Polycystic kidney disease: genes, proteins, animal models, disease mechanisms and therapeutic opportunities

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An increased understanding of the genetic, molecular and cellular mechanisms responsible for the development of polycystic kidney disease has laid out the foundation for the development of rational therapies. Many animal models where these therapies can be tested are currently available. This review summarizes the rationale for these treatments, the results of preclinical trials and the prospects for clinical trials, some already in early phases of implementation.

Keywords: autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, ErbB tyrosine kinase inhibitors, mTOR inhibitors, polycystic kidney disease, vasopressin V2 receptor antagonists.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) are important causes of end-stage renal disease (ESRD), morbidity and mortality in children and adults. ADPKD has two disease loci, *PKD1* and *PKD2* [1–5] that encode the membrane glycoproteins, polycystin-1 and -2 (PC1 and PC2). The C-terminal tail of PC1 interacts with the corresponding region of PC2, G proteins and proteins tethering to the cytoskeleton [6–12]. PC2 is a Ca^{2+} permeable channel [13, 14]. ARPKD has one disease locus *PKHD1* [15–17]. The *PKHD1*-encoded protein, fibrocystin (FC), is also a membrane glycoprotein that may interact with the PCs and also regulate $[Ca^{2+}]_i$ (Fig. 1).

Despite different patterns of inheritance, clinical presentation and typical appearance of the kidneys, ADPKD and ARPKD have some similarities. Very early presentations of ADPKD may be indistinguishable from typical ARPKD, whilst the radiological appearance of ARPKD kidneys at later stages may resemble that of ADPKD kidneys. Both diseases are caused by mutations in proteins located in primary cilia [18–21] and both are characterized by increased rates of tubular epithelial proliferation and apoptosis. Whilst there is general agreement that the cysts in ARPKD derive from collecting ducts, the cysts in ADPKD may arise from all segments of the nephron and collecting ducts. Microdissection studies of ADPKD kidneys in the 1960s and 1970s, however, suggested that collecting ducts are diffusely enlarged and that collecting duct cysts are more numerous and larger than those derived from other tubular segments [22–24]. Studies of kidneys from ADPKD patients have found that most cysts at least 1 mm in diameter stain positively for collecting duct markers [25, 26]. Studies of viable *Pkd1* or *Pkd2* models with postnatal development of cystic disease have shown that most cysts originate from the collecting ducts and distal nephron [27–29]. Cultured epithelial cells from human ADPKD cysts exhibit a much larger cAMP response to 1-deamino-8-d-arginine vasopressin (DDAVP) and vasopressin
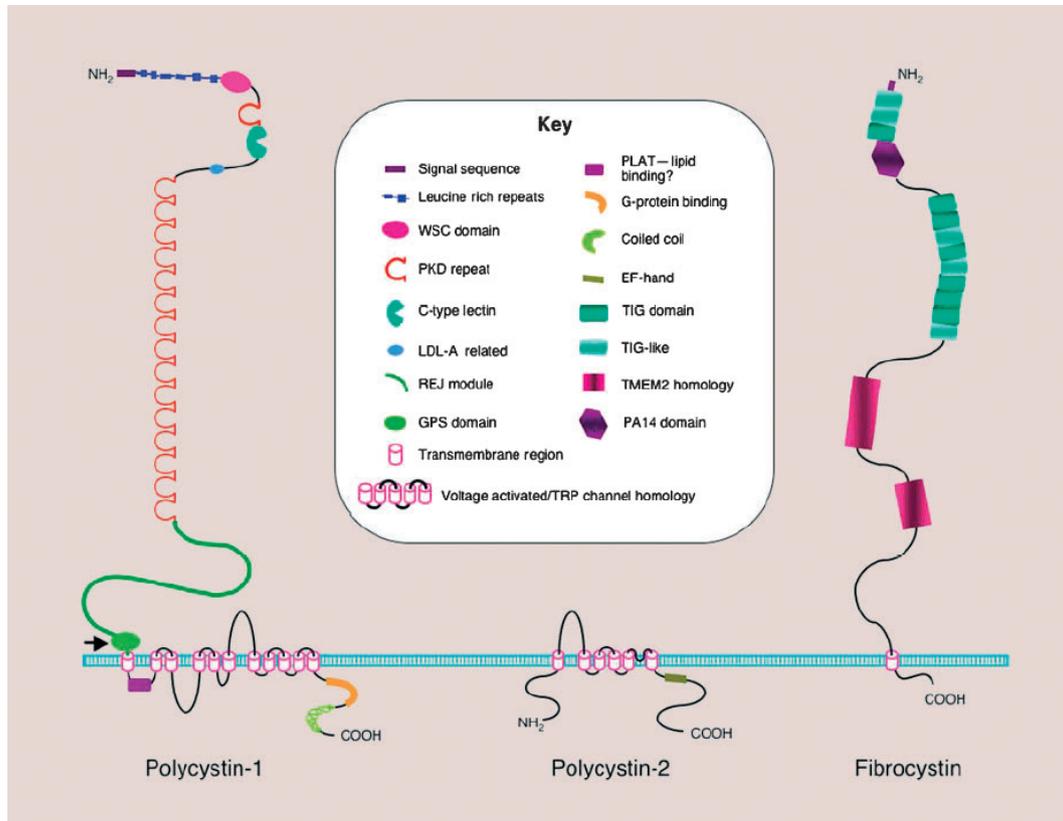
Fig. 1 Diagram of the autosomal dominant polycystic kidney disease proteins, polycystin-1 and 2 and the autosomal recessive polycystic kidney disease protein, fibrocystin. The protein motifs and domains found in these proteins are described in the key. The cleavage site in the GP5 domain of polycystin-1 is arrowed.

than to parathyroid hormone, consistent with a collecting duct origin [30]. A renal concentrating defect is a feature common to human ADPKD and ARPKD and animal models of PKD. This concentration defect is unique, because, contrary to other forms of nephrogenic diabetes insipidus, it occurs despite over-expression of vasopressin V2 receptor and aquaporin 2 (AQP2) [31–34].

