



Genetic mechanisms and signaling pathways in autosomal dominant polycystic kidney disease

Peter C. Harris and Vicente E. Torres

Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA.

Recent advances in defining the genetic mechanisms of disease causation and modification in autosomal dominant polycystic kidney disease (ADPKD) have helped to explain some extreme disease manifestations and other phenotypic variability. Studies of the ADPKD proteins, polycystin-1 and -2, and the development and characterization of animal models that better mimic the human disease, have also helped us to understand pathogenesis and facilitated treatment evaluation. In addition, an improved understanding of aberrant downstream pathways in ADPKD, such as proliferation/secretion-related signaling, energy metabolism, and activated macrophages, in which cAMP and calcium changes may play a role, is leading to the identification of therapeutic targets. Finally, results from recent and ongoing preclinical and clinical trials are greatly improving the prospects for available, effective ADPKD treatments.

Introduction

Polycystic kidney disease (PKD) encompasses a group of inherited disorders that result in cyst development in the kidney in addition to a range of extrarenal manifestations (1, 2). Autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD) are common, simple forms of PKD, in which renal and liver disease account for most of the morbidity. Additionally, a number of syndromic diseases, such as Meckel (MKS), Joubert (JBTS) and Bardet Biedl (BBS) syndromes, have PKD as a major phenotypic manifestation (3). ARPKD has a frequency of approximately 1:20,000, and the typical presentation is of severe PKD detected in utero or in the perinatal period with greatly enlarged kidneys, which is associated with significant neonatal mortality (4). However, ARPKD may first present later in childhood or even in adulthood with less evident renal enlargement and complications of congenital hepatic fibrosis as the major cause of symptomatic disease (5).

Clinical characteristics of ADPKD

ADPKD is the most common form of PKD (frequency 1:400–1:1,000) and one of the most common monogenic diseases (1). The disease is characterized by progressive cyst formation and development during the lifetime of the patient, resulting in bilateral renal enlargement and often end-stage renal disease (ESRD) (1). ADPKD accounts for approximately 4%–10% of ESRD populations worldwide; approximately 30,000 US patients have ESRD resulting from ADPKD (1:3,500 individuals aged 65–69 years) (6). However, the disease course is highly variable and a significant minority of patients do not reach ESRD even in old age, while a small number (<1%) exhibit early-onset disease, with a diagnosis made in utero or in infancy by the identification of enlarged echogenic kidneys (7–9). Clinically significant extrarenal manifestations include a higher frequency of intracranial aneurysms (ICAs), which cause morbidity and mortality by subarachnoid hemorrhage, and severe polycystic liver disease (PCLD), for which resection or other surgery may be required (10, 11).

Most ADPKD patients have an affected parent, but at least 10% of cases can be traced to an apparent de novo mutation (12). Presymptomatic diagnostics of at-risk ADPKD individuals can generally be made by the detection of multiple cysts by renal ultrasound imaging, where specific diagnostic criteria have been defined. More sensitive magnetic resonance (MR) or computed tomography imaging can be helpful in equivocal cases and for longitudinal analysis of disease progression (13). Patients typically only show a significant decline in renal function (measured by estimated glomerular filtration rate [eGFR]) 10 to 15 years before the onset of ESRD. Total kidney volume, measured by MR, may be employed as a measure of disease severity before a detected decline in eGFR and has been used to monitor disease progression in clinical trials (14, 15).

The ADPKD genes, mutations, and disease mechanism

ADPKD is genetically heterogeneous with two loci identified, *PKD1* (16p13.3), which encodes polycystin-1 (PC1), and *PKD2* (4q22), which encodes PC2 (16–19). Further genetic heterogeneity has been suggested; however, a recent study of five apparently unlinked ADPKD families found that three had a *PKD1* and one a *PKD2* mutation. The unresolved case had an atypical presentation with renal atrophy (20). Mutation screening can be of value for ADPKD diagnostics, especially to assess living related donors with equivocal imaging, but also to understand etiology in patients with a negative family history, atypical radiological presentations, early-onset or mild disease, and potentially to define trial/treatment populations (21, 22). Mutation screening of *PKD1* is complex due to segmental duplication of the 5' part of the gene to exon 33, matching six pseudogenes (P1–P6) located approximately 15 Mb further proximal in 16p (17, 23). A high level of similarity with the pseudogenes (98%–99%) means that locus-specific long-range PCR (LR-PCR) products are required to specifically amplify *PKD1* (12).

In groups identified via the renal clinic, *PKD1* accounts for approximately 78% of pedigrees and *PKD2* for approximately 13%, with no mutation detected (NMD) in approximately 9% of cases (24, 25). It is unclear whether all NMD cases are explained by atypical and thus undetected mutations at the known loci or whether, despite recent data (20), a further ADPKD locus exists. *PKD2* may represent up to approximately 25% of mutation characterized cases

