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TRPP2 Channel Regulation

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Abstract Polycystin-2, or TRPP2 according to the TRP nomenclature, is encoded by *PKD2*, a gene mutated in patients with autosomal-dominant polycystic kidney disease. Its precise subcellular location and its intracellular trafficking are a matter of intense debate, although a consensus has emerged that it is located in primary cilia, a long-neglected organelle possibly involved in sensory functions. Polycystin-2 has a calculated molecular mass of 110 kDa, and according to structural predictions it contains six membrane-spanning domains and a pore-forming region between the 5th and 6th membrane-spanning domain. This section first introduces the reader to the field of cystic kidney diseases and to the *PKD2* gene, before the ion channel properties of polycystin-2 are discussed in great detail.

Keywords Polycystic kidney disease · Cation channel · Mechanosensation · Chemosensation · Primary cilia

1 A Brief Introduction to Cystic Kidney Diseases

Cystic kidneys are a cardinal symptom of not only one, but a whole class of diseases comprising the autosomal-dominant and autosomal-recessive forms of polycystic kidney disease, medullary cystic kidney disease, nephronophthisis, Bardet–Biedl syndrome, von Hippel–Lindau syndrome, oro-facial digital syndrome type I and tuberous sclerosis (Witzgall 2005a). Although the age of

onset, the course of the disease, the affected tubular portion and the histopathological presentation vary between the different entities, the basic underlying picture appears to be more or less the same: tubular profiles belonging to the nephrons and the collecting ducts continuously expand. Despite the cloning of most of the mutated genes, the pathogenetic events leading to cyst formation remain a mystery. A reversal of cell polarity, an imbalance between cell proliferation and apoptosis, and changes in the extracellular matrix have been made responsible for cystogenesis but may well represent only epiphenomena (Gallagher et al. 2002). An increasing amount of data points to the involvement of primary cilia, a long neglected extension of the apical membrane of epithelial cells, in cyst formation. Although primary cilia can be found on almost any cell type in the mammalian organism (Andrews and Porter 1974; Latta et al. 1961; Wheatley 1995; Wheatley et al. 1996), they have not attracted a lot of attention for a long time because they were considered an evolutionary relict. Due to the composition of their axoneme of only nine peripheral, but no central, doublets of microtubules, primary cilia are believed to be immotile in most cases, but cells respond to bending of primary cilia with an increase in $[Ca^{2+}]_i$ (Nauli et al. 2003; Praetorius and Spring 2001). It therefore has been proposed that primary cilia serve as mechanosensory organelles which determine the diameter of renal tubules (for a critical discussion of this model see Witzgall 2005a). Most of the proteins associated with cystic kidney diseases have been localized to primary cilia, where at least some of them obviously fulfil distinct functions as transport proteins and ion channels (Witzgall 2005a).

2 Organization and Expression Pattern of the *PKD2* Gene

The positional cloning of the human *PKD2* gene was published in 1996 (Mochizuki et al. 1996), 2 years after the cloning of the distantly related *PKD1* gene (European Polycystic Kidney Disease Consortium 1994). Located on chromosome 4q21–23, the human *PKD2* gene is divided into 15 exons and spreads out over roughly 68 kb of genomic DNA (Hayashi et al. 1997). The murine *Pkd2* gene, which lies on chromosome 5, consists of the same number of exons as the human gene, but it obviously extends over a smaller region (42 kb) of genomic DNA (Park et al. 2000; Pennekamp et al. 1998). No typical TATA box has been identified in the human and murine promoter regions, and a preliminary analysis of the murine *Pkd2* promoter indicates the presence of negative regulatory elements in its upstream region (Park et al. 2000). Mutations in *PKD2* have been identified in approximately 15% of the patients suffering from autosomal-dominant polycystic kidney disease (Peters and Sandkuijl 1992; Roscoe et al. 1993; Torra et al. 1996; Wright et al. 1993), but no obvious mutational hot spots have been found yet. Mutations rather are distributed along the whole length of the gene (Deltas 2001; and http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html).

Two publications have addressed the question of whether a correlation between the genotype and the phenotype of patients exists, and although both reports confirm such a relationship, they differ regarding the basis of the correlation (Hateboer et al. 2000; Magistroni et al. 2003).

The expression pattern of the human *PKD2* gene and its murine and rat orthologues have been investigated repeatedly both by in situ hybridization and immunohistochemistry using different antibodies. A consensus has emerged in so far as the highest expression levels in the kidney were found in the distal nephron portion (Chauvet et al. 2002; Foggensteiner et al. 2000; Markowitz et al. 1999; Obermüller et al. 1999; Wu et al. 1998) which is consistent with the predominant site of cyst formation in *Pkd2* knock-out mice (Wu et al. 1998). On a subcellular level, polycystin-2 was distributed mainly in the basal compartment of distal tubular epithelial cells (Foggensteiner et al. 2000; Markowitz et al. 1999; Obermüller et al. 1999; Wu et al. 1998). In addition to the kidney, polycystin-2 was detected in a wide range of other tissues, but except for the striated ducts of salivary glands (Obermüller et al. 1999), no basal distribution was observed (Foggensteiner et al. 2000; Markowitz et al. 1999; Obermüller et al. 1999).