Autosomal dominant polycystic kidney disease and ARPKD have both been considered to be recessive disorders at a cellular level, with two germline mutations in ARPKD or one germline and one somatic mutation in ADPKD. Complete inactivation of both alleles results in a severe phenotype in each case; perinatal death in human ARPKD and embryonic lethality in *Pkd1* and *Pkd2* mouse knockouts. In most cases of ARPKD, however, less than complete inacti-

vation of both alleles may produce a phenotype [28, 35–39]. Two recently generated mouse models homozygous for a *Pkd1* hypomorphic allele have shown that a somatic second hit is not always required for cystogenesis in ADPKD [28, 29]. Both models provide evidence that diminished expression of native PC1 below a certain threshold is sufficient to induce renal cystic disease. This threshold may be different for various phenotypic manifestations of the disease. In the vascular smooth muscle, reduction of the PC2 level to 50% of normal results in significant alterations in $[Ca^{2+}]_i$ and cAMP, increased rates of cell proliferation and apoptosis, increased contractility and increased susceptibility of the vasculature to hemodynamic stress [37, 40]. A *Pkd2* haploinsufficient state cannot mediate optimal smooth muscle contractility in Drosophila [38]. A 50% PC1 reduction in HEK 293 or MDCK cells by anti-sense oligodeoxynucleotides

induces cell proliferation and premature G1/S-phase transition [39]. Recently, *Pkd2* haploinsufficiency has been associated with an increased rate of cell proliferation in noncystic tubules of *Pkd2*+/- mice [41].

Currently there is no effective therapy for ADPKD or ARPKD. The availability of animal models genetically related to these diseases provides unique opportunities to further the understanding of cystogenesis and develop effective treatments. This article summarizes these recent advances and prospects for novel therapies.

Animal models of polycystic kidney disease

Numerous rodent genetic models of polycystic kidney disease are currently available (Tables 1 and 2). Some arose from spontaneous mutations, others by random mutagenesis, transgenic technologies or gene-specific targeting. These models have been helpful in the identification of disease-causing and modifier genes and elucidation of pathogenic mechanisms. Several of them, particularly the cpk, bpk, orpk, pcy, and *Pkd2*$^{WS25/-}$ mice and the Han:SPRD and PCK rats have been used to test potential therapies (Table 3).

The ideal model for this purpose should be genetically orthologous and reproduce the typical phenotype of human ADPKD or ARPKD. Few, if any, fully meet these requirements. The cpk and bpk mice, that resemble infantile ARPKD, are caused by mutations in *Cys1* and *Bicc1*, genes not known to be associated to human pathology [42]. The Han:SPRD heterozygous rat, frequently used as a model of ADPKD, is characterized by cysts from proximal tubules that do not resemble human ADPKD [43]. The PCK rat, a model caused by a mutation in the ARPKD gene ortholog *Pkhd1*, has a slow progressive phenotype with features of ADPKD and ARPKD [44]. The pcy mouse, caused by a mutation in *Nphp3*, orthologous to the gene mutated in adolescent nephronophthisis, has a slow progressive phenotype that resembles ADPKD [45].

Mice with heterozygous *Pkd1* or *Pkd2* mutations are often normal or have very few cysts (Table 2). The double heterozygote *Pkd2*$^{-/WS25}$ mouse with one *null* and one unstable allele (*WS25*), unlike heterozygotes of *Pkd1* or *Pkd2* knockouts, reliably develops renal cysts within 3 months. Three new *Pkd1* models with postnatal development of polycystic kidney disease

Table 1 Rodent models of polycystic kidney disease

Model	Inheritance	Renal pathology	Progression	Extrarenal pathology	Gene	Protein	Human homologue
Mouse							
Cpk	AR[a]	PT → CD	Rapid	BD[b], P[b]	*Cys1*	Cystin	?
Bpk	AR	PT → CD	Rapid	BD	*Bicc1*	Bicaudal C	?
Jcpk	AD/AR	GI/all tubules	Slow/rapid	BD	*Bicc1*	Bicaudal C	?
Orpk	AR	PT → CD	Rapid	BD, PD	*TgN737*	Polaris	?
inv	AR	PT → CD	Rapid	BA, P, SI	*Invs*	inv	*NPH2*
pcy	AR	CD, nephron	Slow	ICA	*Nphp3*	Nephrocystin-3	*NPH3*
jck	AR	C, OM	Slow	–	*Nek8*	Nek8	?
kat, kat^{2J}	AR	GI, PT	Slow	FD, MS, HC, An	*Nek1*	Nek1	?
Rat							
cy	AD/AR	PT	Slow	L[c]	*Pkdr1*	SamCystin	?
wpk	AR	PT → CD	Rapid	HC	*Mks3*	Meckelin	*MKS3*
pck	AR	CD, DN	Slow	BD	*Pkhd1*	Fibrocystin	*PKHD1*

