channel protein, but is arises when intracellular cations like polyamines and Mg2+
block outward K+ flow.30 The primary structure of 2 transmembrane strands is insufficient to form a complete ion channel, and functional Kir channels are made up of 4 such subunits in a tetrameric complex.31,32 This stoichiometry was confirmed by velocity sedimentation with sucrose density gradients, size exclusion column chromatography, and chemical cross-linking.33 A reference gene sequence (NG016742) spanning 111327–129090 of the original human BAC library clone RP11-174L18, constructed from chromosome 2 of a male blood DNA (AC064852) harbored the Kir7.1 DNA sequence.34 Analysis of the human Kir7.1 gene (KCNJ13) organization revealed 3 exons, 2 introns, and a novel alternative 5’ splice site in exon 2. Kir7.1 cDNA is 1080 bp long consisting of a single ORF (open reading frame) encoding a protein of 360 amino acid residues. Kir7.1 is the most divergent in amino acid sequence among the Kir members. Its sequence shares ~50% homology at the protein level with the closely related Kir4.1 and Kir4.2 ion channels. Human RPE showed that the alternative usage of 2 competing 5’ splice sites in exon 2 gives rise to transcripts encoding either full-length Kir7.1 or Kir7.1S, which is predicted to encode a truncated protein.35 Kir7.1S transcript lacks 236 bp of the 476 bp exon 2 leading to a translation frame shift and introduction of stop codon.36 As a result, the predicted protein (94 aa) is truncated with a shorter C-terminus and has a stretch of 19 aa residues that are not present in Kir7.1. Naturally occurring transcript variants of Kir7.1 form truncated proteins which may co-assemble with Kir7.1 channel subunits and alter Kir7.1 channel properties or surface expression. However, electrophysiology studies in Xenopus oocytes indicated that Kir7.1S does not interact functionally with full length Kir7.1 protein.

One of the key features of Kir7.1 sequence is the presence of phosphorylation sites (discussed below) in the cytoplasmic regions that may play a role in the intracellular trafficking of the protein. Another noteworthy sequence feature is the presence of an N-glycosylation site at position 95 in the extracellular M1-H5 region, which has been shown to control the open probability of another inward rectifier Kir1.1.36 Kir7.1 channel functions as a tetramer; however there is no report of heteromeric assembly of Kir7.1. Immunoreactivity studies demonstrated the presence of Na+-, K+-ATPase on the apical membrane of RPE and choroid plexus22 and suggested the possible functional coupling with Kir7.1 in K+ recycling (Fig. 2).

Biophysical Properties of Kir7.1

Kir7.1 exhibits unusual current-voltage dependence in the slope of the I-V curve as current increases with negative potential compared to other inward rectifier channels.28 Heterologous expression of Kir7.1 shows extremely small ~50fS single channel conductance and an increase in outward current. Negative shift of the membrane potential due mainly to the left shift of electrochemical equilibrium potential upon reduction of extracellular K+ from 5 mM to 2 mM results in an increase in net potassium current (Fig. 2B). An unusual feature of Kir7.1 channel is the large Rb+-to-K+ conductance ratio of the inward current.37 The sensitivity of the channel to Ba2+ and Cs+ is very low with IC50 values of ~1 mM and ~10 mM respectively, which are >10 times greater than other Kir channels.38 Inward rectification of Kir7.1 is weak when extracellular [K+] is low and when extracellular [K+] is high the channel becomes strongly inwardly rectifying (Fig. 2B). This is also reflected by the crossover of the 2 I-V curves at positive potential. The resting membrane potential of human RPE cells is ~74 mV
whereas the zero-current potential of inward rectifier channel is \(-71 \pm 2.1\) for 5 mM \([K^+]_o\) and \(-104 \pm 3.2\) for 1 mM \([K^+]_o\) \(39\).