3 Domain Structure and Intracellular Distribution of Polycystin-2

The *PKD2* gene codes for polycystin-2 (*syn.* TRPP2), a protein with a predicted molecular mass of 110 kDa (Fig. 1). Hydropathy analysis of polycystin-2 predicts six membrane-spanning domains with both its NH_2- and COOH-terminus extending into the cytoplasm (Mochizuki et al. 1996), but so far no experimental data have been presented to support this assumption except for the fact that an antibody directed against the NH_2-terminus of polycystin-2 failed to stain non-permeabilized transfected cells (Cai et al. 1999), which suggests that at least the NH_2-terminus of polycystin-2 is located in the cytoplasm and does not protrude into the extracellular space. Between the 5th and 6th membrane-spanning domain a pore-forming region has been postulated, and the COOH-terminus of polycystin-2 supposedly contains a coiled-coil domain and a Ca^{2+}-binding EF-hand, but again experimental evidence for the presence of these motifs is lacking. Interestingly, the homomerization of polycystin-2 and its interaction with polycystin-1, the protein encoded by the *PKD1* gene, is mediated through its COOH-terminus (Qian et al. 1997; Tsiokas et al. 1997). It will be very useful to find out whether these interactions are modulated by Ca^{2+}.

A controversial debate which has not been conclusively settled yet concerns the intracellular location of polycystin-2. Conflicting results have been brought forward to support the presence of polycystin-2 in the endoplasmic reticulum and in the plasma membrane (for a more detailed discussion see

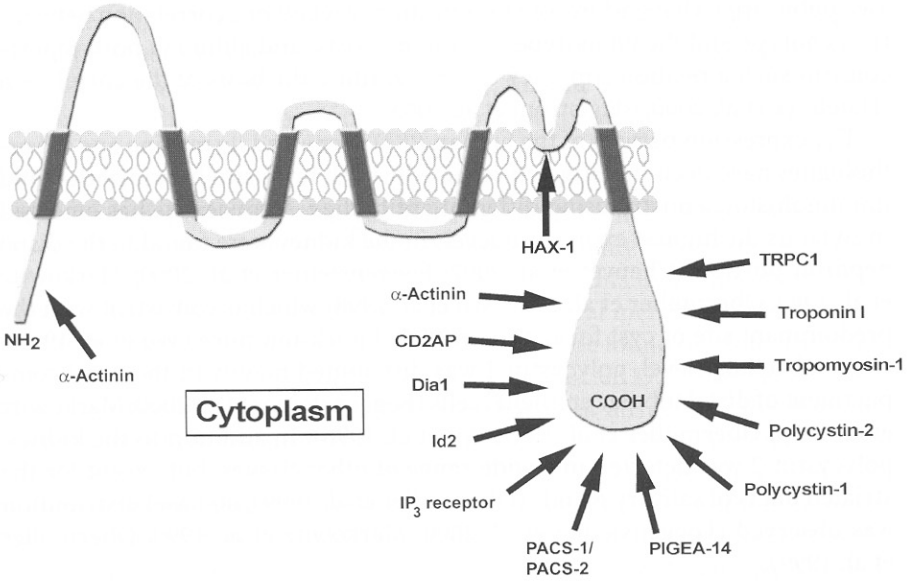

Fig. 1 Putative topology of polycystin-2. Polycystin-2 is predicted to span the membrane six times, with a supposed pore-forming region between the 5th and 6th membrane-spanning domains. The NH_2- and COOH-terminus are believed to extend into the cytoplasm, although these assumptions have not been corroborated experimentally. As can be easily appreciated, the COOH-terminus has received the most attention. It has been shown to interact with polycystin-2 itself (Qian et al. 1997; Tsiokas et al. 1997), with α-actinin (Li et al. 2005a), CD2AP (Lehtonen et al. 2000), Dia1 (Rundle et al. 2004), Id2 (Li et al. 2005b), the IP_3 receptor (Li et al. 2005c), PACS-1 and PACS-2 (Köttgen et al. 2005), PIGEA-14 (Hidaka et al. 2004), polycystin-1 (Qian et al. 1997; Tsiokas et al. 1997), tropomyosin-1 (Li et al. 2003a), troponin I (Li et al. 2003b) and TRPC1 (Tsiokas et al. 1999). The actin cytoskeleton-associated protein α-actinin has also been demonstrated to interact with the NH_2-terminus of polycystin-2, and HAX-1 was shown to associate with the putative pore-forming region of polycystin-2 (Gallagher et al. 2000)

Witzgall 2005b). The intracellular distribution of polycystin-2 has become even more complicated by the fact that polycystin-2 was detected in primary cilia (Pazour et al. 2002; Yoder et al. 2002).