See text for references or available on demand. [a]Focal dilatation of bile ducts in old heterozygotes. [b]In DBA/2J background. [c]Liver cysts in old females. AR, autosomal recessive; AD, autosomal dominant; PT, proximal tubule; CD, collecting duct; Gl, glomeruli; C, cortex; OM, outer medulla; DN, distal nephron; BD, biliary dysgenesis; P, pancreatic cysts or fibrosis; PD, polydactyl; BA, biliary atresia; SI, situs inversus; ICA, intracranial aneurysm; FD, facial dysmorphism; MS, male sterility; HC, hydrocephalus; An, anaemia.

Table 2 Mouse models with *Pkd1* and *Pkd2* targeted mutations

Strain	Mutation	Pkd1$^{-/-}$	Kidney	Pancreas	Blood vessels	Skeleton	Oedema	Heart	Pkd$^{+/-}$
Pkd1^{del34}	Exon 34 deletion	Lethal/perinatal	+	+	nc	+	+	−	Kidney/liver/pancreas
Pkd1^{L}	Exon 43–45 deletion	Lethal	++	++	Leak	nc	+	−	nc
Pkd1^{null}	Exon 4 deletion	Lethal	++	++	nc	++	+	−	Kidney/liver/pancreas
Pkd1$^{del17-21\beta geo}$	Exon 17-	Lethal	++	nc	nc	+	+	Cono-truncal	Kidney/liver
Pkd1^{-}	Exon 2–4 deletion with in-frame lacZ	Lethal	++	++	nc	nc	nc	nc	nc
Pkd1^{-}	Exon 2–6 deletion	Lethal	++	nc	nc	nc	+	Cono-truncal	nc
Pkd1^{-}	Exon 1 disruption	Lethal	++	++	nc	nc	+	−	Kidney/liver
Pkd1^{nl}	Exon 2–11 with aberrant splicing	Viable 40% 1 month 10% >1 year	+-++	+	Dissecting aneurysms	nc	nc	nc	Normal
Pkd1^{L3}	Aberrant transcription and/or splicing	Viable 50% 1–2 months 10% >1 year	+++	+	nc	nc	−	nc	Normal
Pkd1$^{cond/cond}$ *MMTV Cre*	Exon 2–4	Viable	+ (few)	nc	nc	nc	nc	nc	Normal
Pkd2^{-}	Exon 1 disruption	Lethal	+	+	nc	nc	+	+	Kidney/liver
Pkd2^{WS25}	Exon 1 duplication with disruption	Viable	+ adult	+ adult	nc	nc	−	−	Kidney/liver
Pkd2^{-LacZ}	Exon 1 deletion LacZ 'promoter trap'	Lethal	+	+	Randomization w/R pulmonary isomerism	nc	+	+	nc

See text for references or available on demand.

Table 3 Effectiveness of therapeutic interventions in animal models of polycystic kidney disease

| | Rats | | Mice | | | | | |
	Han:SPRD	PCK	orpk	bpk	cpk	pcy	$Pkd2^{-/WS25}$	$Pkd1^{-/-}$
Protein restriction	Yes	–	–	–	–	Yes	–	–
Soy-based protein	Yes	–	–	–	–	Yes	–	–
Flax seed	Yes	–	–	–	–	–	–	–
Betaine	No	–	–	–	–	–	–	–
Bicarbonate/citrate	Yes	No	–	–	–	No	No	–
Amiloride	Yes	No	–	–	–	–	–	–
Acetazolamide	Yes	No	–	–	–	–	–	–
Probucol	–	–	–	–	–	Yes	–	–
Vitamin E	No	–	–	–	–	–	–	–
Paclitaxel	No	–	No	–	Yes	No	–	–
Methylprednisolone	Yes	–	–	–	–	Yes	–	–
Batimastat	Yes	–	–	–	–	–	–	–
Lovastatin	Yes	–	–	–	–	–	–	–
PRARγ agonist	–	Yes	–	–	–	–	–	Yes
ARB	Yes	No	–	–	–	–	–	–
ACEI	Yes	No	–	–	–	–	–	–
c-myc antisense	–	–	–	–	Yes	–	–	–
EGF	–	–	–	Yes[a]	Yes[a]	–	–	–
EGFR TK inhibitor	Yes	No	–	Yes	–	–	–	–
ErbB2 TK inhibitor	–	Yes	–	Yes	–	–	–	–[b]
TACE	–	–	–	Yes	–	–	–	–
c-Src inhibitor	–	Yes	–	Yes	–	–	–	–
MEK inhibitor	–	–	–	–	–	Yes	–	–
Rapamycin	Yes	–	Yes[c]	Yes	–	–	–	–
V2R antagonist	–	Yes	–	–	Yes	Yes	Yes	–

See text for references or available on demand. Cysts are mostly of proximal tubular origin in the Han:SPRD rat and of distal/collecting duct origin in the other animal models. [a]Only when administered in the first week. [b]Inhibits collecting duct dilatation in $Pkd1^{+/-}$ mice. [c]In the orpk rescue mouse model.