There is no evidence of K\(_{ir7.1}\) channel interaction with intracellular molecules like Mg\(^{2+}\) and polyamines at the lining of the channel pore; such interactions generally block K\(^+\) permeation. The competitive blockade of K\(_{ir}\) channels by Mg\(^{2+}\) and polyamines is in general crucial for the control of the magnitude of outward current. However, the K\(_{ir7.1}\) conductance was not affected by changes in either external or internal divalent cation concentrations. This is attributed to the presence of M125 (Arg in all other K\(_{ir}\) channels) in the pore region. When M125 is replaced with R125, channel conductance and Ba\(^{2+}\) sensitivity increases \(-20\) fold and \(-10\) fold respectively. \(20\)

**Identification of Signals in K\(_{ir7.1}\) Protein for Membrane Targeting**

As noted by Pattnaik et al. \(40\) mutation of arginine at position 162 to bulky tryptophan in K\(_{ir7.1}\) does not traffic to the plasma membrane during heterologous expression in CHO-K1 cells. The authors hypothesized that mutant R162W prevents PIP2 binding, which may then prevent translation of the cytoplasmic domain toward the membrane. This study further confirms that phosphoinositides like PIP2 may play a critical role in trafficking of the ion channels to the membrane. Conversely, Zhang et al. \(41\) reported that R162W mutant K\(_{ir7.1}\) channel successfully localized to both the oocyte and Madin Darby canine kidney (MDCK) cell membrane. Oocyte system is a robust system employed for expression of membrane proteins so merely having transmembrane domain may drive the protein to the membrane. On the other hand MDCK cells are widely used for the study of epithelial transport with caution. Given these conflicting data, directed trafficking of K\(_{ir7.1}\) channels to the membrane is poorly studied, and the molecular interactions by R162 residue within the C-terminal remains to be established. \(42\)

In eukaryotic cells, the machinery dedicated to protein folding and secretion is highly conserved. Recent evidence indicated that intrinsic sequence motifs are present in the ion channel proteins that control surface expression by regulating distinct intracellular trafficking steps. \(43\) Since there are few to no reports regarding signals responsible for transport of K\(_{ir7.1}\) protein from the endoplasmic reticulum (ER) to plasma membrane either directly or through the Golgi complex, we will consider potential signals for K\(_{ir7.1}\) protein localization based on the data available for other K\(_{ir}\) ion channels.

**Phosphorylation sites**

Yoo et al. \(38\) reported that phosphorylation is essential for cell surface expression of K\(_{ir1.1}\). Six phosphorylation sites are present

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**Figure 2.** (A) Normal retinal architecture in showing the photoreceptors (PR), subretinal space (SRS), the retinal pigmented epithelium (RPE), the Bruch membrane (BM), and the choroidal vascular network. (B) Representative Kir7.1 current recording showing hyperpolarizing shift in membrane potential on switching extracellular K\(^+\) concentration from 5 mM to 2 mM (Modified from Doring et al. 1998). Gray rectangle represents current activation around the resting membrane potential of RPE cells in response to change in extracellular K\(^+\). (C) Schematic of rod PR. The dark current circulates between the inner and outer segments. The cytoplasmic concentration of cGMP is high, and maintains the cGMP-gated channels in an open state and allows a steady inward current (dark current) to depolarize the photoreceptor. In dark, the K\(^+\) concentration of the subretinal space is approximately 5 mM. Adjacent RPE cell mechanisms active during dark are shown on the right with simplified representation of ion transport. (D) On light exposure, fewer K\(^+\) ions leave the photoreceptors and the K\(^+\) concentration in the subretinal space decreases from 5 to 2 mM. Kir7.1 channels in the apical membrane of RPE recycle efflux K\(^+\). Additionally, the movement of water and Cl\(^-\) is shown. Abbreviations are: Ap – apical, Ba – Basolateral, AQP – aquaporine, KCNQ – M-type K\(^+\) channel, NKCC – Na\(^+\)/K\(^+\)/2Cl transporter, and ATPase – Na\(^+\)/K\(^+\)/ATPase pump. The thickness and length of the arrows depicts the relative magnitude of the driving forces.
in Kir7.1 protein, Casein Kinase II (T321 and T337), PKA (S287) and PKC (S14, S169 and S201) sensitive sites are shown as red filled squares in Fig. 4. Substitution of Casein Kinase II (T321 and T337) and PKA (S287) sites with alanine did not affect the plasma membrane localization of Kir7.1 protein, however the role of other phosphorylation sites remains elusive.

