

# Primary cilia and polycystic kidney disease

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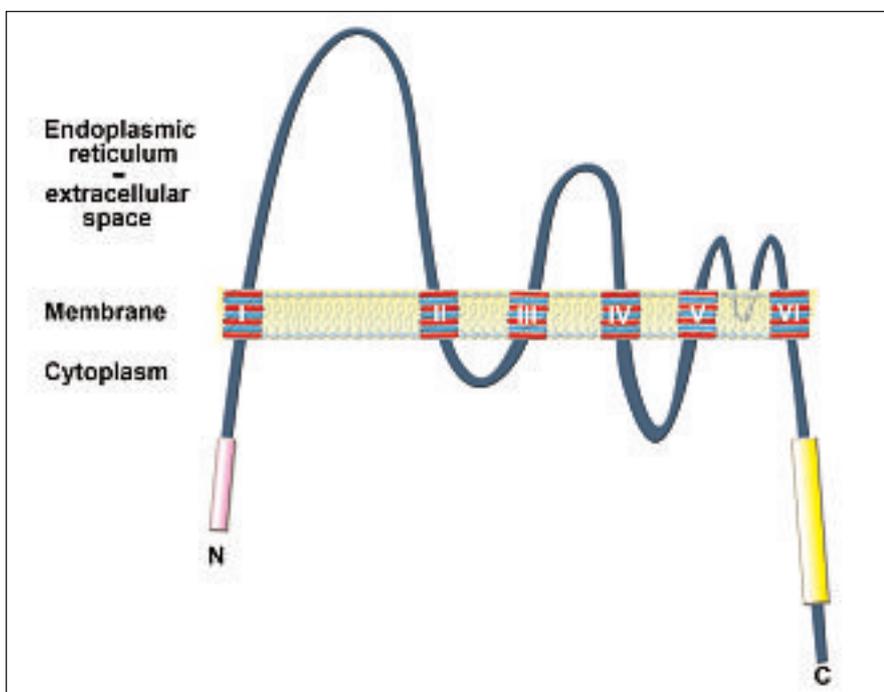
## Situs inversus and polycystic kidney disease converge at primary cilia

Although at first glance our body looks symmetrical, our internal organs obviously are arranged asymmetrically. It goes without saying that the heart is located on the left side and the liver on the right side yet such a seemingly trivial fact poses a very difficult biological question: How is the arrangement of the internal organs, the *situs*, regulated? Development of the organism starts with the fertilized oocyte, a round structure with no obvious asymmetry. At some point during development, however, axis formation occurs: A dorsal-ventral, an anterior-posterior and a left-right axis are established. Defects in left-right axis formation have been observed and are compatible with life, the patients suffer from *situs inversus*, i.e. their heart is located on the right side and the liver is located on the left side. As in many other circumstances, evidence obtained in the mouse has led to important insight into how the left-right axis may be for-

med. One mouse mutant, the *inv* mouse, not only presents with *situs inversus* but also with polycystic kidneys. At the time of its first description it posed a puzzle why both symptoms were present in the same mouse mutant.

Polycystic kidneys are characterized by the continuous formation of cysts, fluid-filled cavities lined by an epithelium. The disease is found in almost 10% of patients suffering from end-stage renal disease, so far no curative therapy is known and many patients finally require dialysis or a kidney transplant (Anonymous, 1991, 1998; European Dialysis and Transplant Association Registry, 1986; European Polycystic Kidney Disease Consortium, 1994; Lowrie and Hampers, 1981; Torra et al., 1995). At a prevalence of at least 1:1,000 autosomal-dominant polycystic kidney disease, one of the forms of polycystic kidney disease, belongs to the most common monogenic diseases affecting patients (Davies et al., 1991; Higashihara et al., 1998). In 1994 muta-