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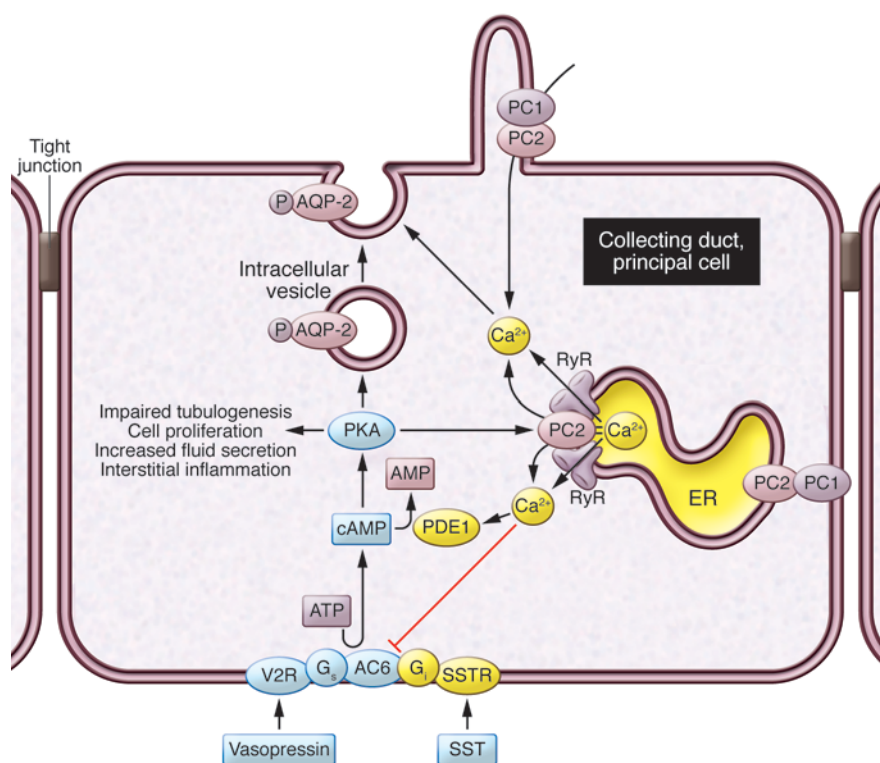
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Table 1
Viable ADPKD mouse models suitable for preclinical trials

Strain	Induction	Target renal tubules/time	Phenotype	Death	Level PC1 ^A	Reference
<i>Pkd1</i> floxed deletion (constitutive)						
<i>fl/-</i>	<i>Ksp-Cre</i>	CD, DT	Kidney^B PC1 loss in target tissue	~P16		136
<i>fl/fl</i>	<i>Pkhd1-Cre</i>	CD	+++CE, 0–1 mo			42
<i>fl/fl</i>	<i>Nestin-Cre</i>	Multiple mosaic	+++CE, P8–P50			141
<i>fl/-</i>	<i>γGT-Cre</i>	PT, CD	+++CE, 0–2 mo; ++F, >1 mo +++CE, P10–P30	~P30		185
<i>Pkd2</i> floxed deletion (constitutive)						
<i>fl/-</i>	<i>γGT-Cre</i>	PT, CD	PC2 loss in target tissue			186
<i>fl/fl</i>	<i>Pkhd1-Cre</i>	CD	+CE, 2 mo +++CE, P8–P50			42
<i>Pkd1</i> floxed deletion (induced)						
<i>fl/fl</i>	<i>CAG-cre/Esr1 +OHT</i>	All <P12	PC1 loss at specific time/target tissue			45
<i>fl/fl</i>	<i>CAG-cre/Esr1 +OHT</i>	All >P14	+++CE, 1 mo			45
<i>fl/fl</i>	<i>Ksp-Cre/ER +tam</i>	CD, DT 4 d	+CE, >3 mo			47
<i>fl/fl</i>	<i>Ksp-Cre/ER +tam</i>	CD, DT >3 mo	+++CE, 1 mo			47
<i>fl/fl</i>	<i>Mx1-Cre/IFN +pt;pC</i>	All P7	+CE, >4 mo			187
<i>fl/-</i>	<i>Mx1-Cre/IFN +pt;pC</i>	All P7	+++CE, 1–10 wk			187
<i>fl/fl</i>	<i>Mx1-Cre/IFN +pt;pC</i>	All 5 wk	+CE, >3–12 mo; +F, >3–12 mo	++LC		187
<i>fl/fl</i>	<i>Pax8^{fl/fl}; TetC-Cre +dox</i>	All 4 wk	+CE, 12 wk; ++CE, 18 wk			44
<i>Pkd2</i> floxed deletion (induced)						
<i>fl/-</i>	<i>Mx1-Cre/IFN +pt;pC</i>	All 6 wk	PC2 loss at specific time/target tissue			187
<i>fl/fl</i>	<i>Pax8^{fl/fl}; TetC-Cre +dox</i>	All 4 wk	+CE, 14 wk +++CE, 18 wk	+LC		44
<i>Pkd1</i> hypomorphic						
n/nl			PC1 global dosage reduction			34
L3/L3			+++CE, 0–1 mo; ++F, >1 mo	~9 mo	~13%–20%	
T3041V/T3041V			+++CE, 0–1 mo; ++F, >1 mo	~P75	~20%	37
R3277C/R3277C			+++CE, 0–1 mo	~P21		54
R3277C/-			+CE, 0–12 mo; ++F, >9 mo	>1 yr	~40%	36
<i>Pkd2</i> hypermutable						
WS25/-			PC2 global dosage reduction	~P28	~20%	36
			+CE, 0–12 mo; +F, >6 mo	>1 yr		33

^AEstimate of functional PC1 generated. ^BCystic expansion (CE) or fibrosis (F), occurring at slow (+) to very rapid (+++) rates of progression. LC, liver cyst; MC, microhamartoma; PC, pancreatic cyst; CD, collecting duct; DT, distal tubule; OHT, 4-hydroxytamoxifen; PT, proximal tubule; tam, tamoxifen; dox, doxycycline. References provided are the earliest published or most informative reference.

