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PERSPECTIVES IN BASIC SCIENCE

Molecular pathogenesis of ADPKD: The polycystin complex gets complex

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Molecular pathogenesis of ADPKD: The polycystin complex gets complex. Autosomal-dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic diseases with an incidence of 1:400 to 1:1000. It is characterized by the progressive development and enlargement of focal cysts in both kidneys, typically resulting in end-stage renal disease (ESRD) by the fifth decade. The cystogenic process is highly complex with a cellular phenotype consistent with "dedifferentiation" (i.e., a high proliferative rate, increased apoptosis, altered protein sorting, changed secretory characteristics, and disorganization of the extracellular matrix). Although cystic renal disease is the major cause of morbidity, the occurrence of nonrenal cysts, most notably in the liver (occasionally resulting in clinically significant polycystic liver disease) and the increased prevalence of other abnormalities including intracranial aneurysms, indicate that ADPKD is a systemic disorder. Following the identification of the first ADPKD gene, *PKD1*, 10 years ago and *PKD2* 2 years later, considerable progress has been made in defining the etiology and understanding the pathogenesis of this disorder, knowledge that is now leading to the development of several promising new therapies. The purpose of this review is to summarize our current state of knowledge as to the structure and function of the *PKD1* and *PKD2* proteins, polycystin-1 and -2, respectively, and explore how mutation at these loci results in the spectrum of changes seen in ADPKD.

AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) IS GENETICALLY HETEROGENEOUS

Mutation to *PKD1* (chromosome region 16p13.3) is the most common cause of ADPKD (∼ 86% cases) with most of the remainder due to changes to *PKD2* (4q22). However, the description of possible unlinked families indicates that at least one other unknown gene may be associated with ADPKD. The clinical phenotypes of PKD1 and PKD2 are closely related; they were only recognized as diseases with different etiologies by genetic linkage analysis in the late 1980s. This phenotypic similarity includes both the nature of the renal disease and the range of extrarenal manifestations (cases of polycystic liver disease and intracranial aneurysms are associated with both genes). However, there are differences: PKD2 is a significantly milder disease in terms of the mean age at diagnosis, a lower prevalence of hypertension, and a later age at onset of end-stage renal disease (ESRD) (PKD1, 54.3 years and PKD2, 74.0 years) [1]. Furthermore, while on average disease severity is similar between males and females in PKD1, PKD2 females have a significantly better prognosis (age at onset of ESRD in males, 68.1 years and in females, 76.0 years). The reason for this difference is unclear [2, 3]. Within both disorders, there is a wide range of intrafamilial phenotypic variability, seen both in terms of the severity of renal disease and manifestations of extrarenal abnormalities, indicating that genetic modifying loci and environment factors significantly influence the course of the disease [2].

Key words: ADPKD, PKD1, PKD2, polycystin-1, polycystin-2.

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THE MUTATION SPECTRUM

PKD1 is a complex gene with 46 exons that generates a large transcript (∼14 kb) containing a long open-reading frame predicted to encode a 4302aa protein. Characterization of the gene structure and identification of mutations has been complicated by genomic duplication of the 5′ region of *PKD1* (to exon 33) such that approximately six copies of *PKD1*-like genes, with various rearrangements relative to *PKD1*, are located in 16p13.1. Sequence similarity of these pseudogenes (many of which encode transcripts but probably not significant protein products) to *PKD1* means that particular anchored, long and locus-specific amplification methods are required to characterize and screen *PKD1* for mutations [4]. *PKD2* has 15 exons, generates a ∼5 kb transcript, and encodes a protein of 968aa.

Both *PKD1* and *PKD2* exhibit marked allelic heterogeneity, with approximately 200 different *PKD1* and over

50 different *PKD2* mutations described [3–5]. The majority of these are unique to a single family, illustrating the complexity of gene-based diagnostics for these disorders and indicating that a significant level of new mutation is occurring [4]. Most mutations are predicted to truncate the protein (due to frame-shifting deletion/insertion, nonsense changes, or splicing defects), but a significant level of in-frame and missense changes have also been described [3, 4]. Mutations are found throughout both genes, although more *PKD1* changes are found in the 3′ half of the gene [4]. This pattern of mutation is consistent with inactivation of one allele but recent genotype/phenotype correlations in *PKD1* suggest that not all mutations may have the same phenotypic outcome. In *PKD1*, mutations 5′ to the median are associated with more severe disease (average age at onset of ESRD at 5′, 53 years and at 3′, 56 years) and a significantly greater risk of developing intracranial aneurysms [2, 5]. This association is not related to mutation type and may be due to the proposed cleavage of polycystin-1 into two different proteins (see later), with mutations to each half having potentially different phenotypic consequences [5, 6]. As yet, no clear phenotype/genotype correlations have been reported for PKD2 [3].

MUTATIONAL MECHANISM

A two-hit mechanism of cyst formation has been proposed for ADPKD (consisting of a germ line mutation to one allele and a somatic mutation to the other). This is an attractive hypothesis which could help explain both the focal nature of cyst development and the striking phenotypic variability seen in most families. Evidence of epithelial cell clonality within individual cysts and the detection of somatic mutations in cells isolated from a proportion of renal and hepatic cysts supports the two-hit hypothesis [7–9]. Furthermore, embryonic renal cyst development in homozygous knockout *Pkd1* or *Pkd2* animals, and particularly the progressive cystic disease seen associated with the $Pkd2^{WS25}$ mutant (that has a *Pkd2* allele prone to inactivation by somatic mutation), are consistent with a two-hit model of cyst development [10, 11]. However, there remain questions as to whether the two-hit mechanism is the only means to generate a cyst and indeed, whether these somatic events may be later events more important for cyst expansion and progression rather than initiation [12].

Persistent or even enhanced immunoreactivity for polycystin-1 or -2 is often detected in cystic epithelia but it is not clear if this signal represents functional protein [13–16]. Recent studies indicate that multiple karyotypic changes resulting in chromosomal imbalances are associated with cyst development, not just loss of heterozygosity (LOH) at the normal ADPKD allele (although LOH at 16p was found at a higher frequency) [17]. Progressive dedifferentiation of cystic epithelia during cyst enlargement in the $Pkd2^{WS25/-}$ model also indicates that cyst development and expansion may be dynamic and the result of more than a simple two-hit process [18]. A situation that illustrates the complexity of cyst development is the finding of somatic *PKD2* mutations in PKD1 cystic epithelia and vice versa, suggesting that cells that are transheterozygous may be prone to cyst development [19, 20]. However, it is apparent that this pattern of mutation cannot fully explain cyst initiation as patients and mice that are trans-heterozygous for a germ line *PKD1* and *PKD2* mutation have been described [21, 22]. Although these patients/animals have more severe disease than cases with either mutation alone, the difference is not dramatic (i.e., not every renal tubular cell gives rise to a cyst). These somatic events to the other ADPKD gene therefore appear to be akin to modifying genetic changes that increase the risk of cyst development (or hasten their progression) rather than cyst initiating events. Another example where a mutation to a second gene (in the germ line) can hasten cyst development and expansion are cases in which both *PKD1* and a tuberous sclerosis gene (*TSC2*) allele are mutated due to a large contiguous gene deletion [23]. These patients have TSC and severe early-onset PKD, indicating a likely synergistic role for polycystin-1 and tuberin (the TSC2 protein) in cyst development [24]. A possible mechanism which could account for this phenotype is the finding that tuberin plays a role in trafficking polycystin-1 to the lateral cell membrane [25].