have been recently reported. The $Pkd1^{nl}$ is a hypomorphic allele caused by the insertion of a neomycin cassette into intron 1 that yields only 13–20% normally spliced $Pkd1$ transcript [28]. Homozygous $Pkd1^{nl}$ mice develop polycystic kidney disease of variable severity, some dying at 1 month of age with massively enlarged kidneys, whilst others with less severe cystic disease survive to 1 year of age. The variability of the phenotype may limit its usefulness for therapeutic trials. The $Pkd1^{L3}$ is another hypomorphic allele caused by the introduction of a *loxP* site

and a *loxP*-flanked *mc1-neo* cassette into introns 30 and 34 of the $Pkd1$ locus that interferes with the transcription and/or splicing machinery and results in a low expression of PC1 [29]. The kidneys of homozygous $Pkd1^{L3}$ mice appeared normal at birth, became cystic and enlarged rapidly during the first 30 days, and gradually decreased in volume afterwards, a course that resembles ARPKD more than ADPKD. A $Pkd1$ mouse line with a floxed allele develops renal and hepatic cysts when crossed to animals expressing Cre or FLPe recombinase [46]. The described renal
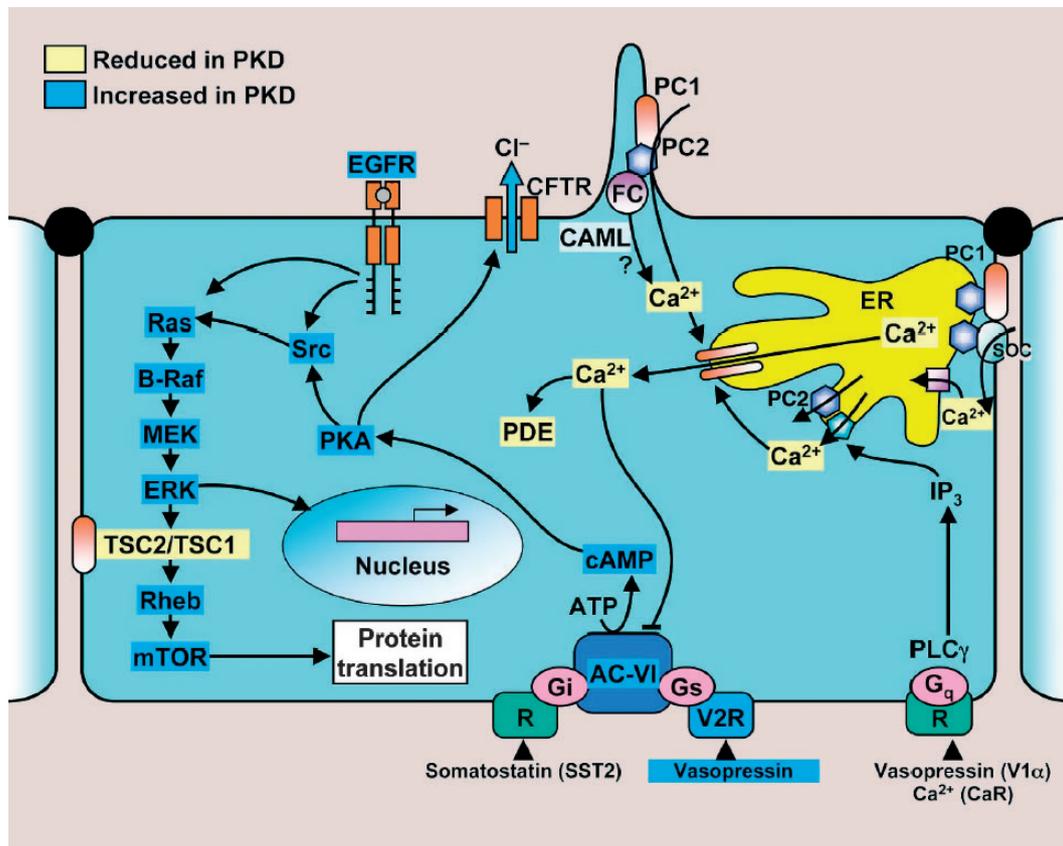
Fig. 2 Diagram depicting the hypothetical downstream effects of disrupting the polycystin pathway. In response to mechanical stimulation of the primary cilium, the polycystin-1/2 complex mediates Ca^{2+} entry into the cell which triggers Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum (ER). Polycystin-2 is also an intracellular Ca^{2+} channel that is required for the normal pattern of $[Ca^{2+}]_i$ responses to agonist stimulation of Gq-coupled receptors. Polycystin-2 physically interacts with TRPC1 and contributes to store operated Ca^{2+} channel (SOC) activity. The cellular content of cAMP is determined by the balance between activities of synthesizing adenylyl cyclases (AC) and catabolizing cAMP phosphodiesterases (PDE) that hydrolyse cAMP to biologically inactive noncyclic 5'-AMP. AC6 is the predominant AC in collecting duct principal cells. AC6 is stimulated by the α-subunit of heteromeric G-proteins (Gαs). It is inhibited by Gαi and by Ca^{2+}. PDE1 in collecting duct principal cells is activated by Ca^{2+}/calmodulin at Ca^{2+} concentrations achieved in response to agonist stimulation. Under conditions of relative Ca^{2+} deprivation cAMP stimulates ERK signalling in a protein kinase A (PKA), Src, Ras, and B-Raf dependent manner. Increased expression, mislocalization and activation of ErbB receptors may also contribute to ERK activation. Phosphorylation of tuberin by ERK may result in dissociation of tuberin and hamartin and activation of Rheb and mTOR.

phenotype with the promoters used so far is very mild and not optimal to test experimental therapies.