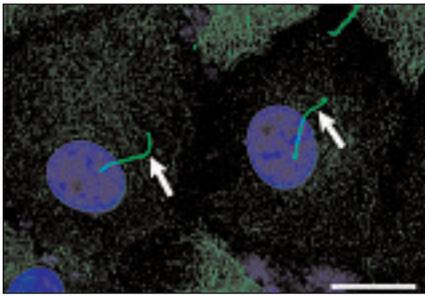
tions in the first gene responsible for autosomal-dominant polycystic kidney disease, *PKD1*, were published (European Polycystic Kidney Disease Consortium, 1994). *PKD1* codes for polycystin-1, a large integral membrane protein of 4,302 amino acids in length and 11 membrane-spanning domains (Hughes et al., 1995; Nims et al., 2003). The NH<sub>2</sub>-terminus of polycystin-1 represents the largest portion of the protein and extends into the extracellular space. It contains many characteristic motifs and is speculated to mediate cell-cell and/or cell-matrix contacts but so far the function of polycystin-1 remains enigmatic. Mutations in the second gene, *PKD2*, were published two years later (Mochizuki et al., 1996). Whereas *PKD1* is mutated in ~85% of the patients, the remaining ~15% suffer from mutations in the *PKD2* gene (Peters and Sandkuijl, 1992; Roscoe et al., 1993; Torra et al., 1996; Wright et al., 1993). Polycystin-2 (Figure 1) also is an integral membrane protein but with 968 amino acids it is much smaller than polycystin-1. It contains the characteristic features of cation channels (Delmas et al., 2004; Koulen et al., 2002). Polycystin-2 traverses the membrane 6 times, the ion-conducting pore is located between the 5th and 6th membrane-spanning domain, and the NH<sub>2</sub>- and COOH-terminus both extend into the cytoplasm. The phenotype of the *Pkd1* and *Pkd2* knock-out mice confirmed that both genes are responsible for the development of polycystic kidneys. One additional, surprising finding in the *Pkd2* [but not in the *Pkd1* (Karcher et al., 2005)] knock-out mice was *situs inversus* (Pennekamp et al., 2002). The connection between polycystic kidneys and *situs inversus* became immediately clear once it was recognized that polycystin-2 is a component of the primary cilium, a hair-like extension of epithelial and many other cell types. Primary cilia are not only found on epithelial cells lining the kidney tubules but also on cells of the primitive node which is suspected to play a central role in breaking left-right symmetry.



**Figure 1. Structure of polycystin-2.** The human polycystin-2 protein is 968 amino acids long and contains 6 transmembrane domains (I-VI), the ion-conducting pore is located between the 5th and 6th transmembrane domain. Both its NH<sub>2</sub>- and COOH-terminus extend into the cytoplasm. Depending on whether polycystin-2 is retained in the endoplasmic reticulum or reaches the ciliary plasma membrane, loops 1, 3 and 5 extend into the lumen of the endoplasmic reticulum or into the extracellular space. A ciliary targeting motif was found at the extreme NH<sub>2</sub>-terminus (pink box, Geng et al., 2006), and a retention signal for the endoplasmic reticulum (ER) was identified in the COOH-terminus (yellow box, Cai et al., 1999).

## Primary cilia, long-neglected organelles

Primary cilia (Figure 2) were documented for the first time in 1898 by Zimmermann (Zimmermann, 1898) but their more precise characterization was not possible until the arrival of the electron microscope and improved



**Figure 2.** Primary cilia on renal epithelial LLC-PK1 cells. Primary cilia (arrows) were visualized with a primary antibody directed against acetylated tubulin and a FITC-conjugated secondary antibody. Nuclei are shown in blue. Bar, 15  $\mu$ m.

techniques for preparing ultra thin sections (Barnes, 1961; Currie and Wheatley, 1966). Although experimental work on primary cilia began at the end of the 1970s (Wheatley, 2005) they were often viewed as rudimentary cell appendages with no function. The primary cilium is found on many different cell types in the mammalian body [(Wheatley et al., 1996), and <http://www.bowserlab.org/primarycilia/cilialist.html>] and occurs as a solitary, non-motile (with the exception of nodal cilia, see below), hair-like cell appendix extending from the basal body. In contrast to motile kinocilia with their 9 peripheral microtubule doublets and one central pair of microtubules, primary cilia lack the central pair of microtubules, the dynein arms, nexin and radial spokes (Figure 3). The plasma membrane of the cell body and of the primary cilium appear to be seamlessly connected, yet a barrier has to exist between them because some proteins can only be found in the primary cilium and not in the surrounding somatic plasma membrane.