When considering the mutational mechanism, it is worth noting that loss of a single allele (haploinsufficiency) may be sufficient in itself to elicit a phenotypic change. Recent studies have shown that $Pkd2^{+/-}$ vascular smooth muscle cells express a lower level of polycystin-2 and have altered intracellular Ca^{2+} homeostasis [26]. In addition, haploinsufficient *Pkd2* mice have reduced survival (not due to renal failure) indicating that a dosage reduction of polycystin-2 itself exerts a phenotypic load [27]. This is, however, not the case with Pkd1 heterozygotes who have a normal life span. Overexpression of functional polycystin-1 via a genomic transgene leads to age-related cysts in the kidney and liver, suggesting that an imbalance in the relative amounts of one polycystin protein can also result in cyst development. One possible explanation is that some polycystin complexes become inactivated due to the stoichiometric imbalance between the two proteins [28]. Given the available data, it seems reasonable to conclude that cystogenesis is a complex process. Cyst development requires a germ line mutation but beyond this, the likelihood of cyst formation is influenced by a number of different factors. These include somatic genetic events at the other (normal) allele, mutations at the other ADPKD gene and possibly a wide array of other genetic loci. In effect, these loci act as

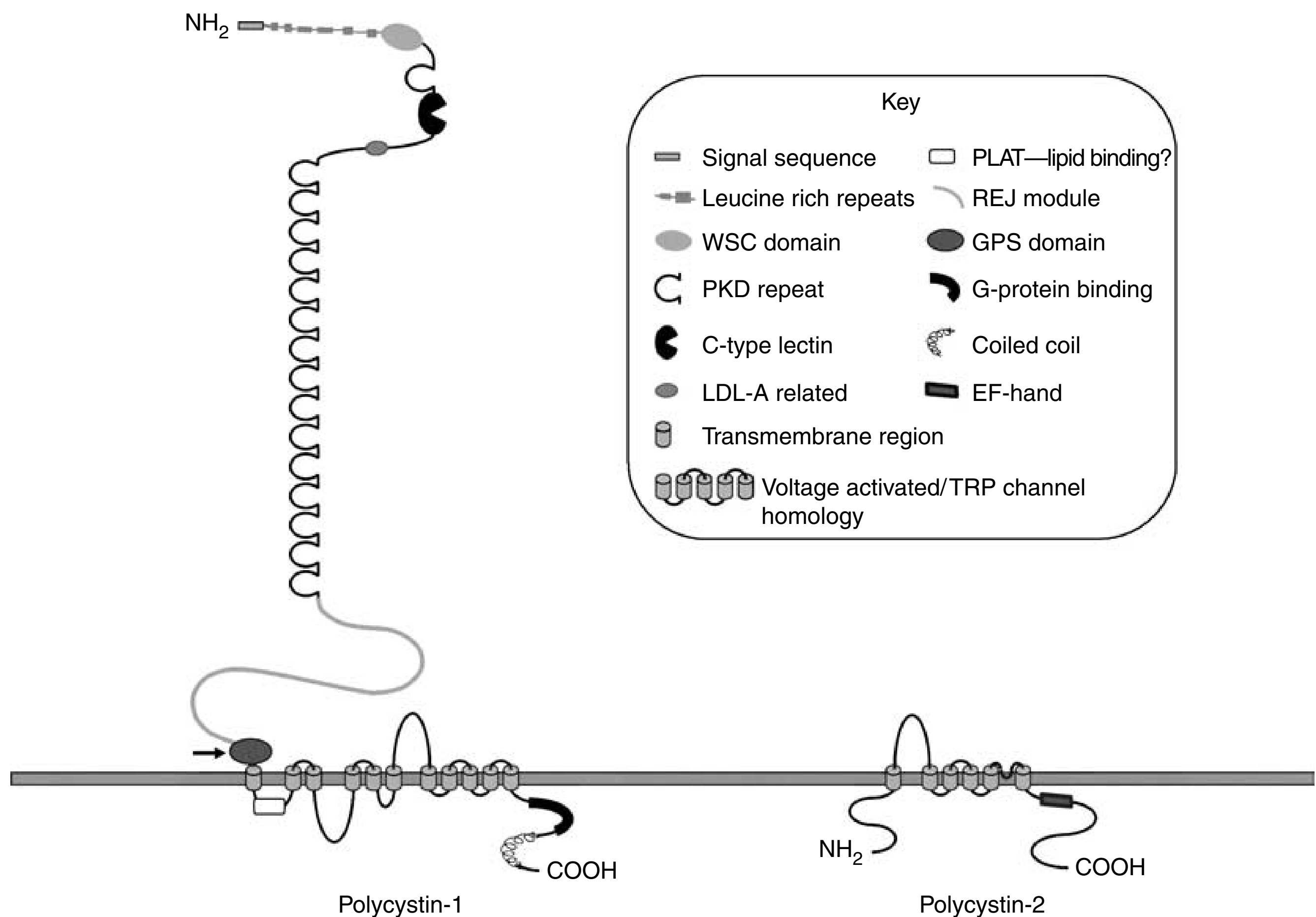


Fig. 1. Predicted structure and topology of polycystin-1 and polycystin-2. The predicted topology and structure of polycystin-1 and polycystin-2 indicates that they are glycosylated integral membrane proteins. Polycystin-1 has multiple structural motifs with predicted protein [leucine-rich and polycystic kidney disease (PKD) repeats], carbohydrate (C-type lectin), and lipid binding (PLAT) capacity. Polycystin-1 has a highly conserved G protein–coupled receptor proteolytic cleavage site (GPS) indicating that it may function as N-terminal and C-terminal products linked by noncovalent bonds. Polycystin-2 has extensive similarity to voltage-gated calcium (VACC) or transient receptor potential (TRP) channels.

modifiers of disease presentation in ADPKD. Beyond the genetic events, stochastic factors probably also influence whether a cell, haploinsufficient for an ADPKD mutation, is diverted into a cystogenic pathway. Another factor which could modify the cystic phenotype is the presence of partially functional (mutant) polycystin-1 protein (e.g., in *Pkd1* del34 mice) [29] or alternatively, nonfunctional mutant protein which could act in a dominant-negative manner (note added in proof).