4 Ion Channel Properties and Pharmacology of Polycystin-2

Due to the sequence and structural similarity of polycystin-2 to α-subunits of voltage-activated calcium channels (Mochizuki et al. 1996) and to the interaction of polycystin-2 with TRPC1 (Tsiokas et al. 1999), it was speculated already early on that polycystin-2 acts as a cation channel. The finding that the closely related protein polycystin-2L acts as a non-selective cation chan-

nel with a large unitary conductance further supported this assumption (Chen et al. 1999). Since then, a large number of publications have reported on the ion channel properties of polycystin-2; they all agree that polycystin-2 represents a non-selective ion channel with a large conductivity of approximately 100 pS. A more detailed presentation of the additional, sometimes contradictory findings is presented in the rest of this section.

The first report on the channel properties of polycystin-2 was based on the expression of the human PKD2 complementary DNA (cDNA) in transiently transfected CHO cells (Hanaoka et al. 2000). Measurements of whole-cell currents showed novel cation currents with slightly outwardly rectifying characteristics. La^{3+} ions, which inhibit non-selective cation channels, and niflumic acid, which inhibits non-selective cation and Cl^- channels, blocked the current in a dose-dependent manner. Consistent with these observations was the finding that polycystin-2 conducted Ca^{2+}, Na^+ and Cs^+ (with permeability ratios of 1.12:1:0.57, P_{Ca2+}:P_{Na+}:P_{Cs+}), but not Cl^- ions. In this publication the remarkable observation was made that polycystin-2 synthesized in CHO cells in the absence of polycystin-1 remained in the endoplasmic reticulum, whereas in its presence it reached the plasma membrane, which correlated with the fact that novel currents were only seen when polycystin-2 was synthesized together with polycystin-1 but not without it. A truncation mutant of polycystin-2 which lacked a large portion of the COOH-terminus including the putative EF-hand (R742X mutant) generated no currents (Hanaoka et al. 2000).

The main observations of this first report were confirmed by another group which expressed the murine Pkd2 cDNA in *Xenopus* oocytes, although there were some differences. Most of the full-length polycystin-2 protein was retained in intracellular membranes, but chemical chaperones and proteasome inhibitors induced its trafficking to the plasma membrane (Vassilev et al. 2001). Whole-cell conductance of *Xenopus* oocytes did not change after injection of the murine Pkd2 cDNA, consistent with the predominant intracellular location of full-length polycystin-2, although the uptake of radioactive Ca^{2+} ions increased fourfold (Chen et al. 2001). Using cell-attached or excised membrane patches, again a novel non-selective cation channel was detected which conducted both monovalent (Na^+, K^+, Li^+, Rb^+, NH_4^+) and divalent (Ca^{2+}, Sr^{2+}, Ba^{2+}) ions and was inhibited by La^{3+} but not nifedipine, a blocker of voltage-gated Ca^{2+} channels and ryanodine and inositol 1,4,5-trisphosphate (IP_3) receptors (Vassilev et al. 2001). This time, however, the channel was slightly inwardly rectifying and no single channel recordings were observed in the presence of Cs^+ (permeability ratios were 0.14:1:0.73, P_{Na+}:P_{K+}:P_{NH4+}, and 0.21:1, P_{Ca2+}:P_{K+}) (Vassilev et al. 2001). The addition of 1 μM Ca^{2+} to the bath solution transiently increased the open probability of polycystin-2, but millimolar concentrations of Ca^{2+} inhibited currents. The R742X mutant displayed a slightly lower conductivity than the wild-type protein, was (almost) impermeable to Ba^{2+} and Sr^{2+} (Chen et al. 2001) and did not respond to the addition of Ca^{2+} (Vassilev et al. 2001). The findings in *Xenopus* oocytes were

extended to non-transfected and transfected inner medullary collecting duct (IMCD) cells in which very similar conductiviti es and permeability ratios for different cations were obtained as in the oocytes (Luo et al. 2003). Finally, synthesis of the full-length polycystin-2 protein in sympathetic neurons together with polycystin-1 led to the accumulation of polycystin-2 in the plasma membrane, where it acted as a non-selective cation channel whose activity could be inhibited by La^{3+} ions and amiloride (Delmas et al. 2004). In contrast to the results obtained with oocytes, however, the P_{Na+}:P_{K+} permeability ratio was 0.98 and not 0.14. The R742X polycystin-2 mutant protein again displayed a somewhat lower conductivity than the full-length protein (90 pS vs 110 ps), but its open probability was markedly higher (Delmas et al. 2004).