**Figure 1**

An abnormal crosstalk of calcium and cAMP signaling disrupts multiple signaling pathways and leads to the cystic phenotype. Activation of calcium-inhibitable adenylyl cyclase 6 (AC6) and inhibition of calcium/calmodulin-dependent phosphodiesterase 1 (PDE1) causes abnormal accumulation of cAMP and activation of PKA. Disrupted intracellular calcium homeostasis interferes with aquaporin-2 (AQP-2) targeting to the apical membrane. Sustained PKA activation of PC2 and RyRs makes these channels leaky and leads to reduced intracellular calcium stores, further driving cAMP/PKA signaling. PKA activation also disrupts tubulogenesis, activates proliferative signaling pathways, stimulates chloride and fluid secretion, and promotes STAT3-induced transcription of chemokines and cytokines. Vasopressin V2 and somatostatin (SST) stimulation of their respective receptors (V2R and SSTR) results in increased cAMP. Gs and Gi refer to guanosine nucleotide-binding proteins s and i, respectively. Yellow indicates proteins that are reduced in PKD; blue indicates proteins that are increased in PKD.

in population-based studies (9). *PKD1* mutation is associated with significantly more severe disease, with an average age at ESRD of 58.1 years compared with 79.7 years for *PKD2* (26). ADPKD displays extreme allelic heterogeneity, with any fully inactivating mutation to a *PKD1* or *PKD2* allele causing ADPKD. In the latest version of the ADPKD Mutation Database (PKDB), 1,272 *PKD1* mutations are described that account for 1,874 families, and 202 *PKD2* mutations are described that cause disease in 438 families (27). For *PKD1*, approximately 65% of mutations are predicted to truncate the protein and approximately 35% are nontruncating (24, 25). Corresponding levels for *PKD2* are approximately 87% truncating and approximately 13% nontruncating; approximately 3% of ADPKD mutations are larger rearrangements involving deletion or duplication of at least one exon (24, 25, 28). Recently, a next-generation sequencing method has been described for ADPKD screening based on sequencing the locus-specific LR-PCR products (29). Such methods can identify unusual mutations such as gene conversions with one of the pseudogenes (29).

Embryonic lethality with cyst development (~E14.5) of mice homozygous for a fully inactivating *Pkd1* or *Pkd2* mutation indicates that complete loss of PC1 or PC2 is incompatible with life, and cystogenesis is associated with protein loss (30, 31). Through the generation of a range of viable ADPKD mouse models with disease of varying severity, we are beginning to understand pathogenesis, and this knowledge is aiding preclinical testing (Table 1). Dominant inheritance, the focal nature of cyst development, loss/mutation of the normal allele of the affected ADPKD gene in cystic cells, and a hypermutable *Pkd2* model (WS25), suggest a two-hit hypothesis of cystogenesis (32, 33). Although somatic mutation may be a means to form a cyst and may be important in cyst progression, there is increasing evidence that cysts can develop with some PC present and that cyst development is a dynamic process (34–37).

Viable ADPKD cases that are homozygous or compound heterozygous for *PKD1* pathogenic variants suggest the presence of hypomorphic alleles (35). Recently, up to 50% of nontruncating changes have been suggested to be hypomorphic, resulting in ESRD at 55 years in patients with truncating *PKD1* mutations and 67 years for those with nontruncating mutations (26, 38). Some cases of early-onset ADPKD, or cases mimicking ARPKD, are due to an in trans combination of two *PKD1* mutations, at least one of which is hypomorphic (35, 39). Studies of a *Pkd1* mouse model with a missense change, p.R3277C, confirmed the hypomorphic nature of this allele and its role in causing early-onset disease (ref. 36 and Table 1). Unilateral parental disomy involving a hypomorphic *PKD2* allele also has been described to cause early-onset ADPKD (40). Mutations in other cystogenes, such as *HNF1B* (associated with the renal cysts and diabetes syndrome) or the ARPKD gene *PKHD1* in combination with a *PKD1* mutant allele have also been suggested to be associated with early-onset PKD (41). Additive cystogenic effects associated with mutations to more than one cystogene have also been suggested by interbreeding of conditional *Pkd1* or *Pkd2* models, or a hypomorphic *Pkhd1* model, with conditional PCLD models *Sec63* or *Prkcsb* (42). In this case, enhanced cystic disease was found with an ADPKD/ARPKD and a PCLD mutation, and reintroducing functional PC1, but not PC2, rescued the phenotype, suggesting a critical role for PC1 in cystogenesis.

A hypothesis is now becoming accepted in which cysts develop below a specific PC threshold, with the dosage of functional PC associated with disease severity (36, 43, 44). Reaching this threshold may occur by a combination of one or more of the following factors: somatic mutation, variants at the ADPKD genes and beyond, stochastic expression differences between cells, and environmental factors such as renal injury (36, 45, 46). In experimental systems, the timing of loss of the second ADPKD allele

