

Mouse models of polycystic kidney disease induced by defects of ciliary proteins

Je Yeong Ko & Jong Hoon Park*

Department of Biological Science, Sookmyung Women's University, Seoul 140-742, Korea

Polycystic kidney disease (PKD) is a common hereditary disorder which is characterized by fluid-filled cysts in the kidney. Mutation in either *PKD1*, encoding polycystin-1 (PC1), or *PKD2*, encoding polycystin-2 (PC2), are causative genes of PKD. Recent studies indicate that renal cilia, known as mechanosensors, detecting flow stimulation through renal tubules, have a critical function in maintaining homeostasis of renal epithelial cells. Because most proteins related to PKD are localized to renal cilia or have a function in ciliogenesis. PC1/PC2 heterodimer is localized to the cilia, playing a role in calcium channels. Also, disruptions of ciliary proteins, except for PC1 and PC2, could be involved in the induction of polycystic kidney disease. Based on these findings, various PKD mice models were produced to understand the roles of primary cilia defects in renal cyst formation. In this review, we will describe the general role of cilia in renal epithelial cells, and the relationship between ciliary defects and PKD. We also discuss mouse models of PKD related to ciliary defects based on recent studies. [BMB Reports 2013; 46(2): 73-79]

INTRODUCTION

Polycystic kidney disease (PKD) is a common genetic disorder and is manifested by defects in renal epithelial cells, resulting in an increase of cell proliferation and cyst formation (1). PKD is a systemic disorder which is characterized by cysts in the kidney, liver and pancreas (2).

ADPKD is caused by mutations of either *PKD1* (85%) or *PKD2* (15%) genes, which encode polycystin-1 (PC1) or polycystin-2 (PC2) respectively (3). PC1 is a large integral membrane protein, which includes a long N-terminal extracellular domain, a short C-terminal intracellular domain, and eleven transmembrane domains, and acts as a G-protein-coupled receptor (4-6). PC1 is localized to primary cilia, lateral domain

of the plasma membrane (7), and regions of cell-matrix interaction (8). The C-terminal, cytoplasmic tail, of PC1 is cleaved to generate products, which interact with transcription factors. PC2 is a membrane protein with six transmembrane domains, and is considered to be a cation channel with specificity for calcium (9, 10). This protein is localized to not only primary cilia (11), but also to subcellular compartments, including the plasma membrane (12) and endoplasmic reticulum. PC1 and PC2 interact via their C-terminus (13) to form transmembrane receptor-ion channel complex in the primary cilia of renal epithelial cells (1, 14). This protein complex regulates intracellular calcium levels through its channel, which is stimulated by fluid flow in the renal tubule, and affects calcium-related pathways. Recent studies have shown that dysfunctions of PC1 and PC2 result in decreased intracellular calcium in renal epithelial cells of PKD (15). Some aberrant signaling pathways have been identified in PKD. Among these, the signaling pathway related to calcium, cyclic AMP (cAMP) and mammalian target of rapamycin (mTOR) is well-known as a major signaling in PKD (16-19). In addition to these pathways, dysregulation of cell cycle, JAK-STAT pathway, canonical and non-canonical Wnt pathway are observed in PKD.

Recent studies suggest that defects of primary cilia are also related to the formation of renal cystic kidneys (20, 21). The cilium acts as a mechanosensor in kidney tubules, in regulating homeostasis of renal epithelial cells. Many signaling components are localized to the membrane of renal primary cilia. Accumulating results show that proteins related to PKD are localized to the primary cilia, and disruptions of ciliary proteins, including intraflagellar transport (IFT), induce PKD (3). These findings indicate that defects of cilia act as a driving force to induce PKD (22).

Consistent with these recent findings, malfunctions of primary cilia, induced by mutations of ciliary proteins, is correlated with PKD. Therefore, it is important to identify the cyst formation mechanism associated with ciliary malfunctions using *in vivo* studies, to develop therapeutic targets for PKD. Here, we will focus on recent advances in understanding the ciliary roles in polycystic kidney and PKD mouse models accompanied by cilia defects.

*Corresponding author. Tel: +82-2-710-9414; Fax: +82-2-2077-7322; E-mail: parkjh@sookmyung.ac.kr
<http://dx.doi.org/10.5483/BMBRep.2013.46.2.022>

Received 24 January 2013

Keywords: Intraflagellar transport, Polycystic kidney disease, Polycystin-1, Polycystin-2, Primary cilia

PRIMARY CILIA AND IFT

The cilium is commonly divided into two groups, motile and non-motile cilia. Motile cilia that have a 9+2 microtubule configuration (3) are mainly located on epithelial cells, and promote the movement of substances. On the other hand, non-motile cilia, known as primary cilia, have a 9+0 microtubule arrangement. Primary cilia projected from the cell membrane act as mechanosensor to sense mechanical forces, growth factors and chemical compounds from extracellular environments (23). Primary cilia have important roles for regulating intracellular signaling, such as Hedgehog (Hh), Wnt, platelet-derived growth factor receptor (PDGFR) α , and mechanosignaling related to PKD1/2 complex (24).

