DISEASE OF THE MONTH

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Genetics and Pathogenesis of Polycystic Kidney Disease

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Polycystic kidney disease (PKD), a common genetic cause of chronic renal failure in children and adults, is characterized by the accumulation of fluid-filled cysts in the kidney and other organs. The renal cysts originate from the epithelia of the nephrons and renal collecting system and are lined by a single layer of cells that have higher rates of cellular proliferation and are less differentiated than normal tubular cells (1). Abnormalities in gene expression, cell polarity, fluid secretion, apoptosis, and extracellular matrix have also been described in PKD, but the mechanism of cyst formation remains incompletely understood (2–6). In recent months, there have been several advances in our understanding of the genetics and pathogenesis of PKD. Genes responsible for autosomal recessive PKD in humans and mice have been cloned, the *PKD2* gene product has been identified as an intracellular calcium release channel, the *PKD1* gene product has been found to regulate the cell cycle, and a neglected cellular organelle, the primary cilium, has emerged as a potential key player in polycystic disease. In this review, we will discuss how the cloning of the human PKD genes and the characterization of animal models have provided new insights into the pathogenesis of PKD. It is hoped that a more thorough understanding of the genetics and pathogenesis of PKD will lead to improvements in diagnosis and treatment.

PKD can be inherited as an autosomal dominant trait (ADPKD) or an autosomal recessive trait (ARPKD) (Table 1). ADPKD is a common disease that occurs in both children and adults, whereas ARPKD is uncommon and occurs primarily in neonates and children. ADPKD is caused by mutations of either the *PKD1* gene on chromosome 16 or the *PKD2* gene on chromosome 4. The gene responsible for ARPKD (*PKHD1*) has recently been identified on chromosome 6. Renal cysts can also occur in association with other genetic diseases (*e.g.*, tuberous sclerosis, von Hippel-Lindau disease, Zellweger syndrome, juvenile nephronophthisis), but these entities will not be discussed further here.

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Autosomal Dominant Polycystic Kidney Disease

ADPKD is one of the most common genetic diseases in humans affecting all ethnic groups worldwide with an incidence of 1 in 500 to 1 in 1,000 (7). The clinical manifestations include abdominal mass, chronic flank or back pain, gross hematuria, urinary tract infection, and urolithiasis. Affected individuals typically present in the third and fourth decade, and ESRD usually occurs within 5 to 10 yr after the development of renal insufficiency. However, presentation in infancy or childhood has also been reported (8,9). In addition to causing progressive renal failure, renal cysts can be complicated by hemorrhage, rupture, infection, nephrolithiasis, and intractable pain. Systemic hypertension is also very common, occurring in more than 75% of patients. Increased BP has been attributed to activation of the renin-angiotensin system, but a primary defect in blood vessels may also exist (10,11).

Although ADPKD is characterized by kidney cysts and renal failure, it should be regarded as a systemic disease. The genes responsible for ADPKD are widely expressed, and mutations can affect a variety of extrarenal tissues (12). Cysts can arise in other epithelial organs, including the liver (75% of patients), pancreas (rare), ovaries, and choroid plexus. The liver cysts originate from the bile ducts and can become infected or hemorrhage but do not cause liver failure; cystic enlargement of the livers can produce symptoms due to mass effects. Other extrarenal manifestations include cerebral and aortic aneurysms, cerebral dolichoectasis, and colonic diverticuli. Cardiac valvular abnormalities include mitral valve prolapse, mitral regurgitation, aortic insufficiency, and tricuspid regurgitation. Left ventricular hypertrophy is common and has been observed in normotensive individuals.

A striking feature of ADPKD is the variability of the phenotype. ADPKD is fully penetrant, meaning that virtually 100% of individuals who inherit a mutated PKD gene will develop renal cysts that can be detected sonographically by age 30 (13). However, the severity of the disease, the age of onset of ESRD, and the spectrum of extrarenal manifestations vary widely between affected individuals, even within the same family (14). Possible explanations for the variable expressivity of the disease are discussed below.

Genetics of ADPKD

ADPKD is genetically heterogeneous and can arise from mutations in two genes, named *PKD1* and *PKD2* (15,16).

Table 1. Characteristics of autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD)

	ADPKD	ARPKD
Inheritance	Autosomal dominant	Autosomal recessive
Incidence	1/500 to 1/1000	1/6000 to 1/40000
Gene (chromosome)	*PKD1* (Chr 16); *PKD2* (Chr 4)	PKHD1 (Chr 6)
Age of onset of ESRD	53 yr (*PKD1*); 69 yr (*PKD2*)	Infancy/childhood usually
Location of renal cysts	All nephron segments	Collecting ducts[a]
Extrarenal manifestations	Hepatic cysts/pancreatic cysts Cerebral & aortic aneurysms Cardiac valvular abnormalities Systemic hypertension	Biliary dysgenesis Hepatic fibrosis Portal hypertension Systemic hypertension
Protein name	Polycystin-1; Polycystin-2	Fibrocystin/Polyductin
Protein size	Polycystin-1: 4302 amino acids Polycystin-2: 968 amino acids	4074 amino acids and alternative shorter forms
Protein structure	Polycystin-1: Integral membrane protein, multiple Ig-like domains, similar to egg jelly receptor Polycystin-2: Integral membrane protein, similar to TRP channel	Transmembrane protein (and possible secreted forms), multiple TIG/IPT domains, as occur in hepatocyte growth factor receptor and plexins
Tissue distribution	Polycystin-1 and -2: Widespread	Kidney, pancreas, and liver
Subcellular localization	Polycystin-1: Plasma membrane, cilia[b] Polycystin-2: Endoplasmic reticulum, cilia	Unknown
Function	Polycystin-1: ? Receptor, forms ion channel when coexpressed with polycystin-2 Polycystin-2: Calcium-activated cation channel	?Receptor

[a] Cysts appear transiently in proximal tubules during fetal development (90).
[b] Based on localization in male-specific sensory neurons in *C. elegans* (66).

Mutations of *PKD1* located on chromosome 16p13.3 are responsible for 85% of cases, whereas mutations of *PKD2* on chromosome 4q21–23 are responsible for 15% of cases. In elderly patients, mutations of *PKD2* are responsible for a higher percentage of cases. Forty percent of PKD patients presenting with ESRD after age 63 have disease linked to *PKD2*, and the rate is 50 to 70% in patients presenting with ESRD after age 70 (17,18). Mutations of *PKD1* and *PKD2* produce identical renal and extrarenal manifestations. However, compared with *PKD1* patients, *PKD2* patients present later in life (median age at diagnosis, 56 *versus* 42), have longer renal survival (median survival to age 69 *versus* 53), and have fewer complications (19). Only 5% of cases due to mutations of *PKD1* are thought to represent new mutations (20). Some families were initially reported to have PKD that is apparently not linked to either *PKD1* or *PKD2*, but recent confirmation of these findings is lacking. One such family actually had bilineal inheritance of both *PKD1* and *PKD2* mutations (21).