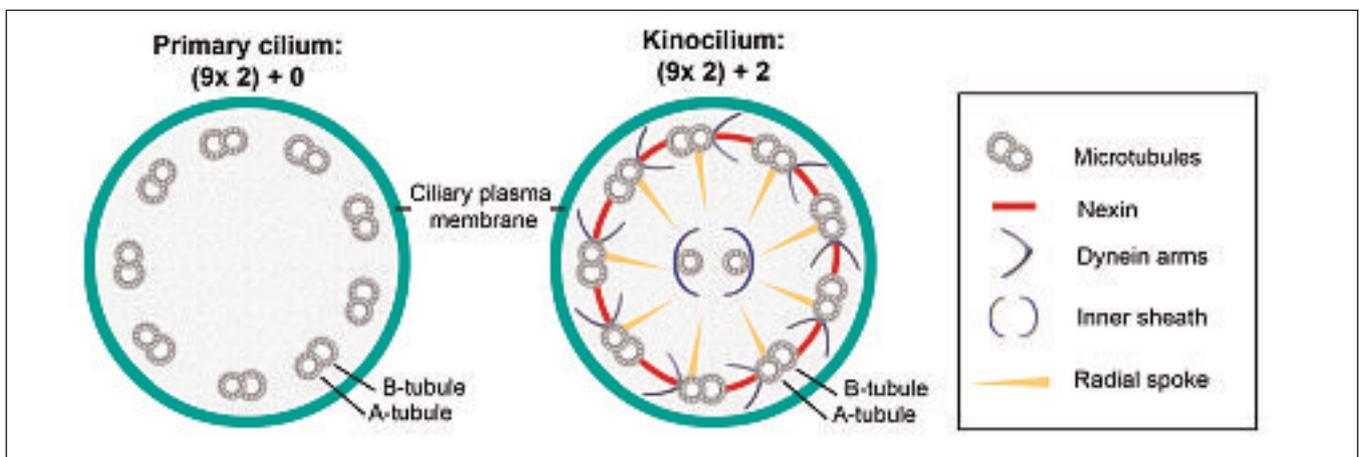
Seminal experiments in the nematode *Caenorhabditis elegans* and with renal epithelial cells

have finally attributed chemo- and mechano-sensory roles to primary cilia. *C. elegans* has a highly developed chemosensory system to find food and to detect mates. The sensory transduction molecules in the worm's chemosensory neurons are located in the sensory cilium which has a structure very similar to that of a primary cilium (Ward et al., 1975; Ware et al., 1975). Barr and Sternberg showed that LOV-1 and PKD-2, the likely orthologues of polycystin-1 and polycystin-2 in *C. elegans*, respectively, are both present in sensory neurons of adult males. PKD-2 is believed to regulate the ability of adult male worms to respond to mating cues that likely involve both chemosensory and mechanosensory components (Barr et al., 2001; Barr and Sternberg, 1999). Mutant worms with defects in LOV-1 and PKD-2 present with the same sensory defects in mating behaviors which argues for a functional connection between the two proteins. Indeed it has been demonstrated by various approaches that polycystin-1 and polycystin-2 also interact biochemically (Casuscelli et al., 2009; Qian et al., 1997; Tsiokas et al., 1997).

Around the same time Praetorius and Spring investigated how MDCK (Madin-Darby canine kidney) renal epithelial cells respond to flow. They produced good evidence that primary cilia act as a flow sensor. When they bent the cilium, the intracellular calcium concentration increased, probably due to the influx of  $Ca^{2+}$  ions through mechanosensitive channels in the shaft or at the base of the primary cilium. Following the influx of extracellular  $Ca^{2+}$  ions,  $Ca^{2+}$  becomes released from inositol-triphosphate ( $IP_3$ )-sensitive stores (Praetorius and Spring, 2001). When the cilia were removed, a flow-induced  $Ca^{2+}$  response was no longer observed (Praetorius and Spring, 2003). Subsequent experiments by the group of Jing

Zhou showed the involvement of polycystin-1 and polycystin-2 in sensing flow (Nauli et al., 2003). The authors suggest that polycystin-1, possibly through the PKD domains in its long extracellular  $NH_2$ -terminus, acts as a mechanosensor and transduces the flow-induced bending of the primary cilium into a molecular signal. Through its interaction with polycystin-2 a local  $Ca^{2+}$  influx in the primary cilium would ensue which would be amplified through  $Ca^{2+}$ -induced  $Ca^{2+}$  release via ryanodine receptors. The changes in intracellular  $Ca^{2+}$  concentrations may modulate various cellular functions such as gene expression, growth, differentiation and apoptosis. Loss or dysfunction of polycystin-1 and polycystin-2 may therefore lead to polycystic kidney disease as a result of the inability of the cells to sense mechanical stimuli that normally regulate tissue morphogenesis.