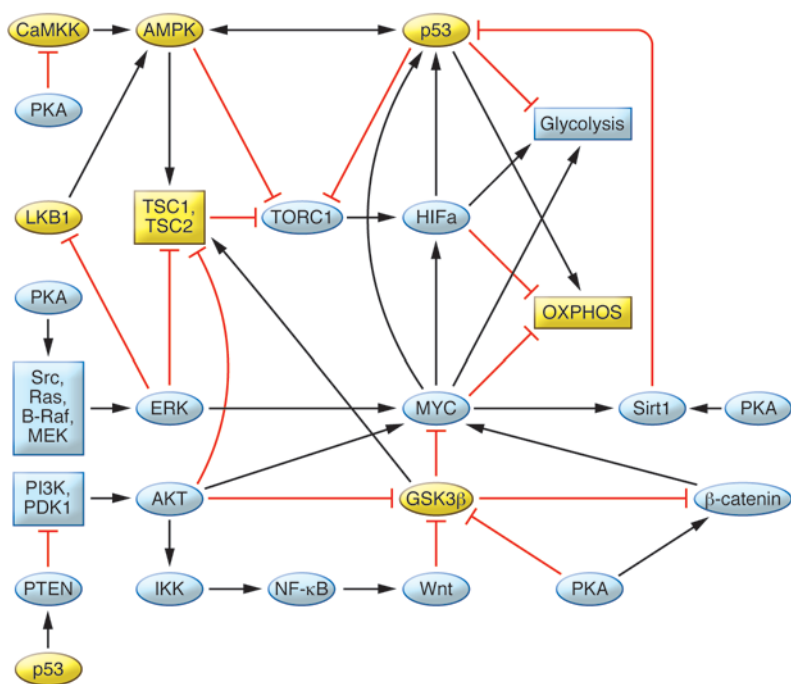


Figure 2

Network of pathways and transcription functions that regulate cell cycle progression, energy metabolism, and cell proliferation and death that are abnormal in PKD. Upregulation of B-Raf/Mek/ERK, PI3K/AKT, and Wnt/ β -catenin pathways and MYC and HIF transcription factors and downregulation of the LKB1/AMPK/TSC pathway, GSK3, and p53 promote aerobic glycolysis and cell cycle progression. Upregulation of MYC and downregulation of p53 exert proapoptotic and antiapoptotic effects, respectively. Downregulation of AMPK stimulates ion transport and fluid secretion. At multiple levels in this network, PKA activity stimulates proliferative and inhibits antiproliferative signals. Yellow indicates proteins that are reduced in PKD; blue indicates proteins that are increased in PKD. OXPHOS, oxidative phosphorylation.

has been shown to significantly influence the severity of cystic disease; mutations before ~P13 in the mouse are associated with much more severe disease than loss after that time (refs. 45, 47, and Table 1). This period corresponds to the completion of renal development in the mouse and suggests that the timing of secondary events may influence disease severity in human ADPKD.

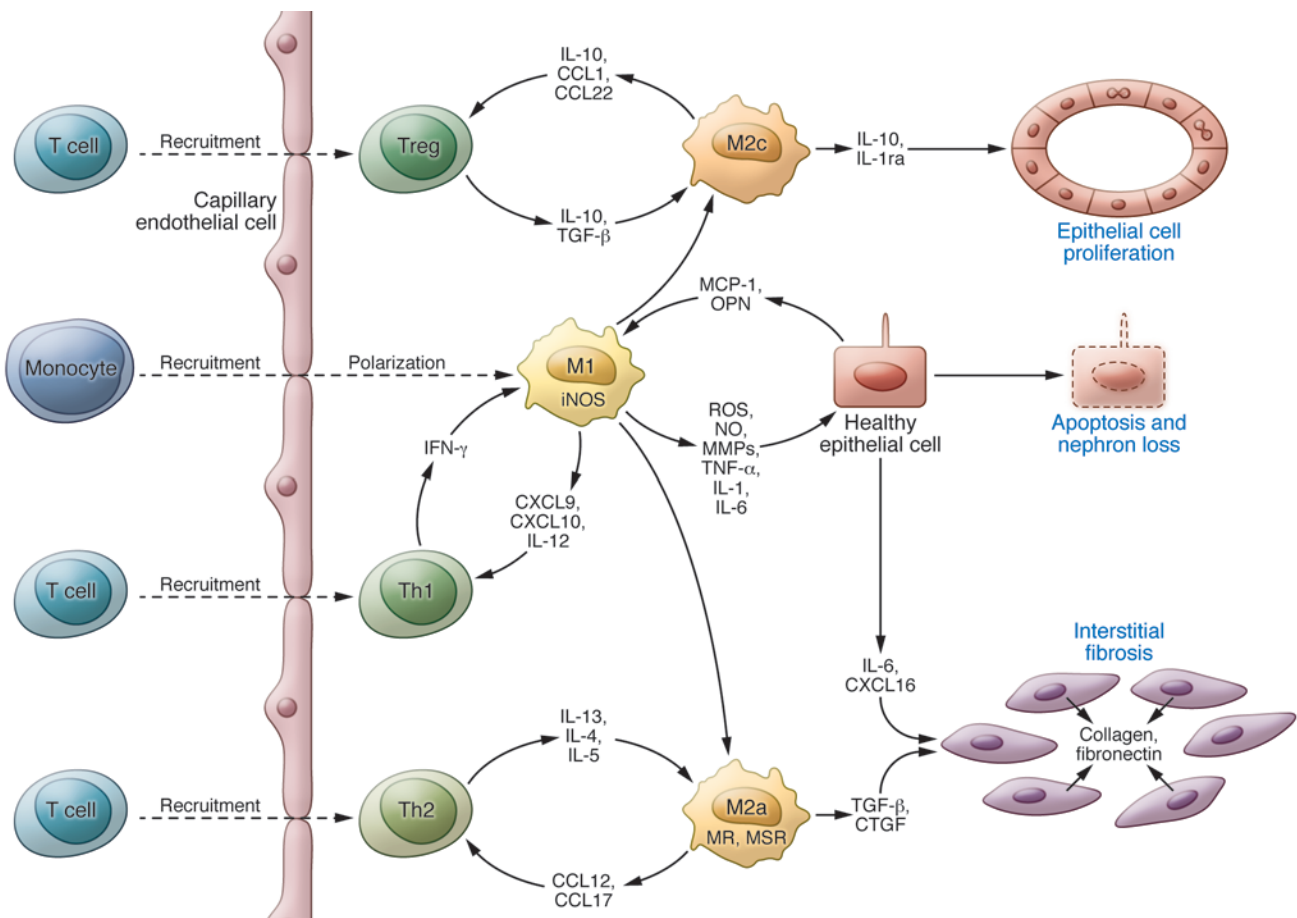
Structure and roles of the ADPKD proteins