The *PKD1* gene is very large, consisting of 46 exons distributed over 52 kb of genomic DNA (22,23). The gene encodes a 14.1-kb mRNA transcript that is translated into a protein composed of 4302 amino acids. Interestingly, the region of the gene extending from exon 1 to exon 33 is duplicated at six other sites on chromosome 16p. The duplicated genes are expressed as mRNA transcripts and may represent pseudogenes (24). Their existence has hindered mutational analysis because it can be difficult to distinguish mutations of *PKD1* from mutations of the duplicated genes. More recently, with the use of long-range PCR, denaturing HPLC (DHPLC), and the protein truncation test, mutations in the duplicated region of the *PKD1* gene have been identified (20,25,26). Rossetti *et al.* (20) have recently completed the most comprehensive survey of *PKD1* mutations to date. Unlike cystic fibrosis, in which a single mutation of *CFTR* occurs in 70% of affected individuals, mutations of *PKD1* can be found throughout the gene. Different types of mutations have been observed including splice site, in-frame, and out-of-frame deletions and insertions, nonsense mutations, and missense mutations. The out-of-frame deletions/insertions and nonsense mutations are very likely to represent inactivating mutations. No correlations between specific mutations and specific clinical manifestations have been identified, but mutations in the 5' end of the gene appear to be associated with earlier onset disease than mutations in the 3' end (27).

The second ADPKD gene, *PKD2*, was cloned in 1996 by positional cloning (28). The *PKD2* gene is located on chromosome 4q21–23 and encodes a 5.3-kb mRNA transcript that is

translated into a 968 amino acid protein. *PKD2* is approximately 25% homologous to a region of the *PKD1* gene. Patients with ADPKD linked to chromosome 4 are heterozygous for inactivating mutations of *PKD2*, proving that *PKD2* is the disease gene (18,28). Mutations have been identified throughout the gene without evidence for clustering (29). Most of the mutations identified to date are truncating mutations (frameshift, splicing, or nonsense mutations) that would be predicted to inactivate the gene product. Only 5% of mutations are missense mutations. The location of mutations in the gene has been reported to have a nonlinear relationship to clinical severity (30). Unlike *PKD1*, the *PKD2* gene is not duplicated, which has simplified the mutational analysis.

ADPKD is a Focal Disease

In a landmark study, Luc Baert (31) microdissected the kidneys of young adults with ADPKD at an early stage of the disease, when the source and extent of the cysts could be identified. He discovered that cysts arise from the tubular portion of the nephron as well as the renal collecting system. However, although all cells of the nephron carry the same germline mutation, only a few cysts arise per nephron. Many nephrons appear completely normal. Therefore, ADPKD is a focal disease that involves only a small fraction of cells in the kidney, even though all cells carry one copy of the mutated gene.

To explain the focal nature of ADPKD, as well as the highly variable expressivity noted previously, a two-hit model of cystogenesis has been proposed (32,33). In this model, a mutated *PKD1* (or *PKD2*) gene is inherited from one parent and a wild-type gene is inherited from the unaffected parent. During the lifetime of the individual, the wild-type gene undergoes a somatic mutation and becomes inactivated. Complete loss of *PKD1* (or *PKD2*) in cells in which second mutations have occurred initiates cyst formation. Because somatic mutations are rare and will only occur in a relatively small number of cells, the formation of cysts will be focal. Studies have shown that renal cysts from ADPKD patients exhibit loss of heterozygosity due to loss of the wild-type allele supporting a two-hit model of cystogenesis (32,33). Thus, while the pattern of inheritance is dominant, the disease occurs by a molecular recessive mechanism. In addition, renal cysts are clonal, consistent with an origin from a single cell that has undergone a somatic mutation (32). Somatic mutations of the *PKD1* and *PKD2* genes have been identified in the cells lining the cysts in both the kidney and liver (32,34–36).

If each renal cyst arises from a discrete second hit, then a relatively high rate of somatic mutagenesis would be required to explain the large number of cysts that are found in polycystic kidneys. Recent studies indicate that the rate of somatic mutations in kidney epithelial cells is approximately 2×10^{-4}, which is more than tenfold higher than in other cells (37). The reason for the high rate of somatic mutagenesis in the kidney is not known. Another prediction of the two-hit model is that homozygous mutations of *PKD1* and *PKD2* would be more deleterious than heterozygous mutations. Indeed, no humans with homozygous germline mutations of either *PKD1* or *PKD2* have been observed, presumably because homozygosity is embryonic lethal.

Lessons From Mouse Models

Orthologues of the human *PKD1* and *PKD2* genes exist in the mouse genome, and knockout mice that lack one or both copies of the *Pkd1* and *Pkd2* genes have been created (11,38–42). Heterozygous mice develop cysts in the kidney or liver late in life, whereas homozygous null mutant mice are embryonic lethal and develop severely cystic kidneys *in utero* (42a, 42b, 42c). Kidney development proceeds normally until embryonic day 14.5, when cysts begin to appear around the glomerular tufts. By birth, the kidneys are massively replaced with cysts. Marker studies reveal that the cysts arise from all segments of the nephron and the renal collecting system. These results demonstrate that loss of *Pkd1* or *Pkd2* is sufficient to cause renal cysts and support the two-hit model.

Further evidence comes from a unique strain of mice carrying a *Pkd2* allele (called WS25) that is prone to genomic rearrangement (38). The WS25 allele produces wild-type protein. However, during somatic life it can rearrange to produce either a null or a wild-type allele. Mice that carry the WS25 allele develop cysts in the kidney during adulthood, and immunostaining with an antibody to the *Pkd2* gene product (polycystin-2) demonstrated staining in tubules but not in the cyst epithelium. This result indicates the *Pkd2* gene is inactivated in cyst epithelial cells and strongly supports the two-hit hypothesis. Compound heterozygous $Pkd2^{WS25/-}$ mice represent the most authentic animal model of human ADPKD established to date.

Although the two-hit hypothesis explains many features of the disease, other genetic mechanisms, such as haploinsufficiency or dominant-negative mutations, have not been excluded, and multiple mechanisms are likely to be involved. For example, it has been shown that cysts can have trans-heterozygous mutations in which individuals that carry a germline mutation of *PKD1* acquire a second hit that involves the other ADPKD gene, *PKD2* (43). Conversely, individuals carrying germline mutations of *PKD2* can have cysts in which there are somatic mutations of *PKD1* (44). Mice with *trans*-heterozygous mutations of *Pkd1* and *Pkd2* exhibit more severe renal cystic disease than would be predicted by a simple additive effect of the cyst formation in singly heterozygous mice (42c). These results suggest that haploinsufficiency of both *PKD1* and *PKD2* may also play a role in cyst formation.