A different approach was used by a third group which first investigated the channel properties of human syncytiotrophoblast cells (González-Perrett et al. 2001). For this, membrane vesicles (apical enriched) of human syncytiotrophoblast cells were reconstituted onto planar lipid bilayers. An endogenous high-conductance, non-selective, voltage-dependent cation channel was detected with a P_{Na+}:P_{K+} permeability ratio of 1.1. Again the channel was inhibited by La^{3+} and high concentrations (90 mM) of Ca^{2+} ions, and also by Gd^{3+} ions and amiloride. The expression of the endogenous *PKD2* gene was confirmed on the messenger RNA (mRNA) and protein level, and an anti-polycystin-2 antibody inhibited this particular channel. Subsequent synthesis of the human polycystin-2 protein in Sf9 insect cells and use of in vitro-translated protein confirmed the data obtained with the human syncytiotrophoblast cells and further demonstrated a P_{Cs+}:P_{Ba2+} permeability ratio of 0.65 (González-Perrett et al. 2001). This group was also able to confirm that the R742X mutant of polycystin-2 acted as a channel, although with a lower conductivity and open probability than the full-length protein (Xu et al. 2003).

A comparable strategy from yet another group, i.e. expression of the human PKD2 cDNA in stably transfected cells (this time the porcine kidney epithelial cell line LLC-PK_1), isolation of endoplasmic reticulum-enriched membrane vesicle and reconstitution onto lipid bilayers again demonstrated that polycystin-2 represented a high-conductance, non-selective, voltage-dependent cation channel (Koulen et al. 2002). Using various organic cations of different sizes, it was estimated that the pore diameter of polycystin-2 was at least 1.1 nm (Anyatonwu and Ehrlich 2005). Another truncated polycystin-2 protein, the L703X mutant, showed a lower conductivity (28 pS vs 114 pS for full-length polycystin-2 in the presence of Ba^{2+}) and required a larger negative membrane potential for activation. Confirming previous results, $[Ca^{2+}]$ up to 1,260 μM increased the open probability of full-length polycystin-2, whereas higher concentrations were inhibitory; no such modulatory activity of Ca^{2+} was observed for the L703X mutant protein (Koulen et al. 2002). Phosphorylation of the serine residue at position 812, possibly by casein kinase 2, appears to increase the calcium sensitivity of polycystin-2 (Cai et al. 2004).

5 Modes of Activation of Polycystin-2

So far only a very limited amount of data are available on how polycystin-2 is activated. In particular it is not known whether there is a natural ligand for this protein. Probably the most exciting data indicate that polycystin-2 (together with polycystin-1) plays an essential role for mechanosensation of kidney epithelial cells by primary cilia (Nauli et al. 2003). There are several open questions as to whether the diameter of renal tubules can be determined by a flow-based mechanism (Witzgall 2005a), but the flow model currently is the most widely discussed one regarding the pathomechanism of cyst formation. Consistent with the biochemical interaction between polycystin-2 and polycystin-1 (Qian et al. 1997; Tsiokas et al. 1997), polycystin-1 was able to reactivate the channel activity of polycystin-2 in lipid bilayer membranes (Xu et al. 2003). The interaction of polycystin-2 with cytoskeletal components such as Hax-1 (Gallagher et al. 2000), troponin I (Li et al. 2003b), tropomyosin-1 (Li et al. 2003a) and α-actinin (Li et al. 2005a) could also be very important in such a scenario, and indeed it was shown for α-actinin (Li et al. 2005a) that it drastically increases the channel activity of polycystin-2. Employing membrane preparations from human syncytiotrophoblast cells, it was demonstrated that the activity of a cation channel, possibly polycystin-2, was modulated by gelsolin and the polymerization status of the actin cytoskeleton (Montalbetti et al. 2005b).

Other factors directly or indirectly modulating the activity of polycystin-2 are the pH value, hydro-osmotic pressure, vasopressin, epidermal growth factor (EGF) and ATP. The channel activity of full-length polycystin-2 (González-Perrett et al. 2002) and its R742X truncation mutant (Chen et al. 2001) was inhibited by an increased $[H^+]$. Using again apical membrane preparations from human syncytiotrophoblast cells, it was also shown that polycystin-2 channel activity can be modulated by hydro-osmotic pressure (Montalbetti et al. 2005a). LLC-PK_1 cells stably transfected with the human PKD2 cDNA were used to demonstrate that polycystin-2 can be activated by vasopressin (Koulen et al. 2002), EGF (apparently through releasing the inhibition by phosphatidylinositol-4,5-bisphosphate) (Ma et al. 2005) and ATP (Gallagher et al. 2006). In addition to acting as a non-selective cation channel itself, polycystin-2 also interacts with the IP_3 receptor and may thereby influence $[Ca^{2+}]_i$. The latter findings deserve note because vasopressin (Torres et al. 2004), EGF (Sweeney et al. 2000; Torres et al. 2003) and ATP (Hooper et al. 2003; Schwiebert et al. 2002) are felt to promote cyst formation. Since most of the mutations in the *PKD2* gene are believed to be inactivating (Deltas 2001), the cyst-promoting activity of vasopressin, EGF and ATP should be prevented as long as functional polycystin-2 is present; only if polycystin-2 is lost by mutations should vasopressin, EGF and ATP be able to induce cystogenesis.