THE POLYCYSTIN PROTEIN FAMILY

Sequencing of the *PKD1* and *PKD2* transcripts allowed the structure of the disease proteins to be modeled (Fig. 1). These structures have been refined by the characterization of orthologs (mouse, rat, and Fugu fish) and an array of homologous proteins characterized in humans (Table 1), other mammals, and more primitive species [30–32]. In addition, laboratory-based structural and functional analysis has helped to refine and confirm the likely structures. Only polycystin-1 and -2 are known to be associated with PKD but the other homologues have provided an insight into the likely ion channel role of polycystin-2–like proteins and the possible proteolytic cleavage of polycystin-1–like proteins. Recent studies of related *Caenorhabditis elegans* and *Drosophila* proteins have suggested a likely functional role for the polycystins in primary cilia or flagella. Of interest, the sea urchin polycystin-1 (suREJ3) and polycystin-2 (suPC2) orthologues have been shown to colocalize at the sperm acrosomal vesicle and coimmunoprecipitate—this could implicate a suREJ3/suPC2 complex in the acrosomal reaction [33].

Polycystin-1 is a large (460 kD), integral membrane glycoprotein (11 transmembrane passes) with an extensive extracellular region containing a number of different domains, many of which are involved in protein-protein or protein-carbohydrate interactions in other proteins and a short C-terminal tail. A possible role in cell/cell and/or cell/matrix interactions has been proposed for this

Table 1. Polycystin-1 and polycystin-2 human homologues

Protein	Gene	Chromosome	Function	Human disease	Reference
Polycystin-1	*PKD1*	16p13.3	? Adhesion; ? channel regulator; ? mechanosensor	ADPKD	[95]
Polycystin-REJ	*PKDREJ*	22q13	? Acrosome reaction	Unknown	[96]
Polycystin-1L1	*PKD1L1*	7p12–13	?	Unknown	[97]
Polycystin-1L2	*PKD1L2*	16q–22–23	?	Unknown	[98]
Polycystin-1L3	*PKD1L3*	16q–22–23	?	Unknown	[98]
Polycystin-2	*PKD2*	4q21–23	Ion channel; LR determination; ? acrosome reaction	ADPKD	[33, 58, 99]
Polycystin-L (polycystin-2L; polycystin-2L1)	*PKDL*(*PKD2L*; *PKD2L1*)	10q24–25	Ion channel	Unknown	[100, 101]
Polycystin-2L2	*PKD2L2*	5q31	?	Unknown	[102]

ADPKD is autosomal-dominant polycystic kidney disease.

protein but, so far, no ligand has been identified with certainty (refer to Table 4). Polycystin-2 has homology with the final six transmembrane domain region of polycystin-1 and topologic and sequence homology with voltage-activated (VAC) and transient receptor potential (TRP) channels [34]. The similarity to the TRP proteins has led to its inclusion (along with other polycystin-2–like proteins) as a new subfamily (TRPP) within the TRP superfamily. Predictions from amino acid sequence (60 N-linked sites are predicted on polycystin-1) and experimental evidence indicates that the polycystins are glycosylated with several different glycol forms of polycystin-1 identified [35]. Preliminary evidence indicates that the C-terminus of polycystin-1 can also be phosphorylated by protein kinase A and a *src*-like tyrosine kinase. Activation of signaling may occur through this modification [36]. Polycystin-2 is constitutively phosphorylated by casein kinase-2 and this may modulate its channel activity [37].

Another posttranslational modification to polycystin-1 that has been proposed is cleavage to generate more than one product. Sequence homology with the latrophilin/CL-1 receptors identified a G-protein coupled receptor proteolytic site (GPS) where the latrophilin/CL-1 proteins are cleaved in the PKD1-like polycystins. Recent data from ectopically expressed polycystin-1 have demonstrated partial cleavage at this site; cleavage did not occur in an array of mutant polycystin-1 proteins with missense mutations in the adjacent receptor for egg jelly (REJ) region [6]. Cleavage at a similar location concurs in the related sea urchin protein suREJ3. By analogy with the latrophilin/CL-1 receptors, the cleaved N- and C-terminal products remain tethered, although in this case the linkage is noncovalent. The functional significance of this cleavage event and whether the two products always remain tethered as part of the same complex are questions that are not yet fully resolved.

Polycystin-1 and -2 are thought to interact via their C-terminal tails to form a complex. The first evidence for this interaction came from yeast two hybrid studies with the interacting region on polycystin-1 within a conserved coiled-coil domain [38, 39]. In addition, polycystin-2 has a region upstream of the heterodimerization domain which appeared to mediate homodimerization. One study also suggested that polycystin-1 could homodimerize via its C-terminus but this was not confirmed in a second study [38, 39]. There is now good biochemical evidence supporting an interaction between the polycystins in vivo (see later).

CELLULAR AND SUBCELLULAR LOCALIZATION OF POLYCYSTIN-1 AND POLYCYSTIN-2

Consistent with the formation of a polycystin complex, there is considerable overlap in the expression pattern of the two proteins. Both proteins are widely expressed in epithelial cells, vascular smooth muscle, cardiac myocytes, and in other locations (see [40] for details). However, there are differences, most notably during development. While polycystin-1 is expressed at the highest level in the embryo and significantly down-regulated shortly after birth, polycystin-2 expression is maintained at a constant level into adult life.

The subcellular localization of both proteins has been most extensively studied in renal epithelial cells with multiple different locations of the proteins identified [40]. There is evidence of polycystin-1 expression at a variety of lateral membrane junctions (tight junctions, adherens junctions, desmosomes), focal adhesions, apical vesicles, and primary cilia [35, 41–47]. In contrast, polycystin-2 has been localized predominantly to the endoplasmic reticulum but also to basolateral plasma membranes, lamellopodia, primary cilia, and mitotic spindles [35, 47–52]. The distribution of these proteins (Table 2) indicates a complex picture that may also be developmentally regulated and suggest that the proteins may sometimes act independently. However, it is worth noting that polycystin-1 expression in the plasma membrane and

Table 2. Subcellular localization of polycystin-1 and polycystin-2 in mammalian cells