Polycystin-1

The proteins encoded by the *PKD1* and *PKD2* genes define a new family, the polycystins, which play important roles in a variety of biologic processes including fertilization, ion translocation, and mechanosensation. Polycystin-1, the product of the *PKD1* gene, contains 4302 amino acids and has a molecular weight of about 500,000 D (23,45,46). As shown in Figure 1, polycystin-1 is an integral membrane protein that is predicted to contain 11 transmembrane segments. The large, extracellular amino-terminal domain contains a unique array of distinct protein motifs, including two leucine-rich repeats flanked by

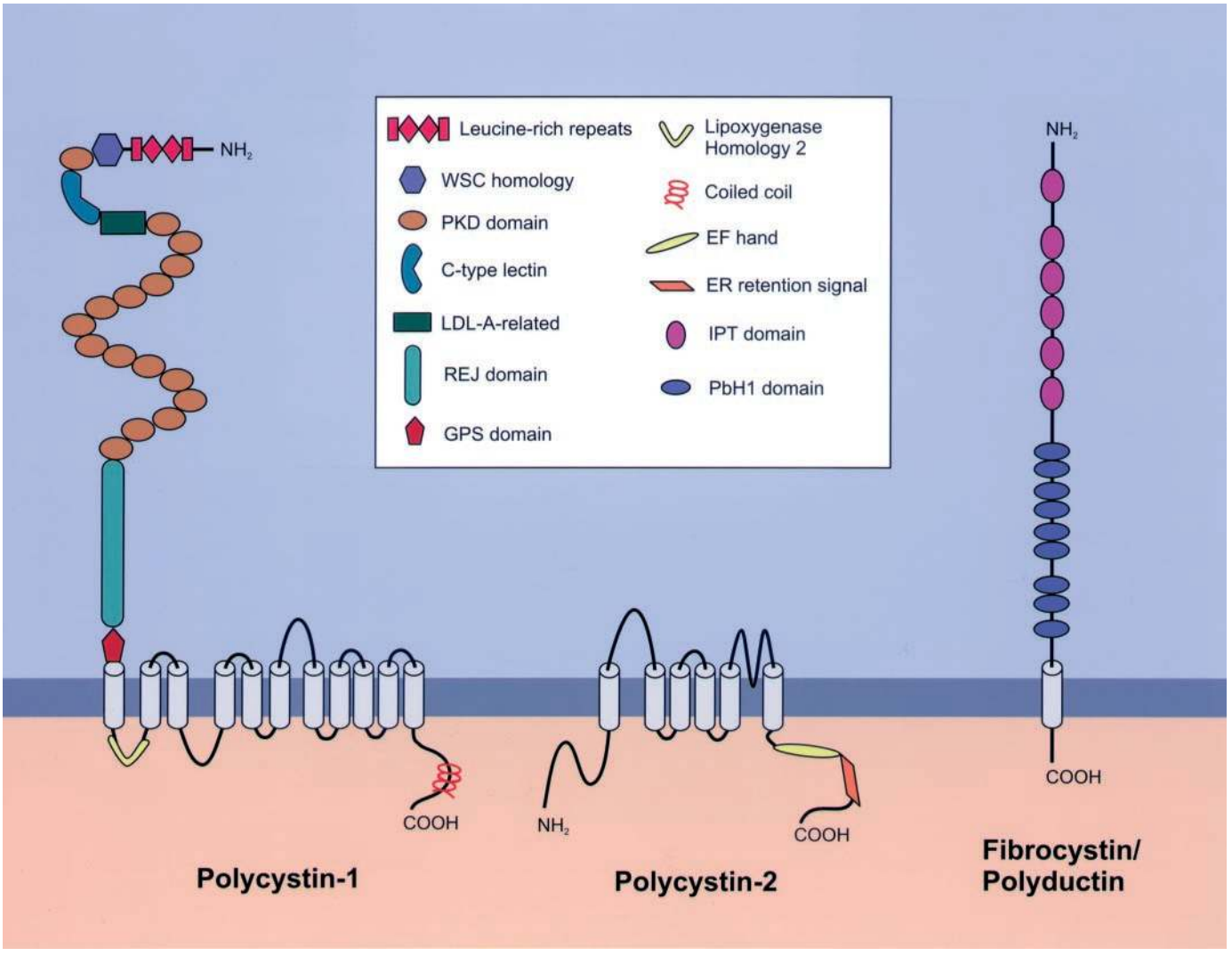

Figure 1. Structures of polycystin-1, polycystin-2, and polyductin/fibrocystin. Thick gray line indicates the membrane bilayer. Tan background indicates cytosol. Blue background indicates extracellular space or ER lumen. Protein motifs are identified in the figure legend. Light gray cylinders represent putative transmembrane segments. Only the membrane-bound form of polyductin/fibrocystin is shown. Structures are not drawn to scale.

cysteine-rich domains, a C-type lectin domain, a WSC domain, and 16 immunoglobin-like domains called PKD repeats. Many of these motifs are involved in protein-protein or protein-carbohydrate interactions, which raises the possibility that polycystin-1 may function as a receptor for an as yet unidentified ligand. Closer to the membrane, there is a region of homology to the sea urchin egg jelly receptor (47) and a potential proteolytic cleavage site (GPS domain) (48,49). Between the first and second transmembrane domains, there is a region of similarity to lipoxygenases (PLAT domain) (49,50). The carboxyl-terminus of polycystin-1 is located in the cytoplasm and contains a coiled-coil domain that mediates protein-protein interactions as well as several potential sites of phosphorylation.

Polycystin-1 is expressed in many tissues, including the kidney, brain, heart, bone, and muscle (51). The subcellular localization of polycystin-1 has been somewhat controversial. However, several studies have identified polycystin-1 in the plasma membrane of tubular epithelial cells, especially in the distal nephron and collecting ducts (52–54). In mature tubules it is primarily in the lateral membrane at sites of cell-cell interaction (55). Consistent with these results, polycystin-1 has been identified in cell junctional complexes, including adherens junctions and desmosomes (56–60). Polycystin-1 is glycosylated (59) and exists in two pools; one is sensitive to endoglycosidase H (Endo H), and another, which is associated with the plasma membrane, is Endo H-resistant (61).

Although the function of polycystin-1 is not well understood, its structure is similar to a family of cell surface receptors that are involved in the acrosome reaction, an essential step in fertilization. The acrosome reaction is an exocytic process in which a large vesicle contained within the sperm head fuses with the plasma membrane and releases its contents into the extracellular medium. The acrosome reaction is trig-

gered by the binding of a ligand in the jelly surrounding the egg to the egg jelly receptor on the sperm head. Activation of the egg jelly receptor results in increased cytosolic calcium and pH and in vesicle fusion. The egg jelly receptors from sea urchin and mammals were found to be homologous to polycystin-1 (47,48,62). Motifs that are conserved between polycystin-1 and one or more egg jelly receptors include the REJ domain, transmembrane segments, C-type lectin domain, PLAT domain, GPS domain, and PKD repeats. The homology with the egg jelly receptors suggests that polycystin-1 may also be a cell surface receptor and that the signaling pathways may be similar. Of note in this regard is the demonstration of proteolytic cleavage at the GPS domain in suREJ3, a polycystin-1 homologue (48). This observation raises the possibility that proteolytic cleavage at the conserved GPS domain in polycystin-1 has a role in polycystin function in the kidney as well. Additionally, a role for polycystin-1 in regulating exocytosis in kidney-derived cells has recently been proposed (64).