At present, the pcy and $Pkd2^{-/WS25}$ mice and the PCK rat appear to be the animal models best suited to test experimental therapies. Given the inherent limitations of available models and animal studies, it seems prudent to confirm observed benefits from experimental therapies in several models before considering clinical trials.

A common cystogenic pathway involving $[Ca^{2+}]_i$ homeostasis

There is general agreement that PC1 and PC2 physically interact and that PC2 is a Ca^{2+} channel that may be regulated by PC1 [13, 14, 47–49]. Whilst PC1 and possibly a small fraction of PC2 are in the plasma membrane, the majority of PC2 appears to be located in the endoplasmic reticulum. The interaction between PC1 and PC2 may occur at the plasma membrane,

between adjacent plasma membrane and endoplasmic reticulum (similar to the conformational coupling of transient receptor potential channels and IP3 receptors) or at both sites. Recent studies have focused on the localization of the PC complex in primary cilia and on its role in mediating Ca^{2+} fluxes in response to mechanical stimulation (Fig. 2). Cells isolated from mice that lack functional PC1 do not show the normal Ca^{2+} influx in response to physiological fluid flow [50]. It has been suggested that cells with only one *PKD* germline mutation are capable to sense luminal fluid shear similar to wild-type cells.

The contribution of the PCs to the regulation of $[Ca^{2+}]_i$ homeostasis likely extends beyond cilia. Overexpression of PC2 in LLPCK cells amplifies the Ca^{2+} release from intracellular stores in response to agonist stimulation [48]. A *Pkd2* haploinsufficient state (i.e. PC2 content half of normal) is sufficient to significantly reduce capacitative Ca^{2+} entry, sarcoplasmic reticulum Ca^{2+} stores and $[Ca^{2+}]_i$ in vascular smooth muscle cells [37]. Reduced PC1 and PC2 levels are accompanied by blunted $[Ca^{2+}]_i$ responses to platelet activating factor in unciliated B-lymphoblastoid cells isolated from ADPKD patients with *PKD1* or *PKD2* mutations [35]. Loss of PC2 localization to the mitotic spindles by knockdown of interacting mDia1 protein blunts agonist-evoked increases of $[Ca^{2+}]_i$ in dividing cells that lack cilia [36]. $[Ca^{2+}]_i$ is reduced in cyst-derived primary-cultured cells form PKD1 kidneys [51] and in collecting ducts isolated from $Pkd1^{+/-}$ mice (O. Devuyst, personal communication).

Compared with normal renal epithelial cells, which exhibit a well polarized mostly reabsorptive phenotype, very low rates of proliferation and apoptosis, and growth inhibition by cAMP, cyst-derived epithelial cells are characterized by abnormal targeting (e.g. apical localization of EGFR) and/or expression (e.g. overexpression of vasopressin V2 receptor) of receptors, predominantly secretory phenotype, increased rates of proliferation and apoptosis, and a proliferative response to cAMP. As lowering $[Ca^{2+}]_i$ in wildtype renal epithelial cells reproduces the altered proliferative response of the cyst-derived cells, whilst addition of Ca^{2+} to cyst-derived cells inhibits the mitogenic

response to cAMP, it seems likely that the dysregulation of the $[Ca^{2+}]_i$ homeostasis caused by mutations in the PCs or FC is responsible for this phenotypic switch [51, 52]. The underlying mechanisms have not been fully elucidated but likely involve the crosstalk of signalling pathways downstream from hormone and growth factor receptors controlling proliferation and survival.

Hormones bind to receptors on the plasma membrane coupled to heterotrimeric G proteins (GPCRs), composed of an α subunit with high affinity for guanine nucleotides and a tightly linked β and γ subunit dimer. When agonist binds to the receptor, GDP is released from the α subunit and replaced with GTP. This results in dissociation of the α subunit from the $\beta\gamma$ dimer, which are free to interact with effectors. Intrinsic GTPase hydrolyses the bound GTP to GDP, allowing reassociation of the α and $\beta\gamma$ subunits. G-proteins are classified by their α-subunits into Gs, Gi, Gq and G12/13 subfamilies. Gαs proteins stimulate and Gαi proteins inhibit adenylyl cyclases (Fig. 2). Gαq proteins stimulate phosphatidylinositol-specific phospholipases, leading to increased levels of intracellular Ca^{2+} and activation of protein kinase C. Gα12/13 proteins regulate Rho and cytoskeletal architecture. Growth factors bind to and induce dimerization and autophosphorylation of tyrosine kinase receptors (TKR) with generation of docking sites for signalling molecules, such as Ras and phosphatidylinositol-3-kinase (PI3K). Mitogen-activated protein kinase/extracellularly regulated kinase (MAPK/ERK) signalling cascades, containing three protein kinases acting in series [MAP kinase kinase kinases (MAPKKK), MAP kinase kinases (MAPKKs/MEKs) and MAP kinases (MAPKs/ERKs)], link Ras to cell proliferation. In the MAPK subfamily ERK1/2, Raf serine/threonine protein kinases (A-Raf, B-Raf, and C-Raf or Raf-1) function as MAPKKK [53]. Activated ERKs translocate to the nucleus and phosphorylate transcription factors that promote proliferation.