PC2 (968 aa; ~110 kDa) is a six-transmembrane, Ca^{2+} -responsive cation channel of the transient receptor potential family (17, 48). PC1 (4,303 aa; ~600 kDa, uncleaved and glycosylated) is a receptor-like protein with a large ectodomain (3,074 aa) that comprises a number of domains involved in protein-protein and protein-carbohydrate interactions, including 16 PKD repeats with an Ig domain-like fold (18, 19). PC1 also has 11 transmembrane domains and a cytoplasmic tail. PC1 and PC2 are thought to interact via their C-terminal tails with the resulting PC complex (the precise ratio of PC1 and PC2 is still debated; refs. 49, 50) thought to play a role in intracellular Ca^{2+} regulation (48, 51, 52). Autoproteolytic cleavage of PC1 at the GPS domain, mediated by a larger GAIN domain, is an important step to form a functional protein (53–55). After embryonic development, the full-length protein is rarely seen, with approximately 130-kDa GPS C-terminal (CT) and two large N-terminal (NT) products; the NT and CT products are thought to stay associated after cleavage (36, 55). Enzyme treatments to remove glycosylation have demonstrated that the two NT products are glycoforms, one mature form that is transmitted through the Golgi and likely surface localized/secreted and one that may be retained in the ER (36, 56, 57).

A number of different localizations of the PCs have been proposed, including localization to the ER (likely a major site of PC2) or to the apical and basolateral membranes, or secretion on microvesicles (exosomes) (56, 58, 59). Although these localizations are likely,

there are several lines of evidence that primary cilia are central to pathogenesis in PKD, making it a ciliopathy (3). *C. elegans* PC homologs are localized exclusively to cilia, and in the Tg737 mouse model of PKD, disease is caused by mutation to IFT88 (60–62), a protein central to intraflagellar transport, which is required for constructing functional cilia. In addition, cyst development results from cilia loss in the kidney, and mammalian PCs localize to the cilium (63–65). The relationship between cilia and PKD is best understood in the syndromic ciliopathies involving PKD (66). For instance, many of the BBS proteins form a complex (the BBSome) that plays a role in trafficking membrane proteins to the cilium, while the MKS/JBTS proteins are proposed to complex at the transition zone at the base of the cilium and form a selective barrier that determines the protein composition of the cilium (66–69).

The precise function of the PC complex on the cilium is a hotly debated and unresolved issue. It has been suggested that the cilium functions as a flow detector, facilitating calcium influx when flow is present and curtailing Ca^{2+} import in response to a lack of flow or loss of the PC complex (70, 71). The nanomechanical properties of the multiple PKD repeats that form the majority of the PC1 ectodomain are consistent with this role (72, 73). This in turn alters multiple signaling pathways triggering characteristic PKD phenotypes, such as increased proliferation and altered secretion (Figure 1). However, there are questions whether PC1 and PC2 are the polycystins regulating the cilia Ca^{2+} compartment (PC1L1 and PC2L1 have been implicated), and whether changes in the cilium can have such global cytoplasmic effects (74). Recent experiments ablating cilia after the inactivation of *Pkd1* or *Pkd2* in the mouse kidney showed that the combined loss results in less severe cystic disease than loss of PC alone (44). This has been interpreted as the PC complex playing a role in regulating an as yet unidentified cilia-based signaling pathway that actively promotes ciliogenesis, although the role of cilia in regulating cell division also needs to be taken into consideration (3).

**Figure 3**

A model for the contribution of macrophages to PKD progression. Activation of signaling pathways and transcription factors (e.g., STAT3, NF- κ B) in cyst-lining cells stimulates the production and release of chemokines (e.g., MCP-1, osteopontin) attracting monocytes, promoting the polarization of invading monocytes and resident macrophages to a proinflammatory phenotype, and activating Th1 lymphocytes with further release of mediators and tissue damage. Opsonization of apoptotic cells by pentraxin-2 and secretion of IL-10 and TGF- β by immunosuppressive regulatory T cells promote the polarization of macrophages to a proproliferative phenotype, releasing antiinflammatory cytokines that induce cell proliferation. Incomplete epithelial healing, ongoing injury, and release of IL-4 and IL-13 by Th2 lymphocytes promote the polarization of macrophages to a profibrotic phenotype, releasing TGF- β and connective tissue growth factor (CTGF), which induces the differentiation of fibroblasts into collagen-secreting myofibroblasts.