Length of protein
Tateno et al. demonstrated that the C-terminus length is a critical determinant for the plasma membrane localization of Kir7.1 protein. They showed that 1–54 aa residues of N-terminus had no effect on the protein transport to the plasma membrane, but reducing the length of the C-terminus to 166 aa residues from the original 204 aa residues, eliminated membrane targeting. Kir7.1 protein truncated at the C-terminus (323–360aa) co-localized with calreticulin, an ER marker, suggested that loss of surface expression of deletion mutants is due to a defect in ER exit.

From ER to golgi complex
Export from ER to the Golgi complex is a key event in intracellular traffic of proteins. N- and C- terminal diacidic motifs consisting of 2 acidic amino acid residues separated by any other amino acid such as DXE or DXD render selective ER export, most likely binding to COPII (Coat Protein II) complexes (Fig. 3). Based on these findings, we identified 3 such diacidic motifs in Kir7.1 protein. The first 7 aa residue long stretch (84–90 aa) is located post TM1, and another 2 (211–213 aa and 338–340 aa) are present in the C-terminus. These are shown as purple diamonds in Fig. 4. The export signals found in Kir7.1 protein share the presence of one or more diacidic motif with the Kir1 (VLSEVDET), Kir2 (FCYENE), Kir3.2 (DQDVESPV and ELETEEE), and Kir3.4 (NQDMEIG) subfamilies. However, Tateno et al. showed that deletion of 37 C-terminus residues that included the diacidic motif (338–340 aa) did not affect localization to the cell surface. In addition, as there are many membrane proteins without diacidic motif that are exported from the ER efficiently, the diacidic motif cannot be the only signal for export from the ER.

RXR, a known ER retention signal, ensures that incorrectly assembled and improperly regulated channel proteins do not reach the cell surface. The RXR motif is present in the N-terminal of Kir7.1 protein at position 16–19 shown in green boxes (Fig. 4). Scaffold proteins like PDZ domain-containing proteins are important for protein complex assembly and may associate with the ion channel proteins early in the secretory pathway, which can facilitate or inhibit ER to Golgi transport. Kir ion channels contain the PDZ binding motif (-S/T-X-V/I/M- for sorting and cluster formation of membrane proteins, but this motif is absent from the C-terminal of Kir7.1 protein.

**Post-golgi trafficking**

The presence of YXXΦ (X is any amino acid and Φ being a bulky and hydrophobic residue) or di-leucine motifs favor clathrin coated vesicles. Di-lysine motifs in the C-terminal bind to COP1 (Fig. 4). Sequence analysis of Kir7.1 protein revealed the presence of YSHI (244–247 aa) in the C-terminal that resembles the consensus sequence YXXΦ signal shown as blue diamonds in Fig. 4. The second sorting motif, the di-leucine motif (LL) was also detected in Kir7.1 protein sequence shown as blue diamonds in Fig. 4. One di-leucine motif is present in the N-terminal (12–13 aa), 2 are present in transmembrane domain 2 (137–138aa and 143–144 aa) and 2 are present in the C-terminal (256–257 aa and 302–303 aa).

It is intriguing that in spite of both tyrosine-based and di-leucine-based peptide sequences classified as basolateral sorting signals Fig. 3, Kir7.1 is trafficked to the RPE apical processes? Two possible scenarios may provide an explanation. In the first scenario, RPE cannot recognize basolateral information in Kir7.1 protein as it does in N-CAM or EMMPRIN. In the second scenario, transcytosis may solve this puzzle as it does in case of Influenza hemagglutinin (HA) (Fig. 3). HA is delivered to the apical surface via an indirect pathway, first being delivered to the basolateral surface, and then to the apical plasma membrane via transcytosis in RPE. Alternatively, Kir7.1 harbor possible unique signals for apical sorting. Schuck and Simons reported that apical sorting motifs are localized in the extracellular or transmembrane domains of proteins, in contrast to the cytoplasm-oriented basolateral sorting motif. Apical sorting signals are much more diverse than basolateral signals and consist of post-translational modifications, rather than distinct peptide sequences. Perhaps
the most extensively studied of such modifications are N- and O-linked glycans (Fig. 3). Döring et al.\textsuperscript{27} reported an N-glycosylation site at position 95 in the extracellular M1-H5 region in Kir7.1 within a consensus sequence of N-X-T/S. We have predicted 2 more potential N-glycosylation sites at position 261 and 335 in Kir7.1.