An exception to the rule that primary cilia are immotile are the nodal cilia in the primitive node, a crucial structure during embryonic development. Just like normal primary cilia they lack a central pair of microtubules but they contain radial spokes and dynein arms. Nodal cilia are capable of a characteristic counterclockwise rotation which occurs for only a few hours during development. This movement is responsible for a fluid flow from right to left across the node which in turn is essential for left-right axis formation (Nonaka et al., 2002; Nonaka et al., 1998). Accordingly the randomization of left-right axis specification has been observed in mice lacking the ciliary transport proteins Kif3a (Marszalek et al., 1999; Takeda et al., 1999) and Kif3b (Nonaka et al., 1998). But how does nodal flow determine the left-right axis in the embryo? One model is based on the observation that two forms of nodal cilia exist – in addition to cilia



**Figure 3.** Structure of primary cilia and kinocilia. Primary cilia only contain 9 peripheral doublets of microtubules whereas kinocilia also contain a central pair of microtubules and additional structural features such as dynein arms and nexin between neighboring microtubules.

with their rotating motion also immotile primary cilia can be found. The immotile primary cilia sense the presence of a yet to be identified morphogen whose asymmetric concentration results from the flow produced by the motile nodal cilia (McGrath and Brueckner, 2003; McGrath et al., 2003). Such a scenario is supported by the observation that the intracellular  $Ca^{2+}$  concentration is higher on the left side of the node (McGrath and Brueckner, 2003). Some cilia-associated proteins like polycystin-2 (Pennekamp et al., 2002), polaris (Moyer et al., 1994; Murcia et al., 2000) and the already mentioned inversin (the protein mutated in the *inv* mouse mutant) (Morgan et al., 1998) obviously are important both during embryonic development in the node and for tubulogenesis in the kidney because their inactivation results in *situs inversus* and polycystic kidneys.

**Polycystin-2, a non-selective cation channel of the primary cilium**

Although it was speculated early on that polycystin-2 acts as an ion channel (Mochizuki et al., 1996), it took several years before formal proof was obtained. Meanwhile it has been firmly established that polycystin-2 belongs to the TRP family of cation channels. It is permeable for mono- and divalent cation and exhibits a large conductivity (Delmas et al., 2004; Koulen et al., 2002). Small concentrations of  $Ca^{2+}$  activate the channel whereas high concentrations of  $Ca^{2+}$  inactivate it. The structural analysis of the COOH-terminus of polycystin-2 has demonstrated the presence of 2  $Ca^{2+}$ -binding sites, one with a low and the other one with a high affinity (Schumann et al., 2009). In the absence of  $Ca^{2+}$  polycystin-2 forms higher-order complexes, possibly trimers, whereas in its presence the complex dissociates. It therefore seems possible that the assembly state of polycystin-2 determines the activity of the channel: In the presence of high  $Ca^{2+}$  concentrations the protein complex disassembles and polycystin-2 no longer functions as a channel.

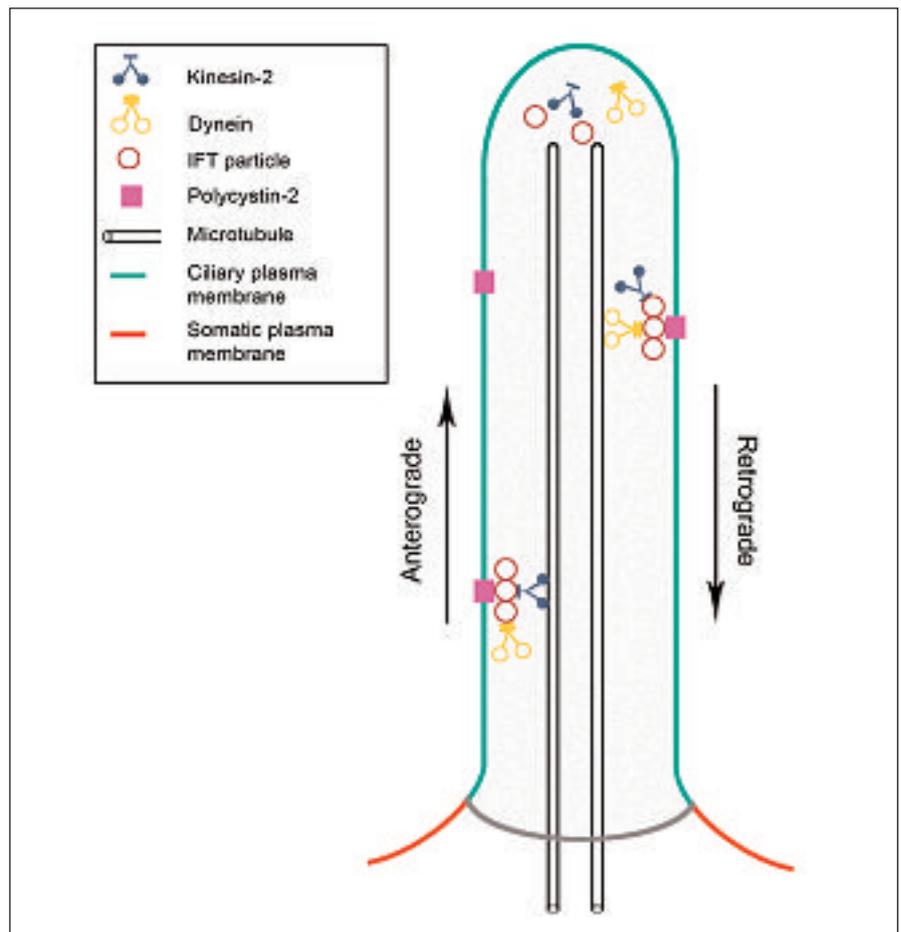
Many interacting proteins have been identified for polycystin-2 which may modulate its activity. For example, Kif3a (Li et al., 2006),  $\alpha$ -actinin (Li et al., 2005) and fibrocystin (Wang et al., 2007) interact with the NH<sub>2</sub>-terminus of polycystin-2, and more than 20 proteins such as polycystin-1 (Qian et al., 1997; Tsiokas et al., 1997), polycystin-2 itself (Tsiokas et al., 1997), PIGEA-14 (Hidaka et al., 2004), Kif3a (Li et al., 2006) and Kif3b (Wu et al., 2006) interact with the COOH-terminus of polycystin-2. Some of the interacting proteins influence the intracellular location of polycystin-2, a controversially discussed topic in the field (Witz-