Regulation of ciliogenesis is critical to maintain functional cilia. The key factor related to ciliogenesis is Intraflagellar Transport (IFT), which is a bi-directional movement multi-protein complex (25). IFT particles are divided into two complexes, A and B. The complex A, known as retrograde transport, moves from ciliary tip to base with dynein, while the complex B, known as anterograde transport, moves from base to ciliary tip with kinesin-2 (26). IFT particles are regarded as vehicles for transporting cargos to regulate cilia assembly, maintenance, and function (27). In addition, these particles have a function in carrying cilia membrane proteins. Consistent with these roles for cilia, cilia defects or mutations of IFT genes are related to human disease, such as PKD, left-right asymmetry defects, cancer, and Bardet-Biedl syndrome (BBS) (24, 28).

RELATIONSHIP BETWEEN CILIA DEFECTS AND POLYCYSTIC KIDNEY DISEASE

In renal epithelial cells, primary cilia protrude into lumen of the renal tubules to sense fluid flow (29). Polycystin-1 (PC1) and polycystin-2 (PC2) are localized to the membrane of cilium, and form a PC1/PC2 complex as a calcium channel. PC1 acts as mechanosensor to detect the bending of cilium induced by flow stimulation that transmits extracellular signals to PC2, which, in turn, induces Ca^{2+} influx to cytosol (11, 30), hence this complex and primary cilia in renal cells regulates intracellular signaling associated with calcium concentration such as the Raf/MEK/ERK pathway, which are increased in PKD (31). Moreover, various signaling molecules related to Hh, PDGFR α , and calcium signaling are localized to the cilia membrane (32). Therefore, defects of cilia or ciliary proteins are sufficient to induce pathological disorders.

Among various diseases, PKD is one which is related to cilia defects. Many proteins whose functions are disrupted in PKD are localized to cilium membrane or to the ciliary basal body (24, 33). Also, disruption of cilia proteins are involved in calcium signaling related to PKD, implicating a relationship between ciliary signaling and cyst formation (34). Consistent with this, the intracellular calcium level is decreased in PKD epithelial cells compared to normal renal epithelial cells (35).

Recent studies have demonstrated that malfunction of renal cilia induces intracellular cAMP, leading to an increase of renal cell proliferation through the Raf/MEK/ERK pathway, known as a hallmark of cystic kidney (36, 37). Therefore, to elucidate the functions of ciliary genes *in vivo* is important to identify the disease inducing mechanisms and therapeutic targets of PKD.

MOUSE MODELS OF POLYCYSTIC KIDNEY INDUCED BY MUTATIONS OF CILIARY PROTEINS

Kidney specific inactivation of *Pkd1*

As we described above, PC1 localized to primary cilia is a major causative gene for the development of polycystic kidneys. In the case of complete knockout of *Pkd1* in the mouse, a complex embryonic lethality is observed: defects in formation of the kidney, pancreas, heart and capillary blood vessels (38-40). However, *Pkd1^{fllox/-}:Ksp-Cre* mice generated by deletion of exons 2-4 by Ksp-Cre are born, but consistently develop cystic kidneys (14). Consistent with this, components related to the MAPK/ERK pathway, blood urea nitrogen (BUN), and cell proliferation, are dramatically increased in the cystic kidney of this mouse (14).

Pkd2^{ws25/-} mice

The *WS25* allele is generated by the integration of an exon 1 disrupted by introduction of a *neo^r* cassette into intron 1 of *Pkd2*, without replacement of the wild-type exon 1 (41, 42). This mutation causes an unstable allele, which increases the somatic mutation rate of *Pkd2* (42, 43). The knockout of *Pkd2* is embryonically lethal between embryonic day 13.5 and parturition, whereas the *Pkd2^{ws25/-}* mouse is born (44). The *Pkd2^{ws25/-}* mouse is an animal model of ADPKD with hepatic cysts and cardiac defects, and exhibits renal failure (44). Studies using this mouse model have shown that renal cilia in the cystic kidney of *Pkd2^{ws25/-}* mouse have normal phenotype (45), but cilia of cystic cholangiocytes are short and malformed (46).

Oak ridge polycystic kidney mice

The oak ridge polycystic kidney (ORPK) mouse resembles human ARPKD with respect to cystic kidneys and hepatic disease (47, 48). This mouse is the first model showing a relationship between polycystic kidney and ciliary malfunction (48, 49). The *orpk* allele was driven by insertion mutation on the intron near the 3' end of the gene, partially disrupting the function and expression of *Ift88* (*Tg737*, *polaris*) (50). Increased levels of PC2 in primary cilia is observed in the kidneys of ORPK mice (49). The *Tg737^{orpk}* mutant mice have polycystic kidneys with shortened cilia on renal epithelial cells (47, 48). In addition to renal disorder, hepatic and pancreatic defects, skeletal patterning abnormalities and hydrocephalus are observed in the ORPK mouse (51-53).