An increased renal accumulation of cAMP is a common feature to most, if not all models of this disease [31, 32, 54]. A number of mechanisms may be responsible (Fig. 2). PC1 may act as a Gi-protein-cou-

pled receptor [11]. A reduction in $[Ca^{2+}]_i$ could stimulate Ca^{2+} inhibitable AC6 [55–58] and/or inhibit Ca^{2+} dependent PDE1 [59–61]. AC6 is the main AC isoform and PDE1, along with PDE4, is the main PDE isoform in the collecting duct principal cells. Some PDE4 isozymes are phosphorylated and inhibited by ERK2 [62], which is known to be activated in PKD. Upregulation of the vasopressin V2 receptor, consistently found in animal models of PKD (cpk, pcy, $Pkd2^{-/WS25}$, and HNF1β knockout mice and PCK rat) [31–34] may play a role. A urinary concentration defect, a feature common to animal models and to human ADPKD and ARPKD, could increase the secretion of vasopressin, the main hormonal modulator of adenylyl cyclase activity in the collecting duct principal cells. Increased plasma vasopressin levels have been reported in ADPKD patients and in animal models of polycystic kidney disease (Refs [63, 64] and V. H. Gattone, personal communication). Vasopressin binds to two GPCRs (V2 and V1a) in principal cells. Binding to the G_s-coupled V2 receptor activates AC6, increases intracellular cAMP levels and activates PKA. Binding to the G_q-coupled V1a receptors stimulates phospholipase C, phosphoinositide hydrolysis and Ca^{2+} release from the endoplasmic reticulum, and may limit the V2-induced effects on water permeability [65].

There is also evidence for Ras/MAPK activation in polycystic kidney disease [66–70]. Apical expression and activation of the EGFR and ErbB2 receptors may contribute to this activation [71, 72]. Altered $[Ca^{2+}]_i$ can also cause Ras activation (e.g. inhibition of the Ras GTPase activating protein CAPRI by reduced $[Ca^{2+}]_i$), but evidence for this at present is lacking. Cyclic AMP, which is increased in polycystic kidneys, can either stimulate or inhibit MAPK/ERK signalling and cell proliferation depending on cell type and context [73–76]. The expression of B-Raf may play a key role in determining these effects. The B-Raf gene encodes multiple protein isoforms, with two main bands on Western blotting, a full-length 95-kDa and a truncated 68-kDa isoform that lacks the amino acids encoded by exons 1 and 2. Cells with high relative expression of 95 kDa B-Raf proliferate, whereas cells not expressing B-Raf or expressing only the short iso-

form are inhibited by cAMP [77–79]. The relative expression of these two isoforms depends on cell type and cellular density (predominantly 95 kDa isoform in subconfluent cells and 68 kDa in confluent cells). Prolonged incubation of wild-type principal cells in a low Ca^{2+} medium or in the presence of Ca^{2+} channel blockers increases 95 kDa B-Raf and allows cAMP activation of a B-Raf/ERK pathway in a PKA-, Src- and Ras-dependent manner [52]. This likely accounts for the stimulatory effect of cAMP on MAPK/ERK signalling, which can be reversed by Ca^{2+} ionophores or channel activators [80–82].

Additional pathways by which extracellular cues detected by the PC complex (including flow) may be transmitted to the nucleus have been proposed (reviewed in Ref. [83]). These include canonical and noncanonical Wnt, JAK/STAT and NFAT pathways. A cleavage event in the GPS domain, separating the extracellular region from the transmembrane part of the protein, may be important for activation of PC1 [84]. It has also been proposed that PC1 may activate transcription directly by cleavage at different sites and translocation of the C-terminal fragments to the nucleus, a process found in other transmembrane proteins and that may be regulated by flow [85, 86]. The role of these pathways in the pathogenesis of PKD remains to be better defined.

Treatment trials in animal models

Evolving insights into the molecular mechanisms of polycystic kidney disease have provided the rationale for preclinical trials in animal models of the disease [87]. Those published as full manuscripts or in abstract form are summarized in Table 3. Technical considerations, such as the requirement for a highly selective, efficient, and durable gene transfer to somatic cells, safety issues, and the observation that overexpression of *PKD1* results in a cystic phenotype cast doubt on the feasibility of gene therapy, at least for ADPKD in the foreseeable future. Treatments directed at reducing the rate of mutations (e.g. antioxidants) are based on the assumption that somatic mutations are important after birth. Low protein diets may help by lowering oxygen consumption, generation of oxygen-free radicals

and renal ammoniagenesis. Other dietary interventions, such as soy-based and flax seed diets, may have a beneficial effect by reducing arachidonic acid and PGE2 synthesis. Lovastatin, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers have a moderate protective effect in some animal models and are frequently used for the treatment of hyperlipidaemia and hypertension in ADPKD. The prominent role of epithelial cell proliferation has stimulated trials of antiproliferative agents, such as taxanes and c-myc antisense with satisfactory results only in certain animal models. PPARγ agonists are protective in two animal models, but their mechanism of action is not known. EGFR tyrosine kinase inhibitors, mTOR inhibitors, vasopressin V2 receptor antagonists and octreotide are currently the therapies best supported by preclinical studies and more likely to enter clinical trials.