gall, 2005). Exogenous polycystin-2 produced in HEK 293 cells (a human embryonic kidney cell line), MDCK cells, LLC-PK1 cells (a porcine kidney epithelial cell line) and HeLa cells (a human cervical carcinoma cell line) is found in a reticular pattern, consistent with its location in the endoplasmic reticulum (Cai et al., 1999; Koulen et al., 2002). The immunofluorescence findings were confirmed biochemically. On their way to the plasma membrane the sugar residues of *N*-glycosylated proteins become modified in the Golgi apparatus in such a way that they cannot be removed by endoglycosidase H any longer. Indeed exogenous polycystin-2 is *N*-glycosylated and still sensitive to endoglycosidase H (Cai et al., 1999; Hidaka et al., 2004). Results from density gradient centrifugations and cell surface biotinylation also support the notion that polycystin-2 is located in the endoplasmic reticulum. An explanation for the intracellular location of polycystin-2 is the presence of a retention signal for the endoplasmic reticulum in the COOH-terminus of the protein (Cai et al., 1999). The findings in the various cell lines have been confirmed with

human and murine kidney tissues (Cai et al., 1999; Koulen et al., 2002). It should also be mentioned, however, that evidence for the presence of endogenous polycystin-2 in the plasma membrane has been presented (Luo et al., 2003; Scheffers et al., 2002). Whatever the final outcome will be, it is generally accepted that a small portion of polycystin-2 escapes from the endoplasmic reticulum and reaches the primary cilium.

**Protein transport to and in the primary cilium**

Little is known how integral membrane proteins such as polycystin-2 reach the primary cilium and are transported in the primary cilium. We believe that most of polycystin-2 is located in the endoplasmic reticulum from where it is transported in a COPII-dependent fashion to the cis-side of the Golgi apparatus. The majority of polycystin-2 is transported back to the endoplasmic reticulum due to a 34-amino acid retention signal in its COOH-terminus (Cai et al., 1999) but a small percentage of the protein escapes and is transported



*Figure 4. Intraflagellar transport. Many proteins, including integral membrane proteins, are actively transported in primary cilia. Transport to the tip of the cilium is mediated by kinesin-2, retrograde transport by dynein. The switch at the tip of the cilium is not understood, furthermore the nature of the barrier at the base of the cilium is unknown.*

to the base of the primary cilium with the aid of a ciliary trafficking motif at its NH<sub>2</sub>-terminus (Geng et al., 2006). The docking of transport vesicles at the base of the primary cilium probably depends on Rab8a, a monomeric G-protein, and on the BBSome, a complex of proteins which are mutated in patients suffering from Bardet-Biedl syndrome (Nachury et al., 2007; Yoshimura et al., 2007).

How is the movement of proteins in the primary cilium regulated? For the assembly and maintenance of the primary cilium a coordinated process called intraflagellar transport (IFT, Figure 4) is necessary (Pazour et al., 2000; Pazour and Rosenbaum, 2002). In *C. elegans* IFT appears to be dispensable for the movement of the polycystin-2 orthologue PKD-2 inside the cilium (Bae et al., 2006; Qin et al., 2005). However, in the flagella of *Chlamydomonas reinhardtii* the retrograde (but not the anterograde) transport component of IFT is essential for the trafficking of the polycystin-2 orthologue CrPKD2. But also in this organism the anterograde part of the IFT plays no role in the movement of CrPKD2 (Huang et al., 2007). Our own studies with LLC-PK<sub>1</sub> cells suggest that the situation is different yet again in mammalian cells.