Subcellular compartment	Cells or tissues	Putative function	Reference
Polycystin-1			
Primary cilium	Mouse collecting duct cells	? Regulator of flow-sensitive channel	[47, 76]
Tight junctions	Adult rat kidney	Junctional permeability; cell polarity	[41]
Desmosomes	Madin-Darby canine kidney	Cell-cell adhesion or signaling	[43, 72, 103]
Adherens junctions	Human fetal kidney; ADPKD cystic cells	Cell-cell adhesion or signaling	[42, 69]
Lateral cell membranes	Human umbilical vein endothelial cells; Madin-Darby canine kidney; mouse collecting duct cells	Cell-cell adhesion or signaling	[44, 68, 104]
Focal adhesions	Human fetal collecting duct cells; ADPKD cystic cells	Cell-matrix adhesion	[45]
Apical vesicles	Human fetal kidney; G401 tumor cells	Recycling compartment	[16, 46]
Endoplasmic reticulum	Transfected COS-7 cells	? Regulation of PC2 channel	[105]
Polycystin-2			
Primary cilium	Human proximal tubular cells; mouse collecting duct cells	Flow-sensitive channel	[47, 76, 83]
Endoplasmic reticulum	LLCPK1; Madin-Darby canine kidney; human embryonic kidney-293; adult human kidney	Endoplasmic reticulum calcium channel	[35, 48]
Basolateral membrane	Adult human or rat kidney	Cell-cell or cell-matrix adhesion	[49, 106]
Lateral cell membrane	Mouse collecting duct cells; inner medullary collecting duct; Madin-Darby canine kidney	Cell-cell adhesion or signaling	[35, 50, 107]
Tight junctions	Mouse collecting duct cells	Junctional permeability; cell polarity	[108]
Lamellopodia	Transfected HeLa and COS7 cells	Cell-matrix adhesion	[51]
Mitotic spindles	HeLa; LLCPK-1	? Cell division	[52]

LLCPK-1, porcine kidney cell line; HeLa, human cervical carcinoma cell line; COS7, African green monkey kidney cell line.

Table 3. Putative functions of polycystin-1 and the role of polycystin-2 interaction in mammalian systems

Function	Model system	PKD1	PKD2	Effect	Reference
Cation channel	Chinese hamster ovary cells	FL	+	Cooperative	[53]
Suppressing proliferation	Madin-Darby canine kidney	FL	?/+	Cooperative (JAK2 activation)	[66]
Inhibiting apoptosis	Madin-Darby canine kidney	FL	?		[65]
Spontaneous tubulogenesis	Madin-Darby canine kidney; inner medullary collecting duct 3	FL or CT	?		[65, 71]
Cell-cell adhesion	Madin-Darby canine kidney; PKD1 transgenic cells	E	?		[67, 68]
Cell migration	Inner medullary collecting duct 3	CT	?		[71]
Cell-matrix adhesion	Human fetal kidney epithelia; ADPKD cystic cells	E	?		[45]
Flow-sensitive ciliary channel	Pkd1-del34 collecting duct cells	E	+	Cooperative	[76]

Abbreviations are: ADPKD, autosomal-dominant polycystic kidney disease; E, endogenous; FL, full-length; CT, C-terminus.

polycystin-2 in the endoplasmic reticulum does not preclude interaction, as coupling between TRP channels in the plasma membrane and inositol-1,4,5 triphosphate (IP_3) receptors in the adjacent endoplasmic reticulum has been described.

Direct evidence of interaction between the polycystins comes from heterologous expression of full-length polycystin-1 and polycystin-2 in Chinese hamster ovary (CHO) cells [53]. The specificity of these findings was shown by the inability of naturally occurring polycystin-1 and -2 mutants, lacking the respective heterodimerization domains to associate. Further studies confirmed the physiologic nature of this interaction by showing that a native complex could be isolated from human kidney and other cultured cells [35, 54]. These studies further demonstrated the existence of polycystin-2 multimers present in native tissues and cells [35]. Treatment with PNGase F (to cleave N-linked sugars) and endoglycosidase H (Endo H) (to differentiate endoplasmic reticulum and plasma membrane fractions) led to the identification of several glycosylated forms of polycystin-1, including Endo H–resistant and sensitive forms. One Endo H–sensitive and a resistant form of polycystin-1 were found to interact with polycystin-2, the latter of which was enriched in plasma membrane fractions and coimmunoprecipitated by polycystin-2 [35]. Membrane fractionation studies provided more evidence for the presence of a polycystin complex in plasma membranes, although over 90% of polycystin-2 was detected in the Golgi and endoplasmic reticulum [35]. Table 3 summarizes the systems in which interaction of the polycystins has been studied and the likely functions of these complexes.

Table 4. Proteins interacting with mammalian polycystin-1 and polycystin-2

Binding protein	Primary method	Putative function	Reference
Polycystin-1			
E-cadherin and catenins	Co-IP	Cell-cell adhesion	[42]
Polycystin-1 (homophilic)	Binding assays (NT)	Cell-cell adhesion	[67]
Intermediate filaments	Y2H (CT)	Cell-cell adhesion or signaling	[103]
Vinculin, paxillin	Co-IP	Focal adhesions	[45]
RGS7	Y2H (CT)	Signaling	[64]
Extracellular matix proteins	Binding assays (NT)	Cell-matrix adhesion	[109, 110]
Siah-1	Y2H (CT)	Protein stability	[111]
Polycystin-2			
TRPC1	Y2H (CT)	Channel	[34]
Polycystin-2 (homophilic)	Y2H (CT)	Channel	[35, 39]
CD2AP	Y2H (CT)	Anchorage to actin	[112]
Hax-1	Y2H (L5)	Anchorage to actin	[51]
Troponin-I	Y2H (CT)	Anchorage to actin	[113]
Tropomyosin	Y2H (CT)	Anchorage to actin	[114]
PIGEA-14	Y2H (CT)	? Endoplasmic reticulum-Golgi transport	[115]
m-Dia1	Y2H (CT)	? Cell division or cell fate	[52]

Abbreviations are: Co-IP, coimmunoprecipitation; Y2H, yeast 2 hybrid; CT, C-terminus; NT, N terminus; L5, loop 5.

Table 5. Extrarenal phenotypes of *Pkd1* and *Pkd2* mouse mutants

Mutant	Exons disrupted	Embryonic lethality	Vascular defect	Cardiac defect	Skeletal defect	Heterozygous phenotype (cysts; age)	Reference
Pkd1							
Del 34	34	+ (E18.5)	–	–	+	renal>9 m.liver>9 m. pancreatic>22 m.	[10, 29, 59]
Pkd1L	4–45	+ (E14.5-15.5)	+ (hem; edema)	–	–	?	[41]
Del 17-21βgeo	1–21	+ (E13.5-14.5)	+ (hem)	+	+	renal>3 m.liver>19 m.	[56]
Null	4	+ (E13.5-16.5)	+ (edema)	–	+	renal>2.5 m.liver>11m. pancreatic>12 m.	[29]
–/–	2–6	+ (E14.5)	+ (hem)	+	–	?	[55]
Null	1	+ (E12.5-birth)	+ (hem; edema)	–	–	renal>3 m.no gross liver cysts	[22]
Pkd2							
WS25 (unstable)	Disrupted exon 1 in IVS1	Live born	–	–	–	WS25+/–renal >2.5 m. (many)liver >2.5 m.	[11, 27]
WS183 (null)	1	+ (E13.5-18.5)	+ (hem; edema)	+	–	+/–renal <3 m. (rare)liver <9 m. pancreatic none	[11, 27]
–/LacZ	1	+ (E12.5-18.5)	?	+	–	?	[57]

Abbreviations are: E, embryonic day; JAK2, Janus kinase 2; hem, hemorrhage.