Deletion of *Ift20* in the mouse kidney

Inactivation of *Ift20* in the mouse kidney using Cre-loxP sys-

tem promotes postnatal cystic kidneys lacking cilia (54). The conditional allele of *Ift20* was generated by targeting exon 2 and 3 (54). Deletion of *Ift20* in kidneys collecting duct cells induces mis-orientation of the mitotic spindle and increases of canonical Wnt signaling, leading to increased renal cell proliferation (54). In addition to this *in vivo* study, there are some evidences showing that *Ift20* is a candidate gene related to PKD. *Ift20* is associated with Golgi complex to regulate cilia assembly and is involved in the localization of PC2 to cilia (55).

Mice with a conditional allele for *Ift140*

Deletion of *Ift140*, subunit of IFT complex A, in renal collecting duct cells leads to renal cyst formation with disruption of the cilia assembly, but this mutation does not have a function on mitotic spindle orientation (56). Several genes which are hallmarks of polycystic kidney and are associated with the canonical Wnt pathway, Hedgehog, Hippo and fibrosis were significantly increased in *Ift140*-deleted kidneys (56).

Kif3α-targeted mouse

Constitutive knockout of *Kif3α*, a subunit of kinesin-II that is critical for ciliogenesis, results in an absence of cilia in the embryonic node, situs inversus, and abnormalities of the neural tube, pericardium and smites (57, 58). This constitutive *Kif3α* knockout mouse shows embryonic lethality before renal organogenesis (59). In contrast, in the case of specific targeting of *Kif3α* in renal tubular epithelial cells, viable offspring were born and developed cystic kidneys (59). These cystic lining epithelial cells have no cilia due to *Kif3α* inactivation, and show increased proliferation, apoptosis, expression of β -catenin, c-Myc, and decreased p21^{Cip1} (59).

Congenital polycystic kidney mouse

The Cystin encoded by *Cys1* is a 145-amino acid cilium-associated protein (60). The congenital polycystic kidney (*cpk*) mice have mutations of Cystin localized to the primary cilia (61-64). This mutant develops renal cysts and biliary dysgenesis and is considered as ARPKD (61). The kidneys of *cpk/cpk* mice have multiple cellular and extracellular abnormalities (63), including increased expression of proto-oncogenes, such as c-myc, c-fos (65, 66), elevated expression of growth factors (67), abnormal expression of genes related to cell adhesion (68), and overexpression of matrix metalloproteinases (MMPs) (69). The renal cystic epithelial cells of *cpk* mice have different length and morphology of cilia (70).

Inversin-targeted mice

The Inversin protein localizes to the primary cilia, cell junctions and nucleus, and acts as a regulator of β -catenin activity and canonical Wnt pathway (71). The inversion of embryonic turning (*inv*) mutation is generated by random insertion of tyrosinase minigene in the OVE210 transgenic line (72, 73). This random insertion disrupts Inversin encoded by *Invs*, which lo-

calizes to primary apical cilia (74). Phillips *et al.* (75) have demonstrated that normal looking monocilia were observed in cystic kidneys of *inv/inv* mice. However, the *inv* mutant shows a reversal of left-right asymmetry, and renal cyst known as diseases induced by ciliary defects (76).

Bbs2-deficient mice

The proteins encoded by Bardet-Biedl syndrome (BBS) genes, except for BBS6, are localized to ciliated organisms (77, 78). BBS2 is one of the proteins that form the complex BBSome, which is localized to the membrane of the cilium and is required for ciliogenesis. Several evidences suggest that BBS4 and BBS6 are expressed in basal bodies of primary cilia (79, 80). The ciliogenic function of the BBS complex is related to the Rab8 GDP/GTP exchange factor, which localizes to basal body and contacts the BBSome (81).

Bbs2-deficient mice are generated by deletion of exons 5-14, and show the phenotype of cystic kidneys (82). Interestingly, renal cilia are observed in Bbs2-deficient mice, but their shape is abnormal compared to cilia of wild-type kidneys (82). In addition to polycystic kidney, this mutant mouse develops retinal degeneration, obesity and olfactory dysfunction (82).

Juvenile cystic kidney mouse

The *jck* allele has a missense mutation in the NIMA (never in mitosis A)-related kinase 8 encoded by *Nek8* (83). NIMA is a serine/threonine kinase in *Aspergillus nidulans*, and has a function in regulating mitosis (84). Published results suggest that *Nek8* is overexpressed in human breast cancers, indicating that *Nek8* has a role in cell proliferation (84, 85).