6
Biological Relevance, Emerging and Established Roles for Polycystin-2

As already elaborated in the introductory remarks and in the preceding section, polycystin-2 is believed to be responsible for the flow-mediated rise in $[Ca^{2+}]_i$ (Nauli et al. 2003) and thereby possibly for determining the correct width of renal tubules. At this point it is not clear how the mechanical stimulus is transmitted through polycystin-2—whether it is a direct action on polycystin-2 or whether other proteins sense the mechanical stress and subsequently act on polycystin-2. Experiments performed with the worm *Caenorhabditis elegans* have provided evidence for a chemosensory role of cilia (Barr et al. 2001; Barr and Sternberg 1999; Haycraft et al. 2001) and it therefore cannot be ruled out that the orthologue of polycystin-2 is activated by chemical stimuli in the worm. The activation of polycystin-2, possibly by phosphorylation, in turn can inhibit the cell cycle through its interaction with the helix-loop-helix protein Id2 (Li et al. 2005b). Such a scenario is consistent with the finding that cyst wall epithelial cells proliferate at a higher rate than regular tubular epithelial cells (e.g. Lanoix et al. 1996; Ramasubbu et al. 1998).

Another surprising finding in the *Pkd2* knock-out mice was the development of situs inversus (Pennekamp et al. 2002), thus indicating that polycystin-2 also plays an important function in left-right axis differentiation. A phenotypic connection between polycystic kidney disease and situs inversus, for which primary cilia may be the key, had already been made earlier for other proteins (Mochizuki et al. 1998; Murcia et al. 2000). The correct placement of the internal organs, e.g. of the heart on the left and the liver on the right, may already be determined very early on (Levin et al. 2002), although the best investigated stage of development represents that of the primitive node. The primitive node is a temporary structure during embryonic development which serves as an essential signalling centre for left–right axis development. Cells in the primitive node elaborate primary cilia, but surprisingly some of these cilia are motile—they are able to rotate, and this rotational movement has been proposed to break the left–right axis (Cartwright et al. 2004). There is another population of cells in the primitive node which are immotile but which (like primary cilia of motile cells) contain polycystin-2. According to the current model, the motile cilia are responsible for generating a gradient for an extracellular signalling molecule which in turn is transduced into an intracellular calcium signal by polycystin-2 (McGrath et al. 2003). It is noteworthy that *Pkd1* knock-out mice do not present with situs inversus, which is consistent with the absence of polycystin-1 in primary cilia of the node (Karcher et al. 2005).

7 Perspective

The emergence of the polycystins and their connection to primary cilia have opened up a whole new arena in the field of cell biology and renal biology. Despite a lot of exciting new evidence, we are still in the early stages of the investigations and many questions remain to be answered. A host of proteins has been described to interact with the COOH-terminus of polycystin-2, and it appears very difficult conceptually to see them all interacting at the same time in this rather short region of the protein. It will be a challenge to sort out the respective interacting domains and to determine whether there are cell type-specific interactions. What about proteins interacting with the rest of polycystin-2, are they involved in directing polycystin-2 to primary cilia? What is the natural ligand for polycystin-2? It remains to be seen whether the stimulation of cells with vasopressin, EGF and ATP is the physiologically relevant stimulus for polycystin-2, how mechanical stimuli are transmitted to and by polycystin-2, and whether polycystin-2 is also involved in chemosensation. We certainly also have much to discover about the signalling events downstream of polycystin-2, and this may lead us to target genes activated by polycystin-2 and ultimately to the "Holy Grail" of the mechanism through which the width of the renal tubules is determined. Obviously the field of cystic kidney diseases, polycystin-2 and primary cilia will keep us busy for quite some time to come.

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References

Andrews PM, Porter KR (1974) A scanning electron microscopic study of the nephron. Am J Anat 140:81–115

Anyatonwu GI, Ehrlich BE (2005) Organic cation permeation through the channel formed by polycystin-2. J Biol Chem 280:29488–29493

Barr MM, Sternberg PW (1999) A polycystic kidney-disease gene homologue required for male mating behaviour in C. elegans. Nature 401:386–389

Barr MM, DeModena J, Braun D, Nguyen CQ, Hall DH, Sternberg PW (2001) The Caenorhabditis elegans autosomal dominant polycystic kidney disease gene homologs lov-1 and pkd-2 act in the same pathway. Curr Biol 11:1341–1346

Cai Y, Maeda Y, Cedzich A, Torres VE, Wu G, Hayashi T, Mochizuki T, Park JH, Witzgall R, Somlo S (1999) Identification and characterization of polycystin-2, the PKD2 gene product. J Biol Chem 274:28557–28565

Cai Y, Anyatonwu G, Okuhara D, Lee KB, Yu Z, Onoe T, Mei CL, Qian Q, Geng L, Witzgall R, Ehrlich BE, Somlo S (2004) Calcium dependence of polycystin-2 channel activity is modulated by phosphorylation at Ser812. J Biol Chem 279:19987–19995

Cartwright JHE, Piro O, Tuval I (2004) Fluid-dynamical basis of the embryonic development of left-right asymmetry in vertebrates. Proc Natl Acad Sci USA 101:7234–7239