## Perspectives

Many novel results on primary cilia and polycystic kidney disease were obtained during the last decade. The intracellular transport of polycystin-2 and other integral membrane proteins from the endoplasmic reticulum to the primary cilium represents a fascinating cell biological problem with many unanswered questions. Where and how does polycystin-2 leave the Golgi compartment? How does it reach the base of the primary cilium? How does it enter the primary cilium? What is the barrier between the ciliary membrane compartment and the surrounding plasma membrane? Answers to these questions promise to provide intriguing insight into fundamental cell biological problems.

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## References

Anonymous. (1991). *Am J Kidney Dis* **18**, 30-37.  
 Anonymous. (1998). *Am J Kidney Dis* **32**, S38-S49.  
 Bae, Y. K., Qin, H., Knobel, K. M., Hu, J., Rosenbaum, J. L. and Barr, M. M. (2006). General and cell-type specific mechanisms target TRPP2/PKD-2 to cilia. *Development* **133**, 3859-3870.

Barnes, B. G. (1961). Ciliated secretory cells in the pars distalis of the mouse hypophysis. *J Ultrastruct Res* **5**, 453-467.  
 Barr, M. M., DeModena, J., Braun, D., Nguyen, C. Q., Hall, D. H. and Sternberg, P. W. (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.  
 Barr, M. M. and Sternberg, P. W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. *Nature* **401**, 386-389.  
 Cai, Y., Maeda, Y., Cedzich, A., Torres, V. E., Wu, G., Hayashi, T., Mochizuki, T., Park, J. H., Witzgall, R. and Somlo, S. (1999). Identification and characterization of polycystin-2, the PKD2 gene product. *J Biol Chem* **274**, 28557-28565.  
 Casuscelli, J., Schmidt, S., DeGray, B., Petri, E. T., Celic, A., Folta-Stogniew, E., Ehrlich, B. E. and Boggon, T. J. (2009). Analysis of the cytoplasmic interaction between polycystin-1 and polycystin-2. *Am J Physiol Renal Physiol* **297**, F1310-1315.  
 Currie, A. R. and Wheatley, D. N. (1966). Cilia of a distinctive structure (9 + 0) in endocrine and other tissues. *Postgrad Med J* **42**, 403-408.  
 Davies, F., Coles, G. A., Harper, P. S., Williams, A. J., Evans, C. and Cochlin, D. (1991). Polycystic kidney disease re-evaluated: a population-based study. *Q J Med* **79**, 477-485.  
 Delmas, P., Padilla, F., Osorio, N., Coste, B., Raoux, M. and Crest, M. (2004). Polycystins, calcium signaling, and human diseases. *Biochem Biophys Res Commun* **322**, 1374-1383.  
 European Dialysis and Transplant Association Registry. (1986). Demography of dialysis and transplantation in Europe, 1984. Report from the European Dialysis and Transplant Association Registry. *Nephrol Dial Transplant* **1**, 1-8.  
 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.  
 Geng, L., Okuhara, D., Yu, Z., Tian, X., Cai, Y., Shibazaki, S. and Somlo, S. (2006). Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *J Cell Sci* **119**, 1383-1395.  
 Hidaka, S., Konecke, V., Osten, L. and Witzgall, R. (2004). PIGEA-14, a novel coiled-coil protein affecting the intracellular distribution of polycystin-2. *J Biol Chem* **279**, 35009-35016.  
 Higashihara, E., Nutahara, K., Kojima, M., Tamakoshi, A., Yoshiyuki, O., Sakai, H. and Kurokawa, K. (1998). Prevalence and renal prognosis of diagnosed autosomal dominant polycystic kidney disease in Japan. *Nephron* **80**, 421-427.  