Another model organism that is providing clues to the function of polycystins is the nematode, *Caenorhabditis elegans*. Polycystin-1 and polycystin-2 homologues in *C. elegans* are essential for the stereotyped mating behavior mediated by a specialized group of ciliated sensory neurons. In *C. elegans*, the polycystin proteins appear to function as mechanosensors (or chemosensors), and their appearance in cilia as well as intracellular membranes has prompted recent interest in the role of the former in mammalian kidneys (65,66) (see below).

The cytoplasmic carboxyl-terminal domain of polycystin-1 has been shown to activate a number of intracellular signaling pathways. Transient transfection of the polycystin-1 carboxyl-terminus activates the Wnt signaling pathway via stabilization of β-catenin and activation of TCF/LEF transcription factors (67). Interestingly, one of the downstream targets for activation by this pathway appears to be the *PKD1* gene itself (68). The expression of β-catenin is downregulated in *Pkd1* mutant mice, an effect that is corrected by the administration of *PPAR*γ agonists (42b). Several studies have suggested that polycystin-1 regulates G protein signaling. The carboxyl-terminal domain of polycystin-1 can directly bind heterotrimeric G proteins and the regulator of G protein-signaling RGS7 (69,70). A recent study has shown that full-length polycystin-1 behaves as a G protein-coupled receptor that activates $G\alpha_{i/o}$ and releases $G\beta\gamma$ subunits (71). Signaling though this pathway is independent of RGS proteins but is antagonized by polycystin-2. G protein signaling pathways regulate processes that are important in cyst formation, such as fluid secretion, proliferation, cell polarity, and differentiation (6). G proteins also appear to be involved in polycystin-1 activation of c-Jun N-terminal kinase and transcription factor AP-1 (72,73).

A characteristic feature of cyst epithelial cells is an abnormally high rate of cellular proliferation (1,3,5). Overexpression of full-length polycystin-1 in MDCK cells inhibits cellular proliferation and suppresses cyst formation (74). In a recent study, Bhunia *et al.* (75) have shown that polycystin-1 has a direct role in the regulation of the cell cycle by inducing cell cycle arrest at the G0/G1 transition. Progression through the cell cycle is controlled by cyclin-dependent kinases (Cdks), and it was found that polycystin-1 inhibits Cdk2 by upregulating its inhibitor, $p21^{CIP1/WAF1}$. Polycystin-1 activates the JAK-STAT signaling pathway in a process that requires polycystin-2 and that leads to activation of $p21^{CIP1/WAF1}$ (75).

Polycystin-2

The *PKD2* gene encodes a protein, polycystin-2, that is composed of 968 amino acids (28). Like polycystin-1, polycystin-2 is predicted to be an integral membrane protein. Polycystin-2 contains six transmembrane segments and intracellular amino- and carboxyl-termini (Figure 1). The transmembrane segments of polycystin-2 are about 50% identical to the last 6 of the 11 transmembrane segments of polycystin-1. Polycystin-2 also shares structural features with transient receptor potential (TRP) channels as well as voltage-activated calcium and sodium channels. The carboxyl-terminal domain contains a motif known as an EF hand that can bind calcium. Polycystin-2 is widely expressed in many tissues, particularly the kidney, heart, ovary, testis, vascular smooth muscle, and small intestine (76,77). In the kidney, polycystin-2 like polycystin-1 is expressed in all nephron segments, with the possible exception of the thin limbs, but is absent from glomeruli.

The structure of polycystin-2 suggested that it might function as an ion channel, and single channel recordings as well as patch clamp analyses have shown that polycystin-2 is a non-selective cation channel that can conduct calcium ions (78–81). Further clues to the function of polycystin-2 have been provided by three types of observations: First, experiments conducted both *in vitro* and *in vivo* have shown that polycystin-2 directly interacts with polycystin-1 (61,81–83). The carboxyl-terminal domain of polycystin-1 contains a coiled-coil motif that binds to the carboxyl-terminal domain of polycystin-2, and deletion of the carboxyl-termini of either protein destroys this interaction. The *in vivo* functional significance of this interaction is supported by the observation that polycystin-1 and polycystin-2 act nonredundantly in the same genetic pathway in *C. elegans* (66). In addition, the interaction of polycystin-2 with polycystin-1 inhibits G protein signaling (71). That polycystin-1 and polycystin-2 interact in a common pathway would explain why mutations of either *PKD1* or *PKD2* produce diseases with identical clinical manifestations.

Second, whereas polycystin-1 appears to be located in the plasma membrane, the majority of polycystin-2 is located in premedial Golgi compartments, primarily the endoplasmic reticulum (ER) (61,81,84). The latter is evidenced by the complete sensitivity of polycystin-2 to deglycosylation with Endo H and by subcellular fractionation studies showing that it colocalizes with ER markers in both cultured cells and native kidney tissue (61,80,84). A domain that retains polycystin-2 in the ER has been identified in the carboxyl-terminus (Figure 1). Co-expression and co-assembly with polycystin-1 have been shown to displace polycystin-2 from the premedial Golgi compartments and allow relocalization to the cell surface in CHO cells (81). Furthermore, recent studies on native polycystin-2 in MDCK cells have found the protein in the Golgi apparatus and at the plasma membrane (85). In cells overexpressing polycystin-1 as a stable transgene, polycystin-2 complexes with both

an Endo H-sensitive and an Endo H-resistant pool of polycystin-1; the latter complex is associated with the plasma membrane (61). Full-length polycystin-1 and truncated forms of polycystin-2 that lack the ER retention domain can be biotinylated on the cell surface, whereas full-length polycystin-2 cannot (59,84).

Finally, several studies have shown that the polycystin-2 channel conducts divalent cations including calcium (78,79,80) and that this activity can be stimulated by calcium on the cytosolic side (80). A recent study further showed that polycystin-2 can amplify calcium release from intracellular stores in response to hormone stimulation that transiently increases cytosolic calcium (80). A naturally occurring human disease mutation altering a highly conserved charged amino acid in the third membrane span results in complete loss of channel activity without apparently altering the expression and interactions of the protein (80,87). Taken together, these studies demonstrate that polycystin-2 activity increases cytosolic calcium, perhaps in local microenvironments, and that the isolated loss of the capacity to translocate calcium results in sufficient loss of function to cause polycystic kidney disease.