A role for the polycystins directly in the vascular disease associated with ADPKD and the increased risk for developing ICAs has been suggested from expression analysis and inducement of vascular events in the *Pkd2*^{WS25/-} and the *Pkd1*^{nl} hypomorphic models (75, 76). A different role for PC1 and PC2 has also been suggested in the vasculature, whereby the ratio of the two proteins regulates pressure sensing, acting through stretch-activated ion channels (77). However, conditional mice generated with loss of PC1 in vascular smooth muscle cells and endothelial cells do not have a clear vascular phenotype (78).

Arrested tubular epithelial cell differentiation in PKD

PC1 and PC2 are dispensable during early stages of nephrogenesis (30, 31) but essential for differentiation of the tubular epithelium during late stages (45, 47) or for recovery from acute kidney injury (AKI) (46, 79). Both are expressed at high levels in murine renal tubules from E14 through P7; the expression of PC1 but not PC2 decreases thereafter (80, 81). The expression pattern correlates with initiation of cysts at ~E14.5 in the pars recta of proximal tubules, rapidly

extending to the cortical and medullary collecting ducts, in *Pkd1*^{null} mice (30). Cyst formation also occurs at ~E14.5 in *Pkd2*^{null} mice, with death by ~E16.5 due to cardiac malformations and failure (31). Cystogenesis occurs more slowly in mice with less aggressive disease caused by a reduction but not complete loss of PC1 (36). In this case, cyst formation parallels the rate of epithelial cell proliferation, which is high in proximal tubules during nephrogenesis but lower than in the distal nephron and collecting duct after maturation (82, 83).

Enhanced apoptosis accompanies increased cell proliferation in polycystic kidneys (84, 85), as occurs during renal development (86–88) and tubular regeneration following AKI (89, 90), where it is important for morphogenesis. An imbalance favoring proliferation over apoptosis contributes to the development of cysts, epithelial hyperplasia, and microscopic adenomas in PKD (91, 92), but enhanced apoptosis may be sufficient to reduce the risk for development of renal cell carcinoma (93). Recent data indicate that further enhancement of apoptosis within cyst linings in a *Pkd1* model is of value in decreasing cystogenesis (94).



Primum movens in PKD: relevance for therapy

Many genes that control proliferation and death during embryonic development (95–97) and tissue regeneration also control cystogenesis in PKD and are constitutively activated (proto-oncogenes) or inactivated (tumor suppressor genes) in cancer cells. These genes regulate a network of growth factors, growth factor receptors, signal transduction pathways, and transcription factors. Activation of proliferative pathways during development or regeneration of nontransformed cells elicits counteracting inhibitory processes to prevent aberrant cell growth and tumor development. In cancer cells, mutations that constitutively activate proto-oncogenes or inactivate tumor suppressor genes circumvent the counteracting measures (98). In PKD, mutations to the *PKD* genes result in persistent expression of developmental genes normally downregulated in mature kidneys and in failure to suppress cell proliferation (99, 100).

Exactly how mutations to *PKD1* or *PKD2* cause the pleomorphic cystic phenotype remains uncertain. This is important because treatments that target primary rather than downstream secondary mechanisms are likely to be more effective. Many hypotheses have been proposed, among them an aberrant cross-talk between intracellular calcium and cAMP signaling (refs. 101, 102, and Figure 1). PC2 is predominantly found in the ER/sarcoplasmic reticulum (SR) where it interacts with ryanodine receptors (RyRs) (103). In the heart, PC2 stabilizes RyR2 in its closed position. Loss of PC2 inhibition results in higher frequency of spontaneous calcium oscillations, reduced SR calcium stores, and heart failure in zebrafish (104). Opening of PC2 and RyRs is modulated by PKA phosphorylation (105, 106). Persistent catecholaminergic stimulation and PKA-induced hyperphosphorylation of RyR2 or RyR1 makes these channels leaky, depleting SR calcium stores and causing heart failure or muscle fatigue, respectively (107, 108). Sustained upregulation of cAMP/PKA signaling in PC1/PC2 cyst epithelial cells may result in leaky PC2 and RyR1 channels and account for the reduced ER calcium stores observed in these cells (75, 103, 109, 110). A review of treatment strategies, preclinical studies, and clinical trials targeting cAMP signaling (Figure 1) has been recently published (111). The following sections review other downstream mechanisms targeted for treatment in PKD.

Growth factors/receptors as targets for PKD treatment

Increased and/or altered expression of growth factors and receptors that regulate ureteric bud (UB) branching and collecting duct elongation in late stages of nephrogenesis (112–118) and promote tubular regeneration after renal injury (119–124) may play a role in PKD pathogenesis. These include members of the EGF family (EGF, TGF- α , heparin-binding EGF, and amphiregulin), HGF and IGF1, and their tyrosine kinase receptors, ErbB1 to ErbB4, MET, and IGF1R, respectively. In most cases, the mechanisms responsible for their upregulation in PKD are not known, but the activation of cAMP response element-binding transcription factor (CREB) and activator protein 1 enhances amphiregulin promoter activity and expression in PC1-mutated cells (125).

ErbB1, ErbB2, and c-MET kinase inhibitors and dietary-induced reduction of IGF1 limit disease severity in various rodent models of PKD (126–129). As in renal development, in which activation of ErbB1 and c-MET act cooperatively to regulate UB branching and mediate maintenance of the mature collecting duct (114), redundancy of these growth factors in PKD development may limit the efficacy of therapies that target only one receptor.