In wild-type kidney, *Nek8* is found in the proximal region of renal cilia, whereas kidneys of the *jck* mutant show that *Nek8* is localized to the entire length of renal cilia (84). The kidneys of *jck* mice have cysts in multiple nephron segments like ADPKD, and increased cAMP levels, which promote renal cell proliferation and fluid secretion (86). Also, the lengthened cilia with overexpression of polycystins are observed in kidneys of *jck* mice (86).

CONCLUSION

The role of the renal primary cilia is under the spotlight, because the disruption of ciliary structures and proteins is associated with polycystic kidney. To determine the mechanism between defects of renal cilia and polycystic kidneys, various cystic kidney mouse models have been generated. Based on many studies, mutation of some proteins localized to primary cilia, such as IFT, leads to cyst formation accompanied by ciliary defects. This mechanism of cyst formation seems to occur via increased renal cell proliferation. Consistent with this, mutations of some proteins localized to ciliary membrane, such as PC1 and PC2, result in the development of cystic kidneys. However, these ciliary membrane proteins, non-IFT family pro-

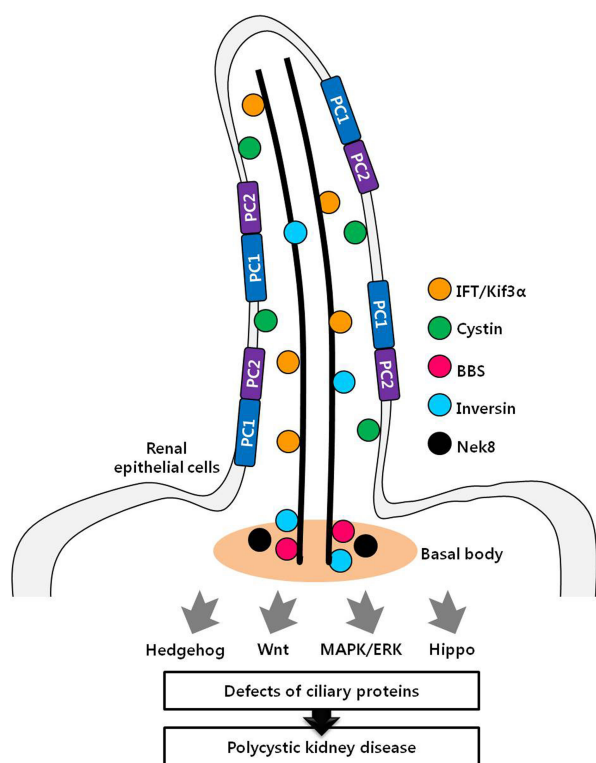


Fig. 1. Localization of ciliary proteins related to polycystic kidney. Many proteins localized to renal cilia are associated with PKD. Disruptions of these proteins induce cystic kidneys through various signaling pathway.

teins, which act as receptor and signaling molecules, seem to have no effect on the regulation of ciliogenesis. Instead of functioning in ciliogenesis, these proteins are localized to ciliary membranes to regulate the cilia function as mechanosensors rather than regulating ciliogenesis. Taken together, dysfunctions of proteins associated with renal primary cilia have an effect on cyst formation of the kidneys (Fig. 1). To identify the exact mechanism of cyst formation induced by ciliary defects, further *in vivo* studies are needed. It is important to reveal the mechanism of cystic kidney development, because a successful treatment for PKD has not yet been developed. Therefore, we expect that these mechanism studies might be used for the selection of novel therapeutic targets of PKD.

Acknowledgements

This Research was supported by the Sookmyung Women's University Research Grants 2010.

REFERENCES

1. Fedeles, S. and Gallagher, A. R. (2012) Cell polarity and cystic kidney disease. *Pediatr. Nephrol.* 2012 Nov 16 [Epub ahead of print] PubMed PMID: 23161205.