Taken together, the trafficking of Kir7.1 to apical membrane of RPE may employ any of the speculated signals discussed above or an altogether different mechanism. Clearly, this provides both an opportunity to understand biology of disease and a challenge to study the traffic route of Kir7.1 and other like proteins in RPE.

**Functional Relevance of Kir7.1 and RPE**

Properly folded Kir7.1 protein reaches the apical membrane of RPE where it maintains the ionic homeostasis of the subretinal space by epithelial transport of ions, metabolites, and fluid between the subretinal space and the choroid.\textsuperscript{35} Illumination of the retina, resulting in a reduction of the K\textsuperscript{+} concentration in the subretinal space from 5 mM to 2 mM,\textsuperscript{50} accompanied with an increase in the volume of the subretinal space. The decrease in the subretinal K\textsuperscript{+} concentration hyperpolarizes the apical RPE membrane and contributes to tight regulation of subretinal space K\textsuperscript{+} homeostasis coupled with Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in active state.\textsuperscript{51} The weak inwardly rectifying property of Kir7.1 provides a compensatory pathway to support the RPE response to either rapidly decrease or increase the subretinal K\textsuperscript{+} concentration. La Cour\textsuperscript{51} reported that decreases in subretinal K\textsuperscript{+} concentration increase the K\textsuperscript{+} permeability of RPE apical membrane, promoting K\textsuperscript{+} efflux. Similarly, Shimura et al.\textsuperscript{24} reported that decreases in extracellular K\textsuperscript{+} concentration increases the slope conductance of Kir7.1. Na\textsuperscript{+} gradient also causes the active transport of Cl\textsuperscript{−} into the cell across the apical membrane via of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{−} co-transporter activity. Nevertheless, the decrease in the subretinal K\textsuperscript{+} concentration decreases uptake of Cl\textsuperscript{−} and results in the hyper polarization of the basolateral membrane to decrease Cl\textsuperscript{−} transport from the cell.\textsuperscript{32} Similarly, light-dependent hyper polarization of the apical membrane reduces the transport rate of apical Na\textsuperscript{+}-HCO\textsubscript{3}\textsuperscript{−} co-transporter and thus causes an intracellular acidification by ~0.35 pH units that facilitates Cl\textsuperscript{−} transport through the basolateral membrane from subretinal space to the choroid direction. This Cl\textsuperscript{−} transport across the cell also supports movement of water in the same direction which holds the retina in the proximity of RPE via suction and prevents retinal detachment. Intracellular acidification may also increase K\textsuperscript{+} efflux and enhance the buffering capacity of subretinal space.\textsuperscript{53}

**Functional Regulation of Kir7.1**

Many cytoplasmic metabolites that interact with the intrinsic amino acid residues have been reported to modulate the Kir7.1 channel. Zhang et al.\textsuperscript{54} showed that renal Kir7.1 ion channel is regulated by cAMP dependent protein kinase A (PKA) and protein kinase C (PKC). They showed that an increase in Kir7.1 current is observed with increasing intracellular cAMP concentration, and is suppressed by the mutating the sole PKA site (S287). The same study also demonstrated that mutation of PKC site S201 inhibits Kir7.1 channel, yet 2 other PKC sites (S14 and S169) do not affect the sensitivity of Kir7.1 channel to PKC.