Signaling pathways as targets in PKD treatment

Many signaling pathways and transcription factors control the development and growth of polycystic kidneys. Because of redundancies, reciprocal reinforcements, and feedback loops, they should be viewed as components of a network rather than as individual axes. A reductionist representation of the network components, their interaction with cAMP/PKA signaling, and their effects on cell cycle and energy metabolism is shown in Figure 2.

Tyrosine receptor kinases and PKA activate the Src/Ras/Raf/MEK/ERK pathway. PKA inhibits Raf/MEK/ERK signaling in wild-type tubular epithelial cells, but in PKD or where intracellular calcium is reduced, PKA activates MEK/ERK in a Src/Ras/B-Raf-dependent manner (130). Src is an advantageous treatment target because it links several pathways activated in PKD (127). The Src/Abl inhibitor SKI-606 (bosutinib) retards cyst growth in nonorthologous models and *Pkd1* heterozygous mice (127, 131), and a phase II clinical trial is currently ongoing (NCT01233869). However, targeting of the Ras/Raf/MEK/ERK pathway has given inconsistent results, possibly due to redundancies with other pathways. PLX5568, a Raf kinase inhibitor, attenuated cyst enlargement in vitro and in a nonorthologous rat model but failed to ameliorate renal enlargement or function and promoted hepatic and renal fibrosis (132). Sorafenib, a Raf kinase inhibitor with activity against vascular endothelial growth factor receptor and platelet-derived growth factor receptor kinases, inhibited cAMP-dependent activation of B-Raf/MEK/ERK signaling, cell proliferation, and growth of ADPKD cysts in vitro (133) but increased cyst growth, cell proliferation, and ERK activation in *Pkd2* conditional knockout mouse livers (134). The MEK inhibitor PD184352 slowed cyst growth in a nonorthologous mouse model (135), but the MEK inhibitor UO126 had no protective effect in *Pkd1* conditional knockout mice (136). These and other studies underline the importance of employing orthologous models (Table 1) for preclinical testing.

There is overwhelming evidence for enhanced mTORC1 signaling in PKD cystic tissues, and preclinical trials of mTOR-inhibiting rapalogs (sirolimus and everolimus) in rodent models have been mostly encouraging. At doses and blood levels achievable in humans, sirolimus and everolimus were effective in a rat model of PKD affecting proximal tubules (137, 138) but not in a model of ARPKD affecting the distal nephron and collecting duct (139). Mice tolerate much higher doses and blood levels than rats and humans, and these high doses of rapalogs were consistently effective in orthologous and nonorthologous mouse models (140, 141). However, the results of clinical trials have been mostly discouraging (142–144) (NCT00346918; NCT00491517; NCT00414440), likely because blood levels capable of inhibiting mTOR in peripheral blood mononuclear cells do not inhibit mTOR in the kidney (145).

Several strategies may overcome the systemic toxicity and limited renal bioavailability of rapalogs. The targeting of sirolimus specifically to the kidney by conjugating it to folate was effective in reducing renal cyst growth and preserving kidney function without toxicity in a nonorthologous mouse model (141). Another approach takes advantage of the mechanism of action of the rapalogs. Phosphatidic acid, a phospholipase D product generated by the hydrolysis of phosphatidylcholine, is required for the association of mTOR with Raptor in mTORC1 and with Rictor in mTORC2. The rapalogs form a complex with FKBP12 that competes with phosphatidic acid for binding to mTOR. A recent study showed



that phospholipase D activity is high in PKD cells and that its inhibition decreases mTORC1 activity and proliferation (146). A third approach is the use of mTOR catalytic inhibitors that are more potent and durable inhibitors of mTORC1 compared with rapalogs and are currently being tested in rodent PKD models (147).

Increased expression of Myc in human and rodent PKD is associated with high rates of proliferation and apoptosis, despite increased expression of the antiapoptotic factor Bcl-2 and unaltered or reduced expression of proapoptotic p53 (148, 149). Proapoptotic effects of Myc, via Arf-mediated inhibition of MDM2 and activation of p53, safeguard against malignant transformation, but Myc may also exert antiapoptotic effects by increasing the expression and activity of the NAD-dependent deacetylase sirtuin 1 (SIRT1), repressing p53 activity (Figure 2 and refs. 150–152). Downregulation of Myc in a nonorthologous mouse model with a Myc antisense morpholino blunted the development of cystic disease both in the kidney and the liver (153). A more recent study has shown that Myc upregulates SIRT1 in *Pkd1*-deficient murine and human ADPKD cells and kidneys. The genetic elimination of SIRT1 or treatment with the pan-sirtuin inhibitor nicotinamide (vitamin B3) or the SIRT1-specific inhibitor EX-527 blocked epithelial cell proliferation, induced p53-mediated apoptosis, and delayed cyst growth in *Pkd1*-null or -depleted embryonic or postnatal kidneys (94).

Drugs that activate AMPK may be beneficial in PKD by inhibiting both cell proliferation and chloride-driven fluid secretion (Figure 2). Metformin inhibited the growth of Madin-Darby canine kidney cysts in collagen gels and cyst growth in metanephric organ cultures and *Pkd1* conditional knockout mice (154). Berberine, an AMPK activator used in traditional Chinese medicine, inhibited the growth of human and mouse ADPKD cystic cell lines (155). Thiazolidinediones, which inhibit mitochondrial respiratory complex I to elevate the AMP/ATP ratio, have been effective in several (156, 157), but not all (158), animal models of PKD. Interestingly, germ-free conditions that markedly inhibit the development of PKD (see below) protected from diet-induced obesity by enhancing AMPK signaling in skeletal muscle and liver (159).