2. Wilson, P. D. and Goilav, B. (2007) Cystic disease of the kidney. *Ann. Rev. Pathol.* **2**, 341-368.
3. Nauli, S. M. and Zhou, J. (2004) Polycystins and mechanosensation in renal and nodal cilia. *Bioessays* **26**, 844-856.
4. 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.
5. Li, X. (2011) Phosphorylation, protein kinases and ADPKD. *Biochim. Biophys. Acta* **1812**, 1219-1224.
6. Delmas, P., Nomura, H., Li, X., Lakkis, M., Luo, Y., Segal, Y., Fernandez-Fernandez, J. M., Harris, P., Frischauf, A. M., Brown, D. A. and Zhou, J. (2002) Constitutive activation of G-proteins by polycystin-1 is antagonized by polycystin-2. *J. Biol. Chem.* **277**, 11276-11283.
7. Chapin, H. C. and Caplan, M. J. (2010) The cell biology of polycystic kidney disease. *J. Cell Biol.* **191**, 701-710.
8. Wilson, P. D., Geng, L., Li, X. and Burrow, C. R. (1999) The PKD1 gene product, "polycystin-1," is a tyrosine-phosphorylated protein that colocalizes with alpha2beta1-integrin in focal clusters in adherent renal epithelia. *Lab. Invest.* **79**, 1311-1323.
9. Ibraghimov-Beskrovnya, O. and Bukanov, N. (2008) Polycystic kidney diseases: from molecular discoveries to targeted therapeutic strategies. *Cell. Mol. Life Sci.* **65**, 605-619.
10. Gonzalez-Perrett, S., Kim, K., Ibarra, C., Damiano, A. E., Zotta, E., Batelli, M., Harris, P. C., Reisin, I. L., Amaout, M. A. and Cantiello, H. F. (2001) Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca²⁺-permeable nonselective cation channel. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1182-1187.
11. 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., Ingber, D. E. and Zhou, J. (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **33**, 129-137.
12. Hanaoka, K., Qian, F., Boletta, A., Bhunia, A. K., Piontek, K., Tsiokas, L., Sukhatme, V. P., Guggino, W. B. and Germino, G. G. (2000) Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* **408**, 990-994.
13. 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.
14. Shibasaki, S., Yu, Z., Nishio, S., Tian, X., Thomson, R. B., Mitobe, M., Louvi, A., Velazquez, H., Ishibe, S., Cantley, L. G., Igarashi, P. and Somlo, S. (2008) Cyst formation and activation of the extracellular regulated kinase pathway after kidney specific inactivation of Pkd1. *Hum. Mol. Genet.* **17**, 1505-1516.
15. Kip, S. N., Hunter, L. W., Ren, Q., Harris, P. C., Somlo, S., Torres, V. E., Sieck, G. C. and Qian, Q. (2005) [Ca²⁺]_i reduction increases cellular proliferation and apoptosis in vascular smooth muscle cells: relevance to the ADPKD phenotype. *Circ. Res.* **96**, 873-880.
16. Tao, Y., Kim, J., Faubel, S., Wu, J. C., Falk, S. A., Schrier, R. W. and Edelstein, C. L. (2005) Caspase inhibition reduces tubular apoptosis and proliferation and slows disease progression in polycystic kidney disease. *Proc. Natl.*