Both extracellular pH (pH\textsubscript{e}) and intracellular pH (pH\textsubscript{i}) regulate the function of the Kir7.1 channel. While Kir7.1 conductance is relatively independent of pH\textsubscript{e} in the range from 6.5 to 9.0, it is strongly inhibited by further lowering the pH\textsubscript{e} below 6.0.\textsuperscript{53-55} On the other hand, Kir7.1 conductance showed a biphasic response to the pH\textsubscript{i}.\textsuperscript{55} Acidification of the cytoplasm from 7.2 to 6.8 activates the Kir7.1 current and further lowering the pH\textsubscript{i} to 6.0 or 5.5 results in a transient activation followed by inhibition.
In contrast, alkalization of the cytoplasm causes a reversible and rapid inhibition. Histidine at position 26 appears to act as the pH sensor of Kir7.1 since mutation of H26 to alanine or arginine affects both proton-induced activation, as well as proton-induced inhibition.

One of the best characterized lipid modulators of Kir channel activity is membrane PIP2 (phosphatidylinositol 4, 5-bisphosphate). PIP2 is found principally in the cytoplasmic leaflet of the plasma membrane that comprises ~1% of plasma membrane phospholipids. Membrane PIP2 abundance is a dynamic entity and is precisely controlled by lipid kinases, phospholipases, and phosphatases. Kir7.1 channels are inhibited upon depletion of membrane (PIP2). The apical membrane of the RPE cell is also the site of many G-protein coupled receptors (GPCR), including α1-adrenergic, muscarinic, and P2Y2. These receptors are linked to Phospholipase C signaling which serves to hydrolyze membrane-bound PIP2. Numerous studies of electrophysiological, biochemical and molecular simulation collectively suggest that PIP2 activates Kir channels directly with variable sensitivity by binding to positively charged residues in the C-terminal cytoplasmic "hot spot." This hot spot in Kir7.1 is located at aa 151–170 residues at the beginning of the C-terminal cytoplasmic domain and contains a cluster of basic residues (R or K). The R162W mutation within the ‘hotspot’ in the KCNJ13 gene converts a basic arginine residue to a bulky tryptophan and is associated with Snowflake Vitreoretinal Degeneration (SVD). Recently, it was inferred that R162W alters the Kir7.1 channel-PIP2 interaction.

Channelopathies Associated with Kir7.1

Genetic alterations in Kir7.1 underlie hereditary ion channel diseases known as channelopathies, and are primarily associated with congenital blindness.

Snowflake Vitreoretinal Degeneration (SVD, MIM 193230) is a developmental and progressive hereditary eye disorder that affects the retina and vitreous. The prevalence of SVD is low (<1/1000000), however the disorder has been described in several families. SVD is one of the vitreoretinal degenerations characterized by early onset cataract, congenital liquefaction of the vitreous humor, and abnormalities of the interface between the vitreous and retina leading to increased risk of retinal detachment and the presence of small yellow-white crystalline deposits in the peripheral retina. Electrophysiological studies reveal an elevated dark adaptation and reduced scotopic waves. Jiao et al. identified the chromosomal location of the gene linked to markers in a region of chromosome 2q36 defined by D2S2158 and D2S2202. Hejtmancik et al. reported that affected members were heterozygous for a missense mutation in the Kir7.1 (KCNJ13), an arg162-to-trp substitution (R162W) that arose from a c.484 C-T transition. This mutation resulted in a non-functional channel in heterologous expression studies and rendered wild-type channel non-functional through a dominant negative mechanism. Some of the variable phenotypes in these heterologous expression studies may be due to the specific expression system. Functionally, R162W mutant Kir7.1 channel may result in the premature depolarization of the RPE cells, resulting in Ca\(^{2+}\) overload and cell death.