WHAT ARE THE FUNCTIONS OF THE POLYCYSTINS?

Insights into the likely functions of the polycystins have come from functional analysis suggested by structural homology (Fig. 1), studies of the polycystin complex in model systems (Table 3), identification of interacting partners (see Table 4 for details), analysis of knockout models (Table 5), and insights from other forms of PKD (Table 6).

Knockout models of ADPKD

A number of different mouse models with targeted disruption to *Pkd1* or *Pkd2* have been described (see Table 5 for details). In all of the homozygous animals, renal development appears to occur normally until embryonic day (E) ~ E14.5, after which renal cysts start to develop [10, 11, 41, 55, 56]. Since "normal" kidneys are formed up to this time, it appears that the polycystins are not required for induction of the nephron but are necessary for the normal maturation and maintenance of tubular architecture. Liver cysts are not detected in homozygotes but pancreatic cysts are seen from E13.5 [10, 11]. Homozygous *Pkd1* and *Pkd2* animals generally die during the embryonic period. The early death of these embryos is not related to the renal disease but may be due to cardiac defects that have been described in some models (see Table 5 for details). An additional common finding is of edema and in some cases, vascular fragility leading to localized and sometimes massive hemorrhage that may also account for the early death of many embryos [41]. *Pkd2*$^{-/-}$ embryos have laterality defects manifesting as a randomization of left/right asymmetry, suggesting a specific defect in the nodal ciliary signaling pathway which establishes asymmetry of the body plan [57]. Recent data indicate that polycystin-2 is expressed in nodal monocilia

Table 6. Cystogenic proteins localized to primary cilia or basal bodies

Gene	Protein	Inheritance	Disease	Species	Situs inversus	Extraciliary locations	References
PKD1	Polycystin-1	Autosomal-dominant	ADPKD	Human	No	Yes (lateral cell junctions; focal adhesions; cytoplasmic vesicles)	[47, 76]; Table 2
PKD2	Polycystin-2	Autosomal-dominant	ADPKD	Human	Yes	Yes (Endoplasmic reticulum; lateral or basolateral membrane; lamellopodia)	[47, 83]; Table 2
PKHD1	Fibrocystin	A	ARPKD	Human	No	Yes (cytoplasmic; microvilli; apical actin web)	[116–118]
OFD1	OFD1	X-linked	OFDS	Human	No	No	[119]
NPHP1	Nephrocystin	AR	NPHP (juvenile)	Human	No	Yes (focal adhesions)	[120, 121]
NPHP2	Inversin	AR	NPHP (infantile); inversin mouse	HumanMouse	Yes	Yes (nucleus, lateral cell junctions)	[122–125]
NPHP3[a]	NPHP3	AR	NPHP (adolescent); pcy mouse	HumanMouse	No	?	[126]
NPHP4[a]	Nephroretinin; nephrocystin-4	AR	NPHP (juvenile)	Human	No	?	[127, 128]
BBS4	BBS4	AR	BBS	Human	Yes	No	[129]
BBS8	BBS8	AR	BBS	Human	Yes	No	[130]
Cys1	Cystin	AR	Cpk mouse	Mouse	No	No	[131]
Tg737	Polaris	AR	Orpk mouse	Mouse	Yes	No	[47]

Abbreviations are: ADPKD, autosomal-dominant polycystic kidney disease; AR, autosomal recessive; OFDS, orofacial digital syndrome; NPHP, nephronophthisis; BBS, Bardet-Biedl syndrome.
[a] Interaction with nephrocystin but ciliary localization has not yet been demonstrated.

and transduces the flow-activated asymmetric Ca^{2+} signal detected at the left border of the node [58]. No laterality defects have been described in *Pkd1*$^{-/-}$ embryos. In contrast, skeletal defects have been reported in several *Pkd1* null animals but not in *Pkd2* knockouts.

In heterozygotes, renal and liver cysts are seen in older animals but the phenotype is generally mild and highly variable between animals [10, 56], with somewhat more severe disease in transheterozygotes. [22] The mildness of the disorder in heterozygotes contrasts with the human condition but may simply reflect the difference in lifespan between humans and mice since ADPKD is generally a late-onset disorder. The development of liver cysts occurs only in heterozygotes (not homozygotes) indicating a central role for the polycystins in maintaining the biliary architecture in the adult rather than during development [27, 59]. Only the *Pkd2*$^{WS25/-}$ compound heterozygous model, with a somatically unstable WS25 allele, develops adult-onset renal cystic disease in a rapid and reproducible way and is presently the only orthologous model of ADPKD in which to test potential therapies [11]. However, conditional knockouts of *Pkd1* represent models of PKD1 that may also be suitable for future therapeutic testing.

Signaling pathways

Functional analysis of cells overexpressing heterologous *PKD1* and *PKD2*, analysis of homology, and identification of interacting partners (Table 4) have implicated the polycystins in a number of key signaling pathways. Polycystin-1 contains a G protein activation sequence (Fig. 1) which binds and activates several classes of Gα subunits providing good evidence that it can function as a G protein coupled receptor. In addition, the C-terminus of polycystin-1 has been shown to promote activator protein-1 (AP-1) transcription via the activation of several classes of G proteins including heterotrimeric G proteins, Rac1 and cdc42 [60, 61]. Unexpectedly, coexpression of polycystin-2 has demonstrated both cooperative and inhibitory effects on polycystin-1 downstream signaling. For instance, ectopic polycystin-2 augments protein kinase C–mediated AP-1 signaling by polycystin-1 yet blocks polycystin-1–triggered G protein coupled signaling in other cells [62, 63]. These results could imply that the interaction between the polycystins results in the differential activation of distinct pathways for specific effector functions in different cells. The C-terminus of polycystin-1 can bind and stabilize RGS7, a regulator of G protein signaling (RGS) molecule which is normally rapidly degraded. [64] The stabilization of RGS7 could in turn modulate a number of G protein–dependent signaling cascades especially those activated by polycystin-1.

Exogenous overexpression of full-length *PKD1* in Madin-Darby canine kidney (MDCK) cells results in a reduced proliferation rate, increased resistance to apoptosis, and the development of branching tubules rather than cysts [65]. This possible role for polycystin-1 may be linked to activation of the Janus kinase (JAK2) and signal transducers and activators of transcription (STAT)1/3 pathway with experimental evidence of the up-regulation of $p21^{waf1}$ leading to cell cycle arrest in G_0/G_1 [66]. Polycystin-2 was not required for JAK2 binding but was necessary for JAK2 activation (by phosphorylation) [66].

Cell-cell/matrix interactions

Homotypic polycystin-1 interactions (via the PKD domains) that could mediate cell-cell contacts have been described [67]. Cell-cell adhesion appears to be disrupted by antibodies to this region that is partially overcome in cells overexpressing polycystin-1 [68]. Heterophilic interactions of polycystin-1 with the E-cadherin/catenin complex have also been reported: these could contribute to the maintenance of normal intercellular adhesion [42, 69]. Cystic ADPKD cells are defective in basolateral membrane trafficking for lipids and certain proteins such as E-cadherin and the low density lipoprotein-receptor (LDL-R) that are retained in the Golgi [70]. It has been postulated that mutant polycystin-1 may bind to E-cadherin leading to its intracellular retention and replacement by N-cadherin [68, 69].