The studies of polycystin-1 and polycystin-2 suggest several possible non-mutually exclusive signaling pathways that are shown in Figure 2. In Figure 2A polycystin-1 is shown in the plasma membrane interacting with polycystin-2 in the adjacent ER. This relationship is reminiscent of conformational coupling between TRP channels in the plasma membrane and IP_3 receptors in the ER (88). Polycystin-1 may function as a receptor for an as yet unidentified extracellular stimulus and signal to the cell interior through its interaction with polycystin-2. The signaling results in activation of calcium channels and increases in cytosolic calcium that trigger exocytosis and changes in gene expression. A defect in exocytosis has been observed in cyst epithelial cells and may be responsible for the mislocalization of some basolateral membrane proteins to the apical membrane that has been found in PKD (4,64). In Figure 2B both polycystin-1 and polycystin-2 are shown in the plasma membrane. Activation of polycystin-1 leads to activation of polycystin-2, which mediates entry of extracellular calcium, producing a rise in cytosolic calcium. Figure 2C shows that polycystin-1 behaves as a G protein-coupled receptor that activates $G\alpha$ and releases $G\beta\gamma$ subunits. Activation of G protein signaling may regulate fluid secretion, proliferation, cell polarity, and differentiation. Figure 2D shows that activation of polycystin-1 leads to activation of JAK-STAT signaling in a process requiring polycystin-2. Activation of STAT1 causes upregulation of $p21^{CIP1/WAF1}$, inhibition of Cdk2, and cell cycle arrest at the G0/G1 transition.

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

ARPKD is less common than ADPKD and occurs in 1 in 6000 to 1 in 40,000 live births (Table 1) (89). ARPKD is characterized by the combination of renal cystic disease and congenital hepatic fibrosis. The renal cystic disease typically begins *in utero* and manifests as fusiform dilatation of the collecting ducts that radiate from the medulla to the cortex (9). During fetal development, cysts also appear transiently in proximal tubules (90). The renal cystic disease is invariably associated with biliary dysgenesis, which is a ductal plate malformation characterized by aberrant intrahepatic bile ducts and portal fibrosis. Gross cystic dilatation of the bile ducts is unusual except in the 6 to 12% of patients with Caroli disease (91). Fibrosis of the pancreas has also been described in some patients (92). Like ADPKD, the clinical presentation of ARPKD is highly variable. ARPKD can present as perinatal, neonatal, infantile, or juvenile-onset disease (9). The variability in the age of onset is due to variable expression of mutations of the same gene as well as the effects of modifier genes and environmental factors rather than mutations of different genes (93,94). Intrafamilial variability is less pronounced than variability between families (95).

The perinatal form of ARPKD presents at birth with bilateral enlarged, hyperechoic kidneys and severe renal failure. Oligohydramnios resulting from poor intrauterine urine output produces Potter facies and pulmonary hypoplasia. Up to 30 to 50% of affected newborns die shortly after birth due to sepsis or respiratory failure (95). Children who do not present until after the first few months of life have less severe cystic involvement and a much better prognosis (91,92). The renal cysts are larger and rounder than in the perinatal form and may resemble ADPKD, which can be excluded by linkage analysis or renal ultrasonography of the parents. Children who survive the neonatal period have a 56 to 67% probability of survival to age 15 without ESRD, and prolonged survival to age 55 has been reported (96–98). However, long-term survivors often develop sequelae of portal hypertension including esophageal varices, hepatosplenomegaly, and hypersplenism (97,98). Other clinical manifestations of ARPKD in children and adults include systemic hypertension (56 to 70% of patients), growth retardation, urinary tract infection, and hyponatremia. Patients with Caroli disease may develop complications of cholangitis and cholangiocarcinoma (98).

Cloning of the ARPKD Gene (PKHD1)

All typical cases of ARPKD are due to mutations of the *PKHD1* gene on chromosome 6p21.1-p12. Genetic linkage to chromosome 6 was first demonstrated in 1994 (99), and subsequent genetic and physical mapping refined the gene locus to a 1-Mb interval (100). Earlier this year, three groups independently cloned the *PKHD1* gene in the critical region on chromosome 6p21.1-p12. Using comparative genomics, Ward *et al.* (101) mapped a rat model of PICD (the *Pck* rat) to a region on rat chromosome 9 that was syntenic with the ARPKD candidate region on human chromosome 6p. Further analysis identified a gene (*PKHD1*) that was mutated in both the *Pck* rat and in humans with ARPKD. Onuchic *et al.* (102) cloned the identical gene by assembling a transcription map of the ARPKD region and identifying a novel transcript that was highly expressed in the kidney. Analysis of this gene identified mutations found only in affected individuals and segregating with the disease phenotype in affected families. Xiong *et al.* (102a) have also recently reported the identification of the human ARPKD gene and mapped the mouse orthologue