Chauvet V, Qian F, Boute N, Cai Y, Phakdeekitacharoen B, Onuchic LF, Attié-Bitach T, Guicharnaud L, Devuyst O, Germino GG, Gubler MC (2002) Expression of PKD1 and PKD2 transcripts and proteins in human embryo and during normal kidney development. Am J Pathol 160:973–983

Chen XZ, Vassilev PM, Basora N, Peng JB, Nomura H, Segal Y, Brown EM, Reeders ST, Hediger MA, Zhou J (1999) Polycystin-L is a calcium regulated cation channel permeable to calcium ions. Nature 401:383–386

Chen XZ, Segal Y, Basora N, Guo L, Peng JB, Babakhanlou H, Vassilev PM, Brown EM, Hediger MA, Zhou J (2001) Transport function of the naturally occurring pathogenic polycystin-2 mutant, R742X. Biochem Biophys Res Commun 282:12511256

Delmas P, Nauli SM, Li X, Coste B, Osorio N, Crest M, Brown DA, Zhou J (2004) Gating of the polycystin ion channel signaling complex in neurons and kidney cells. FASEB J 18:740–742

Deltas CC (2001) Mutations of the human polycystic kidney disease 2 (PKD2) gene. Hum Mutat 18:13–24

European Polycystic Kidney Disease Consortium (1994) The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. Cell 77:881–894

Foggensteiner L, Bevan AP, Thomas R, Coleman N, Boulter C, Bradley J, Ibraghimov-Beskrovnaya O, Klinger K, Sandford R (2000) Cellular and subcellular distribution of polycystin-2, the protein product of the PKD2 gene. J Am Soc Nephrol 11:814–827

Gallagher AR, Cedzich A, Gretz N, Somlo S, Witzgall R (2000) The polycystic kidney disease protein PKD2 interacts with Hax-1, a protein associated with the actin cytoskeleton. Proc Natl Acad Sci USA 97:4017–4022

Gallagher AR, Hidaka S, Gretz N, Witzgall R (2002) Molecular basis of autosomal-dominant polycystic kidney disease. Cell Mol Life Sci 59:682–693

Gallagher AR, Hoffmann S, Brown N, Cedzich A, Meruvu S, Podlich D, Feng Y, Könecke V, de Vries U, Hammes HP, Gretz N, Witzgall R (2006) A truncated polycystin-2 protein causes polycystic kidney disease and retinal degeneration in transgenic rats. J Am Soc Nephrol 17:2719–2730

González-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA, Cantiello HF (2001) Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-permeable nonselective cation channel. Proc Natl Acad Sci USA 98:1182–1187

González-Perrett S, Batelli M, Kim K, Essafi M, Timpanaro G, Moltabetti N, Reisin IL, Arnaout MA, Cantiello HF (2002) Voltage dependence and pH regulation of human polycystin-2-mediated cation channel activity. J Biol Chem 277:24959–24966

Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG (2000) Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. Nature 408:990–994

Hateboer N, Veldhuisen B, Peters D, Breuning MH, San-Millán JL, Bogdanova N, Coto E, van Dijk MA, Afzal AR, Jeffery S, Saggar-Malik AK, Torra R, Dimitrakov D, Martinez I, de Castro SS, Krawczak M, Ravine D (2000) Location of mutations within the PKD2 gene influences clinical outcome. Kidney Int 57:1444–1451

Hayashi T, Mochizuki T, Reynolds DM, Wu G, Cai Y, Somlo S (1997) Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). Genomics 44:131–136

Haycraft CJ, Swoboda P, Taulman PD, Thomas JH, Yoder BK (2001) The C. elegans homolog of the murine cystic kidney disease gene Tg737 functions in a ciliogenic pathway and is disrupted in osm-5 mutant worms. Development 128:1493–1505

Hidaka S, Könecke V, Osten L, Witzgall R (2004) PIGEA-14, a novel coiled-coil protein affecting the intracellular distribution of polycystin-2. J Biol Chem 279:35009–35016

Hooper KM, Unwin RJ, Sutters M (2003) The isolated C-terminus of polycystin-1 promotes increased ATP-stimulated chloride secretion in a collecting duct cell line. Clin Sci 104:217–221

Karcher C, Fischer A, Schweickert A, Bitzer E, Horie S, Witzgall R, Blum M (2005) Lack of a laterality phenotype in Pkd1 knock-out embryos correlates with absence of polycystin-1 in nodal cilia. Differentiation 73:425–432

Köttgen M, Benzing T, Simmen T, Tauber R, Buchholz B, Feliciangeli S, Huber TB, Schermer B, Kramer-Zucker A, Höpker K, Simmen KC, Tschucke CC, Sandford R, Kim E, Thomas g, Walz G (2005) Trafficking of TRPP2 by PACS proteins represents a novel mechanism of ion channel regulation. EMBO J 24:705–716

Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witzgall R, Ehrlich BE, Somlo S (2002) Polycystin-2 is an intracellular calcium release channel. Nat Cell Biol 4:191–197