 Huang, K., Diener, D. R., Mitchell, A., Pazour, G. J., Witman, G. B. and Rosenbaum, J. L. (2007). Function and dynamics of PKD2 in *Chlamydomonas reinhardtii* flagella. *J Cell Biol* **179**, 501-514.  
 Hughes, J., Ward, C. J., Peral, B., Aspinwall, R., Clark, K., San Millan, J. L., Gamble, V. and Harris, P. C. (1995). The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nat Genet* **10**, 151-160.  
 Karcher, C., Fischer, A., Schweickert, A., Bitzer, E., Horie, S., Witzgall, R. and 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.  
 Koulen, P., Cai, Y., Geng, L., Maeda, Y., Nishimura, S., Witzgall, R., Ehrlich, B. E. and Somlo, S. (2002). Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol* **4**, 191-197.  
 Li, Q., Montalbetti, N., Shen, P. Y., Dai, X. Q., Cheeseman, C. I., Karpinski, E., Wu, G., Cantiello, H. F. and Chen, X. Z. (2005). Alpha-actinin associates with polycystin-2 and regulates its channel activity. *Hum Mol Genet* **14**, 1587-1603.  
 Li, Q., Montalbetti, N., Wu, Y., Ramos, A., Raychowdhury, M. K., Chen, X. Z. and Cantiello, H. F. (2006). Polycystin-2 cation channel function is under the control of microtubular structures in primary cilia of renal epithelial cells. *J Biol Chem* **281**, 37566-37575.  
 Lowrie, E. G. and Hampers, C. L. (1981). The success of medicare's end-stage renal-disease program: the case for profits and the private marketplace. *N Engl J Med* **305**, 434-438.  
 Luo, Y., Vassilev, P. M., Li, X., Kawanabe, Y. and Zhou, J. (2003). Native polycystin 2 functions as a plasma membrane Ca<sup>2+</sup>-permeable cation channel in renal epithelia. *Mol Cell Biol* **23**, 2600-2607.  
 Marszalek, J. R., Ruiz-Lozano, P., Roberts, E., Chien, K. R. and Goldstein, L. S. (1999). Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc Natl Acad Sci USA* **96**, 5043-5048.  
 McGrath, J. and Brueckner, M. (2003). Cilia are at the heart of vertebrate left-right asymmetry. *Curr Opin Genet Dev* **13**, 385-392.  
 McGrath, J., Somlo, S., Makova, S., Tian, X. and 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, S. L., Veldhuisen, B., Saris, J. J., Reynolds, D. M., Cai, Y., Gabow, P. A., Pierides, A. et al. (1996). PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272**, 1339-1342.  
 Morgan, D., Turnpenny, L., Goodship, J., Dai, W., Majumder, K., Matthews, L., Gardner, A., Schuster, G., Vien, L., Harrison, W. et al. (1998). *Inversin*, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the *inv* mouse. *Nat Genet* **20**, 149-156.  
 Moyer, J. H., Lee-Tischler, M. J., Kwon, H. Y., Schrick, J. J., Avner, E. D., Sweeney, W. E., Godfrey, V. L., Cacheiro, N. L., Wilkinson, J. E. and Woychik, R. P. (1994). Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. *Science* **264**, 1329-1333.  
 Murcia, N. S., Richards, W. G., Yoder, B. K., Mucenski, M. L., Dunlap, J. R. and Woychik, R. P. (2000). The Oak Ridge Polycystic Kidney (*orp*) disease gene is required for left-right axis determination. *Development* **127**, 2347-2355.  
 Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peranen, J., Merdes, A., Slusarski, D. C., Scheller, R. H., Bazan, J. F., Sheffield, V. C. et al. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* **129**, 1201-1213.  
 Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A. E., Lu, W., Brown, E. M., Quinn, S. J. et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* **33**, 129-137.  
 Nims, N., Vassmer, D. and Maser, R. L. (2003). Transmembrane domain analysis of polycystin-1, the product