The mechanism involved in SVD pathology is still unknown. In principle, the lack of channel activity could be caused by impaired surface expression, which can result from defects in a number of processes, including protein folding, posttranslational modification, assembly, and membrane trafficking, or ER retention and degradation. Zhang et al. showed that mutant Kir7.1 protein is localized to the plasma membrane when over expressed in Xenopus oocytes or MDCK cells. Thus the loss of function in mutant Kir7.1 channels is not due to impaired processing or trafficking to the plasma membrane, but, rather, some other mechanism, such as suppressed channel activity. We previously suggested that there may have been a significant loss of the wild type channel function due to oligomerization with mutant subunits which may affect the assembly of functional tetrameric complexes able to pass the ER quality control machinery. Structural modeling of wild type and mutant Kir7.1 based on the homologous structure of mammalian Kir channel suggested that this mutation causes major structural changes in the vicinity of “hot spot” in KCNJ13 which may be involved in channel activation by PIP2. The cytoplasmic domain may influence channel selectivity either due to a non-selective channel or due to the lack of regulation by PIP2 that will result in non-functional Kir7.1 channel.

Leber Congenital Amaurosis (LCA, MIM 204000) is another loss-of-function homozygous mutation in the KCNJ13. LCA is rare, hereditary disorder that leads to retinal dysfunction and visual impairment from the first year of life. This suggests both impaired retinal development and severe retinal degeneration, which involves both rod and cone photoreceptor pathways. LCA is inherited in an autosomal recessive manner and is caused by 16 different gene mutations, including KCNJ13. LCA accounts for the blindness of more than 20% of those blind from birth.

Sergouniotis et al. recently identified a single loss-of-function homozygous variant c.496C>T (p.R166X) in KCNJ13. R166X results in an early stop codon and produces a truncated Kir7.1 protein lacking most of the cytoplasmic C-terminal sequence. The absence of C-terminal sequence in the mutant would likely lead to mislocalization as discussed above. Another change c.380A>G (p.Q117R) was observed in a patient of white European ancestry with early onset retinal dystrophy and heavily pigmented fundus. Q117 is located within the conserved P-loop domain or selectivity loop. Thus, this mutation is predicted to give rise to either altered K\(^+\) selectivity or complete loss of K\(^+\) permeability by the channel. Another variation c.485G>A (p. R162Q) was identified in the heterozygous state in male patients of Turkish ancestry. While R162 is the site of defect in SVD patients, R162Q had no phenotype as reported for LCA screening. There are also several other genetic variations within KCNJ13 gene reported by Sergouniotis et al. but their functional characteristics are still to be explored. Further studies are necessary to investigate the potential loss or gain-of-function underlying pathophysiology of LCA due to Kir7.1 channelopathy. Moreover, it would be interesting to study the electrophysiological outcome of various genetic variations on RPE cell function.
Open Questions

Despite the progress attained in our understanding of the Kir7.1 channelopathy, several questions remain unanswered. Why does Kir7.1 channelopathy have only a retinal phenotype?49 Within the eye Kir7.1 is also localized to other cell types, yet the phenotype is specific to RPE. How does an RPE apical membrane ion-channel defect generate ERG phenotype, signature of retina electrical output in response to light exposure? Kir7.1 channels are also regulated by membrane cholesterol so future studies should address the role of Kir7.1 in age-related blindness. To address Kir7.1 channel biology, future studies could test the effects of channel knock down (by RNA interference) or channel mutation. Pertaining to Kir7.1 molecule, mutations might alter ion channel function either due to defective trafficking to the membrane, defective channel opening and closing, and/or loss of selectivity for the specific ion. Molecular understanding of the functional abnormalities associated with a specific mutation need to be established for our understanding of the disease pathology. As an example, a mutation of residue 162 to tryptophan results in SVD, whereas in screening for LCA genotype, a mutation to glutamine at the same position is tolerated. Severity in the outcome of the mutation is perhaps a direct measure of severe pathological consequence. Although ion-channel research mostly builds on studies using heterologous expression, stem cell approaches have a remarkable potential, as well. Successful reprogramming of patient fibroblasts to retina iPSCs for disease modeling and drug testing is promising.70–73 IPS cells will be a key for both LCA and SVD modeling, drug discovery and cell therapies using clinically-relevant patient donor samples. When successful, there is a chance to circumvent Kir7.1 channelopathy through drugs or gene or cell therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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