ErbB receptor tyrosine kinase inhibitors

The Erb-B family of receptors is comprised of four members: ErbB 1 (Epidermal Growth Factor Receptor or EGFR), 2, 3 and 4. These receptors and their ligands (EGF, TGFα, amphiregulin and heparin-binding EGF) are important for cell proliferation and differentiation during development. On binding of a ligand, ErbB receptors dimerize and undergo autophosphorylation of tyrosine residues in the intracellular domain. This creates binding sites for signalling molecules leading to the activation of the Ras/MAP kinase signalling pathway and a proliferative response. ErbB receptors frequently form heterodimers. EGFR/ErbB2 heterodimerization has been shown to reduce EGFR complex internalization by endocytosis and contribute to its hyperactivity. Increased levels and activation of EGFR and ErbB2 have been reported in several human malignancies including renal cell carcinoma.

Evidence from a number of laboratories suggests that EGFR, ErbB2 and their ligands promote tubular epithelial cell proliferation and contribute to cyst formation. Although the renal expression of EGF is down regulated in cpk and pcy [88–90] mice and in Han:SPRD rats [91], the expression of TGFα, amphiregulin and heparin-binding EGF are increased in

proximal tubules of models of rapidly progressive recessive disease [92, 93]. EGFR and Erb-B2 (an EGFR-related tyrosine kinase receptor) are overexpressed, mislocalized to the apical membrane and capable of binding EGF and initiating a signalling cascade that results in cell proliferation [71, 94–96]. It has been suggested that increased shedding of growth factors 'upstream' into the urine could exert proliferative effects distally on abnormal collecting tubule epithelia. Renal cyst fluids from ADPKD, ARPKD, and murine and rat models of polycystic kidney disease contain EGF-like peptides in mitogenic concentrations [97–99]. Combined immunoprecipitation of EGF and TGFα abolishes most of the mitogenic activity. EGF and TGFα are cystogenic in vitro [100–102]. Transgenic over-expression of TGFα [103] or Erb-B2 [104] induce renal cyst development in mice. Expression of TGF-α as a transgene accelerates the progression of polycystic kidney disease in pcy mice [71]. Genetic and/or pharmacological (EKI-785, EKB-569) inhibition of EGFR tyrosine kinase activity in vitro and/or in vivo markedly inhibit the development of cystic disease [105, 106] in the bpk and orpk mice, two models of rapidly progressive recessive disease. The protective effect is more moderate in the Han:SPRD rat [107]. A selective Erb-B2 tyrosine kinase inhibitor (HKI-272) inhibits cystogenesis in the PCK rat [108], whereas EGFR tyrosine kinase inhibitors have been ineffective [109], possibly because EGFR is not overexpressed in the kidneys of this animal model. The administration of an Erb-B2 tyrosine kinase inhibitor (AG825) to $Pkd1^{+/-}$ mice has been reported to reduce cystic expansion as determined by stereological measurement of collecting tubule diameters [110].

Other small molecules targeting kinases activated downstream from ErbB have also been effective. PD184352, a MAPKK/MEK inhibitor, had a protective effect in pcy mice. WY-606, a Src inhibitor, inhibited cystogenesis in bpk mice and PCK rats [111]. The therapeutic potential of ErbB and other kinase inhibitors in the treatment of PKD deserves further consideration. Whether these compounds can be safely and effectively administered over decades, as it would be required for the treatment of this disease, remains an important question.

mTOR inhibitors

Tuberous sclerosis is a disease caused by mutations in one of two genes, *TSC1* and *TSC2* encoding for hamartin and tuberin. *TSC2* is adjacent to *PKD1*. Patients with the contiguous *PKD1-TSC2* gene syndrome exhibit a more severe form of polycystic kidney disease than those with ADPKD alone [112]. This observation suggests a convergence of signalling pathways downstream from PC1 and tuberin. Hamartin and tuberin physically interact and together function as a GTPase activating protein that maintains Rheb (Ras homologue enriched in the brain) in an inactive GDP bound state and inhibit downstream signalling from Rheb via mTOR (target of rapamycin). Phosphorylation of tuberin by ERK causes dissociation from hamartin and activation of Rheb and mTOR and may explained the activation of mTOR observed in polycystic kidneys [113]. Tuberin has also been shown to interact with PC1 and may be important for its targeting to the plasma membrane [114, 115]. Studies in three rodent models of PKD have shown that rapamycin significantly retarded the rate of cyst expansion and protected renal function [115–117]. These observations have led to clinical trials of rapamycin and everolimus in ADPKD, now in early phases of implementation [118].

Vasopressin V2 antagonists

The vasopressin effect (via V2 receptors) on adenylyl cyclase in principal cells, the collecting duct origin of cysts in ADPKD, ARPKD and nephronophthisis, and the cystogenic effect of cAMP provided a strong rationale for preclinical trials of vasopressin V2 receptor (VPV2R) antagonists. Encouraging results with the VPV2R antagonist OPC31260 were initially reported in the cpk mouse, a murine model of rapidly progressive PKD [33]. Subsequent studies showed that OPC-31260 lowered renal cAMP and markedly inhibited disease development and progression in the three animal models orthologous to human PKD (PCK rat, ARPKD; *pcy* mouse, adolescent nephronophthisis; *Pkd2*$^{WS25/-}$ mouse, ADPKD). As OPC-31260 is a weak antagonist of the human VPV2R, additional studies were performed to determine whether tolvaptan (OPC-41061), a

potent and selective human vasopressin V2 receptor antagonist [119], is also capable of inhibiting the development of PKD. Tolvaptan, like OPC-31260, significantly lowered the renal cAMP levels, kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices and inhibited Ras and ERK activation. As previously observed with OPC-31260, the administration of tolvaptan did not inhibit the development of fibropolycystic liver disease, consistent with the absence of vasopressin V2 receptors in the liver. A recent study has shown that high water intake by itself also exerts a protective effect on the development of polycystic kidney disease in PCK rats likely resulting from the suppression of vasopressin.