Polycystin-1 has also been localized to focal adhesions and may form part of a focal adhesion complex mediating cell-matrix adhesion, branching tubulogenesis and cell migration [45, 65, 71, 72]. Cystic PKD cells display increased adhesiveness to collagen I mediated by integrins ($\alpha_2\beta_1$) suggesting that polycystin-1 and/or polycystin-2 may influence integrin-mediated signaling [45]. In addition, phosphorylation could act as the molecular switch determining whether polycystin-1 is enriched in cell-cell or cell-matrix complexes [73].

Channel activity

The close resemblance of polycystin-2 (and polycystin-2-like proteins) to TRP-gated channels has led to a number of studies attempting to demonstrate that polycystin-2 can reconstitute a Ca^{2+} channel. There is now good evidence that polycystin-2 conducts divalent cations, including Ca^{2+}, although the major cellular site of channel activity remains controversial [53, 74–76]. A further question is how much polycystin-2 channel function depends on the presence or activity of polycystin-1. In artificial lipid bilayers, recombinant polycystin-2 can reconstitute Ca^{2+} channel activity in the absence of polycystin-1; polycystin-1 appears to stabilize or activate polycystin-2 channel activity in this system [77]. In another study, however, whole cell currents were only induced by coexpression of polycystin-1 and polycystin-2; mutants unable to interact did not induce channel activity suggesting that this interaction was crucial. [53] In this system (CHO cells), polycystin-1 was required to transport polycystin-2 to the plasma membrane. In contrast, a third study found that polycystin-2 had the characteristics of a calcium-activated, high-conductance endoplasmic reticulum channel with a role in the release of intracellular Ca^{2+} from the endoplasmic reticulum [75]. One common theme from all these studies is that polycystin-2 plays a role in increasing cytoplasmic levels of Ca^{2+}. Consistent with this function, cells haploinsufficient for *Pkd2* have a lower resting level of intracellular Ca^{2+} and store-operated Ca^{2+} (SOC) channel activity is also down-regulated [26]. The ability of polycystin-1 to reconstitute channel activity by itself or form part of a channel pore, possibly with other subunits, has not yet been shown. In some systems, heterologous expression of full-length or C-terminus of polycystin-1 alone can activate a nonselective calcium current [78] which could be independent of polycystin-2 [79].

IS THERE A COMMON CILIARY CYSTIC PATHWAY?

Most vertebrate cells express a single nonmotile cilium (primary cilium) with a characteristic "9 + 0" microtubule structure arising from a basal body or centriole (see Fig. 2). In the kidney, all tubular epithelial cell types (except intercalated cells) express a cilia projecting into the lumen of the tubule. Although this organelle had been recognized for over 100 years, until recently its precise function was obscure [80]. Data from a number of different sources now suggest that the primary defect in various forms of PKD, including ADPKD, may relate to abnormalities of ciliary structure or function. Compelling evidence has come from animal models, protein localization studies (see Table 6 for details), disruption of ciliary proteins, and direct functional evidence in model systems.

The first clue directly linking PKD and primary cilia came from the *orpk* mouse model of PKD when it was discovered that the defective protein, polaris, is orthologous to the *Chlamydomonas* intraflagellar transport (IFT) protein IFT88 [81]. Mice null for the gene *Tg737* or with a hypomorphic allele, *Tg737^{orpk}*, have left-right axis defects and absent or stunted primary cilia [81]. Other early evidence linking primary cilia to PKD came from the study of *C. elegans* orthologues of the ADPKD genes, *lov-1* and *PKD2* [31]. Mutants of these genes have specific male mating behavior defects related to a failure of mechanosensation mediated by a specific set of ciliated sensory neurons. These are also the major sites of expression of the *C. elegans* polycystins [31]. Functional evidence directly linking the induction of structural ciliary abnormalities to the subsequent development of PKD has come from the demonstration that a kidney-specific conditional knockout disrupting a ciliary motor subunit gene, *Kif3a*, in tubular epithelial cells results in a cystic phenotype [82]. Simultaneously, a number of proteins associated with PKD in both mice and/or humans have been localized to primary cilia or to the basal body including polycystin-1 and polycystin-2 (see Table 6) [47, 83]. Recently, an insertional mutagenesis screen for cystic mutants in Zebra fish larvae has revealed selective enrichment for the orthologues for several IFT proteins and *pkd2*. This finding adds further weight to the primary importance of ciliary abnormalities in PKD pathogenesis [84].