Lanoix J, D'Agati V, Szabolcs M, Trudel M (1996) Dysregulation of cellular proliferation and apoptosis mediates human autosomal dominant polycystic kidney disease (ADPKD). Oncogene 13:1153–1160

Latta H, Maunsbach AB, Madden SC (1961) Cilia in different segments of the rat nephron. J Biophys Biochem Cytol 11:248–252

Lehtonen S, Ora A, Olkkonen VM, Geng L, Zerial M, Somlo S, Lehtonen E (2000) In vivo interaction of the adapter protein CD2-associated protein with the type 2 polycystic kidney disease protein, polycystin-2. J Biol Chem 275:32888–32893

Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M (2002) Asymmetries in H^+/K^+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. Cell 111:77–89

Li Q, Dai Y, Guo L, Liu Y, Hao C, Wu G, Basora N, Michalak M, Chen XZ (2003a) Polycystin-2 associates with tropomyosin-1, an actin microfilament component. J Mol Biol 325:949–962

Li Q, Shen PY, Wu G, Chen XZ (2003b) Polycystin-2 interacts with troponin I, an angiogenesis inhibitor. Biochemistry 42:450–457

Li Q, Montalbetti N, Shen PY, Dai XQ, Cheeseman CI, Karpinski E, Wu G, Cantiello HF, Chen XZ (2005a) Alpha-actinin associates with polycystin-2 and regulates its channel activity. Hum Mol Genet 14:1587–1603

Li X, Luo Y, Starremans PG, McNamara CA, Pai Y, Zhou J (2005b) Polycystin-1 and polycystin-2 regulate the cell cycle through the helix-loop-helix inhibitor Id2. Nat Cell Biol 7:1202–1212

Li Y, Wright JM, Qian F, Germino GG, Guggino WB (2005c) Polycystin 2 interacts with type I inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca^{2+} signaling. J Biol Chem 280:41298–41306

Luo Y, Vassilev PM, Li X, Kawanabe Y, Zhou J (2003) Native polycystin 2 functions as a plasma membrane Ca^{2+}-permeable cation channel in renal epithelia. Mol Cell Biol 23:2600–2607

Ma R, Li WP, Rundle D, Kong J, Akbarali HI, Tsiokas L (2005) PKD2 functions as an epidermal growth factor-activated plasma membrane channel. Mol Cell Biol 25:8285–8298

Magistroni R, He N, Wang K, Andrew R, Johnson A, Gabow P, Dicks E, Parfrey P, Torra R, San-Millan JL, Coto E, van Dijk M, Breuning M, Peters D, Bogdanova N, Ligabue G, Albertazzi A, Hateboer N, Demetriou K, Pierides A, Deltas C, St George-Hyslop P, Ravine D, Pei Y (2003) Genotype-renal function correlation in type 2 autosomal dominant polycystic kidney disease. J Am Soc Nephrol 14:1164–1174

Markowitz GS, Cai Y, Li L, Wu G, Ward LC, Somlo S, D'Agati VD (1999) Polycystin-2 expression is developmentally regulated. Am J Physiol 277:F17–F25

McGrath J, Somlo S, Makova S, Tian X, Brueckner M (2003) Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell 114:61–73

Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning MH, Deltas CC, Peters DJM, Somlo S (1996) PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 272:1339–1342

Mochizuki T, Saijoh Y, Tsuchiya K, Shirayoshi Y, Takai S, Taya C, Yonekawa H, Yamada K, Nihei H, Nakatsuji N, Overbeek PA, Hamada H, Yokoyama T (1998) Cloning of inv, a gene that controls left/right asymmetry and kidney development. Nature 395:177–181

Montalbetti N, Li Q, González-Perrett S, Semprine J, Chen XZ, Cantiello HF (2005a) Effect of hydro-osmotic pressure on polycystin-2 channel function in the human syncytiotrophoblast. Pflügers Arch 451:294–303

Montalbetti N, Li Q, Timpanaro GA, González-Perrett S, Dai XQ, Chen XZ, Cantiello HF (2005b) Cytoskeletal regulation of calcium-permeable cation channels in the human syncytiotrophoblast: role of gelsolin. J Physiol 566:309–325

Murcia NS, Richards WG, Yoder BK, Mucenski ML, Dunlap JR, Woychik RP (2000) The Oak Ridge Polycystic Kidney (orpk) disease gene is required for left-right axis determination. Development 127:2347–2355

Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AEH, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33:129–137

Obermüller N, Gallagher AR, Cai Y, Gassler N, Gretz N, Somlo S, Witzgall R (1999) The rat Pkd2 protein assumes distinct subcellular distributions in different organs. Am J Physiol 277:F914–F925

Park JH, Li L, Cai Y, Hayashi T, Dong F, Maeda Y, Rubin C, Somlo S, Wu G (2000) Cloning and characterization of the murine Pkd2 promoter. Genomics 66:305–312

Pazour GJ, San Agustin JT, Follit JA, Rosenbaum JL, Witman GB (2002) Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. Curr Biol 12:R378–R380