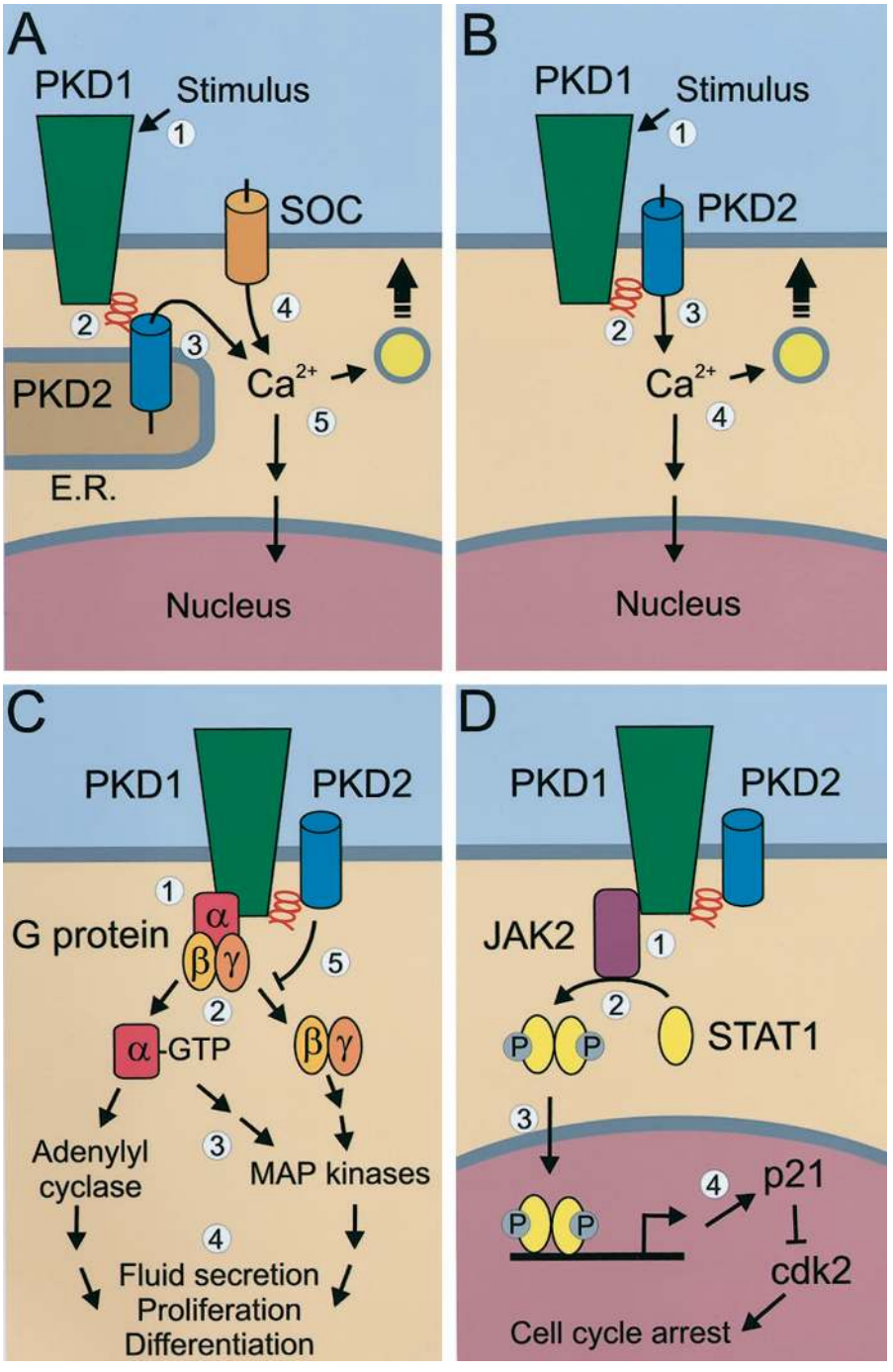

Figure 2. Models of polycystin-1/polycystin-2 signaling. (A) Polycystin-1 is located in the plasma membrane and interacts via a coiled-coil domain (red) with polycystin-2, which is primarily found in the endoplasmic reticulum (ER). Activation of polycystin-1 by an as yet unidentified stimulus (1) leads to activation of polycystin-2 (2). Polycystin-2 forms an ion channel that releases calcium from the endoplasmic reticulum into the cytosol (3). Depletion of calcium from the ER activates store-operated channels (SOC) in the plasma membrane that raise cytosolic calcium further (4). The increase in cytosolic calcium initiates signaling cascades that lead to vesicle fusion and changes in gene transcription (5). (B) At sites where both polycystin-1 and polycystin-2 are located in the plasma membrane, activation of polycystin-2 (1) leads to activation of polycystin-2 (2) and calcium influx across the plasma membrane (3). The rise in cytosolic calcium leads to vesicle fusion and changes in gene transcription (4). (C) Regulation of G protein signaling by polycystins. Polycystin-1 binds and constitutively activates heterotrimeric G proteins (1). Activation of Gα subunits and release of G$\beta\gamma$ subunits (2) affects the activity of adenylyl cyclase, MAP kinases, and other downstream effectors (3) that regulate fluid secretion, proliferation, cell polarity, and differentiation (4). The interaction of polycystin-2 with the coiled-coil domain of polycystin-1 inhibits G protein signaling (5). (D) Regulation of the JAK-STAT signaling pathway by polycystins. Activation of polycystin-1 leads to activation of JAK2 kinase (1) in a process requiring polycystin-2. JAK2 phosphorylates and activates STAT1 (2), which forms homodimers that translocate to the nucleus and bind to the $p21^{CIP1/WAF1}$ gene promoter (3). Upregulation of $p21^{CIP1/WAF1}$ (4) inhibits the cyclin-dependent kinase Cdk2, which leads to cell cycle arrest in G0/G1.

(*Pkd1*) on mouse chromosome 1. Taken together, these results prove that the gene responsible for ARPKD has been identified.

The *PKHD1* gene is very large and consists of at least 86 exons extending over 469 kb of genomic DNA (101,102). The gene undergoes a complex pattern of alternative splicing to generate mRNA transcripts ranging in size from 8.5 kb to 13 kb. Consistent with the sites affected by the disease, the *PKHD1* gene is expressed at high levels in the fetal and adult kidney and at lower levels in the liver and pancreas. In the mouse, *Pkd1* is expressed in renal tubules (102a, 102b) as well as in the bile ducts, blood vessels, testis, and dorsal root ganglia (102b). Mutations of *PKHD1* that have been identified in patients include frameshift, nonsense, and out-of-frame splicing alterations that are consistent with a loss-of-function mechanism. In addition, an abundance of missense variants, the effects of which are less clear, have also been found (101,102). Most individuals in whom two mutations have been identified are compound heterozygotes with one loss-of-function mutation and one predicted missense change or just two predicted missense changes. In the initial reports, one individual with two loss-of-function mutations has been identified, suggesting that the disease may result from loss-of-function of the *PKHD1* gene product (102). Further studies are necessary to determine if there is any relationship between the nature of the mutations and the clinical course of the disease.

Fibrocystin/Polyductin

The protein encoded by the *PKHD1* gene has been named polyductin or fibrocystin and is composed of 4074 amino acids (Figure 1) (101,102). Polyductin/fibrocystin is predicted to be a membrane protein consisting of a large extracellular domain, a single transmembrane segment, and a short carboxyl-terminal tail. A splice variant that encodes a truncated protein lacking the transmembrane segment has also been identified and may encode a secreted form of the protein (102). Polyductin is a novel protein, although it has some similarities to other proteins in the database. The highest similarity is to D86, a protein of unknown function secreted from lymphocytes. Analysis of the predicted amino acid sequence of polyductin identified a signal sequence at the amino terminus. The extracellular domain contains six to eight TIG/IPT domains, which are Ig-like domains that have been identified in cell surface receptors, such as the HGF receptor and plexins, as well as in the Rel family of transcription factors. Between the TIG/IPT domains and the transmembrane domain, there are 9 to 10 PbH1 repeats, which are also found in polysaccharidases (102). Three potential protein kinase A phosphorylation sites were identified in the carboxyl terminus. The structure of polyductin/fibrocystin suggests that it may be a cell surface receptor or secreted protein, perhaps with enzymatic activity. Further studies will be required to identify the subcellular localization and biologic function of polyductin and to determine how mutations of the protein cause disease.

Is PKD a Ciliary Disease?