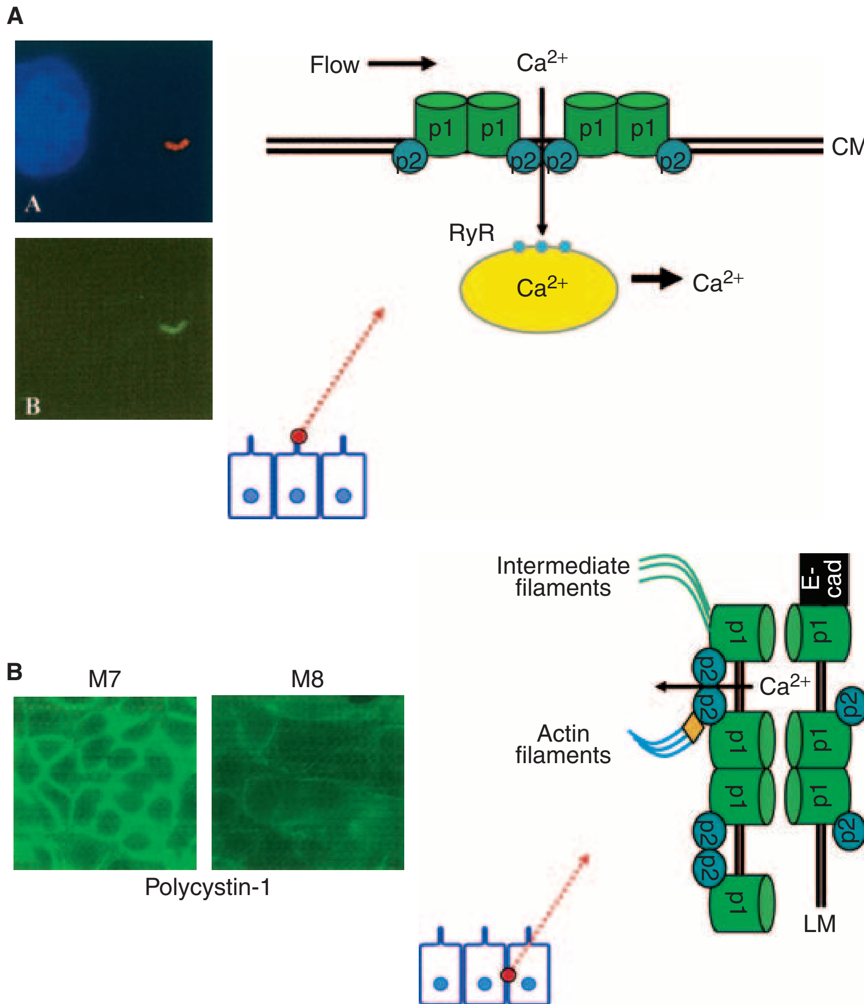


Fig. 2. Two models of polycystin-1/polycystin-2 function. The polycystin-1/polycystin-2 complex could function as a mechanosensory calcium channel localized in primary cilia (*A*) and/or as an adhesion complex with or without E-cadherin (*B*). A solitary primary cilium arising from a human proximal tubular cell (DAPI nuclear stain) can be visualized using an antibody to acetylated tubulin (red) (panel A). Polycystin-1 expression (green) colocalizes with acetylated tubulin expression (panel B). In (panel A), polycystin-1 expression in a *PKD1* transgenic collecting duct cell, M7, is greatly increased compared to that of a control cell, M8. Polycystin-1 is shown outlining the lateral cell borders of a cell monolayer indicating a potential role in mediating cell-cell adhesion. *Cis*- and *trans*-homophilic interactions between the Ig-polycystic kidney disease (PKD) domains of polycystin-1 could mediate cell-cell adhesion and/or signaling. The polycystin complex could be anchored to the cytoskeleton through a number of actin-binding adapter proteins (orange diamond) which link the C-terminus of polycystin-2 to actin filaments and via the direct binding of the C-terminus of polycystin-1 to intermediate filament proteins such as vimentin and cytokeratin (see Table 4). Abbreviations are: p1, polycystin-1; p2, polycystin-2; RyR, ryanodine receptor; CM, ciliary membrane; LM, lateral (plasma) membrane; E-cad, E-cadherin. For other possible locations and functions of the two proteins, please refer to Tables 2 to 4.

A mechanosensory function for renal primary cilia had previously been suggested since flow-induced ciliary bending leads to a rise in intracellular Ca^{2+} [85]. There is now experimental data to indicate that a ciliary polycystin complex acts as the mechanosensor and that the polycystin-2 channel mediates the flow-sensitive influx of extracellular Ca^{2+} [76]. These results suggest that a defect in ciliary mechanosensation due to *PKD1* or *PKD2* mutations results in the disruption of Ca^{2+} influx and this somehow leads to cyst development. Other possible roles for primary cilia have been suggested (e.g., as a sensor of tubular lumen diameter) acting as an important checkpoint in tubule maturation with resultant cystic expansion when defective [86].

One note of caution to an exclusive "ciliary model" of PKD is indicated by a recent study showing that restoration of ciliary length in the *orpk* model by transgenic expression of *Tg737* corrected the left/right axis defects but did not prevent cyst development [87]. It is unclear, however, if the restored kidney cilia are fully functional in this experimental system; it also appears that the transgenic cDNA used does not express all known splice forms of the *Tg737* gene. Nonetheless, an alternative explanation that some splice forms of polaris have extraciliary functions in epithelial cells (analogous to inversin) and that these could be important in suppressing cystogenesis [80]. As shown in Table 6, it should be noted that not all of the PKD proteins are associated with situs inversus (indicative of nodal ciliary dysfunction) and several have been shown to have prominent extraciliary locations as well as significant interactions with nonciliary proteins. Since the timing and prominence of cystic disease can vary greatly between these diseases (e.g., cysts are usually a late and minor feature in nephronopthisis), the extraciliary functions of these proteins could have biologic significance both for cyst formation as well as in the pathogenesis of other features in the diseased kidney (see later).

HOW IS POLYCYSTIN DYSFUNCTION LINKED TO CYST DEVELOPMENT?

Structural and functional analysis of the polycystins has provided clues as to several possible roles for these proteins but do not as yet offer a unifying explanation as to

how the complex might act. Figure 2 shows two possible models through which the polycystin complex could act—as a flow-activated mechanosensitive channel located in primary cilia or alternatively as a ligand-activated and/or mechanosensory adhesion complex involved in mediating cell adhesion. The ciliary function of the polycystin complex has been discussed above but there is also strong evidence that the polycystin complex might mediate cell adhesion. In this regard, it has been shown that the C-terminus of polycystin-2 can bind to several adaptor proteins which bind actin whereas the C-terminus of polycystin-1 has the ability to bind directly to intermediate filaments (Table 4). Both these attachments would anchor the complex firmly to the cytoskeleton and perhaps modulate its function. These two models need not be mutually exclusive since it is possible that more than one defect in polycystin function could be required for cyst development. Potential abnormalities in ciliary function could initiate cyst formation while defects at extraciliary locations could contribute to cyst expansion or some of the other features associated with progression of a cystic phenotype. Experimental models in which the ciliary and extraciliary functions of the polycystin complex can be clearly distinguished would yield further insights into this important question.

The best characterized function of the polycystins is as a Ca^{2+} channel and it seems likely that a disruption of normal intracellular Ca^{2+} homeostasis may be the central defect underlying the ADPKD phenotype. Nevertheless, several different models of PKD have also suggested a central role for another second messenger molecule, cyclic adenosine monophosphate (cAMP), in cyst development. Agonists of cAMP stimulate fluid secretion in collecting ducts and ADPKD cysts [88]. In addition, cells isolated from PKD kidneys have an altered proliferative response to adenylate cyclase agonists with activation of the extracellular-regulated kinase (ERK) signaling cascade compared to an inhibitory effect in normal kidney cells [89]. This phenotypic switch could contribute to the excess proliferation seen associated with PKD. There is also evidence of cross-talk between Ca^{2+} and cAMP signaling pathways in PKD so changes in intracellular Ca^{2+} concentrations could also stimulate an altered response to cAMP [90].

TREATMENT PROSPECTS FOR ADPKD

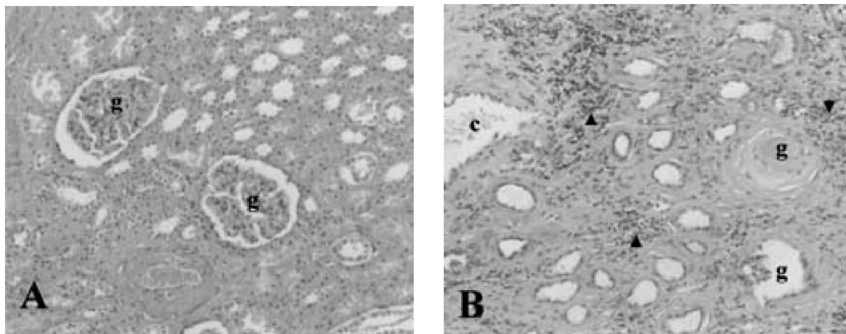
Recently, several promising treatments arising from a greater understanding of the pathophysiology of PKD have been tested in animal models of PKD, including some orthologous to human disease. The compounds tested were designed to target the down-stream effects of cystic transformation, rather than the primary genetic defect itself. Figure 3 shows that cystic transformation may have multiple downstream consequences leading to the typical appearances of the end-stage ADPKD kidney. Pharmacologic strategies targeting these pathways might therefore be useful to preventing or delaying progression to end-stage kidney failure.