- Acad. Sci. U.S.A.* **102**, 6954-6959.
17. Torres, V. E., Bankir, L. and Grantham, J. J. (2009) A case for water in the treatment of polycystic kidney disease. *Clin. J. Am. Soc. Nephrol.* **4**, 1140-1150.
 18. Chang, M. Y. and Ong, A. C. (2012) Mechanism-based therapeutics for autosomal dominant polycystic kidney disease: recent progress and future prospects. *Nephron. Clin. Pract.* **120**, 25-35.
 19. Park, E. Y., Woo, Y. M. and Park, J. H. (2011) Polycystic kidney disease and therapeutic approaches. *BMB Rep.* **44**, 359-368.
 20. Ibraghimov-Beskrovnaya, O. (2007) Targeting dysregulated cell cycle and apoptosis for polycystic kidney disease therapy. *Cell Cycle* **6**, 776-779.
 21. Pan, J., Wang, Q. and Snell, W. J. (2005) Cilium-generated signaling and cilia-related disorders. *Lab. Invest.* **85**, 452-463.
 22. Yoder, B. K., Tousson, A., Millican, L., Wu, J. H., Bugg, C. E., Jr., Schafer, J. A. and Balkovetz, D. F. (2002) Polaris, a protein disrupted in orpk mutant mice, is required for assembly of renal cilium. *Am. J. Physiol. Renal Physiol.* **282**, F541-552.
 23. Mizuno, N., Taschner, M., Engel, B. D. and Lorentzen, E. (2012) Structural studies of ciliary components. *J. Mol. Biol.* **422**, 163-180.
 24. Veland, I. R., Awan, A., Pedersen, L. B., Yoder, B. K. and Christensen, S. T. (2009) Primary cilia and signaling pathways in mammalian development, health and disease. *Nephron Physiol.* **111**, p39-53.
 25. Taschner, M., Bhogaraju, S. and Lorentzen, E. (2012) Architecture and function of IFT complex proteins in ciliogenesis. *Differentiation* **83**, S12-22.
 26. Ishikawa, H. and Marshall, W. F. (2011) Ciliogenesis: building the cell's antenna. *Nat. Rev. Mol. Cell Biol.* **12**, 222-234.
 27. Cao, Y., Park, A. and Sun, Z. (2010) Intraflagellar transport proteins are essential for cilia formation and for planar cell polarity. *J. Am. Soc. Nephrol.* **21**, 1326-1333.
 28. Pedersen, L. B., Veland, I. R., Schroder, J. M. and Christensen, S. T. (2008) Assembly of primary cilia. *Dev. Dyn.* **237**, 1993-2006.
 29. Hurd, T. W. and Margolis, B. (2009) Cystin, cilia, and cysts: unraveling trafficking determinants. *J. Am. Soc. Nephrol.* **20**, 2485-2486.
 30. Praetorius, H. A. and Spring, K. R. (2001) Bending the MDCK cell primary cilium increases intracellular calcium. *J. Membr. Biol.* **184**, 71-79.
 31. Yamaguchi, T., Nagao, S., Wallace, D. P., Belibi, F. A., Cowley, B. D., Pelling, J. C. and Grantham, J. J. (2003) Cyclic AMP activates B-Raf and ERK in cyst epithelial cells from autosomal-dominant polycystic kidneys. *Kidney Int.* **63**, 1983-1994.
 32. Lancaster, M. A. and Gleeson, J. G. (2009) The primary cilium as a cellular signaling center: lessons from disease. *Curr. Opin. Genet. Dev.* **19**, 220-229.
 33. Mahjoub, M. R., Trapp, M. L. and Quarmby, L. M. (2005) NIMA-related kinases defective in murine models of polycystic kidney diseases localize to primary cilia and centrosomes. *J. Am. Soc. Nephrol.* **16**, 3485-3489.
 34. Rydholm, S., Zwart, G., Kowalewski, J. M., Kamali-Zare, P., Frisk, T. and Brismar, H. (2010) Mechanical properties of primary cilia regulate the response to fluid flow. *Am. J. Physiol. Renal Physiol.* **298**, F1096-1102.
 35. Cowley, B. D., Jr. (2008) Calcium, cyclic AMP, and MAP kinases: dysregulation in polycystic kidney disease. *Kidney Int.* **73**, 251-253.
 36. Choi, Y. H., Suzuki, A., Hajarnis, S., Ma, Z., Chapin, H. C., Caplan, M. J., Pontoglio, M., Somlo, S. and Igarashi, P. (2011) Polycystin-2 and phosphodiesterase 4C are components of a ciliary A-kinase anchoring protein complex that is disrupted in cystic kidney diseases. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 10679-10684.
 37. Pan, J., Seeger-Nukpezah, T. and Golemis, E. A. (2012) The role of the cilium in normal and abnormal cell cycles: emphasis on renal cystic pathologies. *Cell Mol. Life Sci.* 2012 Jul 11 [Epub ahead of print] PubMed PMID: 22782110.
 38. Boulter, C., Mulroy, S., Webb, S., Fleming, S., Brindle, K. and Sandford, R. (2001) Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12174-12179.
 39. Lu, W., Peissel, B., Babakhanlou, H., Pavlova, A., Geng, L., Fan, X., Larson, C., Brent, G. and Zhou, J. (1997) Perinatal lethality with kidney and pancreas defects in mice with a targeted Pkd1 mutation. *Nat. Genet.* **17**, 179-181.
 40. Kim, K., Drummond, I., Ibraghimov-Beskrovnaya, O., Klinger, K. and Arnaout, M. A. (2000) Polycystin 1 is required for the structural integrity of blood vessels. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1731-1736.
 41. Wu, G., D'Agati, V., Cai, Y., Markowitz, G., Park, J. H., Reynolds, D. M., Maeda, Y., Le, T. C., Hou, H., Jr., Kucherlapati, R., Edelmann, W. and Somlo, S. (1998) Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* **93**, 177-188.
 42. Wang, X., Harris, P. C., Somlo, S., Batlle, D. and Torres, V. E. (2009) Effect of calcium-sensing receptor activation in models of autosomal recessive or dominant polycystic kidney disease. *Nephrol. Dial. Transplant.* **24**, 526-534.
 43. Wu, G. and Somlo, S. (2000) Molecular genetics and mechanism of autosomal dominant polycystic kidney disease. *Mol. Genet. Metab.* **69**, 1-15.
 44. Wu, G., Markowitz, G. S., Li, L., D'Agati, V. D., Factor, S. M., Geng, L., Tibara, S., Tuchman, J., Cai, Y., Park, J. H., van Adelsberg, J., Hou, H., Jr., Kucherlapati, R., Edelmann, W. and Somlo, S. (2000) Cardiac defects and renal failure in mice with targeted mutations in Pkd2. *Nat. Genet.* **24**, 75-78.
 45. Thomson, R. B., Mentone, S., Kim, R., Earle, K., Delpire, E., Somlo, S. and Aronson, P. S. (2003) Histopathological analysis of renal cystic epithelia in the Pkd2^{WS25/-} mouse model of ADPKD. *Am. J. Physiol. Renal Physiol.* **285**, F870-880.