Energy metabolism as a target for PKD treatment

Warburg described reprogramming of energy metabolism in cancer cells from oxidative phosphorylation to aerobic glycolysis (160–162). Dependency on aerobic glycolysis renders cancer and possibly cyst-derived cells more susceptible to death than control cells after glucose deprivation or interference with glycolysis (163–165). Observations that *Pkd1*^{-/-} mouse embryonic fibroblasts (MEFs) acidify culture medium faster than wild-type cells, have lower glucose and higher lactate and ATP concentrations, and have increased transcription of key glycolytic enzymes suggested a shift of energy metabolism toward aerobic glycolysis in PKD (163). Higher glucose uptake, lactate production, and ATP concentrations in the kidneys of conditional *Pkd1* knockout mice, along with transcriptional deregulation of key glycolytic enzymes in these kidneys and in ADPKD cysts from patients with *PKD1* mutations, provided further support. Glucose deprivation induced apoptosis in *Pkd1*^{-/-} cells (instead of autophagy as observed in wild-type cells), which was blocked by rapamycin. The amelioration of the cystic disease in conditional and hypomorphic *Pkd1* mice treated with the nonmetabolizable glucose analog 2-deoxyglucose (163) and in a nonorthologous rat model treated with the sodium glucose co-transporter inhibitor phlorizin (166) points to aerobic glycolysis as a potential treatment target in PKD.

Activated macrophages as a treatment target in PKD

Three decades ago it was noted that a germ-free environment inhibits cyst development in CFWwd mice (167) and in a model of PKD induced by nordihydroguaiaretic acid; the administration of endotoxin rescued the cystic phenotype (168). Chemokines and cytokines were found at high concentrations in cyst fluid and produced by cyst-lining epithelial cells (169). Recently, alternatively activated macrophages aligned along cyst walls were detected in polycystic kidneys from conditional *Pkd1* knockout and the *Pkd2*^{WS25/-} model (170, 171). Macrophage depletion inhibited epithelial cell proliferation and cyst growth and improved renal function. These observations led to the hypothesis that alternatively activated M2 macrophages contribute to cell proliferation in PKD, as has been described during development (172, 173), recovery from AKI (174, 175), and in cancer (176).

Macrophages appear early in developing organs to eliminate apoptotic cells and secrete trophic factors. In mice, macrophages appear within the renal interstitium between E11.5 and E12 (172) and wrap around tubules as they elongate and become intimately associated with the tubular basement membrane, expressing M2-associated genes found in alternatively activated macrophages. After development, macrophages continue to exert clearing as well as trophic functions essential for repair after tissue injury.

Macrophages differentiate into functional phenotypes depending on the microenvironment, but classifications based on *in vitro* conditions into classically activated M1 and alternatively activated M2a, M2b, and M2c do not adequately reflect the *in vivo* environment. To overcome this limitation, a classification according to the predominant macrophage role in the phases of inflammation (proinflammatory macrophages), epithelial healing (antiinflammatory macrophages), and fibrosis (profibrotic macrophages) has been proposed (177). Antiinflammatory and profibrotic alternatively activated macrophages likely contribute to the progression of PKD (Figure 3).

As described in cancer, a dialog between cystic cells and their inflammatory microenvironment may play an important role in the initiation and progression of PKD. In cancer, this dialog is under the control of two interacting transcription factors, STAT3 and NF- κ B (178, 179). While there is little information on NF- κ B in PKD, evidence supports an important role for STAT3 (180), which has high activity during renal development, following kidney injury, and in polycystic kidneys but is low in normal mature kidneys. Activated STAT3 in cyst-lining cells may, as described in cancer cells, promote transcription of chemokines, cytokines, and growth factors that in turn activate STAT3 on alternatively activated macrophages, resulting in a feed-forward loop that further promotes cystogenesis. Consistent with this, two STAT3 inhibitors, pyrimethamine and S3I-201, inhibited cyst growth in a neonatal and an adult *Pkd1* model (181). A similar protective effect was seen with curcumin, a compound with a broad spectrum of activity that also inhibits STAT3 (182, 183).

In conclusion

Many advances have been made in understanding the pathogenesis of ADPKD since the identification of the disease genes nearly 20 years ago. Despite some questions (44, 74), strong evidence supports a role of cilia and cilia-associated signaling in ADPKD, although the precise role that PC1 plays is not fully resolved. The development of orthologous mouse models that better match the disease course in ADPKD (Table 1) has aided preclinical testing,



although a case for orthologous rat models and possibly even larger animal models can be made. Therapies based on limiting levels of cAMP have shown the most promise so far (111), but it is likely that some of the defective processes and possible treatments highlighted in this Review will also play a therapeutic role. Combination therapies are likely to be necessary to preserve kidney function sufficiently to avoid ESRD and have a low adverse effect profile, as treatment will be needed over many years. Going forward, treatments more proximal to the primary defect are another avenue that should be explored, as in other genetic diseases (184).

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Address correspondence to: Peter C. Harris or Vicente E. Torres, Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA. Phone: 507.266.0541; Fax: 507.266.9315; E-mail: harris.peter@mayo.edu (P.C. Harris), torres.vicente@mayo.edu (V.E. Torres).

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