Cilia are long, thin tubular structures that are present on the surface of most cells (103). Ultrastructurally, cilia consist of a ciliary membrane that is continuous with the cell membrane and a central axoneme that is composed of microtubules (Figure 3). Cilia originate from the basal body, an intracellular organelle related to the centriole. Cilia are generally classified as primary cilia or motile cilia. The axonemes of primary cilia contain nine peripheral bundles of microtubules (9+0 pattern), whereas the axonemes of typical motile cilia, such as those in tracheal epithelia, contain nine peripheral bundles and two central microtubules (9+2 pattern) as well as dynein arms. Renal tubular epithelial cells contain 1 to 2 primary cilia that have a typical 9+0 ultrastructure (104). Primary cilia have been identified in all segments of the nephron from Bowman's capsule to collecting ducts with the exception of intercalated cells (105,106). The primary cilia in the kidney are 2 to 10 μm in length and protrude from the apical cell membrane into the tubule lumen. Some primary cilia are motile, such as those in the embryonic node, but those in the kidney are thought to be immotile (107). Immotile primary cilia may have a chemosensory or mechanosensory function. Although their existence has been recognized for more than a century, primary cilia were often considered vestigial organelles. However, recent studies suggest that disorders of primary cilia may produce polycystic kidney disease.

The involvement of cilia in PKD was first suggested by studies of the *orpk* mouse, which is a mouse model of ARPKD that was created by insertional mutagenesis (108). Homozygous mutant *orpk* mice develop renal collecting duct cysts, biliary dysplasia, and portal fibrosis and usually die within the first week of life. The gene that is mutated in *orpk* mice encodes a novel protein, named polaris, which contains multiple tetratricopeptide repeats that may be involved in protein-protein interactions (109). Polaris is expressed in ciliated cells and localizes to the ciliary axoneme and basal bodies (110). Most cells in the kidney express polaris, and the protein has also been localized to the primary cilia of cultured MDCK cells. In *orpk* mutant mice, the primary cilia in the renal collecting ducts are severely stunted (111).

The function of polaris has been elucidated by studies of homologous proteins in the nematode *C. elegans* and the green algae *Chlamydomonas*. In *C. elegans*, the polaris homologue, named OSM-5, is expressed in the cilia of sensory neurons that are involved in mating (112,113). Male worms that lack OSM-5 form stunted cilia and are unable to mate due to sensory defects. The reason why the cilia fail to develop properly was revealed by studies in *Chlamydomonas*, which contains paired flagella that are structurally related to cilia. These studies have shown that the assembly and maintenance of flagella and cilia involves a process known as intraflagellar transport (IFT). IFT refers to the axonemal transport of large particles or "rafts" that are thought to carry cargo from the base of the flagella to the growing tip. The particles that are transported by IFT are composed of at least 17 subunits, one of which, named IFT88, is homologous to mouse polaris (111). Moreover, mutations of IFT88 completely prevent flagellar

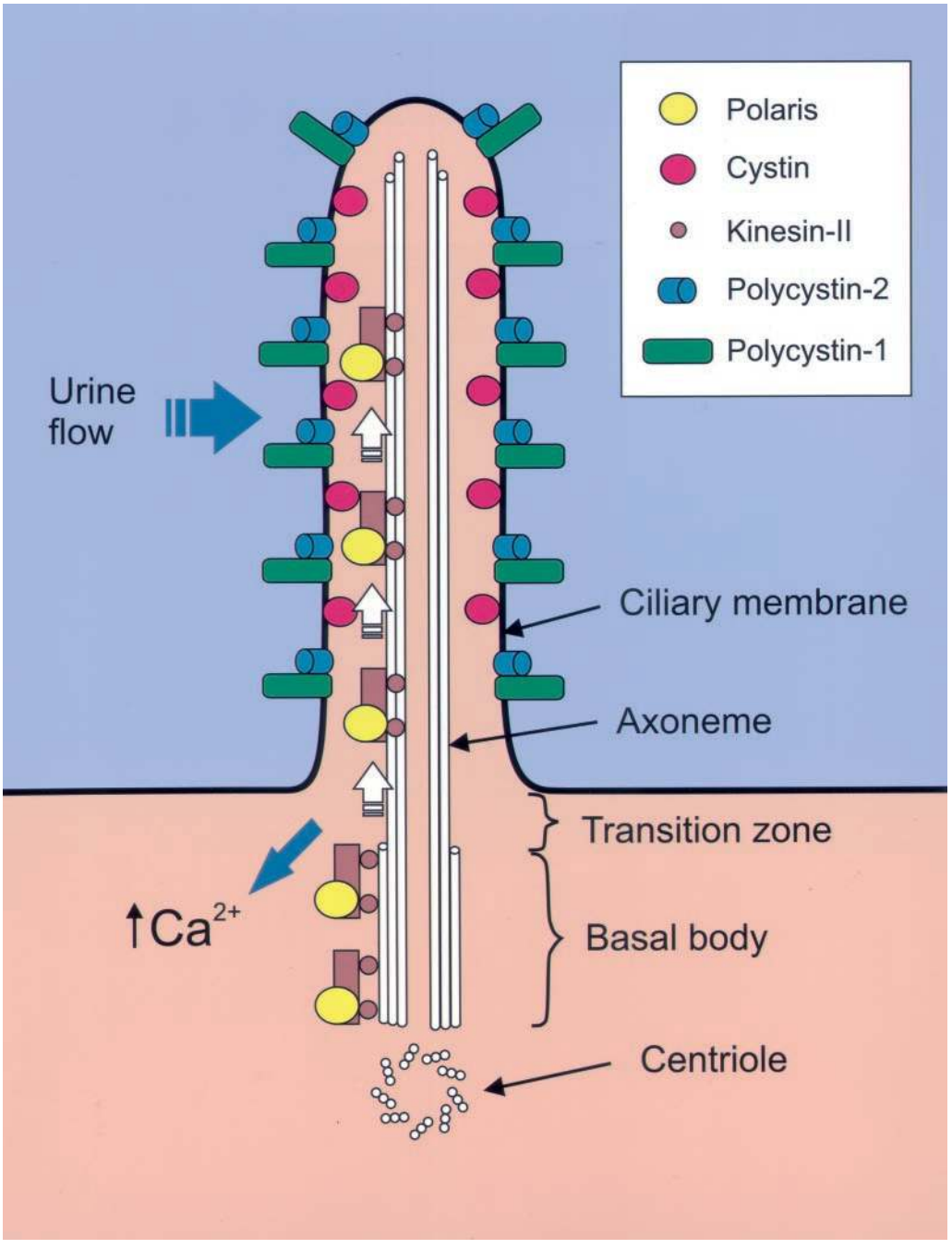

Figure 3. Model of primary cilia structure. Primary cilia are tubular evaginations of the cell surface that are composed of an axoneme surrounded by the ciliary membrane. The axoneme consists of microtubule doublets arranged in a 9+0 pattern and originates from the distal end of the basal body. The basal body contains nine peripheral triads of microtubules and is oriented perpendicular to the neighboring centriole, which is depicted here in cross-section. White arrow indicates outward intraflagellar transport (IFT) of particles (brown) mediated by the motor kinesin-II. Polaris, the product of the gene mutated in *orpk* mutant mice, is a subunit of the IFT particles and is distributed in the ciliary axoneme and the basal body. Cystin, which is mutated in *cpk* mutant mice, is located only in the axoneme and may be anchored to the ciliary membrane by an *N*-myristoyl group. Both polycystin-1 and polycystin-2 have also recently been identified in renal cilia. Blue arrows indicate that bending of renal cilia in response to urine flow stimulates a rise in cytosolic calcium.

assembly. Subsequent studies have revealed that polaris (OSM-5) is also localized to IFT particles and is required for ciliogenesis in *C. elegans* and kidney cells (112–114). These studies suggest that mutations of polaris inhibit the assembly of primary cilia in kidney tubules and that this leads to polycystic kidney disease.