Pennekamp P, Bogdanova N, Wilda M, Markoff A, Hameister H, Horst J, Dworniczak B (1998) Characterization of the murine polycystic kidney disease (Pkd2) gene. Mamm Genome 9:749–752

Pennekamp P, Karcher C, Fischer A, Schweickert A, Skryabin B, Horst J, Blum M, Dworniczak B (2002) The ion channel protein polycystin-2 is required for left-right axis determination in mice. Curr Biol 12:938–943

Peters DJM, Sandkuijl LA (1992) Genetic heterogeneity of polycystic kidney disease in Europe. Contrib Nephrol 97:128–139

Praetorius HA, Spring KR (2001) Bending the MDCK cell primary cilium increases intracellular calcium. J Membr Biol 184:71–79

Qian F, Germino FJ, Cai Y, Zhang X, Somlo S, Germino GG (1997) PKD1 interacts with PKD2 through a probable coiled-coil domain. Nat Genet 16:179–183

Ramasubbu K, Gretz N, Bachmann S (1998) Increased epithelial cell proliferation and abnormal extracellular matrix in rat polycystic kidney disease. J Am Soc Nephrol 9:937–945

Roscoe JM, Brissenden JE, Williams EA, Chery AL, Silverman M (1993) Autosomal dominant polycystic kidney disease in Toronto. Kidney Int 44:1101–1108

Rundle DR, Gorbsky G, Tsiokas L (2004) PKD2 interacts and co-localizes with mDia1 to mitotic spindles of dividing cells. Role of mDia1 in PKD2 localization to mitotic spindles. J Biol Chem 279:29728–29739

Schwiebert EM, Wallace DP, Braunstein GM, King SR, Peti-Peterdi J, Hanaoka K, Guggino WB, Guay-Woodford LM, Bell PD, Sullivan LP, Grantham JJ, Taylor AL (2002) Autocrine extracellular purinergic signaling in epithelial cells derived from polycystic kidneys. Am J Physiol Renal Physiol 282:F763–F775

Sweeney WE Jr, Chen Y, Nakanishi K, Frost P, Avner ED (2000) Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor. Kidney Int 57:33–40

Torra R, Badenas C, Darnell A, Nicolau C, Volpini V, Revert L, Estivill X (1996) Linkage, clinical features, and prognosis of autosomal dominant polycystic kidney disease types 1 and 2. J Am Soc Nephrol 7:2142–2151

Torres VE, Sweeney WE Jr, Wang X, Qian Q, Harris PC, Frost P, Avner ED (2003) EGF receptor tyrosine kinase inhibition attenuates the development of PKD in Han:SPRD rats. Kidney Int 64:1573–1579

Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH II (2004) Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. Nat Med 10:363–364

Tsiokas L, Kim E, Arnould T, Sukhatme VP, Walz G (1997) Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. Proc Natl Acad Sci USA 94:6965–6970

Tsiokas L, Arnould T, Zhu C, Kim E, Walz G, Sukhatme VP (1999) Specific association of the gene product of PKD2 with the TRPC1 channel. Proc Natl Acad Sci USA 96:3934–3939

Vassilev PM, Guo L, Chen XZ, Segal Y, Peng JB, Basora N, Babakhanlou H, Cruger G, Kanazirska M, Ye Cp, Brown EM, Hediger MA, Zhou J (2001) Polycystin-2 is a novel cation channel implicated in defective intracellular Ca^{2+} homeostasis in polycystic kidney disease. Biochem Biophys Res Commun 282:341–350

Wheatley DN (1995) Primary cilia in normal and pathological tissues. Pathobiology 63:222–238

Wheatley DN, Wang AM, Strugnell GE (1996) Expression of primary cilia in mammalian cells. Cell Biol Int 20:73–81

Witzgall R (2005a) New developments in the field of cystic kidney diseases. Curr Mol Med 5:455–465

Witzgall R (2005b) Polycystin-2—an intracellular or plasma membrane channel? Naunyn Schmiedebergs Arch Pharmacol 371:342–347

Wright AF, Teague PW, Pound SE, Pignatelli PM, Macnicol AM, Carothers AD, de Mey RJ, Allan PL, Watson ML (1993) A study of genetic linkage heterogeneity in 35 adult-onset polycystic kidney disease families. Hum Genet 90:569–571

Wu G, D'Agati V ,Cai Y, Markowitz G, Park JH, Reynolds DM, Maeda Y, Le TC, Hou H Jr, Kucherlapati R, Edelmann W, Somlo S (1998) Somatic inactivation of Pkd2 results in polycystic kidney disease. Cell 93:177–188

Xu GM, González-Perrett S, Essafi M, Timpanaro GA, Montalbetti N, Arnaout MA, Cantiello HF (2003) Polycystin-1 activates and stabilizes the polycystin-2 channel. J Biol Chem 278:1457–1462

Yoder BK, Hou X, Guay-Woodford LM (2002) The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. J Am Soc Nephrol 13:2508–2516