The renal selectivity (V2 receptor expression restricted to collecting duct principal cells and endothelial cells) and apparent safety of these drugs in preclinical and clinical studies (for disorders of water retention, such as congestive heart failure and cirrhosis) makes them attractive for clinical trial in ADPKD. Preliminary results of phase II clinical trials have shown that tolvaptan can be administered safely to and is well tolerated by patients with ADPKD and a phase III clinical trial is scheduled to start later this year.

Octreotide

Somatostatin is a cyclic 14 amino acid peptide secreted by pancreatic islets (D cells), gastrointestinal tract, nervous system and thyroid gland. There are five known somatostatin receptor subtypes named sst1 to sst5. The sst2 receptor is a Gi-coupled receptor present in both renal tubular epithelial cells and cholangiocytes. It inhibits cAMP generation in Madin–Darby canine kidney (MDCK) cells and in microdissected rat medullary and cortical collecting ducts and to antagonize the effects of vasopressin in the toad urinary bladder and dog collecting dugs in a manner consistent with inhibition of basal and hormone-stimulated adenyl cyclase. Furthermore, it inhibits chloride secretion in the shark rectal gland in a way suggesting inhibition of adenylcyclase and of post-cAMP events, as well as an inhibition of secretion stimulated by agents that do not generate cAMP. Based on these observations and the described effects of cAMP on cell proliferation and fluid secretion

in polycystic kidneys, a small randomized, cross-over, placebo controlled trial with a long-acting somatostatin analogue (Octreotide-LAR) was performed and found to be effective in slowing renal volume expansion measured by CT [120].

Somatostatin sst2 receptors are also present and somatostatin and its analogues have been shown to inhibit elevated cAMP levels and to decrease fluid secretion and cell proliferation in cholangiocytes. To confirm the preliminary observations on renal growth in this small ADPKD clinical trial and to test whether somatostatin may also be effective in the treatment of polycystic liver disease, a recent study in PCK rats has assessed the effects of octreotide on cAMP levels and the rate of cyst growth and expansion in isolated bile ducts *in vitro* and on hepatic and renal cyst formation *in vivo* [121]. Octreotide markedly reduced the cAMP levels and prevented the cystic expansion of bile ducts in a 3-D culture system. The administration of octreotide *in vivo* significantly lowered cAMP levels in bile ducts and in serum and significantly reduced liver and kidney weights, cyst volumes and fibrosis. These observations provide a strong rationale for further consideration of somatostatin analogues in the treatment of polycystic kidney and liver disease, particularly considering that this class of drugs is already being used for long-term treatment of multiple endocrine tumours with modest side effects.

Clinical trials

In planning for clinical trials for ADPKD, the utilization of renal function as the primary outcome measure immediately becomes an issue. Decades of normal renal function, despite progressive enlargement and cystic transformation of the kidneys, characterize the natural history of ADPKD. By the time the GFR starts to decline, the kidneys are markedly enlarged, distorted and unlikely to benefit from any potential treatment. On the other hand, early interventional trials would require unrealistic periods of follow-up if renal function were to be used as the primary outcome measure.

To address this problem the NIH funded a Consortium for Radiologic Imaging Studies of PKD (CRISP)

to validate surrogate markers of disease progression [122]. The results of this longitudinal study of 241 patients that included magnetic resonance (MR) measurements of total kidney and cyst volumes and measurements of GFR have been recently published. The results of this study indicate that the kidneys grow in nearly all the patients, but the rate of growth varies markedly from patient to patient. The study also shows that the rate of renal growth is a good predictor of functional decline and justifies the utilization of kidney volume as a surrogate marker of disease progression. A number of clinical trials are currently using kidney volume as the primary outcome measure.

HALT-PKD is a large clinical trial sponsored by the NIH to ascertain the value of optimizing renin-angiotensin blockade is currently on going. It consists of two studies, study A in patients with CKD stage 1 or 2 and study B in patients with CKD stage 3. Study A has a two by two design and compares ACE inhibitors alone versus ACE inhibitors and ARBs and two levels of blood pressure control. The primary outcome is change in kidney volume measured by MR. Study B compares ACE inhibition and combined ACE inhibition and angiotensin blockade. The primary outcome measure is doubling of serum creatinine, ESRD or death. Because of the importance of hypertension and cardiovascular morbidity and mortality in ADPKD, secondary outcome measures of HALT include LV mass and RBF measured by MR.

Other ADPKD clinical trials, now ongoing or in early phases of implementation (tolvaptan, octreotide, sirolimus and everolimus) are also using kidney volumes as primary outcome measures.

Conflict of interest statement

No conflict of interest was declared.

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