Interestingly, mice deficient in polaris fail to develop left-right asymmetry, presumably due to defects in the formation of

nodal cilia in early embryogenesis (109). Another mouse model with defects in left-right asymmetry, the *inv/inv* mouse, is interesting in that it too develops renal cysts in addition to abnormalities of nodal cilia (115,116). Transgenic re-expression of inversin, the *inv* gene product, not only rescues the defect in left-right axis determination but also the renal cystic phenotype (115). Recently, abnormalities of left-right axis determination including *situs inversus* and dextrocardia have also been observed in *Pkd2* mutant mice (117). The coupling of defects of left-right axis determination with kidney cysts in several mouse models lends indirect support to the notion that cilia may play a role in renal cystic disease.

The involvement of cilia in PKD is further supported by studies of the *cpk* mouse, which is a well-characterized, naturally occurring recessive mouse model of polycystic kidney disease (118). Homozygous *cpk* mutant mice develop kidney cysts beginning late in gestation and succumb to renal failure within 4 to 5 wk after birth. Recently, Hou *et al.* (119) identified the *cpk* gene by positional cloning and found that it encodes a 145-amino acid protein that is expressed primarily in the kidney and liver, which they named cystin. The structure of cystin is novel and not similar to any proteins in the database. However, the protein contains two potential *N*-myristoylation sites that could anchor it in the membrane. When epitope-tagged cystin is expressed in cultured collecting duct cells, the protein localizes to the primary apical cilia. At higher magnification, cystin can be found along the ciliary axoneme but does not appear to be expressed in the basal body from which the cilium originates. Taken together, the *orpk*, *inv*, and *cpk* models serve to highlight the potential importance of cilia in ARPKD.

Polycystins are Localized in Cilia

Remarkably, cilia may also be involved in the pathogenesis of autosomal dominant PKD. Homologues of polycystin-1 and polycystin-2 have been identified in *C. elegans* (65). These homologues, named LOV-1 (for location of vulva) and PKD-2, respectively, also appear to be important for the function of sensory cilia. GFP fusion proteins containing LOV-1 and PKD-2 co-localize to the cilia of the same sensory neurons that express OSM-5 (66). PKD-2 is also expressed in a punctate pattern in the cell body consistent with localization in the endoplasmic reticulum. As with *osm-5* mutants, worms with mutations of the *lov-1* and *pkd-2* genes exhibit abnormal male mating behavior due to sensory defects (65). *lov-1/pkd-2* double mutants have a similar phenotype, and transgenic expression of *lov-1* does not rescue *pkd-2* mutants (or *vice versa*), indicating that the two genes act nonredundantly in the same pathway (66). In *osm-5* (polaris) mutant worms, the cilia are severely stunted and LOV-1 and PKD-2 fusion proteins accumulate in the stunted cilia (112). In *lov-1* and *pkd-2* mutant worms, the structure of the cilia appears to be normal suggesting that these genes are not required for ciliary assembly (66).

Recently, Pazour *et al.* (120) have identified polycystin-2 in the primary cilia of mammalian renal epithelial cells. In addition to localization in the endoplasmic reticulum, polycystin-2 colocalizes with ciliary tubulin in the cilia of cultured mouse and human kidney cells. Expression in renal cilia is also seen in native kidney tubules. In *orpk* mutant mice that have stunted renal cilia and polycystic kidney disease, the expression of polycystin-2 in the cilia is increased. *Pkd2* mutant embryos, in which embryonic turning and cardiac looping are randomized, show absence of the normally left-sided expression of *nodal* and *Lefty-2* consistent with an abnormality in nodal cilia signaling (117). Recently, Yoder *et al*. (117a) have shown that polycystin-1 is also expressed in renal cilia, where it co-localizes with cystin. These results further support the hypothesis that abnormalities of ciliary function play a role in the pathogenesis of PKD.

Because their function is not known, it is not clear how abnormalities of renal cilia would produce kidney cysts. Cilia in the kidneys of lower organisms are motile and are thought to generate urine flow. However, the sparse occurrence of primary cilia in the mammalian kidney does not favor a propulsive function. Other functions that have been suggested include facilitation of solute reabsorption, concentration of receptors for a urinary ligand, and monitoring of urinary flow (105). In support of the last possibility, primary cilia in cultured renal epithelial cells have been shown to bend in response to flow (107,121). Bending of the cilia in cultured MDCK cells, either mechanically or with flow, stimulates a rise in intracellular calcium concentration (122). These studies suggest that the cilia in the kidney may function as mechanosensors of urine flow, perhaps analogous to the sensory function of cilia in *C. elegans*. Further studies are needed to elucidate the normal function of renal cilia and how alterations in ciliary structure and function lead to cyst formation.

Cilia as Potential Targets for Treatment of PKD

No specific treatment for PKD currently exists, so it is hoped that a more complete understanding of the molecular pathogenesis of the disease will identify novel therapeutic strategies. Even before the potential importance of cilia was recognized, Woo *et al.* (123) demonstrated salutary effects of taxanes in the *cpk* model of ARPKD. Taxanes, such as paclitaxel, promote the polymerization of microtubules and stabilize existing microtubules. Microtubule polymerization is an essential process in ciliogenesis. Treatment of *cpk* mutant mice with paclitaxel (Taxol) retards the progression of PKD and markedly prolongs survival. Some animals survive more than 6 mo with treatment. Other taxanes also inhibit cyst progression, and the salutary effects of these compounds are directly related to their ability to promote microtubule assembly *in vitro* (124). Other investigators have confirmed these results, although the benefit appears to be specific to *cpk* mice (125). These data are consistent with the hypothesis that lack of cystin results in altered ciliary microtubule stability. Therapy directed at increasing microtubule stability is unlikely to affect the mechanosensory defect hypothesized for the polycystins in the cilia. On the other hand, if the role of cilia in ADPKD is confirmed and if polycystin-2 is delivered to the ciliary membrane in the absence of polycystin-1, agonists of polycystin-2 channel activity that are filtered at the glomerulus could play a role in

treating the most common form of ADPKD, that caused by mutations in polycystin-1.

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