- of the polycystic kidney disease-1 (PKD1) gene: evidence for 11 membrane-spanning domains. *Biochemistry* **42**, 13035-13048.
- Nonaka, S., Shiratori, H., Saijoh, Y. and Hamada, H. (2002). Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature* **418**, 96-99.
- Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M. and Hirokawa, N. (1998). Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* **95**, 829-837.
- Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B. and Cole, D. G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol* **151**, 709-718.
- Pazour, G. J. and Rosenbaum, J. L. (2002). Intraflagellar transport and cilia-dependent diseases. *Trends Cell Biol* **12**, 551-555.
- Pennekamp, P., Karcher, C., Fischer, A., Schweickert, A., Skryabin, B., Horst, J., Blum, M. and Dworniczak, B. (2002). The ion channel polycystin-2 is required for left-right axis determination in mice. *Curr Biol* **12**, 938-943.
- Peters, D. J. and Sandkuijl, L. A. (1992). Genetic heterogeneity of polycystic kidney disease in Europe. *Contrib Nephrol* **97**, 128-139.
- Praetorius, H. A. and Spring, K. R. (2001). Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol* **184**, 71-79.
- Praetorius, H. A. and Spring, K. R. (2003). Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol* **191**, 69-76.
- Qian, F., Germino, F. J., Cai, Y., Zhang, X., Somlo, S. and Germino, G. G. (1997). PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat Genet* **16**, 179-183.
- Qin, H., Burnette, D. T., Bae, Y. K., Forscher, P., Barr, M. M. and Rosenbaum, J. L. (2005). Intraflagellar transport is required for the vectorial movement of TRPV channels in the ciliary membrane. *Curr Biol* **15**, 1695-1699.
- Roscoe, J. M., Brissenden, J. E., Williams, E. A., Chery, A. L. and Silverman, M. (1993). Autosomal dominant polycystic kidney disease in Toronto. *Kidney Int* **44**, 1101-1108.
- Scheffers, M. S., Le, H., van der Bent, P., Leonhard, W., Prins, F., Spruit, L., Breuning, M. H., de Heer, E. and Peters, D. J. (2002). Distinct subcellular expression of endogenous polycystin-2 in the plasma membrane and Golgi apparatus of MDCK cells. *Hum Mol Genet* **11**, 59-67.
- Schumann, F. H., Hoffmeister, H., Schmidt, M., Bader, R., Besl, E., Witzgall, R. and Kalbitzer, H. R. (2009). NMR-assignments of a cytosolic domain of the C-terminus of polycystin-2. *Biomol NMR Assign* **3**, 141-144.
- Takeda, S., Yonekawa, Y., Tanaka, Y., Okada, Y., Nonaka, S. and Hirokawa, N. (1999). Left-right asymmetry and kinesin superfamily protein KIF3A: new insights in determination of laterality and mesoderm induction by kif3A-/- mice analysis. *J Cell Biol* **145**, 825-836.
- Torra, R., Badenas, C., Darnell, A., Nicolau, C., Volpini, V., Revert, L. and 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.
- Torra, R., Darnell, A., Cleries, M., Botey, A., Revert, L. and Vela, E. (1995). Polycystic kidney disease patients on renal replacement therapy: data from the Catalan Renal Registry. *Contrib Nephrol* **115**, 177-181.
- Tsiokas, L., Kim, E., Arnould, T., Sukhatme, V. P. and Walz, G. (1997). Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc Natl Acad Sci USA* **94**, 6965-6970.
- Wang, S., Zhang, J., Nauli, S. M., Li, X., Starremans, P. G., Luo, Y., Roberts, K. A. and Zhou, J. (2007). Fibrocystin/polyductin, found in the same protein complex with polycystin-2, regulates calcium responses in kidney epithelia. *Mol Cell Biol* **27**, 3241-3252.
- Ward, S., Thomson, N., White, J. G. and Brenner, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J Comp Neurol* **160**, 313-337.
- Ware, R. W., Clark, D., Crossland, K. and Russell, R. L. (1975). The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. *J. Comp. Neurol.* **162**, 71-110.
- Wheatley, D. N. (2005). Landmarks in the first hundred years of primary (9+0) cilium research. *Cell Biol Int* **29**, 333-339.
- Wheatley, D. N., Wang, A. M. and Strugnell, G. E. (1996). Expression of primary cilia in mammalian cells. *Cell Biol Int* **20**, 73-81.
- Witzgall, R. (2005). Polycystin-2--an intracellular or plasma membrane channel? *Naunyn Schmiedebergs Arch Pharmacol* **371**, 342-347.
- Wright, A. F., Teague, P. W., Pound, S. E., Pignatelli, P. M., Macnicol, A. M., Carothers, A. D., De Mey, R. J., Allan, P. L. and Watson, M. L. (1993). A study of genetic linkage heterogeneity in 35 adult-onset polycystic kidney disease families. *Hum Genet* **90**, 569-571.
- Wu, Y., Dai, X. Q., Li, Q., Chen, C. X., Mai, W., Hussain, Z., Long, W., Montalbetti, N., Li, G., Glynne, R. et al. (2006). Kinesin-2 mediates physical and functional interactions between polycystin-2 and fibrocystin. *Hum Mol Genet* **15**, 3280-3292.
- Yoshimura, S., Egerer, J., Fuchs, E., Haas, A. K. and Barr, F. A. (2007). Functional dissection of Rab GTPases involved in primary cilium formation. *J Cell Biol* **178**, 363-369.
- Zimmermann, K. W. (1898). Beiträge zur Kenntnis einiger Drüsen und Epithelien. *Arch Mikr Anat Entwicklungsmech* **52**, 552-706.

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anti-Cardiac Actin  
anti-Non-Filamentous Actin  
anti-Nuclear Actin  
anti-Drebrin  
anti-Actin Related Protein T1  
anti-Actin Related Protein T2

*Antibodies to Junction Proteins*

anti-p0071 Protein, mouse monoclonal  
anti-p0071 Protein, guinea pig serum  
anti-ARVCF, mouse monoclonal  
anti-ARVCF, guinea pig serum  
anti-Desmoglein 4, guinea pig serum

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