Many of the animal models used so far have not been orthologous to ADPKD but an effective treatment in one model may prove to be useful in ADPKD given the common phenotypic changes found in most forms of PKD. Epidermal growth factor (EGF) and a related molecule, transforming growth factor-a (TGF-a), have been shown to promote epithelial cell proliferation and cyst development in PKD. Cyst fluids contain mitogenic quantities of these growth factors; moreover the EGF receptor (EGFR) and EGFR-related tyrosine kinase receptor (Erb-B2) are overexpressed and mislocalized to the apical membrane in ADPKD and other rodent models of PKD. Compounds that inhibit EGFR tyrosine kinase activity markedly inhibit cyst development and progression in three of these models [91]. However, it remains to be seen how effective these compounds will be in models orthologous to ADPKD since a deleterious effect was recently reported with treatment in the *Pck* model of ARPKD [92]. Another possible treatment, pioglitazone (a thiazolidinedione), prolonged survival of $Pkd1^{-/-}$ embryos and reduced cardiac defects and renal cystogenesis when administered maternally [55]. This compound could correct some of the downstream effects of polycystin dysfunction, such as the reduced levels and mislocation of β-catenin. Improved endothelial function was also noted following prolonged treatment of $Pkd1^{+/-}$ mice but its effect on cyst development in the heterozygote has not yet been reported.

Promising results using a new drug (OPC31260), designed to reduce intracellular concentrations of cAMP in PKD by targeting the vasopressin V2 receptor (VPV2R), the major cAMP agonist in the collecting duct, have also been reported. Treatment dramatically inhibited disease development, halted and even caused regression of established disease in the *PCK* rat (model of ARPKD) and *pcy* mouse (model of nephronophthisis NPHP3) [93]. Furthermore, treatment of the $Pkd2^{WS25/-}$ model of ADPKD with OPC31260 also markedly inhibited renal enlargement and cyst progression in this model [94]. OPC31260 appears to be a safe treatment with remarkably few side-effects, possibly due to the restricted expression of VPV2R to the principal cells of the collecting duct. It seems likely that one or more of these compounds will be tested in human ADPKD in the near future, opening a new chapter in the history of this disorder.

CONCLUSION

The molecular analysis of ADPKD has begun to clarify the complex phenotype of this condition. However, it has also revealed the complexity of the genes and proteins



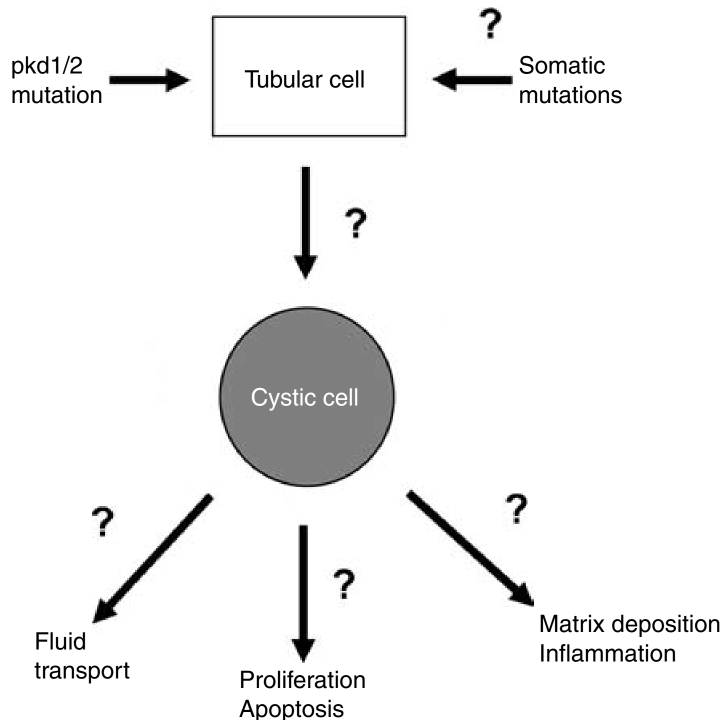


Fig. 3. Pathophysiology of disease progression in the cystic autosomal-dominant polycystic kidney disease (ADPKD) kidney. Compared to the normal kidney (*A*), the end-stage ADPKD kidney (*B*) is characterized by glomerular sclerosis, interstitial fibrosis, and tubular atrophy associated with a prominent inflammatory cell infiltrate (arrowheads). As shown in (B), apart from tubular cysts (c), the glomeruli (g) may be cystic or sclerosed (magnification ×100). A schematic model for disease progression in ADPKD kidney shows that it is likely that somatic mutations in *PKD1*, *PKD2*, or other genes (e.g., *TSC2*) and/or stochastic factors trigger the entry of a predisposed cell bearing a germ line *PKD1* or *PKD2* mutation into a cystic pathway resulting in a number of downstream deleterious effects on fluid transport, cell turnover, and interstitial scarring. The initiation and impact of these processes could in turn be modulated by nonallelic ("modifying genes") or environmental factors acting at each stage (indicated by ?).

that are involved, their likely multiple roles and the many possible downstream consequences of mutation. Nevertheless, we now have a greater understanding of ADPKD pathophysiology and a number of new treatment options. The rapid pace of the advances being made hold great promise for patients with this previously untreatable disease. To summarize, (*1*) polycystin-1 and polycystin-2 are cardinal members of a new protein family (the eight members that have been identified so far can be subdivided into "PKD1-like" and "PKD2-like" proteins); (*2*) a variety of functions have been attributed to polycystin-1 but there is general consensus that polycystin-2 functions as a cation channel; (*3*) significant phenotypic differences between *Pkd1* and *Pkd2* knockout mice indicates that these proteins have overlapping as well as independent functions; (*4*) a number of key signaling pathways regulated by the polycystins, especially controlling cell proliferation and fluid secretion, have been described; (*5*) there may be several mutational mechanisms underlying cyst formation in ADPKD; (*6*) abnormalities of ciliary structure and function may underlie the PKD phenotype; and (*7*) several new therapies showing promise in rodent PKD models merit testing in humans.

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NOTE ADDED IN PROOF

Lantinga-van Leeuwen et al have recently demonstrated that haploinsufficiency of Pkd1 can itself lead to cyst formation (*Human Molecular Genetics* 13:3069–3077, 2004).

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