 46. Stroope, A., Radtke, B., Huang, B., Masyuk, T., Torres, V., Ritman, E. and LaRusso, N. (2010) Hepato-renal pathology in *pkd2ws25/-* mice, an animal model of autosomal dominant polycystic kidney disease. *Am. J. Pathol.* **176**, 1282-1291.
 47. 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.
48. 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.
49. Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L. and Witman, G. B. (2002) Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr. Biol.* **12**, R378-380.
50. Lehman, J. M., Michaud, E. J., Schoeb, T. R., Aydin-Son, Y., Miller, M. and Yoder, B. K. (2008) The Oak Ridge Polycystic Kidney mouse: modeling ciliopathies of mice and men. *Dev. Dyn.* **237**, 1960-1971.
51. Zhang, Q., Davenport, J. R., Croyle, M. J., Haycraft, C. J. and Yoder, B. K. (2005) Disruption of IFT results in both exocrine and endocrine abnormalities in the pancreas of Tg737(orpk) mutant mice. *Lab. Invest.* **85**, 45-64.
52. Banizs, B., Pike, M. M., Millican, C. L., Ferguson, W. B., Komlosi, P., Sheetz, J., Bell, P. D., Schwiebert, E. M. and Yoder, B. K. (2005) Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development* **132**, 5329-5339.
53. Cano, D. A., Murcia, N. S., Pazour, G. J. and Hebrok, M. (2004) Orpk mouse model of polycystic kidney disease reveals essential role of primary cilia in pancreatic tissue organization. *Development* **131**, 3457-3467.
54. Jonassen, J. A., San Agustin, J., Follit, J. A. and Pazour, G. J. (2008) Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease. *J. Cell Biol.* **183**, 377-384.
55. Follit, J. A., Tuft, R. A., Fogarty, K. E. and Pazour, G. J. (2006) The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol. Biol. Cell* **17**, 3781-3792.
56. Jonassen, J. A., SanAgustin, J., Baker, S. P. and Pazour, G. J. (2012) Disruption of IFT complex A causes cystic kidneys without mitotic spindle misorientation. *J. Am. Soc. Nephrol.* **23**, 641-651.
57. 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. U.S.A.* **96**, 5043-5048.
58. 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.
59. Lin, F., Hiesberger, T., Cordes, K., Sinclair, A. M., Goldstein, L. S., Somlo, S. and Igarashi, P. (2003) Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 5286-5291.
60. Tao, B., Bu, S., Yang, Z., Siroky, B., Kappes, J. C., Kispert, A. and Guay-Woodford, L. M. (2009) Cystin localizes to primary cilia via membrane microdomains and a targeting motif. *J. Am. Soc. Nephrol.* **20**, 2570-2580.
61. Yoder, B. K., Hou, X. and Guay-Woodford, L. M. (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.
62. Chiu, M. G., Johnson, T. M., Woolf, A. S., Dahm-Vicker, E. M., Long, D. A., Guay-Woodford, L., Hillman, K. A., Bawumia, S., Venner, K., Hughes, R. C., Poirier, F. and Winyard, P. J. (2006) Galectin-3 associates with the primary cilium and modulates cyst growth in congenital polycystic kidney disease. *Am. J. Pathol.* **169**, 1925-1938.
63. Hou, X., Mrug, M., Yoder, B. K., Lefkowitz, E. J., Kremmidiotis, G., D'Eustachio, P., Beier, D. R. and Guay-Woodford, L. M. (2002) Cystin, a novel cilia-associated protein, is disrupted in the cpk mouse model of polycystic kidney disease. *J. Clin. Invest.* **109**, 533-540.
64. Preminger, G. M., Koch, W. E., Fried, F. A., McFarland, E., Murphy, E. D. and Mandell, J. (1982) Murine congenital polycystic kidney disease: a model for studying development of cystic disease. *J. Urol.* **127**, 556-560.
65. Cowley, B. D., Jr., Smardo, F. L., Jr., Grantham, J. J. and Calvet, J. P. (1987) Elevated c-myc protooncogene expression in autosomal recessive polycystic kidney disease. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8394-8398.
66. Cowley, B. D., Jr., Chadwick, L. J., Grantham, J. J. and Calvet, J. P. (1991) Elevated proto-oncogene expression in polycystic kidneys of the C57BL/6j (cpk) mouse. *J. Am. Soc. Nephrol.* **1**, 1048-1053.
67. Nakamura, T., Ebihara, I., Nagaoka, I., Tomino, Y., Nagao, S., Takahashi, H. and Koide, H. (1993) Growth factor gene expression in kidney of murine polycystic kidney disease. *J. Am. Soc. Nephrol.* **3**, 1378-1386.
68. Rocco, M. V., Neilson, E. G., Hoyer, J. R. and Ziyadeh, F. N. (1992) Attenuated expression of epithelial cell adhesion molecules in murine polycystic kidney disease. *Am. J. Physiol.* **262**, F679-686.
69. Rankin, C. A., Suzuki, K., Itoh, Y., Ziemer, D. M., Grantham, J. J., Calvet, J. P. and Nagase, H. (1996) Matrix metalloproteinases and TIMPS in cultured C57BL/6j-cpk kidney tubules. *Kidney Int.* **50**, 835-844.
70. Winyard, P. and Jenkins, D. (2011) Putative roles of cilia in polycystic kidney disease. *Biochim. Biophys. Acta.* **1812**, 1256-1262.
71. Gerdes, J. M., Davis, E. E. and Katsanis, N. (2009) The vertebrate primary cilium in development, homeostasis, and disease. *Cell* **137**, 32-45.
72. Yokoyama, T., Copeland, N. G., Jenkins, N. A., Montgomery, C. A., Elder, F. F. and Overbeek, P. A. (1993) Reversal of left-right asymmetry: a situs inversus mutation. *Science* **260**, 679-682.
73. Guay-Woodford, L. M. (2003) Murine models of polycystic kidney disease: molecular and therapeutic insights. *Am. J. Physiol. Renal Physiol.* **285**, F1034-1049.
74. Morgan, D., Eley, L., Sayer, J., Strachan, T., Yates, L. M., Craighead, A. S. and Goodship, J. A. (2002) Expression analyses and interaction with the anaphase promoting complex protein Apc2 suggest a role for inversin in primary cilia and involvement in the cell cycle. *Human Mol. Genet.* **11**, 3345-3350.
75. Phillips, C. L., Miller, K. J., Filson, A. J., Nurnberger, J.,

- Clendenon, J. L., Cook, G. W., Dunn, K. W., Overbeek, P. A., Gattone, V. H., 2nd and Bacallao, R. L. (2004) Renal cysts of *inv/inv* mice resemble early infantile nephronophthisis. *J. Am. Soc. Nephrol.* **15**, 1744-1755.
76. Sugiyama, N., Tsukiyama, T., Yamaguchi, T. P. and Yokoyama, T. (2011) The canonical Wnt signaling pathway is not involved in renal cyst development in the kidneys of *inv* mutant mice. *Kidney Int.* **79**, 957-965.
77. Mykytyn, K., Mullins, R. F., Andrews, M., Chiang, A. P., Swiderski, R. E., Yang, B., Braun, T., Casavant, T., Stone, E. M. and Sheffield, V. C. (2004) Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate *Bbs4* in flagella formation but not global cilia assembly. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 8664-8669.
78. Li, J. B., Gerdes, J. M., Haycraft, C. J., Fan, Y., Teslovich, T. M., May-Simera, H., Li, H., Blacque, O. E., Li, L., Leitch, C. C., Lewis, R. A., Green, J. S., Parfrey, P. S., Leroux, M. R., Davidson, W. S., Beales, P. L., Guay-Woodford, L. M., Yoder, B. K., Stormo, G. D., Katsanis, N. and Dutcher, S. K. (2004) Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* **117**, 541-552.
79. Ansley, S. J., Badano, J. L., Blacque, O. E., Hill, J., Hoskins, B. E., Leitch, C. C., Kim, J. C., Ross, A. J., Eichers, E. R., Teslovich, T. M., Mah, A. K., Johnsen, R. C., Cavender, J. C., Lewis, R. A., Leroux, M. R., Beales, P. L. and Katsanis, N. (2003) Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* **425**, 628-633.
80. Kim, J. C., Badano, J. L., Sibold, S., Esmail, M. A., Hill, J., Hoskins, B. E., Leitch, C. C., Venner, K., Ansley, S. J., Ross, A. J., Leroux, M. R., Katsanis, N. and Beales, P. L. (2004) The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat. Genet.* **36**, 462-470.
81. 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. and Jackson, P. K. (2007) A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* **129**, 1201-1213.
82. Nishimura, D. Y., Fath, M., Mullins, R. F., Searby, C., Andrews, M., Davis, R., Andorf, J. L., Mykytyn, K., Swiderski, R. E., Yang, B., Carmi, R., Stone, E. M. and Sheffield, V. C. (2004) *Bbs2*-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 16588-16593.
83. Liu, S., Lu, W., Obara, T., Kuida, S., Lehoczy, J., Dewar, K., Drummond, I. A. and Beier, D. R. (2002) A defect in a novel Nek-family kinase causes cystic kidney disease in the mouse and in zebrafish. *Development* **129**, 5839-5846.
84. Sohara, E., Luo, Y., Zhang, J., Manning, D. K., Beier, D. R. and Zhou, J. (2008) Nek8 regulates the expression and localization of polycystin-1 and polycystin-2. *J. Am. Soc. Nephrol.* **19**, 469-476.
85. Bowers, A. J. and Boylan, J. F. (2004) Nek8, a NIMA family kinase member, is overexpressed in primary human breast tumors. *Gene* **328**, 135-142.
86. Smith, L. A., Bukanov, N. O., Husson, H., Russo, R. J., Barry, T. C., Taylor, A. L., Beier, D. R. and Ibraghimov-Beskrovnaya, O. (2006) Development of polycystic kidney disease in juvenile cystic kidney mice: insights into pathogenesis, ciliary abnormalities, and common features with human disease. *J. Am. Soc. Nephrol.* **17**, 2821-2831.